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Liquid crystals and emulsions in the formulation of drug carriers

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Abstract

Targetting an encapsulated drug towards a diseased organ, with the possibility to control its release at the right place and at the right time, is one among numerous questions asked to the new nanotechnologies. We present a partial answer with the example of recently elaborated lipidic nanocapsules. Liquid crystals play a role in the preparation techniques, and possibly in the delivery mechanisms of the active principle in situ. We also know that liquid crystalline behaviours are involved at the membrane level and are essential in the cell machinery. Drug nanocarriers could find their way, as do viruses, in the liquid crystalline context of cell organization. ***To cite this article: P. Saulnier et al., C. R. Chimie 11 (2008).***

Résumé

Cibler un médicament encapsulé vers un organe malade, avec la possibilité de contrôler sa libération à l'endroit précis et au juste moment, est l'une des questions posées aux nouvelles nanotechnologies. Nous présentons une réponse partielle, avec l'exemple de nanocapsules lipidiques récemment élaborées. Les cristaux liquides jouent un rôle dans les techniques de préparation et peut-être dans les mécanismes de libération du principe actif in situ. Nous savons aussi que des comportements cristallins liquides sont à l'œuvre au niveau des membranes et dans la machinerie cellulaire. Les nanovecteurs de médicaments pourraient ainsi trouver leur chemin comme le font les virus, dans le contexte cristallin liquide de l'organisation cellulaire. ***Pour citer cet article : P. Saulnier et al., C. R. Chimie 11 (2008).***

Keywords: Nanoparticles; Liquid crystals; Phase inversion; Bicontinuous systems; Fast dilution

Mots-clés : Nanoparticules ; Cristaux liquides ; Inversion de phase ; Systèmes bicontinus ; Dilution rapide

1. Introduction: drug administration at the right place and time

The local delivery of medicaments at precise times and according to an optimized kinetics [1,2] is a very ancient dream, which was clearly expressed some 30 years ago at least [3,4]. This perspective becomes today one of the main challenges in pharmaceutical research [1,2,5,6]. The pharmacologically active molecules must be present at the level of cells and tissues involved in a disease, and only there, if possible, to avoid side effects and further complications. This requires the preparation of microscopic *drug carriers* [6] easily

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dispersed in the living body, insuring the protection of the active molecules, and also their controlled release just before attachment to the target cells, so that the endeavour is simply considerable [1,6].

Actually, there are very different purposes for drug encapsulation at the micrometric or nanometric scales, and each one must be considered separately. For instance, encapsulation modifies and generally improves *bioavailability* of medicaments after administration, with smaller concentrations observed over a much longer period, and this is better accepted by the organism. It is worth remembering that preparation methods could modify the chemistry and the activity of the drug itself, and precise tests are required.

To entrap an active principle within an envelop is a protection for the drug itself, against defenses of the organism, but encapsulation also is a protection for organs themselves, against the side effects of the drug. Particle destruction by cells is frequent and occurs at different times after administration, for instance in the liver, by the highly phagocytic Küpffer cells [1]. Protection against such cells might be improved by an appropriate coating of particles, which make them stealthy or ''furtive'', when covered by hydrophilic polymers such as polyethylene glycols, for instance. But great problems remain, and for instance the creation of suitable ligands able to link particles to target cells, with a good efficiency, even when particles are stealthy, and tests are required, not simply in cell cultures, but within the living organism. Note that solutions exist, since viruses perdure, owing to the fact that some of them find their target cells, despite the immune system and other defenses.

In this research program, the main advances came from colloid physics and chemistry [1,5,6]. Among colloids, *liquid crystals* are well-defined states of matter, structurally and thermodynamically, with sharply defined transitions, first order, or second order, but well detected. These liquids are anisotropic at rest, due to a spontaneous alignment of molecules, for reason of least encumbrance, i.e. a minimization of excluded volumes, whereas molecules diffuse as in liquids. Liquid crystals are known for applications in electronic devices, such as displays in computers, for instance.

What is less known is the presence of liquid crystals in living cells, in membranes, in chromosomes and in extracellular compartments of tissues, where ordered liquid secretions are essential in the first differentiation steps of extracellular matrices, as recalled in several contributions of this issue. Liquid crystals are present in large subdomains of colloidal textures, emulsions, froths and water–lipid systems. Liquid crystals also are involved in essential steps of drug carriers' preparation [10], in the form of micro- or nanoparticles, and we shall consider the case of lipid nanocapsules obtained by the phase inversion method.

