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# A PERCOLATION MODEL FOR LATERAL DIFFUSION IN CHOLESTEROL-PHOSPHOLIPID MIXTURES

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**A simple path model is developed for the lateral diffusion in a cholesterol-phospholipid monolayer. The diffusion constant jumps, as seen by photobleaching recovery near 20%mol cholesterol on both sides of the chain melting transition, is then explained by a two-dimensional percolation argument.**

It is well known that incorporation of cholesterol in a phospholipid bilayer is accompanied by a number of thermodynamic sequels: drop of the chain melting transition enthalpy [1] and temperature [2], a large increase of its width [1,3] and a phase separation between cholesterol-rich and cholesterol-poor domains [4–5]. A number of lattice models has successfully explained some of these behaviors [6], and also proposed phase diagrams. On the other hand, Rubenstein et al. [7] have clearly demonstrated that the lateral diffusion constant ($D$) undergoes substantial jumps at $c$ around 18–20% ($c$ = molar concentration of cholesterol in the phospholipid mixture): a 10-times increase if $T < T_c$ ($T_c$, chain melting temperature) and a 2–3-times decrease if $T > T_c$. Other measurements have shown comparable results [8].

Since $D$ behaves peculiarly on both sides of $T_c$, the problem is very difficult to handle theoretically. From the fact that the phosphatidylcholine rippled bilayer structure seems to disappear around $c \sim 20\%$, as seen by freeze-fracture electron microscopy, it has been proposed [9,10] that the $D$ jump ($T < T_c$) is caused by a one-dimensional percolation type phenomena in which pure lipid strips act as difficult-to-cross areas. Unfortunately, these ripples are strongly preparation dependent [9]; moreover, it is not clear how one can interpret such long range order patterns if $c$ is large since phase separation in these systems means only a few hundred molecule domains, as shown by large ($> 2°C$) transition width. In fact, calorimetric measurements of Estep et al. [4] seem to indicate that no detectable pretransition ripple structure exists for $c > 3.6\%$. Finally, we note that this theory gives no account of the $T > T_c$ behaviors. However, the recent computer simulation of Snyder and Freire [11] has certainly proved that some two-dimensional continuous percolation phenomena may be invoked, at least for $T < T_c$.

Our approach is based on a lattice model that we have recently developed [12] in order to describe the complete cholesterol-phosphatidylcholine mixture thermodynamics, especially the two peak specific heat data of Refs. 1 and 4. This model shows a phase separation on both sides of $T_c$, a fact that has probably been seen by Cherry et al. [13]. For $c < 22\%$, each site of a triangular lattice may hold one of these three molecular patterns: (1) an all-*trans* phospholipid, (2) a melted phospholipid or (3) a cholesterol-phospholipid aggregate formed by one cholesterol and 3.5 (or 7 chains) lipids in a less frozen state than (1) if $T < T_c$, or in a less melted state than (2)

for $T > T_c$. Since these aggregates are nearly circular (see Fig. 1A of Ref. 14), and since phase separation occurs readily before c = 18%, we can represent the system as in Fig. 1.

If a molecule diffuses laterally in this system, it must pass between the sites, on a lattice segment. We define three types of segments or 'between-two -sites passages': firstly, when the two nearby sites are occupied by melted lipids, the segment is called easy because these molecules are flexible and easily movable; secondly, if the lipids are in the gel state, the segment is called difficult, because both the lattice and the molecules are rigid; lastly, if the segment joins two cholesterol aggregates, it is called intermediate since the corresponding lattice and aggregate lipid chains are not so rigid. We neglect the free lipid-aggregate segments as they represent a fraction of approx. $1/\sqrt{N_0}$ ($N_0$, average size of the phase separated domains) of the total number of segments in a well separated system, and also because this boundary is not a probable path, having an 'easy' domain on one side. We now define $x$ as the concentration of segments which are intermediate ($c = x/(1 + 3.5x)$). The travelling molecule will pass through domains of identical kinds of segments: easy, difficult or intermediate domains (Fig. 2). Note that since the lifetime of a domain picture like Fig. 2 is $\geqslant N_0$ times the lifetime of aggregates, and since this latter lifetime is long compared to the time it takes to cross one segment (see below), we may consider that the travelling molecule sees a static domain picture which allows percolation to occur. The intermediate domains will contain a fraction $x$ of all the segments in the system. The mean size of the domains is certainly > 260 molecules [15], with the size of the aggregate domains probably growing with $c$.

