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Flocculation of Egg-Phosphatidylcholine Liposomes under Drift Flow Brought on Gravity

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Flocculation behavior of large egg-phosphatidylcholine liposomes caused by Ca^{2+} under the influence of gravitational force due to the density difference between liposomes and medium has been studied. The greater the difference in density, the more closely packed are the structures of the flocs obtained.

The importance of the membrane-membrane interaction between phospholipid membranes has been pointed out by many researchers in such varied fields as cell biology, biophysics, physical & colloid chemistry and so on.[1-4] Up to the present, studies on the membrane-membrane interaction of acidic phospholipids have been extensively conducted from various standpoints, including the coagulation behavior of liposomes in colloid chemistry. Being different from other phospholipids, phosphatidylcholine (PC) has a hygroscopic nature, so that it's liposomes show high resistivity against coagulation/fusion, whose repulsive force is usually called "hydration force" or "steric force".[5] Egg-PC liposomes used in this experiment show negative electrophoretic mobilities because of the presence of a small amount of acidic lipids, which is usually found in naturally occurring materials. The liposome-liposome interaction in this case is mainly determined by the summation of three kinds of forces; the repulsive electrical double layer force, attractive Van der Waals force, and the hydration force. Because of the existence of hydration force, the interaction potential between egg PC-liposomes has only a shallow potential minimum even if the electrostatic double layer potential disappears. This brings particle-size dependency to the flocculation behavior of PC liposomes.[6] This means that PC liposomes with less than about 300 nm diameters never show flocculation behavior at the point of zero zeta potential ($[CaCl_2]=10^{-3}$ mol dm^{-3}). On the other hand, larger sized liposomes (diameter: above 320 nm) can flocculate at this Ca concentration. This critical particle size is well explained by the theory of colloid stability with parameters such as the Hamaker constant and the intensity factor and the decay length of hydration force obtained by J.L. Lis et al. [2] Not only at the zero zeta-potential point, but also above ca. 0.1 mol dm^{-3} $CaCl_2$, large PC liposomes can form flocs because of a reduction of electrostatic repulsion through the thinning of the electrical double layer. The same phenomenon was observed by using Mg^{2+}, Ba^{2+}, La^{3+} but not by Na^+, Cs^+. This fact is proof that the flocculation is due to the reduction of

electrostatic repulsion and not to specific interaction between metal ions and liposomes. Thus, there are no high potential barriers before the shallow minimum when liposomes approach each other in such high ion concentration regions and the flocculation is caused by the diffusion (transportation) limited process but not a reaction limited one.

Fundamentally, aggregation or the flocculation process is a dynamical phenomenon so that besides the interactions mentioned above, any kind of hydrodynamic interaction can contribute to it.[7] It is desirable to clarify such dynamical phenomena in various situations, because the liposome aggregation occurs, in many cases, under external force fields such as gravity or flow field caused by hydrostatic pressure gradients, etc. In these cases, liposome particles move not only in isotropic Brownian motion but also in additional velocity.

In this study, we examined the flocculation process of the large PC liposomes (diameter: 1-2 μm), caused by Ca^{2+} ([Ca]=0.36 mol dm^{-3}) under the condition of uniaxial drift velocity due to the differences in density between liposomes and medium. The liposomes were prepared by the vortex mixing method with filtration (2 μm pore size filter) to exclude larger ones and dialysis (1 μm pore size filter) to exclude smaller ones, and had 1-2 μm diameters, The flocculation process was measured by a microscope (x400, Olympus IMT-2) with a video and image-analyzing system and the figure of the flocs was evaluated by calculating the area fraction of the projected figure of flocs to a 2D-plane, i.e., the area fraction Φ=(area of body of flocs)/(area in a circumscribed circle of the flocs)(see Fig.1). The drift movement of liposomes was generated by changing the density of the medium through a variation of the mixing ratio of D_2O/H_2O. So, in addition to isotropic Brownian motion, the liposomes move, through buoyancy by gravity, toward the upper surface of a glass cell (a micro-electrophoretic glass cell with 0.5 mm thickness) and the focus of the microscope was fixed on the plane a bit below the upper surface. After more than 3-4 h, the flocculated figures were frozen. The important point is that once the formed figure of flocs is frozen, there is no reconfiguration to other

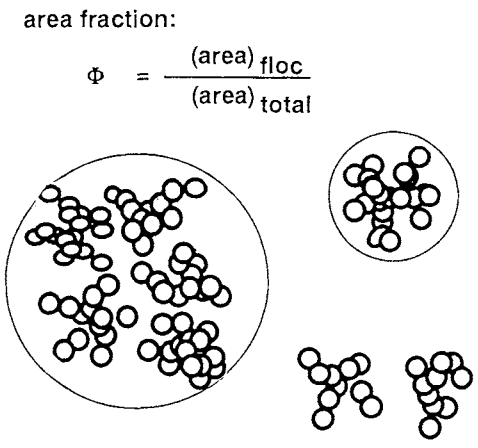

Fig. 1. Calculation of area fraction.