2. Several types of particles in pharmaceutical research

Polymers and amphiphilic components are the main ingredients of drug carriers, and several kinds can be recognized: matrices, micro- and nanospheres, micro- and nanocapsules, liposomes, etc.

Hydrophilic active principles can be incorporated in *matrices* of biodegradable polymers or block-copolymers such as PLA or poly-L,D lactide, or $[-O-C^{*}H(CH_3)-CO-]_n$; PGA or poly-glycolide, or $[-O-CH_2-CO-]_n$; and PLAGA or poly-D,L lactide-*co*-poly-glycolide; all these polymers have two extremities, an acid or an alcohol. Small crystals of the active principle can be suspended by mechanical stirring in these polymers dissolved in dichloromethane, and coacervation is obtained by a progressive addition of an inducer, which is another polymer, a silicone oil or a petroleum ether for instance [6]. These polymer matrices separate in the form of particles, either microspheres, or nanospheres, according to their sizes.

If the active principle is hydrophobic, an organic solution of the drug and of the polymer is prepared and emulsified in water, with a tensioactive agent, under mechanical stirring. Solid microspheres of the polymer matrix are obtained after solvent evaporation.

Similar polymers often serve to prepare *capsules* with an outer wall and one or several cavities filled with oil or water for instance, according to the solubility of the drug. The main techniques to prepare these drug carriers are emulsions of oil droplets in water (o/w) or the reverse (w/o), and multiple emulsions, for instance an emulsion of oil drops in water, which in turn is emulsified in oil (o/w/o) or the reverse (w/o/w). One can prepare the corresponding micro- or nanocapsules, with polymers in one compartment and drug in the other one. The polymer is stabilized into a durable capsule, but remains biodegradable [6].

Among well known drug carriers, *liposomes* are produced in general by sonication of lamellar phases of amphiphilic compounds in presence of water [3–5]. Amphiphilic molecules show two parts in general, one hydrophilic and one hydrophobic. The main example is that of phospholipids in cell membrane, usually extracted from egg yolks or soybeans. Most proteins in cell membranes are amphiphilic also. Purely synthetic examples of amphiphilic compounds are the

innumerable surfactants produced by industry, and also diverse polymers associating blocks of different solubilities. Amphiphilic molecules form one or several types of micelles, spherical, elongated, direct or inverse, and various phases often complex, occupying defined areas in phase diagrams, but some phases cannot exist for diverse reasons, often steric, the molecular shape being not always compatible with the geometry of such assemblies. Bilayers are frequent and form different systems such as simple or concentrically enclosed vesicles and many other sets of concentric cylindrical bilayers in myelin figures. The vesicles, separated or enclosed, correspond to liposomes, and serve as carriers of water-soluble drugs lying in vesicles or between bilayers, whereas amphiphilic drugs arrange within the bilayers themselves.

3. Difficulties encountered in the preparation of drug carriers

We see that most particles and liposomes are produced by techniques which require high energies due to mechanical stirring or sonication, and use a lot of organic solvents. They must be eliminated, but extraction techniques based on diffusion or evaporation are long and difficult, with a cost of energies which is very high. The elimination of solvents remains far from complete and new techniques were devised to avoid these points in microparticle preparation, spray-drying for instance [6]. Aerosols are prepared by nebulization of solutions and droplets dried in warm air. The resulting particles are separated from air charged with solvent, but the involved energies remain considerable also and air needs to be purified.

Progress was expected from another technique, when an inert gas is placed under supercritical conditions, and continuously transformed into a liquid, which behaves as a solvent for active principles and polymers, but the required energies by such techniques and their costs remain very high. We have some hopes to improve the situation by the production of a new type of lipid nanocapsules, where liquid crystals play an essential role in preparation methods and could offer cheaper costs.

4. Phase inversion techniques and preparation of lipidic nanocapsules

We studied several examples of lipidic nanocapsules, but here we only consider two of them. In the first one, we used an oil which is a by-product from petroleum, a mixture of saturated hydrocarbons, known under names such as vaselin oil or paraffin (from Cooper, Melun, Fr.). This oil was emulsified under magnetic stirring, in salted water (NaCl), by a non-ionic surfactant C18E6, which is a polyethylene glycol stearate $HO-(CH_2-CH_2-O)_6-OC-C_{17}H_{35}$, (from Stéarinerie-Dubois, Boulogne, Fr.). This milky emulsion of oil in water, first prepared at room temperature, was further heated progressively to transform into a transparent one and finally to give another milky emulsion, but inverse with water droplets in oil. This was observed in a thermostated stage, Mettler FP 52, by simple light microscopy (Fig. 1), in natural light or between crossed polars.