Now, for $T < T_c$, $D$ will be small and certainly increasing in $c$ until a continuous network of 'intermediate domains' will be formed across the area; then, a sudden percolation-like jump in $D$ must happen since an easy and complete path will exist. However, for $T > T_c$, $D$ will be large and

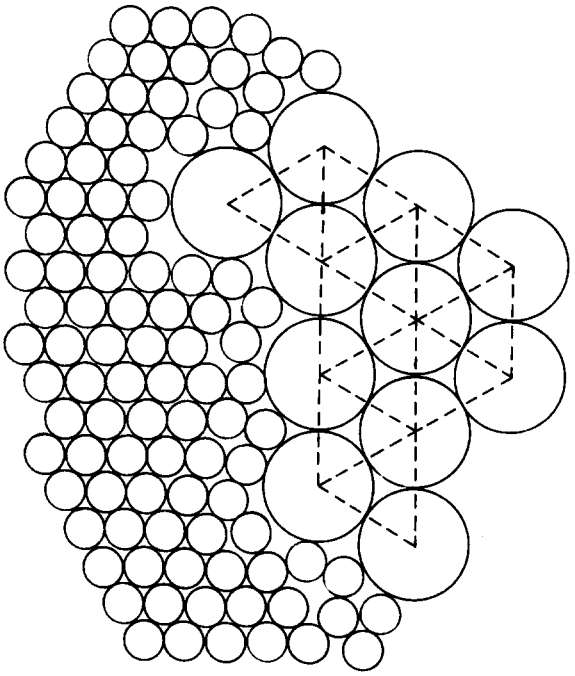

Fig. 1. A schematic view of the possible lattice structure near a phase separation boundary. Small circles represent free phospholipids whereas large circles represent the almost circular cholesterol-phospholipid (1 : 3.5) aggregates. A diffusing molecule near the boundary will choose between small or large circle areas: thus the boundary is not important. Holes are not likely to occur in a real system: they are artifacts of the construction method.

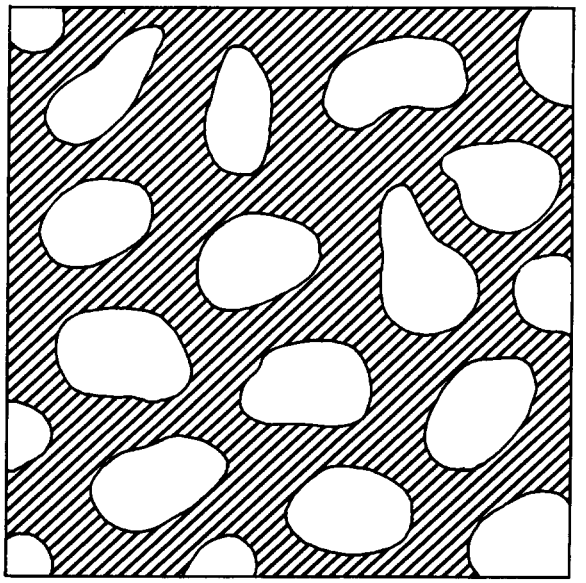

Fig. 2. Picture of a domain-separated system. Unshaded patches represent cholesterol-rich domains on a phospholipid surface. These patches are said to be intermediate areas for diffusion on a difficult ($T < T_c$) or easy ($T > T_c$) phospholipid surface.

almost constant in $c$ until a continuous network of 'intermediate domains' will form an inevitable barrier between the two end sides of the large area; then, $D$ will begin to drop proportionally to the barrier thickness. Both these phenomena will occur at the same $c = c^{\star}$ since they are produced by a similar continuous percolation event; note that even for $c > c^{\star}$, $D(T > T_c) > D(T < T_c)$, because the aggregate lipids are not in a purely intermediate state between the gel and melted states.The resulting qualitative $D(c)$ diagram (Fig. 3) is well supported by the measurements of Rubenstein et al. [7]. The reason why NMR measurements do not support these $D$ jumps [16] is obvious here: the time this technique allows for lateral diffusion is too short for the travelling molecule to cross more than 1–2 patches, i.e. for percolation phenomena to occur. However, those measurements clearly show an anomaly in $D$ around $c \simeq 5\%$, a phenomenon probably associated with the occurrence of lateral phase separation in this region as proposed by us [12].

