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Self-organising structures of lecithin

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Abstract. Modern concepts of the self-assembly of amphiphiles are considered on the example of self-organising structures of the natural lecithin. Binary, ternary and multicomponent systems are discussed. A considerable part of the review is devoted to the peculiarities of self-organisation of this phospholipid in non-aqueous media and to the role of polar organic solvents. Virtually all of the structures formed by lecithin are examined: micelles, swollen micelles, microemulsions, emulsions, organogels, vesicles (liposomes), and lyotropic liquid crystals. In each specific case, attention is drawn to the dependence of self-assembly at the macroscopic level on interactions at the molecular level, shape of molecules, and their solvation and packing at the interface. The self-organising lecithin structures formed in the interfacial area of immiscible liquids in the course of unrestricted adsorption from the bulk of non-aqueous solution are considered. The bibliography includes 282 references.

I. Introduction

The term 'self-organising structures' has appeared in physical and colloid chemistry rather recently. It was borrowed from molecular biology where it serves to describe the mode of cellular structure assembly from constituent molecules. Nowadays, much attention is being given to the elucidation of principles and mechanisms underlying the aggregation of substances and their association into supramolecular systems.

Lecithin is the best known of all natural surfactants. It is widely distributed in living nature, being a basic structural component of the lipid matrix of biological membranes and membranous organelles, and finds uses in food industries, cosmetics, pharmacology, and biotechnology as an effective dispersive and emulsifying agent. All this explains the keen interest of investigators from different fields of science in studies of lecithin properties, its self-organisation, the structures formed, and their transformation.

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Lecithin forms monomolecular layers, bimolecular films, liposomes and vesicles, liquid crystals, emulsions and microemulsions, and organogels. The systematic studies, which are underway in many laboratories virtually through all this century, have provided a tremendous body of experimental material on its structural organisation in various media. Numerous monographs and reviews devoted to phospholipids and surfactants describe the specific properties and phase behaviour of lecithin; however, no generalising consideration and analysis of its self-organisation have been done until now. The present review is an attempt to fill in this gap.

II. Composition of the lecithin molecule

Lecithin belongs to the class of phospholipids. The content of phospholipids in living cells varies from 15 mass % to 50 mass % (dry mass), whereas in the nerve tissue its level is as high as 60 mass % – 80 mass %.[1–4] Lecithin dominates over other phospholipids in marine invertebrates, plants, and animals.[1–5] In animal organs and tissues, lecithin is mostly contained in the nerve tissue, liver, heart, lungs and blood.[2,4] The content of lecithin often exceeds that of any individual phospholipid.[1–5]

The principal natural sources of lecithin are egg yolk and soybeans. The yolk accounts for 30% – 35% of the egg mass of which $\sim 1/3$ is lecithin.[4] The content of phospholipids in the soybean oil is much lower ranging from 1.5% to 2.5%. Nonetheless, soybeans are the major industrial source of lecithin. The soybean lecithin accounts for as much as $\sim 90\%$ of the annual world lecithin production of 145 000 tons.[6] This is explained by the large scale of soybean oil production. For example, 16 m tons of this oil was produced in 1989 – 1990.[4] Accordingly, the phospholipids, which are obtained as by-products in the soybean oil purification, accounted for about 300 000 tons of which 40% was lecithin.[4]

Lecithin is an ester of glycerol in which two hydroxy groups at positions 1 and 2 are esterified by higher fatty acids, whereas the third hydroxyl is esterified by phosphoric acid to which a choline residue is attached (Fig. 1). Owing to the presence of phosphate residue, phospholipids are sometimes called 'phosphatides', while lecithin is widely known as 'phosphatidylcholine'.

The fatty acid composition of the egg yolk and soybean lecithins is presented in Table 1. It is seen that the latter is more unsaturated. Usually, a saturated fatty acid residue is at the position 1, while the residue of an unsaturated acid occupies the position 2.[8] In its physicochemical properties, the egg yolk lecithin is close to 1-palmitoyl-2-oleyl-*sn*-glycero-3-phosphatidylcholine[9]

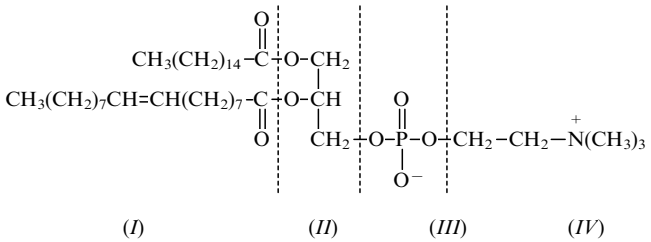


Figure 1. The structural formula of 1-palmitoyl-2-*sn*-glycero-3-phosphatidylcholine, an analogue of egg-yolk lecithin. (*I*) fatty acid residues; (*II*) glycerol residue; (*III*) phosphate group; (*IV*) choline.

(Fig. 1). No close synthetic analogues of the soybean lecithin have been suggested to date.

Table 1. Fatty acid composition of natural lecithins.

Fatty acid composition						Molecular mass	Ref.
$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$		
Soybean lecithin							
12.2	0.4	2.7	10.7	67.2	6.0	773	10
Egg lecithin							
35.8	1.5	18.3	37.4	6.8	–	766	11

One should pay attention to the following important fact. The fatty acid composition of natural phospholipids is heterogeneous (Table 1); however, it does not exhibit any substantial variation from sample to sample and is little dependent on the lecithin source. An important consequence is that all investigators deal with natural phospholipids, which are sufficiently close by their composition. This allows an adequate comparison of the results obtained at different times and in different laboratories.

III. The factors determining self-organisation

The lecithin molecule carries two non-polar hydrocarbon chains and a voluminous polar region composed of residues of three molecules (Fig. 1). The lecithin molecule is neutral in the pH range of 3–12.[7] The combination of two large moieties of opposite polarities occurs definite effects on all the physicochemical properties of lecithin. For instance, it cannot exist in the molecular form in media of any polarity.[12,13] Lecithin is either concentrated at the interface or self-assembled into micelles or liquid crystals in the bulk solution.

1. Hydrophobic effects

The existence of two hydrocarbon chains drives the lecithin molecule out of the water towards the interface or stimulates its association with other molecules. In aqueous solution, these chains are surrounded by water molecules which, owing to additional hydrogen bonds, are less mobile and more structured than the bulk-distributed solvent molecules. Therefore, the dissolution of non-polar compounds leads to an entropy (S) decrease, which proves to be rather significant. Being of entropic nature, this effect is known as 'hydrophobic'.[14] The resulting change in the free energy may be expressed as

$$\Delta G = \Delta H - T\Delta S, \tag{1}$$

where H is the enthalpy, and T is the temperature. Changes in the standard free energy upon transition from aqueous solution to oil may be estimated using the equation, proposed[15–17] for the calculation of the hydrophobic effects in the adsorption of surfactants

$$\Delta G_a^\circ = -C_a(38.3n_{CH_3} + 31.9n_{CH_2} + 18.6n_{CH} + 1.9n_C), \tag{2}$$

where C_a is the adsorption coefficient equal to 106 J mol^{-1} Å^{-2}, n is the number of the respective hydrocarbon groups, the factors involving n are the areas of the cavity surface (in Å^2) formed around the group indicated by the subscript. According to Eqn (2), the absolute value of ΔG_a° for the lecithin molecule containing the hydrocarbon chains of 15–17 carbon atoms can be higher than 100 kJ mol^{-1}.

2. Thermodynamic description of self-organisation

The contact of hydrocarbon chains with water can also be excluded by the aggregation of amphiphilic molecules. This may result in the formation of various structures. Their common feature is that the surface is covered by polar groups shielding the inner volume filled with non-polar groups. Micelles are the best known example. As a matter of fact, the thermodynamic description of the self-organisation is based on the approach † proposed for micelle formation by Tanford[19] and supplemented with molecular geometry by Israelachvili *et al.*[20,21] that permitted its application for any self-organising structures.

Let us represent the process of aggregation of one type non-ionogenic surfactant in the form of a reversible chemical reaction

$$N\,\mathrm{B} \rightleftharpoons \mathrm{M},$$

where B and M stand for the surfactant molecule and micelle, respectively; N is the aggregation number. The condition of aggregative equilibrium in a micellar system coincides with the condition of equilibrium of a reversible chemical reaction that was first deduced by Gibbs[18,22]

$$N\mu_1 = \mu_N, \tag{3}$$

where μ_1 and μ_N are the chemical potentials of a compound in the monomeric and aggregated states, respectively. Taking into account the expression for the chemical potential

$$\mu = \mu^\circ + RT\ln X,$$

one may transform Eqn (3) into

$$N\mu_1^\circ + NRT\ln X_1 = \mu_N^\circ + RT\ln X_N, \tag{4}$$

where μ_1° and μ_N° are the standard chemical potentials of a compound in the monomeric and aggregated states, respectively; X_1 and X_N are the mole fractions of this compound in each state, R is the gas constant, and T is temperature. The equation does not include the activity coefficients, since the interactions between non-aggregated molecules and between aggregates were not considered.

In this case Eqn (4) at $N \to \infty$ gives an approximated equation

$$\Delta G_N^\circ = -A_N^\circ \simeq RT\ln X_{CMC}, \tag{5}$$

where ΔG_N° is the change of the standard free energy during the aggregation of surfactant, A_N° is the standard affinity for micellisation,‡ X_{CMC} is the critical concentration of micelle formation.

In colloidal chemistry, Eqn (5) is often used to calculate the standard free energy of micelle formation.[14,18,23] The critical micelle concentration is easily found from the experimental property — composition dependence of surfactant solutions. In the scale of molar fractions, the standard substance concentration is assumed to be unity.[18]

One can estimate the magnitude of ΔG_N° for dipalmitoyllecithin. For this phospholipid, the critical micelle concentration in water is 4.6×10^{-10} M.[24] Substitution of this value into Eqn (11)

† It should be noted that the approach to the description of micelle formation is not uniform. To this end, various models were proposed in the literature. They are exhaustively discussed in the substantial monograph by Rusanov.[18] The present review provides a schematic account of the basic concepts of the thermodynamics of micellisation.

‡ The affinity for micellisation corresponds to the chemical affinity for reaction in thermodynamics (for details, see Ref. 18).

gives the standard free energy of micelle formation of 53 kJ mol^{-1} (at 25 °C). In order of magnitude, this value is comparable with the standard free energy of adsorption.[16,17]

If the dependence of ΔG_N° on temperature and pressure (p) has been established (for which purpose experimental values of CMC under different conditions are required), it is possible to calculate the volume-related (V_N°) effects accompanying micellisation and the standard thermodynamic functions of aggregation, *i.e.* entropy (S_N°) and enthalpy (H_N°).

Analysis of the standard thermodynamic functions alone does not permit the estimation of the aggregate size or the prediction of the type of the structures formed. Therefore, the description of the processes underlying the self-organisation of amphiphiles usually includes the consideration of intermolecular interactions.[14,18,20,21,25–28] In the standard free energy of aggregation, two groups of components are distinguished: standard energies of attractive and repulsive interactions. A balance of these interactions determines the optimal packing of amphiphilic molecules, which in turn defines the type of self-organising structures.

3. The dimensionless packing parameter

It has been proposed [20,21,25] to characterise the packing of amphiphilic molecules in aggregates by three parameters: the optimal area A_0 occupied by one molecule at the interface, the volume V of hydrocarbon chains, and the maximal length L_{max} to which the chains are extended in the *trans–trans*-conformation. Criteria of such packing are derived from the following consideration.

The aggregation number, *e.g.* spherical, of micelles with radius r may be expressed as

$$N = 4p\frac{r^3}{3V}. \qquad (6)$$

It is assumed [18,20,21,25] that the molecules are incompressible, and that the radius of spherical aggregates cannot be larger than L_{max}. In this case,

$$r = 3\frac{V}{A_0} \leqslant L_{max}. \qquad (7)$$

This allows transformation of Eqn (7) into

$$N = 4p\frac{r^2}{A_0}. \qquad (8)$$

The criterion of amphiphile micellisation is derived from Eqn (8). Provided that the above-stated conditions are met, the molecules whose size satisfies the requirement

$$S_p = \frac{V}{A_0 L_{max}} \leqslant \frac{1}{3}, \qquad (9)$$

will organise themselves into spherical aggregates.

The criteria of all other types of structures (Fig. 2) are established in a similar way.

The criterion S_p is commonly called 'the dimensionless packing parameter', since the $V/(A_0L_{max})$ ratio is dimensionless. This term will be used further in this review.

Numerous studies [20,21,25–30] showed that the consideration of molecular geometry alone makes it possible to predict the character of the structural organisation of amphiphiles. In a number of instances, the theoretical aggregation number and micelle size are in good quantitative agreement with the experimental values. Nonetheless, the estimation of the magnitude of the dimensionless packing parameter is far from being simple. The major difficulties are related to the determination of real A_0 and L_{max} values. Calculations based on molecular models give an S_p parameter which differs markedly from its real value.[31] This discrepancy is due to the fact that the amphiphile structures are not in the molecular form but in the solvated state. The solvation shell imposes definite steric constraints on the packing of molecules.

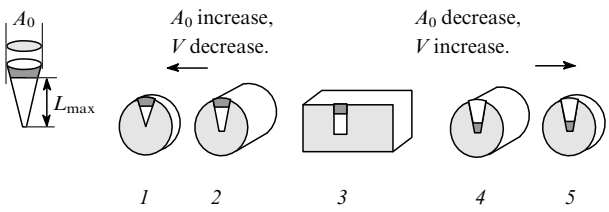


Figure 2. Types of structures formed by amphiphiles and the corresponding values of the dimensionless packing parameter S_p: (*1*) 1/3 (micelles); (*2*) 1/2 (hexagonal structures); (*3*) 1 (lamellar structures; (*4*) 2 (inverted hexagonal structures); (*5*) 3 (reverse micelles).

Accordingly, these should be accounted for in the parameters V, A_0, and L_{max}. In order to make realistic estimates, one should know the number of solvent molecules in the solvation shell as well as its structure.[31–33]

Nowadays, the parameter S_p is used basically for qualitative estimates. Based on simple geometric concepts, the use of the dimensionless packing parameter permits prediction of the type of self-organising structures and the direction of phase transformations. The effects of compounds on the structure of amphiphiles are interpreted with due account for their additivity.[29,30,34] In other words, the use of S_p makes it possible to approach the development of a system with the required structural organisation without resort to complex, arduous, and time-consuming studies (see, for example, Ref. 35).

IV. Binary systems

1. Lecithin – water

Natural lecithins in their molecular form are virtually insoluble in water (the solubility is of the order of 10^{-10} M).[12] Nevertheless, they are hygroscopic. Sometimes, even azeotropic distillation with benzene lasting many days fails to remove water completely. Therefore, lecithins, even after thorough drying, usually contain ~1 molecule H_2O per phospholipid molecule.[10] Lecithin is insoluble in aqueous solutions, but rather swells. Depending on the magnitude of water/lecithin ratio, various liquid-crystalline structures are formed. This property has been given the name of 'lyotropic mesomorphism'.

a. Liquid crystals

Binary phase diagrams of egg lecithin – water and soybean lecithin – water systems are presented in Fig. 3. Their superposition shows that the diagrams are not fully analogous, which is due to the difference in the fatty acid composition of two lecithins.