At room temperature, the oil droplets were dispersed in water, well visible between slide and coverslip, in the microscope, as shown in Fig. 1a. They disappeared by heating (Fig. 1b) and transformed into the inverse phase (aqueous droplets in oil, as in Fig. 1d), but we had two situations:one passes either directly from Fig. 1b to d, or there is an intermediate phase, with myelin figures differentiated in the course of the inversion phase, as shown in Fig. 1c, in natural light (c_1), or between crossed polarizers (c_2); myelin figures vanished progressively before the transformation into the inverse phase.

In Fig. 2, we show also two situations for the conductivity measurements. Passing from the situation of an oil in water (o/w) to the reverse one (w/o), we observed a global decrease of conductivity, which was reversible and the curve was simply sigmoidal, when water and oil percentages were equal with a 5% proportion of surfactant. On the contrary, for a water/oil ratio (WOR) of 40%, and a 10% proportion of surfactant, there was first a resistivity peak, followed by a conductivity one. The resistivity peak was shown to correspond to the presence of a lamellar phase, some 60 years ago by Winsor [13,14], in comparable situations. The proportions of ingredients were different in the two cases, and transition temperatures also, as in Fig. 2a and b. In our preparations, water evaporates at the periphery of the coverslip and we do not know precise compositions. But in all cases we pass from oil droplets in water to the reverse.

At the phase inversion, when the system is transparent, the oil droplets join and form a continuum interlaced with the water phase, which itself remains continuous for a certain time, so that, in the absence of myelin figures, the system is said to be bicontinuous. Later on, with a further temperature increase, the water phase disjoins into separate droplets, whereas continuity is maintained within the oily phase. The reverse transformations are observed when temperature is

