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Water diffusion in lecithin–water and lecithin–cholesterol–water lamellar phases at 22°

C.M. GARY-BOBO, Y. LANGE and J.L. RIGAUD
Laboratoire de Physiologie Cellulaire, Collège de France, Paris 5e (France)
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SUMMARY

The diffusion of water (^{3}HHO) was studied in lecithin (from egg yolk)–water lamellar phases, at 22°, as a function of their water content, Φ_W. Depending upon Φ_W, two diffusion rates can be distinguished, which are related to both sterical factors and hydration water in the phase. The results obtained in the lecithin–cholesterol–water systems support this interpretation.

Recent developments in the structure analysis of lipid–water systems have led to much speculation about their possible role in biological functions, but very little experimental work has been done to test these possibilities. However, the lipid–water systems appear to be useful models for membrane permeability studies. They offer the possibility of carrying out permeability measurements in systems of which not only the chemical composition but the structure is known at the molecular level, and therefore of making fruitful correlations and progress in the understanding of permeability processes. This note describes preliminary water diffusion measurements in the lecithin–water and lecithin–cholesterol–water lamellar phase at 22°.

Lecithin was extracted from egg yolk according to the method of Singleton *et al.*[1] and checked for purity by thin-layer chromatography. The phases were prepared by mixing a known weight of lecithin and water under vacuum. Homogeneity of the phases was obtained by forcing the mixture back and forth through a tube about 5 mm long and 1 mm in diameter joining two syringes. After 48 h, the phases appeared stable; there was no significant difference between diffusion rates measured in a phase 48 h after preparation and in the same phase 2 weeks later.

The measurement of the diffusion coefficient D of water (^{3}HHO) in the lamellar gels was made in the following way: 1-mm-thick lucite slabs were pierced with a central hole 1.2 mm in diameter. The slabs were stacked in a hollow cylinder so that their central holes formed a capillary 5 cm long. The capillary was filled with the phase and closed at one end. The other end was covered for a short period of time (1–2 min) with filter paper

impregnated with a ^{3}HHO solution of suitable specific activity, so as to create a plane source of "infinitesimal" thickness, containing sufficient amounts of radioactivity. The filter paper was then removed and the end of the capillary closed by a tight-fitting glass cover slip. After enough time had elapsed (from 4 to 24 h, depending on the composition of the phase) for the ^{3}HHO to diffuse through the first 2 cm of the capillary, the slabs were separated and their content analysed for ^{3}HHO. For diffusion in a semi-infinite cylinder, when a given amount M of a substance is deposited as a plane source at one end ($x = 0$) at time $t = 0$, the concentration, c, of the substance at time t at a distance x from the plane source is given by the relation[2] $c = M/(\pi Dt)^{1/2} \cdot e^{-x^2/4Dt}$. In our experimental conditions, x was taken as the distance from the middle of each slab to the plane source. A plot of $\log c$ *versus* x^2 resulted in a straight line of slope $1/4Dt$, from which D was calculated using the method of least squares. A more detailed description of the method will be given elsewhere.

The structural analysis by X-ray diffraction of lecithin–water and lecithin–cholesterol–water systems has been performed by Reiss-Husson[3] and Lecuyer and Dervichian[4]. They found that at room temperature (22°) both systems exhibited a lamellar structure within a wide range of water content, from about 10 to 45% for lecithin–water systems and from 10 to 35% in systems where the lipid part is composed of equimolecular quantities of lecithin and cholesterol. From X-ray diffraction data, the respective thicknesses of the lipidic and aqueous layers may be computed: the former decrease and the latter increase with the increase of the water content in the structure. In such a lamellar phase, water can diffuse in both the lipidic and the aqueous layers. The diffusion rate of water in hydrocarbons is far from negligible[5]. However, in view of the very slight solubility of water in lipids, it may be assumed, at least as a first approximation, that the ^{3}HHO diffusion measured exclusively reflects the diffusion in the water layers.

In Fig.1, the ^{3}HHO diffusion coefficient D is plotted as a function of the water content, Φ_W, in g/g of the phase. It can be seen that D increases with Φ_W. However, it is

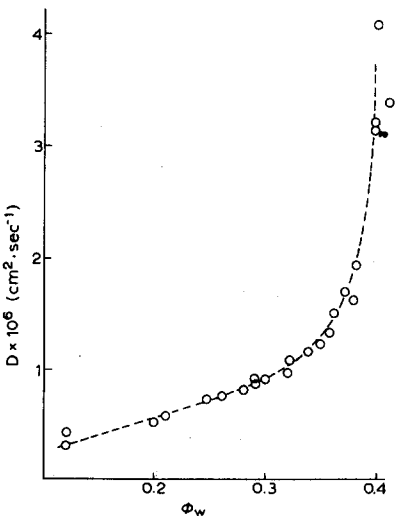

Fig.1. Water diffusion, D, in lecithin–water lamellar phases as a function of water content, Φ_W.

possible to distinguish between two regions in the curve. For Φ_W, in the range from 0.10 to 0.30, D increases slowly with Φ_W. Above 0.30, the rate of increase of D with the water content is much faster.