The lamellar liquid-crystalline mesophase L_α § is predominant in lecithin – water mixtures. It is formed by numerous parallel lamellae arranged into a bimolecular layer (Fig. 3). The polar groups of phospholipid molecules are localised on the bilayer surface, while their hydrocarbon chains fill the inner volume. Water molecules form a hydration shell around the polar groups and an aqueous interlayer between lamellae.

At room temperature, a homogenous lamellar liquid-crystalline mesophase L_α exists when the water content is in the range from 9 mass % – 10 mass % to 43 mass % – 45 mass % for egg lecithin and from 7 mass % to 35 mass % for soybean lecithin. In sealed tubes, the mesophase L_α is preserved up to 220 – 240 °C. At higher temperatures, it melts to form an isotropic liquid composed apparently of reversed micelles (L_2 phase). When the water content exceeds the indicated limits, the lamellar mesophase L_α separates into the mesophase L_α and aqueous solution. This is the region of liposome and vesicle formation.

At low water levels (< 10 mass %), the formation of other liquid-crystalline mesophases is observed: lamellar L_β, hexagonal H_{II}, and cubic Q_α (Fig. 3). Note that the non-bilayer mesophases

§ Liquid-crystalline mesophases are designated according to Luzzati.[36]

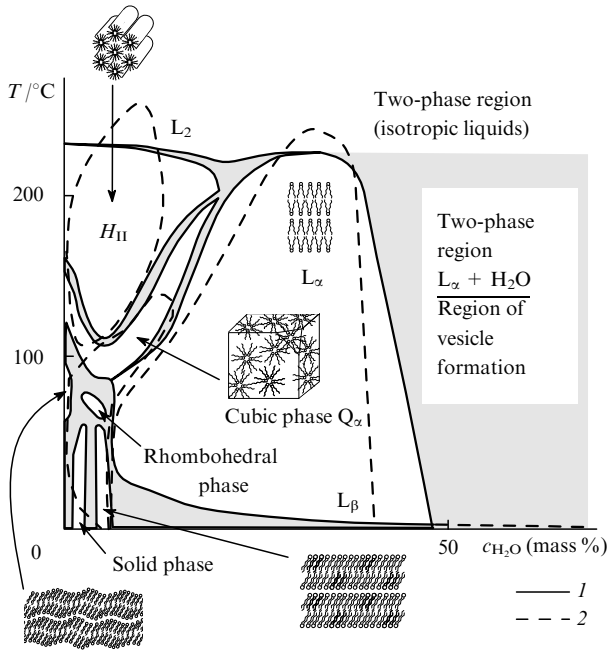


Figure 3. Binary phase diagrams of egg (*1*) and soybean (*2*) lecithins.[10, 13, 58] The basic types of liquid-crystalline structures are shown as schematic insets. For comments, see the text.

H_{II} and Q_α occur solely at higher temperatures (> 90 °C). The phase transition $L_\alpha \rightarrow Q_\alpha \rightarrow H_{II}$ is induced by the different bilayer expansion in the polar and non-polar regions. Its expansion is larger in the region of hydrocarbon chains due to the thermal activation of *cis–trans*-transitions of C–C bonds.[25, 27, 28] As a result, the molecule acquires the shape of inverted cone, *i.e.* the magnitude of the dimensionless packing parameter S_p becomes > 1, and phase transition occurs. The transition $H_{II} \rightarrow L_2$ takes place in sealed tubes at temperatures above 220 °C.

When a dry phospholipid is left in air, it absorbs water vapour and adopts the lamellar liquid-crystalline mesophase L_a. The quantity of absorbed water is dependent on the air humidity (partial pressure of water vapour). The maximum absorption is 20–21 H_2O molecules per molecule of egg phospholipid.[37, 38] According to different estimates,[13, 39–41] the full hydration shell contains from 33 to 39 water molecules, though it is formed only when lecithin is in a direct contact with water.

Hydration is a multistage process. The first solvent molecules are absorbed fairly rapidly. The absorption time constant is below one minute.[42] The rate of this process considerably decreases as the hydration shell is filled, and in the final stage the absorption time constant is as high as ~ 40 min.[42]

The water of hydration is believed to consist of three or four types. The first one or two H_2O molecules linked to a lecithin molecule are regarded as the 'inner' or 'strongly bound' water.[43–45] The use of molecular models[46] has shown that the energy of their interaction with phosphorylcholine is as high as 30–40 kJ mol^{-1}, while that of the interaction of H_2O molecules with each other in bulk water is only 25 kJ mol^{-1}. Further addition of water induces formation of the basic hydration shell around the polar region of the lecithin molecule. This water type is called 'bound' water because of a markedly lower mobility of H_2O molecules in comparison with liquid water. According to the reported estimates,[45, 47-50] the quantity of 'bound' water varies from 5 to 11 molecules. The next type is the 'included' or 'trapped' water comprising 4–11 H_2O molecules.[41, 43, 45, 49, 50] In the final stage, water molecules form mostly an aqueous interlayer between the bimolecular layers. As regards its physicochemical properties, this water virtually does not differ from that in the bulk of aqueous solutions, and this was the reason for calling it 'free' water.

The first 4–5 water molecules are attached to the phosphate group by means of hydrogen bonds and are localised in the region of phosphorylcholine (Fig. 1). The subsequent molecules of the polar solvent penetrate deep enough to reach the hydrocarbon chains and form hydration shells around their carbonyl groups.[51–53]

The changes in the parameters of the bimolecular layer in the lamellar mesophase L_α of egg and soybean lecithins induced by the variation of the water level are shown in Fig. 4. It is seen that under the same conditions, for the lamellar mesophase the period in egg lecithin determined by electron diffraction (curve *1*) is somewhat larger than that in soybean lecithin (curve *4*). This difference is explained by the higher unsaturation of the latter (Table 1). A detailed picture of osmotic swelling is offered by the example of egg lecithin. The absorption of water leads to the thickening of the layer comprising the bimolecular layer and an aqueous interlayer (curve *1*), as well as to the increase in the area A_0 per lecithin molecule in the bilayer (curve *3*). At the same time, a decrease is observed in the thickness of the layer formed by the hydrocarbon chains L_{max} (curve *2*). The observed thickening of lamellae is achieved through hydration of the polar region of lecithin and the formation of the water interlayer between lamellae.

The area A_0 and the layer thickness L_{max} have been reported[56] to vary in the opposite ways. This results in a virtual independence of the dimensionless packing parameter S_p of the water content. This finding explains the stability of the bilayer structure and the persistence of the lamellar mesophase (L_α) over a wide range of water concentrations.[56]

In conclusion, it should be noted that the presence of 1 mass % – 3 mass % of admixtures of ionogenic substances is conducive to a dramatic increase in the extent of lecithin swelling in aqueous solutions (Fig. 4, points *5*).[12, 55, 57, 58] In the presence of ionogenic compounds the thickness of water interlayers may be 4–6 times larger than of lamellae. In this situation, the interval of the lamellar mesophase (L_α) occurrence may be considerably extended due to the shift of the right-hand boundary in the binary phase diagram (Fig. 3) towards higher H_2O concentrations (sometimes, up to 85 mass %).[55] This effect is explained by the electrostatic repulsion of lamellae from each other. This is

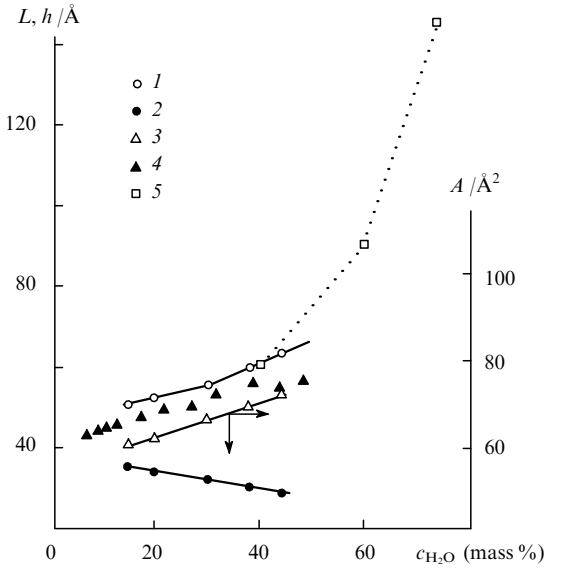


Figure 4. The lamellar mesophase (*L*) period determined from the results of X-ray diffraction (*1*, *4*, *5*), thickness of the hydrocarbon portion of lamellae (*h*) (*2*), and the area occupied by a lecithin molecule in the bimolecular layer (*A*) (*3*).[10, 13, 54, 55] Egg lecithin (*1–3*), soybean lecithin (*4*, *5*), mixture containing 1.5 mass % of cetyltrimethylammonium bromide (*5*).

confirmed by the absence of the extremal swelling of lecithins in solutions of inorganic salts.[55]

b. Liposomes and vesicles

In the phase diagram region corresponding to the binary system phase L_α – water (Fig. 3), the lecithins placed into water are swelling to form characteristic structures known as 'myelin figures' (Fig. 5). They were first described by Virchow[59] in 1854. The appearance of myelin figures indicates that an amphiphile adopts the form of a lamellar mesophase L_a.[60]

The formation of myelin figures is associated with the expulsion of lamellae from the bulk of a substance as a result of their swelling (for more details, see Refs 61 – 63). The coaxially arranged bimolecular layers form cylinders which possess high optical anisotropy. The process of swelling is illustrated by a series of photographs shown in Fig. 5. The final stage appears to be highly significant for the problem under discussion. It comprises disintegration of myelin figures into spherical and oval particles (Fig. 5 *c*), whereas the substance bulk is transformed into a sort of paste as a result of swelling. Microscopic examination in the light transmitted through crossed polaroids reveals that the pasty mass formed has a pseudoisotropic texture with oily streaks (Fig. 5 *d*). This type of texture is characteristic of the lamellar mesophase L_α.

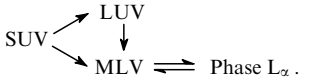
Under mechanical impacts (even weak ones), *e.g.*, shaking, the swollen mass is dispersed in the excess of the aqueous solution. Electron microscopy showed[64] that such dispersions, known nowadays as 'liposomes' or 'vesicles',¶ were formed (like an onion bulb) by numerous closed lamellae.

Papahadjopoulos[65] proposed the following classification of vesicles by size (μm): 0.1 – 100 — multilamellar vesicles (MLV); 0.02 – 0.1 — small unilamellar vesicles (SUV); 0.1 – 1.0 — large unilamellar vesicles (LUV); up to 50 — giant vesicles (GV); 0.02 – 0.1 — small oligolamellar† vesicles (SOV); 0.1 – 1 — large oligolamellar vesicles (LOV).

The vesicle size is determined by subsequent mechanical impact, its intensity and duration. The diameter of vesicles produced under standard conditions is usually within the limits of 1 – 4 μm.

Spontaneous formation is observed only in the case of MLV. All other vesicle types can be produced through external impact. The largest energy is required for the production of SUV. This is due to the increase in the free energy during dispersion (Fig. 6).

The increase in the free energy as the vesicle size decreases is caused by an abrupt increase in the interface surface. Consequently, the unilamellar vesicles SUV and LUV turn out to be thermodynamically unstable systems, though they prove to be stable kinetically. With time, the unilamellar vesicles are fused to form multilamellar structures, *i.e.* the system recovers to its initial state. The transitions occurring may be schematically represented as follows:



The result is the separation of a homogeneous dispersion into an aqueous solution and a sediment composed of a mixture of multilamellar liposomes and lamellar mesophase L_α.

Most of the basic techniques for producing vesicles were developed in the 1970s. Their description may be found in a number of surveys (see, *e.g.*, reviews[66 – 70]). In the subsequent years, diverse modifications of these techniques were proposed. Information on the basic methods of vesicle preparation, as well as their advantages and drawbacks are listed in Table 2, which also gives values of the inner water volume, accounted for when

¶ The term 'liposome' is mostly used in the technical literature. In the scientific literature, dispersions of phospholipids are commonly called 'vesicles'.

† The term 'oligolamellar' means that the vesicular envelope is composed of one to several bimolecular layers. This vesicle type is intermediate between the unilamellar and multilamellar vesicles.

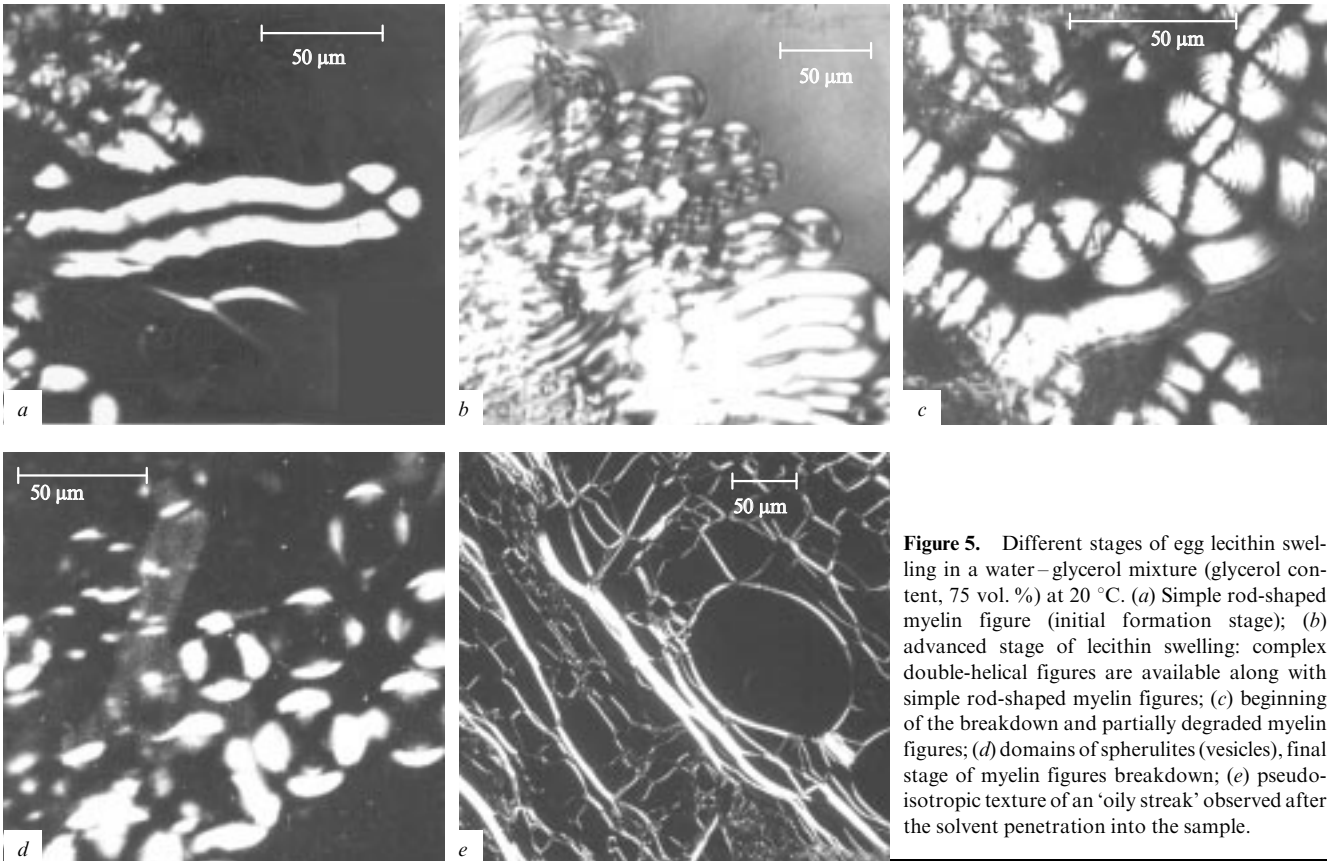


Figure 5. Different stages of egg lecithin swelling in a water – glycerol mixture (glycerol content, 75 vol. %) at 20 °C. (*a*) Simple rod-shaped myelin figure (initial formation stage); (*b*) advanced stage of lecithin swelling: complex double-helical figures are available along with simple rod-shaped myelin figures; (*c*) beginning of the breakdown and partially degraded myelin figures; (*d*) domains of spherulites (vesicles), final stage of myelin figures breakdown; (*e*) pseudoisotropic texture of an 'oily streak' observed after the solvent penetration into the sample.