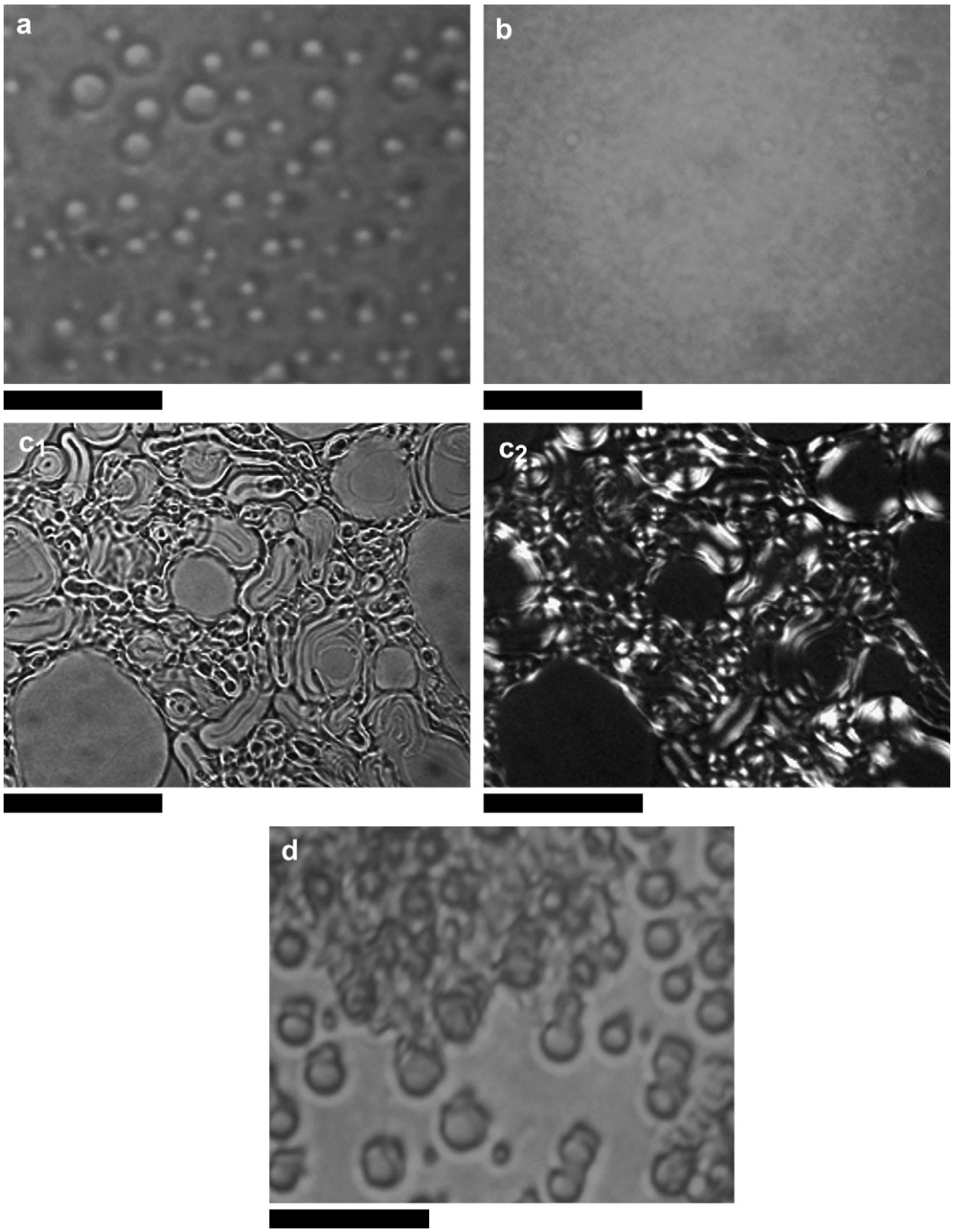

Fig. 1. Successive steps in the transformation of an emulsion of paraffin oil droplets in salted water, in presence of the C18E6 surfactant. (a) Oil droplets in water at room temperature; (b) transparent emulsion in the first stage of the inversion phase at ?°C; (c) myelin figures observed during the inversion phase, either in natural light (c_1) or between crossed polars (c_2); (d) inverted emulsion of water droplets dispersed in an oil continuum.

progressively decreased, and several thermal cycles can be repeated, but conductivity curves maintain their shape, though they are slightly shifted toward lower temperatures [12].

To obtain our lipidic nanocapsules, we heated the initial emulsion of oil droplets in water up to the inverted phase and repeated *three thermal cycles* from the direct emulsion to the inverse one, and we came

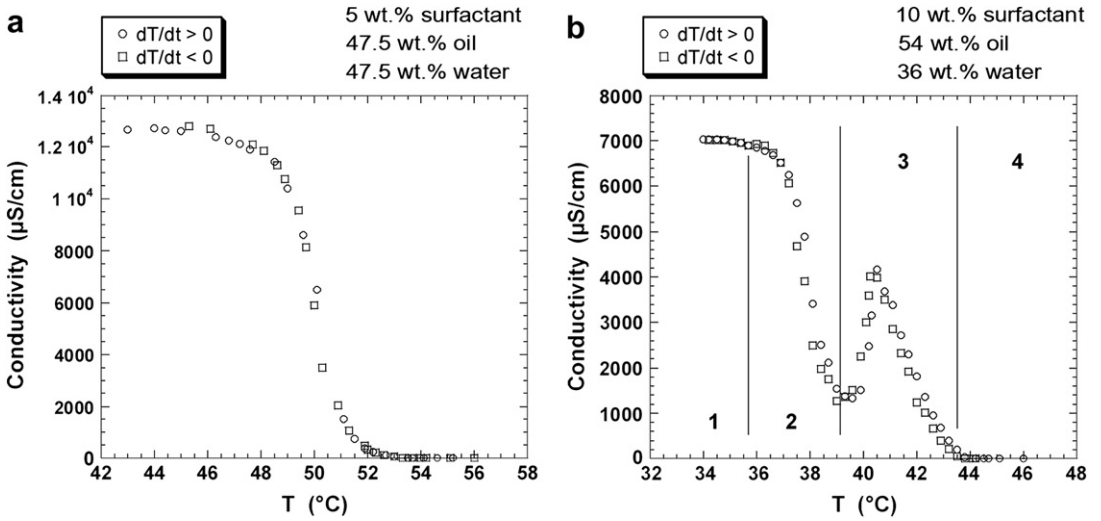

Fig. 2. Conductivity measurements plotted against temperature T (°C), in an emulsion of oil droplets in water (o/w) at room temperature, transforming into an inverse emulsion (w/o), when temperature is increased ($dT/dt > 0$) or decreased ($dT/dt < 0$). Depending on the water/oil ratio (WOR) and the proportion of surfactant, the curve is either sigmoid (a) or presents a peak (b). Four intervals (1–4) can be distinguished in case (b), and correspond to the direct emulsion (1), the phase inversion (2, 3) and the inverse emulsion (4), and the presence of a peak in (3). Courtesy of Dr. N. Anton.

back to the transparent bicontinuous phase. Then, we diluted rapidly the system by cold water, close to 0 °C and the result was a set of nanocapsules with a lipidic core, which sedimented slowly, forming a rigid and gel-like precipitate, translucent and bluish. This gel made of separate but strongly adhering nanoparticles, without coalescence, was stable for months (and years possibly).