In order to interpret the data more easily, it is convenient to consider the diffusion coefficient relative to water only, *i.e.* the ratio $D_{aq} = D/\Phi_W$. This relative coefficient, D_{aq}, may be related either to the known geometrical parameter in the phase, *i.e.* to the thickness of the aqueous layer, d_{aq}, or to the possible physicochemical interaction of water with the polar groups, *i.e.* the state of water in the phase. In Fig.2, D_{aq} has been plotted as a function of d_{aq} computed from the data of Lecuyer and Dervichian[4]. In Fig.2, the distinction between the two regions is even more evident than in Fig.1: D_{aq} appears to be approximately constant and equal to $0.3 \cdot 10^{-5}$ cm$^2 \cdot$ sec^{-1}, independent of the thickness of the water layer up to 25 Å (which corresponds to $\Phi_W = 0.30$). In the range of 25 to 35 Å, D_{aq} increases 3-fold, approaching the value of the self-diffusion coefficient of water in bulk, $2.3 \cdot 10^{-5}$ (ref. 6). There is apparently no direct relationship between D_{aq} and d_{aq}. However, it must be kept in mind, as pointed out by Lecuyer and Dervichian[4], that in the case of phospholipids with relatively large hydrophilic groups such as lecithin, the aqueous layer does not represent a pure water region, but a hydrophilic region containing the whole water *plus* the long choline phosphate groups of the lecithin. Since the length of these groups is 10 Å when fully expanded, it could be considered that as long as d_{aq} does not exceed 20 Å, the water region is crowded by these bulky polar groups which impede water diffusion. Only above 20 Å can there be a pure water region where diffusion can speed up, as is apparent in Fig.2.

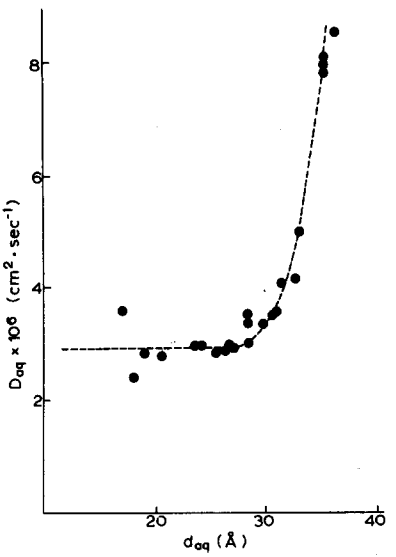

Fig.2. Water diffusion relative to water, D_{aq}, in the lecithin–water lamellar phase as a function of the thickness of the aqueous layer, d_{aq}.

Beside steric hindrance considerations, the interpretation of water diffusion in lamellar phases has to take into account the state of water in the hydrophilic region. In a study of the water sorption isotherms of egg yolk lecithin at 25°, Elworthy[7] has

determined that 1 g of lecithin binds 0.48 g of water at saturation, a quantity which corresponds to a phase containing 32% of water. By differential scanning calorimetry, Chapman *et al.*[8] have shown that up to 20% of the water in a lecithin–water mixture is bound and cannot freeze. These facts suggest that the diffusion could be interpreted in more physicochemical terms. It may be considered that as long as the water in the phase is in this particular state of hydration, all diffusion is dominated by strong interactions with the polar groups and remains constant. The diffusion rate would only increase if "free" water appeared in the system.

The results obtained on lecithin–cholesterol–water systems seem to give some support to this interpretation. The introduction of cholesterol does not appreciably modify the structure of the lecithin phase. The evolution of d_{aq} with Φ_W in a system containing equimolar quantities of cholesterol and lecithin closely follows that of pure lecithin systems[4]. However, the diffusion rates observed in the presence of cholesterol are always faster than in the pure lecithin–water phase. More characteristic is the fact that the range of Φ_W (or d_{aq}) in which D_{aq} is constant is much smaller, and the rapid increase in the diffusion rate starts for Φ_W values of about 0.15–0.20, compared to 0.30 in the case of the pure lecithin system. This difference is readily explained by the fact that the number of polar groups is halved, the amount of hydration water is therefore smaller, and free water appears sooner in the system.

A more detailed study of water diffusion in lipid–water systems as a function of spacing, the number of polar groups and temperature is currently in progress in this laboratory. The results will make possible quantitative correlations between diffusion and these parameters.

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