Table 2. Characterisation of methods for vesicle preparation.

Method	Basic stages	Type of vesicles prepared	Inner volume,[a] /litre mol^{-1}	Advantages	Disadvantages	Ref.
Swelling of lecithin in water						
in a flask	1. Dissolution of lecithin in organic solvent. 2. Evaporation of lecithin solution in a rotary evaporator. 3. Addition of water solution to the dry phospholipid. 4. Shaking, stirring, and sonication of lecithin mixture with water solution.	MLV	1 – 4	The simplest and most accessible method.	1. Heterogeneity of vesicle size. 2. Non-reproducibility of vesicles shell thickness (number of lamellae).	64, 67, 80
on a support	1. Dissolution of lecithin in organic solvent. 2. Transfer of solution onto a special flat support. 3. Drying of solution in vacuum. 4. Addition of water solution to the dry phospholipid.	SUV, LUV, LOV, GV	No data	A simple method for preparing monolayer vesicles.	1. Necessity to add charged surfactants to lecithin. 2. Influence of support on vesicle preparation.	81, 82
Sonication of lecithin-containing solutions	1. Preparation of multilamellar vesicles. 2. Sonication of prepared vesicles.	SUV	0.2 – 1.5	Uniformity of vesicle size.	1. Intensive sonication. 2. Presence of metal from the dispenser tip. 3. Small volume of inner aqueous solution. 4. Impossibility of incorporating high-molecular-mass compounds into vesicles.	83, 84
Phase reversal	1. Dissolution of lecithin in organic solvent. 2. Addition of water to the dry phospholipid. 3. Emulsification of the mixture by sonication. 4. Concentration of the mixture at 300 – 400 mm Hg up to the gel formation. 5. Complete removal of organic solvent from the mixture at 700 mm Hg.	LUV, LOV	up to 10	A large volume of inner aqueous solution.	Use of organic solvent.	68, 85
Dispersion of lecithin with detergents	1. Preparation of multilamellar vesicles. 2. Addition of detergent to vesicles. 3. Removal of detergent from the mixture (dialysis, gel-filtration, adsorption, ultrafiltration, centrifugation).	SUV, LUV	up to 4	Suitability for reconstructing lipid-protein complexes.	Use of detergents.	86 – 88
Injection of solution	1. Dissolution of lecithin in organic solvent. 2. Injection of solution with a syringe into a warm water solution.	SUV, LUV, LOV	$\sim$ 0.5 (in the case of SUV)	A fast and simple method for preparing monolayer vesicles.	1. Rapid coagulation of phosphatidylcholine vesicles. 2. Extended range of sizes. 3. Use of organic solvent. 4. Rather high temperatures.	89 – 92
Double emulsification	1. Dissolution of lecithin in n-hexane. 2. Preparation of water-in-oil emulsion in the presence of surfactant. 3. Partial evaporation of n-hexane. 4. Second emulsification in the aqueous solution with surfactant. 5. Removal of surfactant and n-hexane.	SUV	–	–	Large surfactant content.	93

[a] The volume of aqueous solution in the vesicle core surrounded by the phospholipid layer shell characterises the solubilising capacity of vesicles and is expressed in litres of aqueous solution per mole of phospholipid.

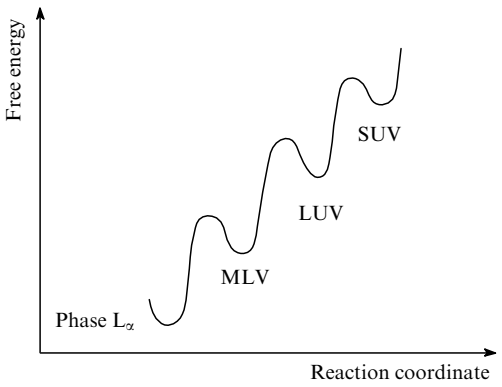


Figure 6. Free energy of a dispersion system for different vesicle types.[66]

incorporating drugs, proteins, DNA, *etc*. into vesicles. The techniques employed for the preparation of unilamellar vesicles are illustrated in Fig. 7.

The mechanism underlying the vesicle formation is not as yet fully understood. Numerous questions remain still unanswered.[71] The most universal model was designed by Lasic.[66, 72] It relies on the aggregation of the preformed bimolecular 'blocks' into vesicular structures.

Soon after their discovery, vesicles became very popular as models of biological membranes, and they remain active performers of this role until now. Vesicles allow the investigation of the transport of various compounds across membranes, membrane fusion, as well as the reconstitution of lipoprotein complexes.[67, 73–76] The vesicles have acquired wide use in medicine, pharmacology, genetic engineering, cosmetics, and food industries. Today, there are bright prospects for their application in the development of highly specific systems for the targeted transport of drugs and genetic material.[76–79]

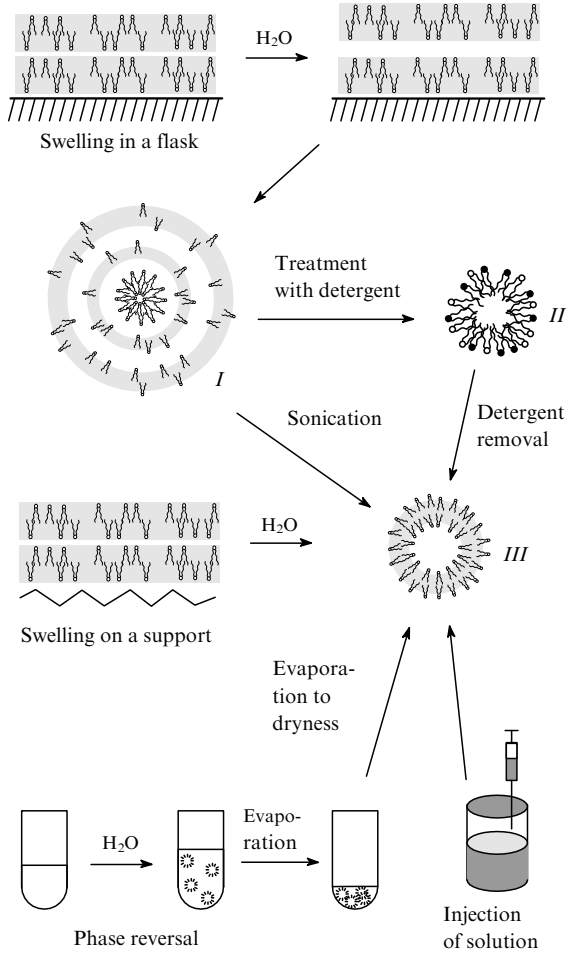


Figure 7. Basic techniques for preparing monolayer vesicles. See Table 2 for details. (*I*) MLV; (*II*) mixed micelles; (*III*) SUV.

2. Lecithin — organic solvent

Owing to its amphiphilic nature, lecithin is soluble in virtually all organic solvents, except for acetone.[4, 12, 13] The type of self-organising lecithin structures in solutions is determined by the nature of the solvents. The latter may be divided into two large groups. The first group includes for the most part non-polar solvents in which lecithin forms reverse micelles; the second group comprises polar solvents whose molecules (like water) form numerous hydrogen bonds generating three-dimensional arrays. In these media, the lecithin molecules self-organise to form liquid crystals and display lyotropic mesomorphism.

a. Reverse micelles

The phenomenon of micellisation was studied mostly for egg lecithin.[94, 95] The critical micelle concentrations have been determined for its solutions in *n*-heptane and benzene. In both cases, the formation of micelles is initiated at a lecithin content of 0.01 μM.[11, 95, 96] Table 3 lists the aggregation numbers N in different solvents, dielectric constants (ε) of solvents, as well as conditions of their determination and relevant methods.

Elworthy and McIntosh [97] have attempted to relate the micelle formation to the dielectric constant of a medium. The magnitude of ε was varied by the addition of water or benzene to ethanol. It was found that no aggregates were present in 93% ethanol (ε = 29.0). The absence of micellisation was explained by the balance of the solvent interactions with the polar and non-polar lecithin regions. These authors have assumed a medium with ε = 29.0 to be the dividing line. The increase in the solvent polarity must initiate the formation of direct micelles, whereas its decrease must stimulate the reverse micelles.

Analysis of the data presented in Table 3 does not reveal any direct correlation between N and ε. In studies of micellisation, more justified seems to be the approach, according to which the solvent influences the molecular form of lecithin in its aggregates. Certain evidence for this effect may be found in Ref. 95.

In their mixtures with lecithin, non-polar solvents accumulate in the region of hydrocarbon tails that is conducive to a higher effective volume and eventually to the magnitude of the dimensionless packing parameter S_p exceeding unity. For example, the addition of alkanes to the lamellar mesophase L_α (Fig. 3) results in its transition to the inverted hexagonal mesophase H_{II}.[98, 99] No formation of micelles is observed when the solvent molecules are localised not only near a certain individual group, but occupy the space along the entire lecithin molecule. For instance, under definite conditions such a situation was observed in benzene. Cooling of concentrated solutions led to their thickening. Relevant studies [100, 101] showed that in this case the reverse micelles are replaced by structures similar in their composition to the lamellar mesophase L_α. In such structures, the benzene molecules are localised in the regions of both the hydrocarbon tails and the polar groups of lecithin and also fill the interlamellar layers. According to the reported results,[100–103] the localisation of benzene in the polar region is explained by the interaction of benzene with the functional groups of the phospholipid.

b. Non-aqueous liquid crystals

The formation of the lamellar mesophase L_α by lecithin in a water-containing glycerol was first described by Rinne,[114] though this report remained mostly unnoticed. The first systematic studies of non-aqueous liquid crystals in ethylene glycol, as well as in alkanediols and low-molecular-mass polyethylene glycols were carried out by Friberg *et al.*[115–119]

Table 3. Micellisation of egg lecithin in organic solvents.

Solvent	Lecithin concentration / mM	ε^{-1} (Ref. 113)	T / °C	N	Method determination of N	Ref.
n-Heptane	⩾0.01	1.883	25	5	Interfacial tension	11
Benzene	< 0.1	2.284	25	4 – 6	Osmometry	94, 97, 104
	> 1	2.284	25	> 70	"	97,104
	13	2.284	40	45	"	105
	10 – 100	2.284	37	15	"	106
	13	2.284	20	~80	Light scattering, viscosimetry, diffusion	46
Toluene	3.7 – 15	2.379	25; 40	65; 82	Osmometry	107
	3.7 – 15	2.379			Light scattering	107
o-Dichlorobenzene	15.6	9.93	30	20±5	Ultracentrifugation	108
Chloroform	10 – 130	4.72	1.5 – 44	~3	NMR	109
Methanol	3 – 13	32.63	20	3	Light scattering	110
	6.7 – 27	32.63	25	1	Osmometry	111
Ethanol	3 – 13	24.55	20	9	Light scattering	110
Butanol	3 – 13	17.1	20	24	"	110
Hexanol	3 – 13	13.3	20	28	"	110
Diethyl ether	7 – 10	4.27	25	~40	Ultracentrifugation	112

The interest in mixtures of phospholipids with organic solvents was related, on the one hand, to the problem of perturbation of the intact lipid matrix under the influence of toxic solvents, *e.g.* ethanol. On the other hand, the investigation of lecithin proved to be in the wake of the studies aimed at elucidating the nature of the self-assembly of surfactants in media of different polarity. Nowadays, there are sufficiently settled views on the nature and mechanisms underlying the self-assembly of amphiphiles in organic media.

In organic media, like in water, lecithin can form different types of liquid crystals,[120, 121] depending on its concentration and temperature. The lamellar mesophase L_α is the most characteristic of this phospholipid at room temperature. Excess quantities of glycerol and ethylene glycol cause swelling of lecithin with the formation of myelin figures that further disintegrate into multilamellar vesicles.[60, 63, 122]

The physicochemical properties of solvents, in which the formation of liquid crystals is observed, are listed in Table 4. It also carries characteristics of some solvents, that makes it possible to reveal distinctive features determining the aggregation of amphiphiles.

Analysis of these tabulated data shows that the liquid-crystalline state of lecithin occurs in high-polarity media with a considerable cohesion between the solvent molecules. The latter is characterised by the dipolar angular correlation index and the cohesion parameter.[113, 123, 124] The ability of solvents to form hydrogen bonds is determined by their donor and acceptor numbers.[113, 123] The cohesion is seen to be the strongest in the case of water, formamide, glycerol, and ethylene glycol. It is caused by the intermolecular hydrogen bonding. Of note are the maximum values of acceptor numbers of solvents in which liquid-crystalline structures are formed. This may mean that the donor properties are significant for the self-assembly of lecithin.

The above peculiarities of polar organic solvents provided the grounds for suggesting that the necessary condition of the amphiphile self-organisation is the occurrence of hydrogen bondings in the medium.[120, 121, 125, 126] This suggestion was convincingly confirmed in the experiments with formamide, methylformamide, and dimethylformamide.[120] Successive methylation of the amino group in these solvents results in the total

Table 4. Viscosity (10^{-1} / Pa s), surface tension (σ / mN m^{-1}), dielectric constant (ε), dipolar angular correlation parameter (g), cohesion parameter ($\sigma / V^{1/3}$/mN m^{-1}), donor number (DN) and acceptor number (E_t) of solvents.