We studied another emulsion associating an *oil*, the Labrafac® WL 1349 made of triglycerides of capric and caprylic acids, with an average molecular weight of 512; the Lipoid® S75-3, a soybean *lecithin*, made of 69% of phosphatidylcholine and other phospholipids; another surfactant which was the Solutol® HS 15, a mixture of polyethylene glycol 660 hydroxystearate (C18E15), and free polyethylene glycol 660 [7,8]. This preparation differed from the preceeding example, by the use of natural triglycerides, rather than a petroleum by-product, longer chains of PEG and the presence of free PEG chains. We prepared several mixtures with different compositions for the exploration of the phase diagram, but we recommend the following ones, as being optimal for the preparation of lipidic nanocapsules: Labrafac® (1.028 g), Lipoïd® (0.075 g), Solutol® (0.846 g), NaCl (0.089 g) and pure water (2.962 g). This mixture was prepared under magnetic stirring, at room temperature, to obtain an emulsion of oil in water. After heating at a rate of 4 °C/min, under magnetic stirring, we observed a short interval of tranparency at temperatures close to 70 °C, and obtained at 85 °C the inverted phase (water droplets in oil). Then three cycles of cooling and heating were applied between 85 and 60 °C at the same rate of 4 °C/min, around the phase inversion zone, and finally a fast dilution in cold water at a temperature close to 0 °C gave a transient suspension of sedimenting nanocapsules, transforming into a kinetically stable gel [7–9].

Ternary diagrams of ‘‘feasibility’’ were established for both types of emulsions in the space of relative concentrations (oil, surfactant and water) at room temperature, and we also reported the phase transitions by changing temperature. The useful phases were characterized by several techniques: electric conductivity, differential scanning calorimetry, rheology, interfacial tensiometry (Langmuir balance and pendent drop), photon correlation spectroscopy and diverse microscopic techniques, atomic force microscopy, transmission electron microscopy (TEM and cryo-TEM) and also polarizing microscopy for birefringent phases.

The production of reproducible lipidic nanocapsules strongly depended on composition of the initial emulsion of oil droplets in water [9]. We explored the ternary phase diagram for feasibility (Fig. 3). There are large areas where phases obtained after rapid dilution were devoid of structures. Micelles were observed in some areas but clearly differed from nanocapsules confined in a well delimited domain of

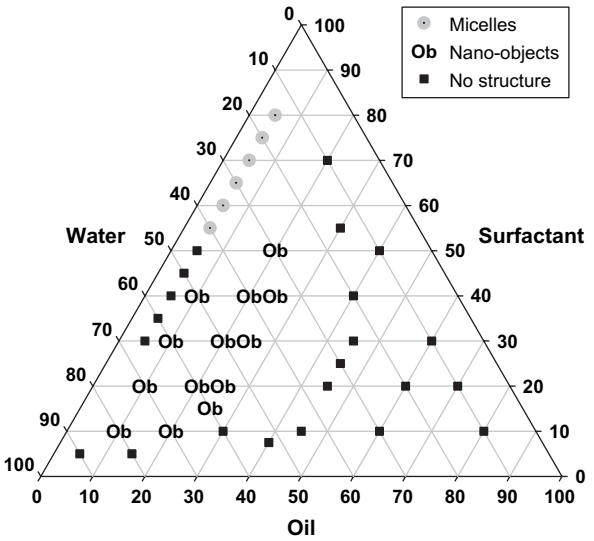

Fig. 3. Influence of the initial proportions of water, oil and surfactant on the production of lipidic nanocapsules (Ob), after three thermal cycles in the phase inversion zone, and a rapid dilution in cold water (reproduced with permission after Ref. [9]).