We define $c^{\star\star}$ by the concentration where the random placing of small domains on a large area may not be done further without overlapping; this is the point where the phase separated domains, with a thermodynamically determined mean size, will begin to form large patches. It is clear that $c^{\star} \gtrsim c^{\star\star}$. Finegold and Donnel [17] have found that for small circles, this limit corresponds to $50.27 \pm 0.20\%$ of the large area covered by circles. For us, it means that 50.27% of the segments are of the intermediate kind (that is $x = 0.5027$). Thus, for $c = c^{\star} \gtrsim 18.18\%$, an easy path ($T < T_c$) will begin to form on the large area (Fig. 3).

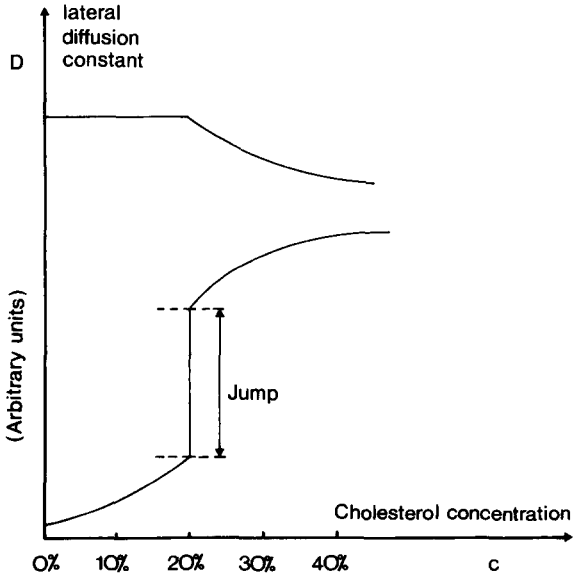


Fig. 3. Qualitative aspects of the $D$ vs. $c$ diagram for the system described in the text. Agreement with Ref. 7 is obvious. We see a percolation-like jump at $c \gtrsim 18\%$.

Obviously, to be applicable, this model necessitates a number of conditions. First, the impurity molecule must be the center of a rigid aggregate, because the travelling molecule must not pass through it. Second, this aggregate must phase separate into quite pure domains in which a quasi-lattice may be defined in order to evaluate the number of segments or passages between the aggregates. Third, the domains have to be large enough for us to neglect their perimeter compared to their surface ($1/\sqrt{N_0} \ll 1$). It is also probably necessary to use a percolation based on the number of segments and not on the relative area of domains.

This very phenomenological but microscopically founded model localizes well the $c^{\star}$ value and predicts adequately the main aspects of the $D(c)$ diagram for the cholesterol-phosphatidylcholine systems. In this case, the conditions seem to be respected. The aggregate hypothesis [12] is certainly well suited since the one lattice site characteristic crossing time (approx. 40 $\mu$s) is much shorter than the possible aggregate lifetime (which is probably $\leqslant 30$ ms since [18] stated that the more stable 1:1 aggregates, present at $c \geqslant 22.2\%$ [12], have a lifetime between 30 ms and 10 s). This aggregate is also rather circular [14] and phase separates in pure domains, which satisfy conditions two and three. Application of this model to other systems is easy, but not many diffusion constant measurements have been made on protein-phospholipid or cholesterol-sphingomyelin systems for example. However, a percolation critical study near $c \sim 19\%$ ($T < T_c$) is certainly worthy since the results of Rubenstein et al. show a very steep change in this region. Computer simulation should be a good way to investigate further on the lateral diffusion near $c = 5\%$ (the phase separation region) and 20% (the percolation region).

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