Solvent	η	σ	ε	g [a]	$\frac{\sigma}{V^{1/3}}$ (see [b])	DN [c]	E_t [d]
Liquid crystals of lecithin are formed							
Water	0.894	72	78.3	2.61	27.7	33.18	63.1
Formamide	3.30	58.2	111.0	2.04	–	24	56.6
Glycerol	945	63	42.5	2.16	15.1	19	57.0
Ethylene glycol	16.9	47.7	37.7	2.13	12.5	20	56.3
Liquid crystals of lecithin are not formed							
Ethanol	1.078	21.9	24.55	3.01	5.7	32	51.9
Acetone	0.304	22.67	20.70	1.21	5.57	17.0	42.2
DMSO	1.996	42.86	46.68	1.08	11.2	29.8	45.1
DMF	0.796	35.2	36.7	1.00	–	26.6	43.8

Note. Abbreviations: DMSO, dimethyl sulfoxide; DMF, dimethyl-formamide. The solvents in which liquid crystals are formed are characterised by the formation of three-dimensional networks of H-bonds; ethanol favours linear chains; in other solvents formation of networks of H-bonds is not characteristic.

[a] The dipolar angular correlation parameter indicates the deviation of the electric permittivity from the magnitude that the liquids would have at the same values of polarisability and dipolar moment in the absence of their association. Another meaning: g is the inverse energy of reactive interaction of dipolar molecules; therefore, this parameter is the index of hindrance to their free rotation induced by dipole-dipole interactions. For non-associated solvents, $g \simeq 1$. When the liquids are strongly structured and the dipoles are parallel one to another, $g > 2$.[113, 123] [b] The cohesion parameter proposed by Gordon;[124] V is the molar volume. For solvents with high cohesion, $\sigma/V^{1/3} > 12.5$ mN m^{-2}. [c] The donor number of Gutman serves for the quantitative estimation of the basicity of individual solvents.[113, 123] [d] The acceptor number proposed by Dimrot and Reihardt [113] for the quantitative estimation of the acidity of individual solvents.[113, 124]

closing of the H-donor centre. It was found that upon transition from formamide to methylformamide, the interval of the lamellar mesophase L_α occurrence is sharply reduced, whereas in dimethylformamide, in which no hydrogen bonds are available, the liquid-crystalline structures are not formed.

It should be noted that the self-organisation of lecithin into the liquid-crystalline state can occur in not all media with intermolecular hydrogen bonds. In this case, an important role is played by the character of hydrogen bonding. A good example are the aliphatic alcohols in which micellisation was found to occur (Table 3), but no liquid crystals were revealed (Table 4). The peculiarity of the aliphatic alcohols is that they form linear hydrogen bonds, which link molecules into chains.[113, 123] The formation of liquid crystals is observed in solvents (water, formamide, glycerol, and ethylene glycol) with three-dimensional networks consolidated by H-bonds.

Studies of non-aqueous liquid crystals by the NMR and X-ray methods have provided conclusive data on the diminished structural ordering of lecithin molecules upon substitution of organic solvents for water.[126–128] This is ascribed to the decrease in the solvophobic effect in the series water > formamide, glycerol ≫ ethylene glycol.[129] This inference is also confirmed by the fact that micellisation of the same surfactants is initiated in polar solvents at concentrations ~ 100 times larger than those in aqueous solutions.[23, 126] As a result, the standard free energy of aggregation calculated by Eqn (5) proves to be smaller by $11-12$ kJ mol^{-1}. Interestingly, the increment per methylene group remains unchanged.[23]

In the lamellar mesophase L_α, polar solvents form a solvation shell around the polar lecithin region and an interlamellar layer, but they do not penetrate as far as the region of hydrocarbon tails. Investigation of ethylene glycol-containing systems by NMR revealed the existence of three types of its molecules.[116, 118, 128] The first type is realised when a single diol molecule is attached to the phosphate group of the phospholipid via hydrogen bonds and turns out to be the least mobile. The second type includes up to seven diol molecules. They form the basic solvation shell on the polar portion of the lecithin molecule. Further addition of ethylene glycol induces the third type whose characteristics do not differ from those of the pure solvent. Apparently, the third solvent type comprises free diol molecules localised in the interlamellar layers. The above division of the ethylene glycol molecules into three types in the lamellar mesophase L_α is consistent with what has been proposed for water (see above).

The role of specific solvent in the non-aqueous liquid crystals may be performed by esters of fatty acids and polyethylene glycol. Friberg *et al.*[130] observed the formation of the lamellar mesophase L_α after mixing lecithin with tetra- or heptaethylene glycol laurate in the absence of any other additives. The period of lamellar structure determined by the method of small-angle electron diffraction diminished with the increase in the content of synthetic surfactant. The same effect took place when the temperature was increased. It has been assumed that the alkyl group of polyethylene glycol laurate is incorporated into lamellae between the phospholipid molecules, while the polyethylene residue acts as a solvent by forming a sort of solvation shell around the polar lecithin region.

The ability of phospholipids to exist in the form of lamellar mesophase L_α in the presence of a variety of organic solvents is of high biological significance.[131] Plant seeds, yeast cells, fungal spores, as well as some microorganisms undergo dehydration during drying. Addition of water causes osmotic swelling and leads to the recovery of their vital functions. A similar cycle is observed in plants preserved in the dehydrated state during the winter season. The removal of water and lower temperatures induce the phase transitions $L_\alpha \rightarrow L_{\beta'} \rightarrow$ crystalline phase (Fig. 3). Hydration causes the reversal to the lamellar mesophase L_α. This reverse transition is the usual cause of perturbations in intact biological membranes.[131] To prevent negative consequences of drying, the living systems have devised a mechanism of stabilisation of the biomolecular structure of the lipid matrix consisting in the replacement of water by polyols (ethylene glycol, glycerol), disaccharides (trehalose and sucrose), or an amino acid (proline).[131]

In this respect, the best studied is the phase behaviour of trehalose-containing systems.[132–134] The stabilisation seems to consist in the substitution of water and the binding of trehalose hydroxyls to the phosphate group of phospholipids through hydrogen bonds. In this case, the range of the lamellar mesophase L_α occurrence is extended towards smaller solvent concentrations and lower temperatures. The latter circumstance allows polyols, disaccharides, and proline to act as cryoprotectors.[131]

V. Ternary systems

In a ternary system containing lecithin, water, and oil, the lecithin molecules are localised at the oil–water interface. Their interaction with two solvents from different phases rather than with one offers additional possibilities for varying the parameters A_0, L_{max}, V, and S_p.

1. Water-containing systems

a. Swollen micelles

In the case of natural lecithins, the formation of micelles takes place only in organic solvents. Therefore, the further discussion is devoted to the reverse micelles.

Lecithin retains its hygroscopy in the dissolved state, which determines the absorption of water from air by its non-aqueous solutions.[135, 136] This leads to the dissolution of relatively small quantities of H_2O, from 15 to 30 molecules per phospholipid molecule.[12, 47, 94, 95, 136-138] The addition of water induces changes in the form of reverse micelles. The spherical shape of aggregates is preserved in aromatic (benzene, toluene) and chlorinated (chloroform, tetrachloromethane) solvents. On the other hand, there is a large group of organic solvents in which the spherical micelles are transformed into cylindrical ones under the influence of traces of aqueous solution (see below).

The first molecules of absorbed water are used to form a hydration shell around the polar head of the lecithin molecule. The hydration pattern has much in common with that described in Section IV.1.a. The differences reside in the number of water molecules: the hydration shell of lecithin in the reverse micelles includes somewhat smaller solvent quantities than, *e.g.*, that in the lamellar mesophase L_α.[95, 138] The 'free' water forms an aqueous core of the aggregates. As the amount of water increases, the radius of the aggregates increases, which provided the ground for calling such micelles 'swollen'.

The swollen micelles are water droplets surrounded by a monomolecular lecithin layer. Their radius can vary from 10 to 100 Å.[94] In benzene, for example, the diameter of the water core at the maximum level of the solubilised H_2O is 50 ± 15 Å, whereas in carbon tetrachloride it is 30 ± 5 Å.[137]

The existence of the water core makes it possible to solubilise inorganic and water-soluble organic substances in the non-polar systems with swollen micelles. It is also possible to introduce proteins and to carry out enzymatic reactions in such systems, and this seems to be promising for biotechnological applications.[94, 139]

b. Organogel

Addition of small quantities of water to non-aqueous lecithin solutions induces their thickening and formation of a gel phase (organogel). The list of organic solvents, in which this phenomenon takes place, includes over 50 names. The presence of organogel in organic solvents was first observed by Scartazzini and Luisi[140] in 1988. An analogous phenomenon was simultaneously and independently observed in a system of immiscible liquids. It was induced by the transfer of water into a non-aqueous solution of lecithin upon application of an electric field.[141]

The formation of lecithin organogel begins at the level of egg and soybean lecithins of $4-7$ g litre^{-1} ($\geqslant 550$ μM).[140, 142] After the complete dissolution of water, the organogel is outwardly

indistinguishable from the initial solution; it is homogeneous, transparent, and optically isotropic. Differences are revealed upon mechanical impact. The lecithin organogel forms a jelly-like mass. Its viscosity is strongly temperature-dependent. On heating, the organogel is transformed into a non-viscous solution, though it regains its gel-like state on cooling. In other words, jellification is a thermally reversible phenomenon.

An organogel stabilised with lecithin appears to be a thermodynamically stable system. This view is supported by its spontaneous formation. Such a stability is time-limited; the appearance of oxidation products induces degradation of an organogel; their presence also prevents the gel phase formation.[140]

The occurring phase and pseudophase transitions, as well as the dependence of static viscosity (η_0) on the number of water molecules (η) per lecithin molecule are shown in Fig. 8 on the example of soybean lecithin solution in n-decane.[142] The initial

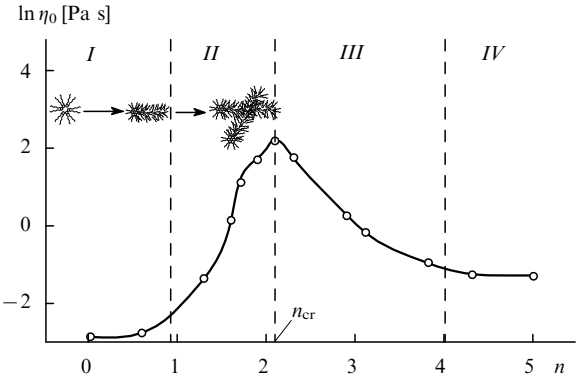


Figure 8. Dependence of the static viscosity of soybean lecithin solution in n-decane (10 mg ml^{-1}) on the number of added water molecules per lecithin molecule (n). The dashed lines indicate the boundaries between different phase and pseudophase states in solution: (*I*) Newtonian fluid; (*II*) organogel; (*III*) non-viscous solution + sedimented organogel; (*IV*) non-viscous solution + solid sediment. The graph was plotted from the data reported in Ref. 143.

water-free solution containing spherical reverse phospholipid micelles displays properties of a Newtonian fluid.[143] Upon addition of water, the static viscosity increases proportionally to the quantity of introduced solvent according to the equation

$$\eta_0 = \eta_s(1 + {}^{\circ}f)\,, \tag{10}$$

where η_s is the viscosity of the initial solution, C is the constant, and f is the solvent volume fraction. The range of linear dependence is not large. Deviation from the linearity is observed when the quantity of added water exceeds 0.5 H_2O molecules per lecithin molecule. Within the range from one to two water molecules, η_0 increases more than 10^4 times (Fig. 8). Other rheological parameters (loss modulus G' and storage modulus G'') also abruptly increase.[143, 144] When the magnitude of η_0 reaches its maximum, the separation of the gel phase into a non-viscous non-aqueous solution and a dense gel begins. In other words, until a certain level, the water acts as a thickener, and then it plays the role of coagulant. It was proposed to characterise this critical concentration by the parameter n_{cr} [140, 143] indicating the number of water molecules per lecithin molecule, which corresponds to the maximal water quantity at which the most viscous organogel is formed. The n_{cr} values of non-polar solvents, in which the soybean lecithin forms organogel, are the following: n-hexane, 3; n-octane, 2; n-decane, 2; n-dodecane, 1; 2,3-dimethylbutane, 4; oct-1-ene, 4; octa-1,7-diene, 7; isooctane, 3; cyclohexane, 6; cyclodecane, 12; ethyl laurate, 4; dibutyl ether, 6; tripropylamine, 4; and tributylamine, 2.

The lecithin organogel does not belong to the class of Newtonian fluids. It displays the properties of a viscoelastic (Maxwellian) body.[143, 144] The rheological behaviour of such bodies is simulated by a spring-piston model, whereas the dependence of dynamic moduli on the frequency of harmonic oscillations is described by the equations [143]

$$G'(\omega) = \frac{G_0\omega^2\tau^2}{1+\omega^2\tau^2}\,, \tag{11}$$

$$G''(\omega) = \frac{G_0\omega\tau}{1+\omega^2\tau^2}\,, \tag{12}$$

where ω is the angular frequency equal to $2\pi\nu$ (ν is the harmonic oscillation frequency), τ is the structural relaxation time, and G_0 is the static modulus. At fast deformations, the lecithin organogel displays properties characteristic of elastic bodies, whereas at slow deformations its properties resemble those of fluids. These states are characterised, respectively, by the static modulus (the high-frequency storage modulus) G_0 and the zero shear viscosity (Newtonian viscosity limit) η_0 (Fig. 8).

Investigations of the lecithin organogel by the methods of light scattering, small-angle neutron scattering, dielectrometry, rheology, electron microscopy, and IR and NMR spectroscopy carried out primarily by Schurtenberger *et al.*[144–149] and by other researchers [137, 150, 151] have ascertained changes occurring in the reverse micelles upon addition of water.

Water causes one-dimensional growth rather than swelling of spherical aggregates, which leads to the appearance of giant rod-like micelles (Fig. 8). The size of aggregates increases from 30 to 400–700 Å and may eventually be as high as thousands of angstroms.[149] The transformation of micelles occurs within a narrow range of water concentrations. Addition of 1–3 water molecules per lecithin molecule often proves to be sufficient (Fig. 8). The giant rod-like micelles entangle and form three-dimensional networks.

By its structural organisation and rheological properties, the lecithin organogel resembles semi-dilute solutions of polymers.[152, 153] A number of other surfactants were found to form gels in aqueous [152–162] and organic [158, 163–168] solutions in the absence of polymeric compounds. This provided the ground for segregating micellar gels into a special group. Cates [169–171] proposed to consider the flexible rod-like micelles as the regular components of the gel phases of amphiphiles. They play the role of polymeric (long-chain) molecules. That is why the giant rod-like micelles are known in the literature as 'polymer-like' or 'worm-like' micelles. Formation of a three-dimensional network of entangled aggregates in the bulk of solutions determines the visco-elastic properties of the gel-like phases of amphiphiles.

The dynamics of polymer-like micelles was considered by Cates [169–171] from two different standpoints. On the one hand, aggregates may be regarded as stable flexible rod-like particles, for which a reptation mechanism is applicable. The latter has been proposed for the description of the diffusion of chain molecules in a structured medium.[172, 173] The migration occurs in an imaginary tubular cavity the dimensions of which are determined by the interactions with the neighbouring molecules. A close analogy may be the movement of individual snakes in their ball. Diffusion in the gels is quantitatively characterised by the relaxation time τ_{rep} required for the particle to migrate at the distance equal to its length.