nano-objects (Ob). The dimensions of nanocapsules and their variations also depended of composition within this domain of feasibility (Fig. 4). Finally, we studied these nanocapsules by cryo-transmission electron microscopy, in thin films of vitrified water at very low temperatures, without ice crystals, a strictly amorphous environment, as tested by electron diffraction. The nanocapsules were spherical, with different diameters ranging from 25 to 60 nm or more (Fig. 5). The

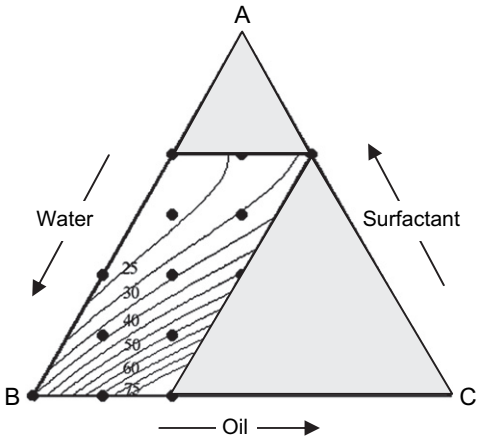

Fig. 4. Calculated lines of equal average diameter from 25 to 75 nm in the zone of nanocapsule production. The solid circles correspond to compositions of our initial mixtures, which gave stable lipidic nanocapsules (reproduced with permission after Ref. [9]).

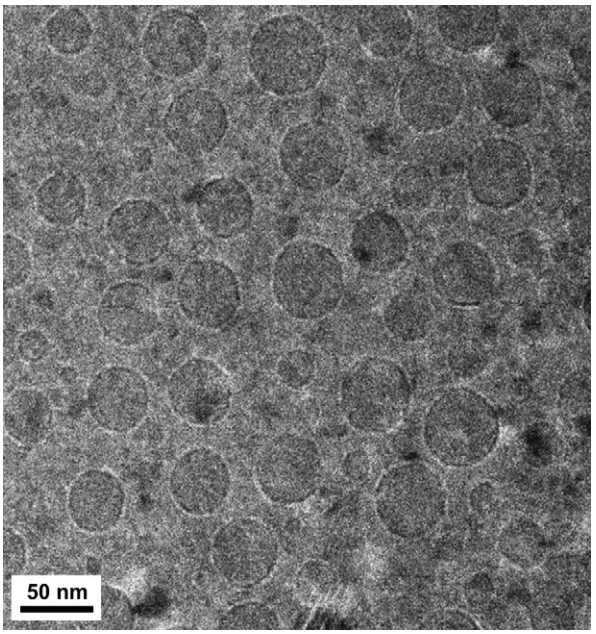

Fig. 5. Electron micrograph of a thin film of lipidic nanocapsules, vitrified by ultrarapid cooling at ultralow temperature, after cryo-transfer to the cryoTEM. Bar: 50 nm (courtesy of Dr. Lambert).

lipid core was homogeneous, limited by an electron dense interface, and an external clear layer. Capsules were surrounded by a large continuum which seemed to correspond to the large quantities of added water and free polyethylene glycol.

5. Discussion: liquid crystals in cells, tissues and medicaments

As recalled in Section 1, *liquid crystals* are present in *living organisms* and, for instance, in biological membranes and in certain chromosomes. There are also *stabilized liquid crystals* in biological systems, which correspond to phases where fluidity was abolished by a sol–gel transition, for instance, and this is the case of many extracellular matrices, with 3D-geometries and symmetries closely related to those observed in certain classes of liquid crystals. In biological membranes also, there are stabilized areas, which are not fluid, and for instance at the level of junctions between cells.

Pharmaceutical preparations use a rich diversity of molecules and polymers, either natural or not, which also show *liquid crystalline behaviours*, and this is mainly the case of *water–lipid systems*, obtained with amphiphilic components. A lot of pharmacologically active molecules are amphiphilic and form liquid crystalline phases in presence of water, amiodarone for

instance [15] and this is an essential point in the perspective of nanoparticle preparation [16]. Many water–lipid systems are used to prepare diverse creams. Many polymers serve as excipients and are known to spontaneously align in the appropriate conditions to form liquid crystalline phases, with many examples such as hydroxypropyl-cellulose and xanthan [17,18]. Liquid crystalline polymers can be stabilized by sol–gel transitions, suggesting diverse applications.