On the other hand, micelles cannot be regarded as stable formations. They are dynamic structures that are in the process of continuous reversible dissociation and recombination. The amphiphilic molecules pass into solution, reform associations or become involved in exchanges between aggregates;[14, 23] the aggregates themselves dissociate reversibly into smaller associations. The stability of micelles is characterised by their lifetime τ_b, which is the mean time span between an aggregate formation and its breaking into two parts.

Naturally, the dynamics of polymer-like micelles is determined by the ratio of times τ_{rep} and τ_b. It was proposed to distinguish between the two extreme cases.[169–171]

1. $\tau_b \gg t_{rep}$. The processes of dissociation and recombination are inhibited to such an extent that the micelles may be considered as stable particles whose movement in gel systems follows the reptation mechanism. Such systems may be described within the framework of net theories of polymers.

2. $\tau_b \ll t_{rep}$. The dynamics of the processes depends on both the reversible dissociation/recombination of micelles and their diffusion via the reptation mechanism. In this case, the time of structural relaxation is determined by the dependence $\tau \simeq (\tau_b \tau_{rep})^{1/2}$.

The nature of dynamic processes may be clarified by the analysis of concentration dependences of the rheological parameters.[169–171] The theoretical values of the exponents of lecithin concentrations corresponding to the reptation mechanism and those of polymer-like micelles in the case of G_0 and η_0 are listed in Table 5 along with some experimental values.[143, 144] The variations of G_0 and η_0 are considered as functions of lecithin and water concentrations since the formation and properties of organogels are determined by both components. The best correlation between the experimental and theoretical data is achieved for the dependence of the rheological parameters on lecithin concentration. However, in this case too, a substantial discrepancy is observed. A better fit to experimental data is achieved by employing a more complex model of dissociation/recombination of micelles, though noticeable differences are still found.[144, 170] The participation of water in the self-assembly of lecithin is believed [143] to be a possible cause of the deviation of parameters from the values calculated on the basis of theory of polymer-like micelles. Usually, the role of solvent in the formation of amphiphilic gels is passive. In a number of cases, the addition of water leads to shortening of cylindrical reverse micelles [174] or to the degradation of organogels.[167] The structure-forming function of H_2O is manifested only in non-aqueous solutions of lecithin. This is the principal difference of lecithin organogels from the gel phases of other amphiphiles. A molecular mechanism of the lecithin self-assembly into polymer-like micelles has been developed and substantiated.[142, 175, 176] It is considered below in the discussion of the role of polar solvents.

Table 5. Exponents of concentrational dependences.

Theoretical values		Experimental values	
Reptation mechanism	Polymer-like micelles	Data from Ref. 144	Data from Ref. 143
Concentrational dependence of G_0			
2.25	2.25	2.0±0.1	11
Concentrational dependence of η_0			
3.75	3.5	1.9±0.1	18

In conclusion, it is noteworthy that lecithin organogels have good perspectives for the practical use in cosmetics and pharmacology. Preliminary studies have demonstrated the possibility of incorporation of biologically active compounds, medicinal preparations, and enzymes into lecithin organogels. High viscosity of lecithin organogels makes their use particularly promising as the basic components of creams and ointments for cosmetic purposes, and in the therapy of skin diseases and burns. A number of successful applications of such organogels have been reported.[177–179]

c. Emulsion

Emulsions are not ranked among thermodynamically stable systems, though they possess the so-called 'kinetic stability'.[180–183] In the absence of surfactants, dispersions are extremely unstable and rapidly separate into layers of initial liquids. The coalescence (fusion) of droplets can be prevented, first of all, by a shell formed by amphiphilic molecules adsorbed at the interface of two immiscible liquids. The scope of this review does not go as far as surveying all the factors determining the stability of emulsions; it is confined to the consideration of peculiarities inherent in lecithin.

Lecithin belongs to the group of effective emulsifiers, *i.e.* emulsions are preserved for an extended time period after dispersion. Since this phospholipid is more soluble in non-aqueous media, one could expect formation of an emulsion of the 'water-in-oil' type in accordance with the empirical rule of Bancroft.[184] Nonetheless, in the presence of lecithin, the usual case is dispersion of oil in water.[185] Furthermore, in some instances, spontaneous emulsification takes place.[186]

Fairly stable emulsions are formed at an oil–water ratio of 0.4–0.6 and a lecithin concentration above 0.5 mass %.[185] The size of droplets is dependent on the lecithin concentration [187] and varies from 5 to 15 μm.[185, 187]

Lecithin emulsions are characterised by high viscosity levels close to that of gel-like systems. The dramatic growth of viscosity (η) is frequently related to the formation of the lamellar mesophase L_α.[160, 188] This is fully justified in the case of lecithin. Numerous studies [12, 17, 58, 95, 185–187, 189–198] have shown that the shell of emulsion droplets is made of a number of bimolecular layers. When present at high concentrations, multilamellar structures can be observed in a polarisation microscope even at a small magnification, owing to anisotropy of the lamellar mesophase L_α. The appearance of the L_α-phase induces a sharp increase in the emulsion stability (see, *e.g.* the papers [17, 95, 185–187]). Groves *et al.*[198] believe however that the shell of emulsion droplets does consist of the monomolecular lecithin layer, while the stability is attributed to the formation of multilayer vesicles separating oil droplets.

It should be noted that the multilamellar shell effectively prevents the coalescence of emulsion drops, though it does not prevent flocculation (agglutination).[185, 187] In the course of time, emulsions are observed to separate into a layer of aqueous solution and a concentrated emulsion phase. The multilamellar shell may be regarded as an effective structural-mechanical barrier.[199] Surfactants form usually a monomolecular film around emulsion drops. The more complex structural organisation of lecithin is associated with its proneness to self-assembly into liquid-crystalline structures at the interface of immiscible liquids. The preferential formation of the lamellar mesophase L_α is due to the cylindrical shape of molecules. The insufficient resistance to flocculation is explained by the electroneutrality of the lecithin molecule. This resistance is enhanced by the addition of ionogenic surfactants. Furthermore, the addition of phospholipids carrying other polar groups modifies the dimensionless packing parameter S_p. This enables one to change the emulsion type and enhance its stability, which is widely used for practical purposes.

d. Liquid-crystalline structures

There are numerous studies on ternary systems (see, *e.g.*, Ref. 200); however, no whole phase diagram for the lecithin–water–oil system has been constructed until now. This circumstance does not allow discussion of the general pattern of phase transitions as a function of concentration, although certain features of the phase behaviour of lecithin may be considered.

Most of the studies centred on the transformation of the lamellar mesophase L_α into the inverted hexagonal mesophase H_{II}. The increased interest in this problem was initiated by the hypothesis of the important role of non-bilayer structures in processes occurring in living cells, such as their fusion, endo- and exocytosis, as well as transport of proteins and nucleic acids across biological membranes.[25, 27, 201–205] It was presumed that the elucidation of the basic factors determining the phase transition $L_\alpha \rightleftharpoons H_{II}$ would allow a better understanding of the mechanisms for the certain cell processes.

As can be seen in the binary phase diagram (Fig. 3), lecithin occurs mostly in the form of lamellar mesophase L_α. Its transition to the non-bilayer state (the cubic or the inverted hexagonal mesophase H_{II}) takes place on heating. Non-polar solvents induce

this phase transformation at room temperature. For example, the molecules of n-alkanes, the effects of which were most extensively studied by numerous investigators, penetrate between the hydrocarbon chains of phospholipids organised into bimolecular lamellae. This penetration is accompanied by increased lateral pressure in the region of hydrocarbon chains, which results in higher values of the dimensionless packing parameter S_p (Fig. 2) and in the occurrence of the phase transition $L_\alpha \rightarrow H_{II}$.

A thermodynamic approach to the description of phase transition has been developed by Gruner *et al.*[206–208] According to this approach, the free energy of a system is the sum of three components: the elastic component including all intermolecular interactions, the hydration component, and a component accounting for the packing of the hydrocarbon chains of amphiphilic molecules. Their theoretical estimation proves to be highly problematic because of the complexity of such systems; therefore, phase transitions are considered phenomenologically in terms of the 'spontaneous', known also as 'equilibrium' or 'inner', curvature radius R_0 of the monomolecular layer of amphiphiles. When $R_0 \rightarrow \infty$, the amphiphile molecules aggregate spontaneously into the lamellar mesophase L_α; when R_0 has a finite value, the monolayer is curved, which leads to the formation of spherical (cubic mesophase) or cylindrical (hexagonal mesophase) aggregates. Specifically, solubilisation of alkanes in the lamellar mesophase L_α induces the decrease of R_0. In this case, the deformation progresses towards the aqueous solution, which corresponds to the transition to the mesophase H_{II}.[27, 28, 209]

The lamellar mesophase (L_α) of lecithin solubilises about 8 mass % of n-alkanes.[99, 210–212] The critical alkane concentration, which induces the phase transition $L_\alpha \rightarrow H_{II}$, is determined by the size of alkane molecule, the degree of phospholipid unsaturation, the lecithin–water ratio, and temperature. The ability of hydrocarbons to induce phase transitions diminishes as the molecular mass increases.[28, 99, 211, 212] This effect correlates with the decrease in the adsorption and the penetration of n-alkanes into bimolecular layers.[213–215] In addition, at low water content, non-polar solvents are known to reduce the temperature of the phase transition $L_\alpha \rightarrow H_{II}$ (Fig. 3).[209] Unsaturation of lecithin favours phase transformations.[99, 211, 212] For example, a smaller quantity of hydrocarbon is required for the phase transition $L_\alpha \rightarrow H_{II}$.[209]

All of the above-discussed effects of non-polar solvents may be explained in the framework of the model of Gruner *et al.*,[206–208] on the one hand, and by the transformation of the cylindrical form of the phospholipid molecule into a conical one, on the other. Clearly, the polar solvents will act in the opposite manner. By forming the solvation shell of functional groups, they increase the effective cross-sectional area of the lecithin polar region. This leads to a decrease in the magnitude of the dimensionless packing parameter S_p, which in turn induces the reversal of phase transitions (Fig. 2). For example, the dissolution of water in a non-aqueous solution of lecithin with inverted spherical micelles (L_2-phase) initiates the transformation of aggregates into the lamellar mesophase L_α. Lindman *et al.*[210] observed a more complex phase behaviour. Successive addition of water to the mesophase H_{II} obtained at a definite lecithin–n-alkane ratio induced the following transitions: $H_{II} \rightarrow L_\alpha \rightarrow H_{II}$. The phase transition $L_2 \rightarrow L_\alpha$ usually proceeds with concomitant precipitation.[4, 13, 15, 17, 94, 135, 136] This effect has found its practical application in the technology of lecithin isolation from soybean oil.[4]

2. Non-aqueous systems

In the discussion of the binary systems, it was shown that the ability of lecithin to self-assemble is preserved upon substitution of water by polar organic solvents. This property of lecithin is observed also in the ternary system lecithin–polar organic solvent–oil, though such systems have been studied to a lesser extent. For example, no reports devoted to liquid crystals are available, although some mention of their existence can be found in the relevant literature. Systematic studies were carried out solely on lecithin organogels. It should be noted that the formation of microemulsion was observed in non-aqueous ternary systems, whereas in the presence of water only emulsions could be prepared.

a. Organogels: a molecular model of self-assembly

Solubilisation of trace quantities of polar organic solvents in a non-aqueous solution of natural lecithin leads, as in the case of water, to its thickening.[142, 175, 176] Being a Newtonian fluid, the initial solution is transformed into a viscous-elastic gel-like mass displaying properties of a Maxwellian body. The gel-forming solvents and their critical concentrations n_{cr}, at which the maximal level of viscosity is achieved, are listed in Table 6. Comparison of parameters n_{cr} shows that the gel-inducing efficiency decreases in the series glycerol > water > formamide > ethylene glycol. In some of its properties, the diol occupies an intermediate position between the gel-forming and gel-non-forming additives.[142]

Similarly to water, polar organic solvents induce one-dimensional growth of the reverse spherical micelles. These are transformed into giant flexible cylindrical aggregates. The molecular mechanism of the lecithin self-assembly into polymer-like micelles has been proposed and substantiated in a number of reports [142, 175, 176] from IR and NMR spectroscopic studies of the interactions of different polar organic solvents with the phospholipid.

Table 6. Formation of lecithin organogels and physicochemical properties of solvents.[142, 175]

Solvent	n_{cr}	$10^3\,\eta$ /Pa s	σ /mN m^{-1}	ε	Solubility [a] (%)
Lecithin organogel is formed					
Water	3.6–3.8	0.894	72	78.3	0.01
Formamide	4.6–4.8	3.30	58.2	111.0	0.01
Glycerol	1.7–1.9	945	63	42.5	–
Ethylene glycol	> 5	16.9	47.7	37.7	–
Lecithin organogel is not formed					
1,3-Propanediol	–	39.2	49.2	35.0	0.02
Ethanol	–	1.078	21.9	24.55	0.2
DMF	–	0.796	35.2	36.7	0.08

Note. The organogel-forming solvents and 1,3-propanediol are characterised by the formation of three-dimensional networks of H-bonds; ethanol favours linear chains, while DMF is not characterised by formation of H-bonds. [a] Solubility in squalene; data from Ref. 221.

Jellification takes place upon the addition of glycerol, formamide, and ethylene glycol, which form three-dimensional networks through hydrogen bonds. In this case, non-aqueous liquid crystals are also formed. Nonetheless, if the self-assembly of lecithin into liquid-crystalline structures is induced by the solvophobic effects, the roles of polar organic solvents and water in the formation of polymer-like micelles turn out to be different.

This inference is implied by the fact that the addition of 1–3 solvent molecules per phospholipid molecule (parameter n_{cr}) is sufficient for inducing the gel formation. The totally filled hydration shell of lecithin comprises up to 30 water molecules. In other words, the quantity of polar solvent in polymer-like micelles is insufficient even for the total filling of their solvation shells. Consequently, the polar solvent plays a different role in jellification, which should be regarded as structure-forming.

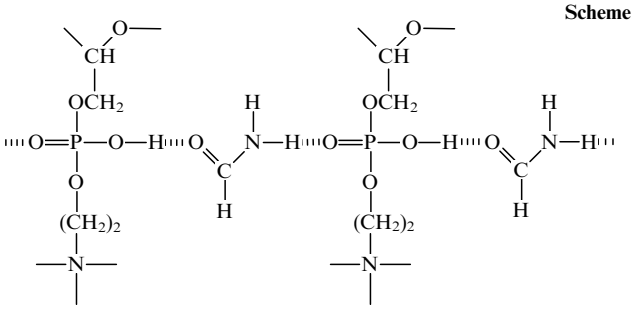
Comparison of structurally close polar organic solvents may provide a better understanding of the mechanism for the self-organisation of lecithin. The full list of the additives studied to date may be found in Refs 142, 175. We shall confine ourselves to

considering the effects of 1,3-propanediol, ethanol, and dimethylformamide (Table 6).