Liquid crystals are anisotropic liquids, so that they are fluid and *often birefringent*. This is easily observed in polarizing microscopy, between slide and coverslip, in myelin figures, for instance, as in Fig. 1c$_2$, examined between crossed polars. The fluidity is directly recognized when slight pressures are exerted onto the coverslip. However, *some liquid crystals are not birefringent, though they are anisotropic*, as this is the case for solid crystals, diamonds or NaCl crystals for instance, which are cubic. There are also *cubic liquid crystals*, where a fluid bilayer forms a periodic surface with cubic symmetries, so that such phases are not birefringent; they are physically anisotropic, but optically isotropic. Diffusion of molecules was revealed by RMN experiments, so that the amphiphilic molecules move within such bilayers arranged into a cubic lattice [19]. However, their distribution is anisotropic, since they lie normally to the bilayer. Such systems are bicontinuous, not birefringent, but anisotropic. In liquid crystals, we also have phases called *sponges*, which are very similar bicontinuous systems, but without the regular cubic periodicities. Note that biological counterparts of cubic water–lipid systems and of sponges also exist in cells, when internal membranes rearrange into such complex surfaces [20].

We encountered a similar situation in our bicontinuous emulsions, that we transformed into a set of separate nanocapsules, by a rapid addition of water, at a temperature close to 0 °C. At the initial temperature, in the bicontinuous phase, the interface between the oily and the aqueous phases mainly contains PEG–stearates in the two examples, plus phospholipids in the second one, so that these molecules are aligned normally or somewhat obliquely with respect to this interface, and the order parameter is not zero, but not equal to one as it would be for a perfect order. This interface is therefore a 2D-liquid crystal, due to this anisotropy and to its fluidity. Actually the liquid character of this interface is not directly demonstrated in our material, but is necessary, since its topology is changed simply by cooling, when the bicontinuous system transforms into one which is not, with its division into separate nanocapsules, each one showing its own lipidic core, as in Fig. 5. This requires a rehandling of the interface at each separation, and we suppose a fluidity of the interface comparable to that observed when two spherical oil droplets coalesce into a larger one, which rapidly refinds a spherical shape. Such topological recombinations of the interface require fluidity, even we cannot observe it directly at the nanoscales.

Such rehandlings of interfaces are common at all scales and this is a general character of liquids, in soap bubbles for instance detaching from soap films, and also in cell membranes with exo- or endocytosis. Similar recombinations of membranes occur when certain viruses penetrate cells, or when they bud outside, after repeated duplications of their genetic material.

Diverse kinds of rehandling between cell membranes and bilayers limiting nanocapsules or liposomes will have to be considered by pharmacologists. A simple vesicle able to coalesce with the outer membrane suffices for drug penetration in the cytoplasm, if the drug is water soluble, but if this drug is amphiphilic and present in the vesicle bilayer, it will diffuse within the outer cell membrane after coalescence. A double liposome associating two concentric vesicles could lead to penetration of a simple vesicle within the cytoplasm, able later to coalesce with the membrane of internal organelles. Such double penetrations allow one to consider several targets for active principles, according to their solubilities. With such methods, lipophilic principles could visit either the outer membranes, or the organelle membranes, and the water-soluble ones could be directed towards the hyaloplasm or towards the internal compartments of organelles. Such rehandlings are made easier by diverse molecules, simple ones and proteins. Here we simply introduce a topological meaning of the term ‘‘target’’ at the cell level, whereas it is usually considered as purely geographical, within the internal landscape of tissues and organs.

Let us end with the fact that among pharmacologically active molecules, there are many amphiphilic components, and some of them form bicontinuous systems. Two examples were studied, the amiodarone used in heart therapy and the palmitoyl-timolol malonate, a prodrug of timolol, a medicament for glaucoma [21]. Both were poorly soluble in water at ordinary temperature. When dispersed in water, after heating, different liquid and transparent phases were obtained at concentrations much higher than the CMC. These transparent phases were stable at room temperature and often coexisted with a lamellar phase nucleating at an interface separating two of these transparent phases, which were considered as inverse bicontinuous sponges [22]. This suggested that the lamellar phase observed at the

transition between the two sponges was thermodynamically stable, whereas the sponges themselves were metastable. It would be interesting to look for such an interface at the inversion zone in our systems, since it could be a nucleation site for myelin figures, that we believe to be inappropriate for the generation of lipidic nanocapsules [11].

More generally, we think that liquid crystalline phases are present in most emulsions devised to prepare drug carriers, and that their knowledge could be essential in several thermodynamical questions. Myelinic phases in such systems were likely to introduce difficulties in our preparations. Similarly it was shown that amiodarone in presence of NaCl at concentrations close to 10 mg/ml, as in human blood, formed lamellar phases, but such bilayers resist as cell membranes and easily extend across capillaries, all that with deleterious effects [23]. The liquid crystalline models suggest new ideas for drug encapsulation and targeting, but also must be taken into account to understand a variety of undesired side effects.

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References

[1] J.-P. Devissaguet, P. Couvreur, F. Puisieux, CNRS—Images de la Recherche 1, in: P. Rigny, P. Tambourin, D. Thoulouze (Eds.), De la matière au Vivant: les systèmes moléculaires organisés, vol. 268, 1994, p. 217.
[2] J.-P. Behr, CNRS-Images de la Recherche 1, in: P. Rigny, P. Tambourin, D. Thoulouze (Eds.), De la matière au Vivant: les systèmes moléculaires organisés, vol. 268, 1994, p. 221.
[3] G. Gregoriadis, P.D. Leathwood, B.E. Ryman, FEBS Lett. 14 (Apr 1971) 95.
[4] G. Gregoriadis, Biochem. Soc. Trans. 3 (1975) 613.
[5] J. Delattre, P. Couvreur, F. Puisieux, Les liposomes, aspects technologiques, biologiques et pharmaceutiques, Inserm, Tec and Doc, Lavoisier, Paris, 1993.
[6] J.-P. Benoit, H. Marchais, H. Rolland, V. Vande Velde, in: S. Benita (Ed.), Microencapsulation: Methods and Industrial Applications, Marcel Dekker Inc., New York, 1996, p. 35.
[7] B. Heurtault, P. Saulnier, B. Pech, J.E. Proust, J. Richard, J.-P. Benoit, Lipidic Nanocapsules: preparation process and use as Drug Delivery Systems Patent WO02688000, 2000.
[8] B. Heurtault, P. Saulnier, B. Pech, J.E. Proust, J. Richard, J.-P. Benoit, J. Pharm. Res. 19 (2002) 875.
[9] B. Heurtault, P. Saulnier, B. Pech, M.-C. Venier-Julienne, J.-E. Proust, R. Phan-Than-Luu, J.-P. Benoit, Eur. J. Pharm. Sci. 18 (2003) 55.
[10] P. Izquierdo, J. Esquena, T.F. Tadros, C. Dederen, M.J. Garcia, N. Azemar, C. Solans, Langmuir 18 (2002) 26.
[11] R. Pons, I. Carrera, J. Caelles, J. Rouch, B. Panizza, Adv. Colloid Interface Sci. 106 (2003) 129.
[12] N. Anton, P. Saulnier, A. Béduneau, J.-P. Benoit, J. Phys. Chem. B 111 (2007) 3651.
[13] P.A. Winsor, Trans. Faraday Soc. 44 (1948) 376.
[14] P.A. Winsor, in: G.W. Gray, P.A. Winsor (Eds.), Liquid Crystals and Plastic Crystals, 1, Ellis Horwood, London, 1974, p. 199.
[15] Y. Bouligand, F. Boury, J.-M. Devoisselle, J.-C. Gautier, D. Girard, J.-E. Proust, Langmuir 14 (1998) 542.
[16] A. Lamprecht, Y. Bouligand, J.-P. Benoit, J. Controlled Release 84 (2002) 59.
[17] P. Zugenmaier, in: D. Demus, J.W. Goodby, G. Gray, H.W. Spiess, V. Vill (Eds.), Handbook of Liquid Crystals, vol. 3, VCH Verlagsgesellschaft, Weinheim, Allemagne, 1998, p. 453.
[18] G. Maret, M. Milas, N. Rinaudo, Polym. Bull. 4 (1981) 291.
[19] J. Charvolin, A. Tardieu, in: L. Liébert (Ed.), Liquid Crystals, Solid State Physics, vol. 14, Academic Press, 1978, p. 209.
[20] Y. Bouligand, in: J.-F. Sadoc (Ed.), Geometry in Condensed Matter Physics, World Scientific, 1990, p. 193.
[21] B. Pech, J.-E. Proust, Y. Bouligand, J.-P. Benoit, Pharm. Res. 14 (1997) 37.
[22] Y. Bouligand, F. Boury, B. Pech, J.-P. Benoit, J.-C. Gautier, J.-E. Proust, Liq. Cryst. 26 (1999) 1281.
[23] G.H. Ward, S.H. Yalkowsky, J. Parenter. Sci. Technol. 47 (1993) 161.