These solvents are structurally close to ethylene glycol, glycerol, and formamide. However, they cannot induce jellification in non-aqueous solutions of lecithin. The IR and NMR studies have shown that the water molecules,[142, 175, 176, 216, 217] like those of gel-forming organic solvents,[142, 175, 176] concentrate in the region of the phospholipid polar group by linking to it via hydrogen bonds. 1,3-Propanediol, ethanol, and dimethylformamide also bind with the phosphate residue, though this binding is substantially weaker. The essence of distinctions between the effects produced by the additives that can and cannot induce gel formation may be readily demonstrated by comparing dimethylformamide and formamide.

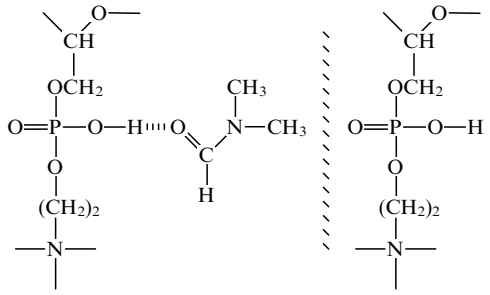
The molecule of the former carries an acceptor centre (C=O group) and hence can form H-bonds with the donor phosphate group (P–OH). Formamide has larger possibilities since its molecule contains both acceptor (C=O group) and donor (NH_2 group) centres. This allows its linking to the P–OH and P=O groups of phosphate via hydrogen bonding. The polymer-like micelles have been assumed [142, 175] to form when the molecules of solvents interact simultaneously with the neighbouring lecithin molecules. A possible variant of the structure with formamide is presented in Scheme 1.

Scheme 1



The basic distinction between formamide and dimethylformamide, which prevents simultaneous binding of the latter with two lecithin molecules, is that the methyl groups exclude the formation of hydrogen bonds (Scheme 2).

Scheme 2



By forming hydrogen bonds with the adjacent phospholipid molecules, formamide acts as a bridging agent. An important aspect is that the phosphate carrying both acceptor and donor groups can also take part in the establishment of several H-bonds.[113, 123] It has been assumed [142, 175] that the self-assembly of lecithin into polymer-like micelles is possible because of the formation of extended chains made up of solvent molecules and phospholipid phosphate residues.

Linear networks of hydrogen bonds exert a stabilising effect. This inference was prompted by the comparison of lecithin with didodecyldimethylammonium bromide. This ammonium salt forms, like lecithin, reverse cylindrical micelles, but without participation of H-bonds. The entanglement of micelles produces in the bulk of solutions a similar three-dimensional network, though such micelles do not possess any viscoelastic properties.[218, 219] Hoffmann [160] has found that the time of structural relaxation (τ) is 10–20 μs in solutions of didodecyldimethylammonium bromide. After addition of polar solvents to lecithin organogels, *i.e.* after the appearance of hydrogen bonds, the τ value increases abruptly and amounts to several seconds in the vicinity of n_{cr}.[142, 143]

The formation of a bridged structure is dependent on the disposition and orientation of solvent molecules relative to the lecithin phosphate group. A possible reason for the lack of jellification after the addition of 1,3-propanediol and ethanol, which can form multiple H-bonds, is thought [141, 175] to be their insufficiently high polarity (Table 6). It determines both the distribution character of substances in systems of immiscible liquids and their localisation in the interface area.[161, 180, 220, 221] The lecithin molecule has different polarity regions (Fig. 1). The variation of the dielectric constant [from $\varepsilon(H_2O)$ to ε(oil)] in the monolayer of lecithin occurs not by jumps but rather in a stepwise mode.[222–224] The molecules of penetrated compounds are localised in different regions along the lecithin molecule according to their polarity.[225] The highest polarity is characteristic of the phosphate group. The probability of a solvent molecule being located near the phosphate group increases with higher solvent polarity. In the series glycerol – ethylene glycol – 1,3-propanediol – ethanol, the dielectric constant diminishes, whereas the solubility in the non-aqueous phase increases (Table 6).[221, 224] The use of the ^{31}P NMR method has shown that upon transition from the gel-forming to gel-non-forming additives, the hydrogen bond with the lecithin phosphate group is considerably weakened. This is usually favoured by its curving.[226]

b. Non-aqueous microemulsion

Lecithin is known not to form microemulsions in aqueous ternary systems. Microemulsification was found to occur in mixtures with water replaced by ethylene glycol. It is unknown whether or not microemulsions are formed with other polar solvents, since the system soybean lecithin – ethylene glycol – n-decane is the only one that has been described to date.[228, 229] Microemulsions are characterised in greater detail below. Now, we shall consider the picture of non-aqueous microemulsion formation, as reported by Friberg *et al.*[227, 228]

The area of microemulsion existence in the system lecithin – ethylene glycol – n-decane is shown in Fig. 9.[227] At soybean lecithin concentrations of 6 mass % – 10 mass %, microemulsification was observed in mixtures where the diol level increased from 30 mass % to 80 mass %. The size of droplets immediately after their preparation was 100–150 nm. Such a water-free microemulsion displayed a limited stability: the separation into immiscible layers began after 10–28 days. Its stability was enhanced with the increasing content of phospholipid and ethylene glycol.

A distinctive feature of microemulsions is their stability during indefinitely long time periods, since they belong to the thermody-

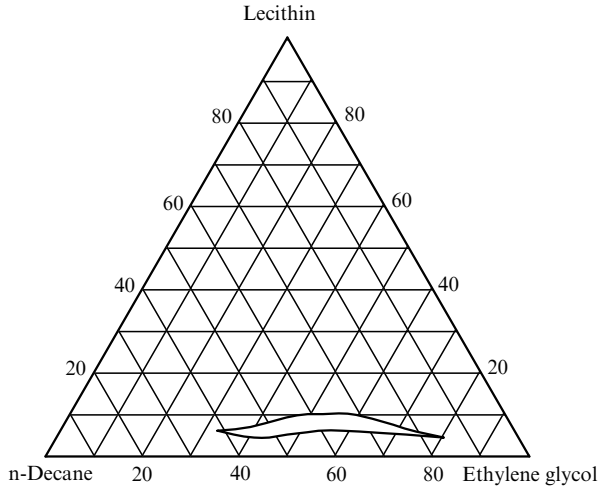


Figure 9. Position of the microemulsion region in the triangular phase diagram of the system n-decane—ethylene glycol – lecithin.[227, 228]

namically stable systems. The separation of a three-component system with time into immiscible layers casts some doubts on the validity of its reference to the microemulsion systems.

Friberg and Podzimek [227] have pointed out the absence of common boundaries between the microemulsional and micellar regions in the phase diagram. This also differentiates the non-aqueous emulsion of lecithin from the known microemulsion systems. The reason for such differences still cannot be identified, since the lecithin microemulsions with ethylene glycol have not as yet been studied to the extent usually required for such purposes. It is hoped that studies of non-aqueous systems along these lines will be reported soon.

VI. Multicomponent mixtures

Mixtures of one or two solvents and different amphiphiles with lecithin are acquiring diverse practical applications. These are already so numerous that merely their listing would require considerable space. The present section is devoted to the basic criteria used for the selection of appropriate components of practically important systems, such as microemulsions and emulsions possessing enhanced stability. In this approach, much attention is given to mixtures of lecithin with other phospholipids and a number of surfactants having important practical and physiological significance.

1. Microemulsions

The term 'microemulsion' is used for a dispersion of microdroplets of one liquid in another.[229] This does not, however, necessarily reflect the essence of given dispersive systems and is far from providing their adequate characterisation. The definition proposed by Danielsson and Lindman [230] is gaining popularity over recent years. They refer to microemulsions the water–oil–amphiphile systems that form homogeneous, optically isotropic, and thermodynamically stable liquid solutions. Generally, microemulsions are prepared by adding a third solvent (co-solvent) or a low-molecular surfactant (co-surfactant). It should be noted that the number of ternary systems in which microemulsification can occur is limited. Lecithin belongs to the group of amphiphiles incapable of stabilising the microemulsion phase in the absence of co-solvents or co-surfactants.

The principal difference of emulsion from microemulsion resides in the higher stability of the latter. To understand the reason for different stability, let us express the variation of free energy during dispersion of one liquid in another as

$$\Delta G = \gamma \Delta A - T\Delta S,$$

where γ is the interfacial tension at the oil–water interface, A is the interfacial area between immiscible liquids, and S is the entropy of dispersion. Microemulsification is accompanied by a dramatic increase in the A area. The system is thermodynamically stable when $\Delta G < 0$. In other words, the increase in A must be totally compensated. It has been shown [231–233] that in some cases this may be done at the expense of the increase in entropy in the course of dispersion. Apparently, the increment ΔA is compensated by the decrease in the interfacial tension. So, in microemulsions containing alkanes, the magnitude of γ diminishes sometimes from ~ 50 mN m^{-1} down to 10^{-4} mN m^{-1},[23,139] whereas in emulsions it does not drop below 1 mN m^{-1}.

The distribution of microemulsion phases in a three-component phase diagram plotted in the triangular coordinates is presented schematically in Fig. 10 along with the insets showing different dispersive systems. The phase state in the system is determined by the ratio of its components. The single-phase microemulsion is usually localised near the central part of the diagram. The decrease in the amphiphile concentration induces the separation into bi- or tri-phase mixtures. These were first described and classified by Winsor [234], and ever since are referred to by his name.

All the multiphase systems are divided into three types (Fig. 10). Winsor I (WI): microemulsion is in contact with the organic phase. Winsor II (WII): microemulsion is in contact with the aqueous solution. Winsor III (WIII) microemulsion separates the organic and water phases. Transitions between states are induced by changes in the system composition or temperature. The most common are the transitions WI $\rightleftharpoons$ WIII $\rightleftharpoons$ WII. When the amphiphile quantity proves to be sufficient for the total filling of the interface between immiscible liquids, a one-phase microemulsion is formed instead of WIII. In this case, the 'phase behaviour' is often complicated by the appearance of lamellar mesophase L_α.[23,228,235] In the phase diagram, its region is in contact with the region of one-phase microemulsion (Fig. 10).

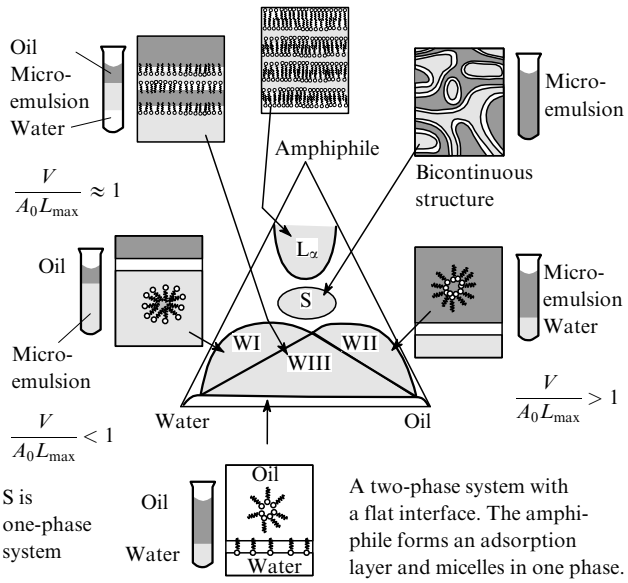


Figure 10. Microemulsion phases in the ternary phase diagram and schematic drawings of dispersive systems. For comments, see the text.

A reversible transition from WI to WII is accompanied by the reversal of the sign of curvature $C' = 1/r$ (r is the droplet radius) of the amphiphile monolayer localised at the interface separating two immiscible liquids. For the oil-in-water dispersions, the curvature C' is assumed to be positive, *i.e.* the monolayer is curved relative to the water. In this case, in the WII system, $C' < 0$. The transition from WI to WII must proceed via an intermediate state with $C' \approx 0$ at $r \to \infty$. Though the structures formed in this process occur less frequently than the microdrop dispersions, they nonetheless attract the attention of investigators owing to a number of their specific properties. For example, at $C' \approx 0$, one-phase, homogeneous mixtures are produced with the largest content of immiscible liquids, which is highly important for practical purposes. The structures formed at $r \to \infty$ have come to be known as sponge-like or bicontinuous. They are characterised by the interpenetration of the aqueous and non-aqueous phases with a constant dynamic inversion of 'water-in-oil' into 'oil-in-water' structures and *vice versa*. This is explained by the proximity of value of the elasticity modulus (K) for an amphiphile monolayer to kT,[23] which makes the structures sensitive to thermal oscillations. For comparison, the magnitude of K for the condensed monolayers of amphiphiles on the water surface is close to $100\,kT$,[236] while in the bimolecular films of egg lecithin it amounts to $10\,kT$.[237] Interestingly, the introduction of a bipolar amphiphile (an amphiphile with two functional groups separated by an extended hydrocarbon chain) into lecithin vesicles was observed to reduce the value of K down to $(1.0-0.1)\,kT$ with the concomitant fast and large-amplitude fluctuations of the bimolecular layer resulting in the appearance of diverse vesicle shapes.[237,238] A similar pattern of dynamic rearrangements seems to take place in the bicontinuously organised microemulsions.

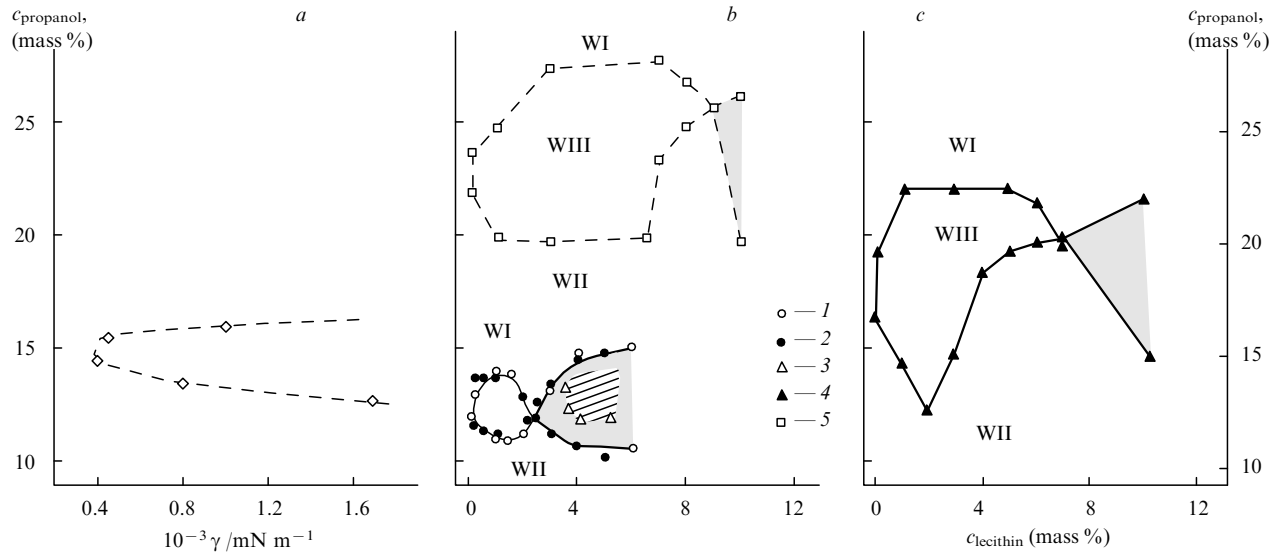


Figure 11. The interfacial tension between aqueous solution and alkane in the system soybean lecithin – water solution of propanol – n-hexane as a function of the propanol content in water (30 °C) (*a*), and the phase diagrams of the ternary system soybean lecithin – aqueous solution of propanol – n-alkane (25 °C) (*b*, *c*). The ordinate, propanol content in water; the abscissa, lecithin concentration in n-alkane. The water : n-alkane ratio = 1 : 1. The region of lamellar L_α phase is hatched; the regions of one-phase microemulsion are marked with grey colour. The system contains n-hexadecane (*1* – *3*), n-tetradecane (*4*), and n-dodecane (*5*). The diagrams were plotted from the data of Refs 241 (*2*, *3*) and 245 (*1*, *4*, *5*).

The role of an amphiphile in the microemulsion systems appears to be crucial. It forms a monomolecular film that separates two immiscible liquids and thereby creates conditions for mixing and simultaneous solubilisation of solvents and compounds with opposite polarities. The resultant balance of molecular interactions turns out to be very sensitive to experimental conditions and the composition of mixtures. Even a slight variation of the latter factors may induce the transitions WI $\rightleftharpoons$ WIII $\rightleftharpoons$ WII. On the other hand, phase transitions may also be initiated by changing the form of amphiphile molecules from the inverted to the normal conical shape. In this case, the magnitude of the dimensionless packing parameter must vary successively from 1/3 to 3 (Fig. 2). The shape of molecules cannot change so strongly spontaneously; this may be done only under the action of a number of factors. These include temperature, electrostatic interaction between charged groups, hydration, and the penetration of solvents and co-surfactants into the monomolecular layer of amphiphiles. The magnitude of the dimensionless packing parameter may be regulated by one or all of these factors. They shift the equilibrium in the required direction and induce formation of any of the possible microemulsions or initiate their successive transformations (Fig. 10). Natural lecithin is well-suited for the consideration of the basic regularities of such transitions.

The lecithin molecule preserves its cylindrical form at different water – non-polar solvent ratios, as well as over a wide temperature range.[56] Besides, lecithin does not reduce the oil – water interfacial tension below 1 mN m^{-1}.[11,17,95] As a result, no formation of microemulsion is observed in the ternary lecithin – water – oil systems. In this case, the only possibility is to prepare emulsions.

First systematic studies of the conditions of microemulsification in the multicomponent systems containing lecithin were carried out by Shinoda *et al.*[239–243] It was shown that microemulsions are formed upon addition of low-molecular-mass aliphatic alcohols to the ternary system soybean lecithin-water-hexadecane. The addition of co-surfactants induced a sharp decrease in the interfacial tension (Fig. 11). The process of microemulsification was markedly dependent on the alcohol polarity. For example, the addition of butanol led to the formation of reverse swollen micelles. The preparation of Winsor mixtures and one-phase microemulsions was found to require the presence of ethanol or propanol. The latter was more effective, as microemulsification occurred at its lower concentration. The phase states in the ternary systems with propanol at two levels of soybean lecithin are shown in Fig. 12. It is noteworthy that the area of one-phase microemulsion with bicontinuous organisation is considerably extended with higher phospholipid content.

Thorough investigation of the dependence of microemulsification on the length of lecithin hydrocarbon chains, molecular mass of n-alkanes, and hydrophilicity of aliphatic alcohols has enabled Kahlweit *et al.*[244] to reveal a number of regularities making it possible to determine *a priori* the composition of microemulsions intended for different purposes. It was shown that the shorter hydrocarbon chains in lecithin, the more hydrophobic co-surfactants should be used. This is achieved, for example, by substituting butanol for propanol. In the case of didecyllecithin, a further increase in the hydrophobicity of alcohols is required. To this end, the addition of equal amounts of butanol and pentanol was proposed. The increase in the molecular

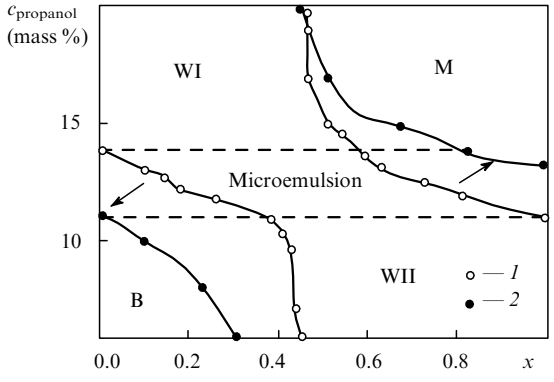


Figure 12. Phase diagram of the system soybean lecithin – water solution – n-hexadecane at 25 °C. The ordinate, propanol content in water. Lecithin concentration in n-alkane: 1 mass % (1) and 3 mass % (2); the arrows indicate the expansion of the microemulsion region with higher lecithin levels. The horizontal dashed lines indicate the boundaries of the single-phase microemulsion. M, non-aqueous phase; B, aqueous phase. The diagram was plotted from the data of Refs 242 and 243.

mass of n-alkanes favours microemulsification in the presence of natural lecithins. This is illustrated by Fig. 11 *b*, *c*.[241, 242, 245] On going from n-dodecane to n-hexadecane, the microemulsification is seen to occur at a lower propanol content. Furthermore, a one-phase microemulsion is obtained at a smaller lecithin concentration. The reduction of the molecular mass of n-alkanes and the replacement by aromatic hydrocarbons require a higher hydrophilicity of the aliphatic alcohol. For example, upon transition to n-hexane, propanol proves to be ineffective as co-surfactant because of higher solubility. Preparation of microemulsions requires its replacement by ethanol.

Kahlweit *et al.*[244] have proposed to use mixtures of alcohols for the fine regulation of phase equilibria. One should bear in mind that aliphatic alcohols exert toxic effects on living systems. This fact restricts essentially the practical use of microemulsions stabilised by natural lecithins. Non-toxic mixtures containing 1,2-alkanediols as co-surfactants were proposed.[246] Besides, n-alkanes were replaced by the non-toxic ethyl oleate. Comparative studies of 1,2-alkanediols have revealed that the optimal conditions for microemulsification in mixtures containing soybean lecithin are created in the case of 1,2-pentanediol whose efficiency is comparable with that of propanol.

In conclusion, it should be said that today the relevant literature carries rather definite concepts of the structure and mechanisms of microemulsion formation. As indicated in a number of recent reviews,[139, 160, 247–251] the period of intensive studies is giving way to wide and diverse practical applications of microemulsions. The spectrum of their uses proves to be tremendously broad, ranging from chemical and biochemical technologies to functional technologically important fluids, food, cosmetic and medicinal preparations. In this context, it seems appropriate to mention basic advantages that make microemulsions attractive for most varied fields.

1. Universal solvents. As microemulsion systems comprise aqueous and non-aqueous phases, they allow simultaneous solubilisation of both polar and non-polar substances. Consequently, it becomes possible to prepare mixtures of compounds that otherwise cannot exist in direct contact as solutes.

2. Microreactors. In bicontinuous structures, each droplet or domain contains all of the reagents included in a given mixture, which makes it possible to consider them as individual reaction systems. Owing to the size of such microreactors, microemulsions are promising for the synthesis of nanomaterials.

3. Highly effective extraction and reaction systems. High dispersiveness ensures a tremendous interfacial area between immiscible liquids. This intensifies the processes whose rates are limited by migration across the interface or are determined by contacts of substances belonging to different phases.

2. Mixtures with phospholipids and surfactants

In the discussion of emulsions, it was noted that although lecithin is an effective emulsifier, its effect still proves to be insufficiently great. Numerous extensive studies of mixtures of this phospholipid with different substances were, therefore, aimed at enhancing the stability of the emulsions finding most varied practical applications. The present review does not centre on the consideration of numerous experimental data reported to date. Instead, it focuses on the basic principles of the selection of co-emulsifiers illustrated by a number of concrete examples.

The stabilising effect of lecithin is associated with the formation of a multilamellar shell around each emulsion droplet which acts as an effective structural-mechanical barrier preventing coalescence. Despite this, the resistance of droplets to flocculation is not sufficiently high. To eliminate this drawback, methods should be employed that would prevent contacts between the emulsion droplets.

The distance between the bimolecular layers of lamellar mesophase L_α is enlarged by electrostatic repulsive forces (see Fig. 4). Consequently, in order to avert the flocculation of an emulsion stabilised by neutral lecithin, it is necessary to introduce ionogenic additives. This is achieved through the employment of natural phospholipids, which introduce no toxic properties and do not perturb the biocompatibility with living systems, and by the addition of different ionogenic surfactants.

The set of phospholipids that can acquirie an electrical charge via dissociation is rather limited. It includes phosphatidylserine, phosphatidylinositol, and phosphatidic acid. Their addition is conducive to a substantial increase in the resistance of lecithin-stabilised emulsions to flocculation.[58, 185, 194] These phospholipids have been found to possess different efficiency. This is illustrated by a diagram in Fig. 13. This diagram presents the half-lifetimes of oil droplets from the moment of their contact to fusion measured in model experiments. Accordingly, as regards their efficiency, the anionic phospholipids may be arranged in the following series: phosphatidylinositol < phosphatidylserine < phosphatidic acid. The addition of the latter to lecithin produces the strongest stabilising effect.

Ionogenic surfactants exert analogous effects. A good example are the salts of bile acids, which play an important physiological role in the processes of fat digestion in the human and animal intestine.[252] The major bile ingredients are lecithin and bile acid conjugates, *e.g.*, of cholic acid with taurine and glycine. Their simultaneous presence provides for the emulsification of lipids coming with food. In order to achieve an effective functioning of lipases and subsequent uptake of fatty acids by the intestine walls, the emulsion must be composed of as small droplets as possible and be maintained in the non-flocculated state for a few hours.

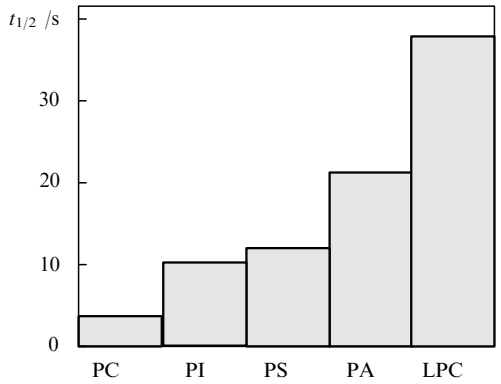


Figure 13. Resistance of oil droplets in water to coalescence.
The non-aqueous phase contains soybean lecithin (PC, 0.1 mM), mixture of lecithin with phosphatidylinositol (PI, 30 mg ml^{-1}), phosphatidylserine (PS, 25 mg ml^{-1}), phosphatidic acid (PA, 15 mg ml^{-1}), and lysophosphatidylcholine (LPC, 20 mg ml^{-1}). The ordinate, the half-lifetime of droplets from the moment of their contact to fusion. The graph was plotted according to the data of Ref. 194.

The highly significant physiological role of the lecithin mixtures with salts of bile acids stimulated a keen interest shown by different groups of researchers.[187, 253–256] Studies of the emulsification process *in vitro* revealed that nature had devised a highly effective system. For instance, model experiments [187] showed that the addition of sodium taurocholate to the egg lecithin-stabilised emulsions led to the further increase in their stability. Electrophoretic measurements confirmed that this effect was induced by the appearance of charges on the droplet surface. In addition, sodium taurocholate promoted the formation of a finer dispersion. After its addition, the size of emulsion droplets decreased three to four times.[187] The relevant literature carries indications [257] that the presence of sodium cholate can stabilise even microemulsions, although the pertinent processes and the phase behaviour of systems have been little studied.

Another possibility for enhancing the stability of emulsions and modifying their type is offered by the regulation of the dimensionless packing parameter S_p. Lecithin, having an S_p

parameter close to unity, does not possess a molecular geometry optimal for the formation of curved surfaces. This explains wide practical use of additives modifying the dimensionless packing parameter S_p. The surfactants with a large polar region and single hydrocarbon chains diminish S_p, which favours the formation of 'oil-in-water' emulsions, while those with small functional groups and two hydrocarbon radicals induce the formation of 'water-in-oil' emulsions. Let us consider how this is realised in practice.

Lysolecithin, *i.e.* a lecithin derivative in which one of the two fatty acid residues is missing, often serves as an amphiphilic additive with a large polar head. As can be seen from the diagram in Fig. 13, the neutral lysolecithin induces a sharp increase in the stability of lecithin-stabilised emulsions. Its efficiency proves to be substantially higher even than that of ionogenic phospholipids.

As a stabilising additive, lysolecithin has the advantage of being a lecithin derivative. It seems more convenient to perform partial hydrolysis of this phospholipid instead of adding other co-emulsifiers. It has been proposed to use group A phospholipases for this purpose. The enzymatic treatment of lecithin has been shown to result in a substantial increase in the stability of emulsion systems.[258–260]

The type of emulsion may be changed by the addition of phosphatidylethanolamine. This phospholipid is a lecithin analogue whose molecule includes a quaternary nitrogen carrying three hydrogen atoms instead of three methyl groups. This determines a smaller cross-sectional area of the polar region. As a consequence, the molecule has a conical shape, and $S_p > 1$. Contrary to lecithin, phosphatidylethanolamine forms mostly non-bilayer liquid-crystalline structures, in particular an inverted hexagonal mesophase H_{II}.[13, 28, 58, 73] Its mixing with lecithin increases the S_p parameter. This leads to the eventual transformation of direct emulsions into their reverse type.[58, 261]

The efficiency of co-emulsifiers increases when both factors are brought into play, *i.e.* when the droplet surface is charged and the variation of the dimensionless parameter is directional. Sometimes, it is problematic to differentiate them since the molecules of different substances are always different in their shape, while electrostatic interactions affect the magnitude of S_p. The interrelation of these factors may be analysed on the example of lecithin mixtures with sodium taurocholate.

In the study of emulsification, Fillery-Travis *et al.*[187] analysed only the effects associated with electrical charges of droplets. As a matter of fact, the hydrophobic taurocholate anion cannot penetrate inside the bimolecular layer and is most likely localised in the region of the lecithin polar groups. With this distribution, the polar region is expanded, which leads to lower values of the S_p parameter. The decrease in the magnitude of S_p is also favoured by the mutual electrostatic repulsion of molecules in the lateral plane. The mixing of lecithin with taurocholate in the monolayer makes the average molecule acquire the reversed cone shape, which favours the surface curvature and explains the experimentally observed formation of smaller dispersion droplets.[187]

It should be noted that the quantitative estimation of the S_p parameter variation in mixtures is a complex, if at all realistic, task. We can predict a general character of changes in the molecular form. The variation of surfactant concentration in mixtures with lecithin induces a series of successive structural rearrangements,[253–256, 262] which are indicative of a gradual variation of S_p. It seems impossible to establish *a priori* the concentration limits of the existence of different structures. Therefore, appropriate compositions for specific practical purposes are selected on an empirical basis. For example, the German company 'Lucas Meyer', a major producer of soybean lecithin, offers various lecithin-based compositions[263] including lysolecithin, charged phospholipids, and phosphatidylethanolamine as ingredients. Tests of lecithin-based mixtures in technological processes and as components of various systems made it possible to determine their composition ranges for the optimal use in food industry, cosmetics, pharmacology, and paint and varnish production.

VII. Self-organisation of lecithin at the interface of immiscible liquids in the course of adsorption

The investigation of three- and multi-component systems comprising immiscible liquids has shown that their properties and phase behaviour are determined by the state of the amphiphile layer at the water–oil interface. Despite the important role of interfacial films, the problem of lecithin self-assembly in the interfacial regions has not received due attention. Most of the studies were devoted to the monomolecular layer,[264–271] though even those were not numerous because the priority was always given to the air–water interface.

However, it should be noted that the specifics of experiments with monolayers, such as a limited amount of amphiphile that is added directly at the oil–water interface and the mechanical compression–expansion cycles brought about with the help of a mobile barrier, rule out the occurrence of spontaneous processes. A more suitable approach to the study of the self-organisation phenomena is offered by the method based on the investigation of the course of adsorption, which is not limited by any external factors, after bringing a non-aqueous lecithin solution and water into direct contact, and on the analysis of structures formed in the process of equilibrium onset. Such studies were mostly carried out at the Institute of Chemistry (the Far-Eastern Branch of the Russian Academy of Sciences). The basic results pertinent to the topic of this review are discussed in this section.

Numerous studies were devoted to egg lecithin adsorption from the bulk of n-alkanes at the alkane–water interface.[11, 17, 95, 272–274] These papers report values of basic thermodynamic and kinetic parameters of egg lecithin adsorption, such as the standard free energy of adsorption (G°_{ad}), diffusion coefficient (D), and the rate constant of lecithin adsorption (β) from the non-aqueous phase at the n-alkane–water interface: $G^\circ_{ad} = -47.6$ kJ mol^{-1}, $D = (2.0\pm0.5)\times10^{-10}$ m^2 s^{-1}, $\beta = 5\times10^{-7}$ m s^{-1}. The diffusion coefficient is calculated from the equation

$$\Pi = RTC\left(\frac{Dt}{\pi}\right)^{1/2},$$

where Π is the interfacial pressure equal to the difference of interfacial tensions at the oil–water interface in the presence and the absence of lecithin, C is the phospholipid concentration in the non-aqueous solution, t is the time of contact between two immiscible liquids. The diffusional kinetics is characteristic of the initial stages of the adsorption layer formation. As the interface is filled, the diffusional kinetics gives way to adsorptional events. In other words, the entry of the substance into the interfacial film becomes the limiting stage of the process.[95]

The character of the interface filling may be estimated from the dependence of the area per lecithin molecule at the n-alkane–water interface on the concentration of lecithin in the non-aqueous solution (Fig. 14).

Analysis of the peculiarities of the interfacial processes allowed differentiation of at least three concentration ranges in which different types of the adsorption layer are formed.

Dilute solutions (10^{-7}–10^{-6} M). Lecithin possesses a high surface activity. The change in the interfacial tension begins from the lecithin concentration of about 10^{-7} M. Such a high activity is explained by the large polar region of lecithin (Fig. 1), which does not allow its molecules to remain in the monomeric state in non-polar media, and by the high hydration energy determining the preferential localisation of the phospholipid molecules at the oil–water interface. Thus the standard free energy of lecithin adsorption proves to be of the same order as the hydration energy.[17, 93]

Relevant studies have shown that within the framework of the classical thermodynamic theory of Gibbs, lecithin adsorption may be described only for strongly dilute solutions. The basic equation used for this purpose is

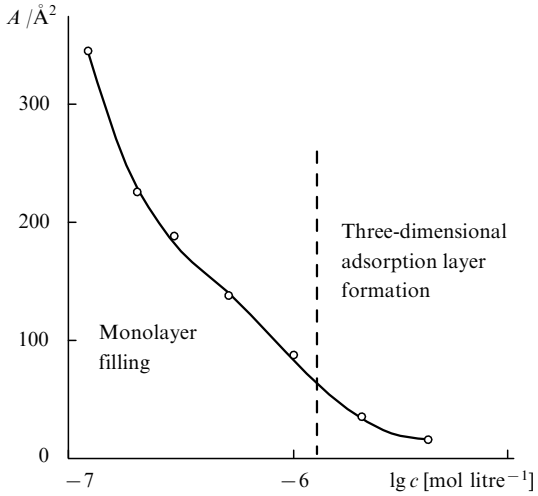


Figure 14. Dependence of the area per egg lecithin molecule at the n-decane – water interface on the concentration in the non-aqueous phase. The dashed line indicates an approximate boundary of the transition from the two-dimensional to three-dimensional filling of the interface. The A values were calculated by the Gibbs equation (3). The interfacial tension was determined using the droplet profile technique which allows measurements after the equilibrium onset in the system (15 – 17 h).

$$d\Pi = \frac{RT}{AN_A} d\ln C\,, \tag{13}$$

where N_A is the Avogadro number. The Gibbs equation (13) makes it possible to estimate the area per lecithin molecule in the adsorption layer and to establish thereby the character of the filling of the interface separating immiscible liquids (Fig. 14). At the egg lecithin concentrations ranging from 10^{-7} to 10^{-6} M, the characteristics of its adsorptional layer have been found to be close to those of the monolayer.[17, 95, 272 – 274]

Intermediate solutions ($> 10^{-6} – 10^{-2}$ M). At the lecithin concentrations above 10^{-6} M, its quantity at the oil – water interface exceeds the level required for the monomolecular filling.[17, 95, 273, 274] Two-dimensional interfacial films are giving way to the formation of three-dimensional ones. In concentrated solutions, they become distinguishable even at small magnification.[95, 273] Until now, no special physicochemical techniques have been employed to study the structure of the interfacial layer. This problem is addressed only in rare accounts of experimental observations.

The interfacial structures have been found to display optical anisotropy,[95, 275] which is indicative of their possible liquid-crystalline organisation. This is consistent with the results from studies of the emulsion droplet shell. More definite ideas of the interfacial layer structure may be derived from experiments on the thinning of non-aqueous films separating two aqueous solutions. It has been shown [95, 214, 276, 277] that the structure of the adsorption layer resembles the lamellar mesophase L_α. Such a structural organisation is implied by a discrete thinning similar to the successive 'removal' of layers with a thickness approximately equal to that of the bimolecular layer.[214, 277]

It is noteworthy that the formation of three-dimensional structures at the interface of immiscible liquids is not characteristic of surfactants. Generally, the multilayer filling of the interfacial surface takes place, as confirmed by numerous checks of the validity of the Gibbs equation (13). In the case of lecithin, self-assembly of its molecules into more complex structures has been revealed. Hydration of this phospholipid is believed [95, 273, 274] to be the driving force of this process. Kinetic studies [95, 273, 274] of the absorption from the bulk of non-aqueous solutions have shown that on reaching the oil-water interface, a lecithin molecule is retained there because of its high hydrophilicity resulting from its hydration with up to 30 water molecules, which considerably reduces its solubility in the non-polar phase. Lecithin gradually accumulates near the oil – water interface, and when its level becomes higher than that required for monolayer filling, the formation of more complex structures begins.

Concentrated solutions ($\geqslant 10^{-2}$ M). The interfacial processes spread inside the non-aqueous phase and modify their character. Figure 15 is a photograph illustrating the liquid phase separation near the oil – water interface.[274, 278] Its appearance in the form of a thin interfacial film is revealed a few minutes after the contact of non-aqueous lecithin solution with water. The film grows thicker, though irregularly. Its surface reveals protrusions directed towards the non-aqueous phase. At a certain moment the film is disrupted allowing a liquid influx to the surface.

Studies of the interfacial processes have led to the conclusion [274, 278] that it was not a separate liquid phase that was isolated, but rather a dilute non-aqueous solution with a reduced lecithin content. The phospholipid is concentrated in a different, gel-like film in which it is later transformed into a floccular sediment. The gel and the sediment are localised directly on the surface of the water support.

The experimental findings have been used as a basis for developing a mechanism of the interfacial processes [274, 278] that

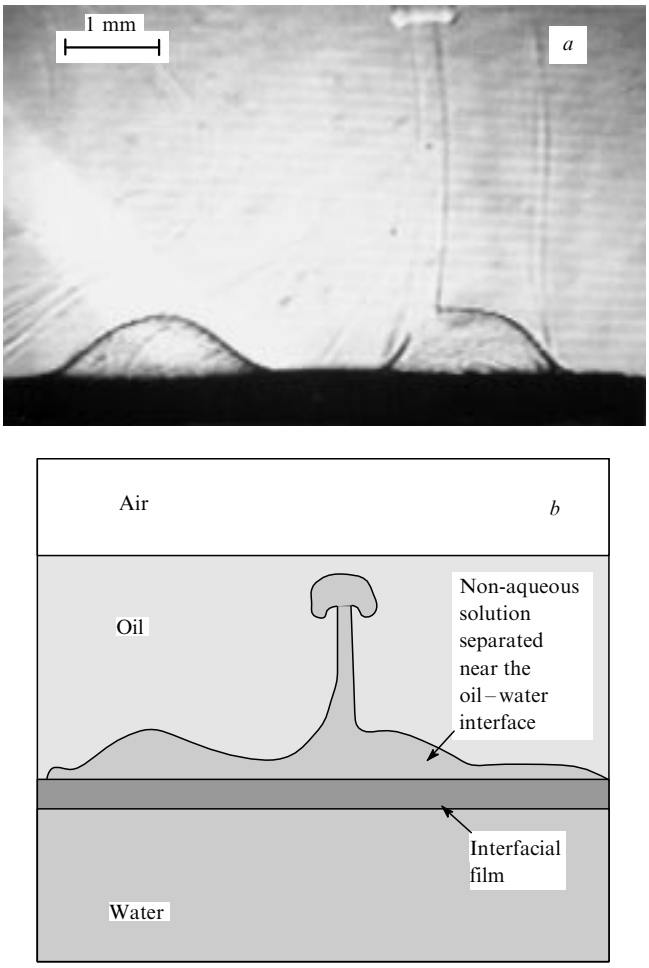


Figure 15. A photograph of the segregated liquid phase in a non-aqueous solution of lecithin near the alkane-water interface at the moment when the liquid is jetting to the alkane surface (*a*) and a schematic representation of the cell used for experiments (*b*).
The photograph was taken after a 5.4 min contact of immiscible liquids. Illumination was done with a halogen lamp. For distinct revelation of the segregated liquid phase, the photograph was taken through partially crossed polaroids. Soybean lecithin concentration in n-decane, 10 mg mlitre^{-1}.

relies on the transformation of reverse spherical lecithin micelles into polymer-like aggregates after addition of trace amounts of water. This transformation involves a number of phase and pseudophase transitions: the formation of organogel and its subsequent separation into a compact gel and a dilute non-aqueous solution, and transformation of the gel bulk into a solid sediment (Fig. 8).

In a system of immiscible liquids, the above-mentioned phase and pseudophase transitions are recognised by the appearance of the corresponding phases near the oil–water interface. The respective processes are induced and develop with time owing to the water supply from the adjacent aqueous solution. Since H_2O is insoluble in the non-polar phase, the role of carrier seems to be performed by the lecithin itself. The possible mechanism of such processes may be as follows.

A lecithin molecule attaches water molecules by virtue of hydration at the interface with subsequent desorption of the hydrated molecule and its migration into the bulk of non-aqueous solution, where H_2O is redistributed among non-hydrated lecithin molecules, which induces the transformation of spherical micelles into the polymer-like ones.

It is remarkable that the phase transitions take place not in the adsorption layer but spread considerable distances to occur inside the non-polar phase (Fig. 15). Similar situations are created artificially by bringing into contact two immiscible liquids with one containing a substance that is also well soluble in the second liquid. Its migration may be caused by solubilisation. This may lead to successive phase transitions determined by the phase behaviour of individual amphiphiles.[180, 279] In the lecithin-containing systems, the migration of water to the non-polar phase is due to different causes, the principal being the hydration of the phospholipid.

The self-organising structures of lecithin modify the interface of immiscible liquids and endow it with new properties. This is especially clearly manifested under external impacts. The application of electric fields has been shown [17, 95, 274, 280, 281] to lead to the decreased interfacial tension and the emergence of electrodynamic instability at a field strength nearly 100 times as low as that inducing analogous phenomena in the absence of lecithin. Furthermore, the application of a voltage has been found to induce some phenomena that did not occur beforehand: dispersion of a planar interface, the formation of inverted emulsion, and jellification of the non-polar phase. The latter is of interest for practical uses. The substantial decrease in the electric field strength, inducing dispersion of water in oil, allows realisation of processes under mild conditions without high-voltage equipment. Another advantage is that the dispersions formed are preserved only in external electric fields. This makes possible regulation of the processes of the formation and degradation of dispersions.

VIII. Conclusion

The above consideration of the lecithin self-assembly in different media has shown that today it is possible to present a sufficiently full picture of its structures and their phase transformations. One could hardly expect it to be otherwise, since a tremendous number of studies have been devoted to this phospholipid. The investigation of specific aspects of the self-organisation of natural lecithin often gave impetus to the development of new research directions. Liposomes are a good example.

The development of preparative techniques and the use of dispersions as models of cell membranes *in vitro* were for the most part based on lecithin-containing systems. When *in vivo* applications of liposomes were brought onto the agenda, they revealed a number of drawbacks of lecithin among which the principal disadvantage was fast degradation of liposomal structures by cell systems and their insufficient specificity towards the cells selected as targets for biologically active compounds. The efforts aimed at enhancing the stability and targeting the liposomes that involved chemical modification of the lecithin molecule and its complexation with different compounds have resulted in the development of a new generation of liposomal transport systems for biologically active compounds and drugs that has laid the foundation for one of the most rapidly developing directions in biochemistry and biomedicine.[69, 70, 76–79] Nowadays, further ways for the application of the principles of lecithin self-organisation for devising novel structures are being actively debated in the literature. Specifically, there is a view [76] that the next stage in the perfection of liposomes will be the development of virus-like systems that would allow a deep insight into the problem of genetic information transfer that is indispensable for the therapy of hereditable diseases or targeted transformations of cell structures for the needs of biotechnology.

The investigation of the self-organising lecithin structures has resulted in the development of a novel type of hemocompatible polymeric material. The basic stages of this work and the application of the principles of lecithin self-assembly have been described in the recent review by Chapman.[282] An impetus to the development of this scientific field has been given by the discovery of blood non-coagulation in the lecithin-containing systems. Much work was done to study self-organising structures, such as monomolecular films, liposomes, and liquid crystals that are extensively used as cell membrane models. At the first stage, polymers were produced by polymerisation of the lecithins containing diacetylene bonds in its hydrocarbon chains. It has been found that the incorporation of choline alone proves to be sufficient for making the polymeric materials hemocompatible.

The above-cited examples provide evidence that the potential of the investigation of self-organising lecithin structures has not been exhausted, despite the many-year record of systematic studies. On the contrary, new perspectives seem to appear in this field. They are for the most part related to the study of the lecithin self-assembly in mixtures with biologically active substances, *i.e.* to the investigation of structures that are still closer to the living cell systems.

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