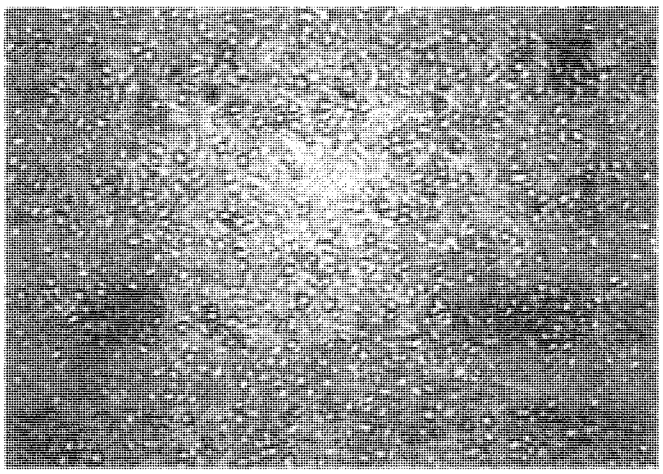

Fig. 2. Microscopic image of liposomes. [Ca]= 0.03 mol dm^{-3}, D_2O= 100 %.

figures at least 12 h. This ensures the possiblity of the clarification of the relationship between the figure of the liposome flocs and the liposome-liposome interaction.

Figure 2 shows the microscopic image of the liposome at 4 h after mixing the liposome dispersion with $CaCl_2$ solution (D_2O fraction=1); the resultant concentration of Ca is 0.036 mol dm^{-3}. At this Ca concentration, the liposomes have a positive surface potential and also the thickness of the electrical double layer is large enough to stabilize the liposomes as a single particle. The liposomes gather beneath the upper surface, and they never make flocs but move vigorously at random. This result indicates that the liposomes never flocculate only through the compression by such a density difference.

However, at more concentrated conditions of Ca, the liposomes show flocculation phenomenon. Figure 3 shows the microscopic images of flocculated egg-PC liposomes for four types of media with different densities which were prepared by four different mixing ratios of the D_2O/H_2O (D_2O fraction: 1, 0.7, 0.4, and 0) in 4 h after the mixing of liposome suspension with $CaCl_2$ solution. From the figure we can easily realize that the different shaped flocs are formed. Different medium densities give different buoyant velocities to liposomes but most of the liposomes are gathered near upper the glass wall in 4-5 h.

If the flocs are formed by only Brownian motion, the figure of the flocs should be the same but the time scale of flocculation varies depending on the density deference. Thus, the experimental results imply that the difference in the shape of flocs is attributable to the velocity difference. These floc figures are easily broken into single liposome particles by the reduction of the Ca concentration through dilution of the solution. This fact clearly indicates that there are no high potential barriers

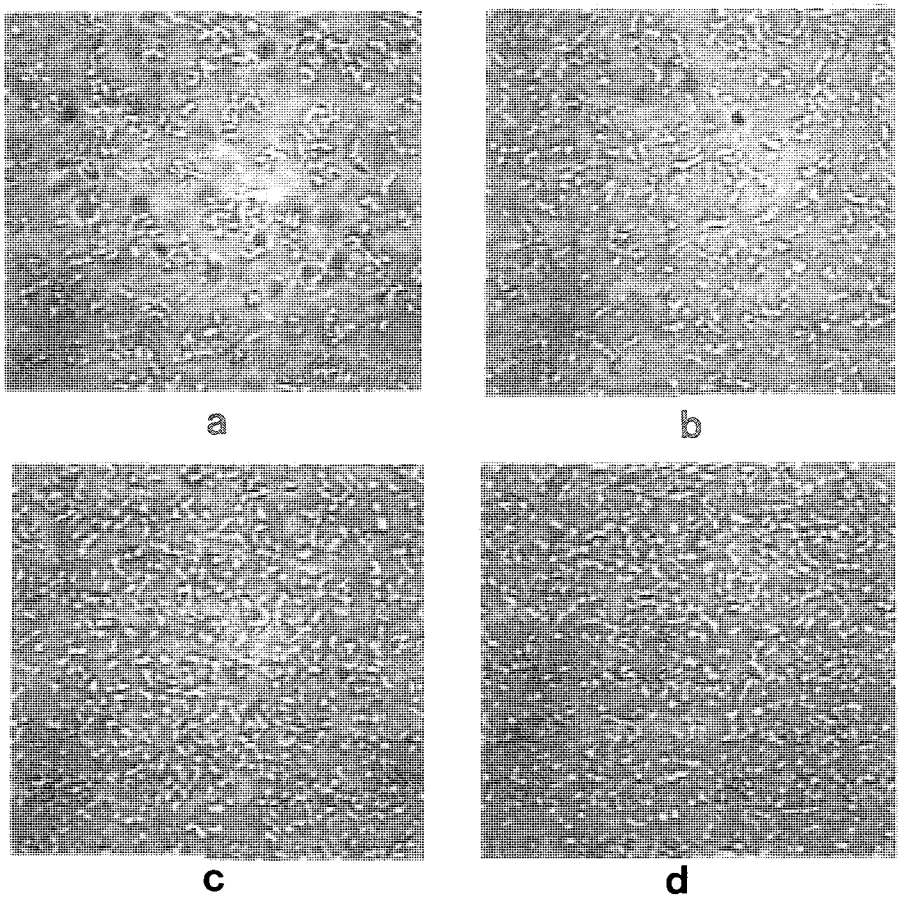

Fig. 3. Microscopic view of liposome flocs.
D_2O; a: 0.0, b: 0.4, c: 0.7, d: 10. [Ca]= 0.36 mol dm^{-3}.

when liposomes make a separation.

Figure 4 shows the area fraction (Φ) for each case in figure 3. According to the increase in the medium density, the Φ has a higher value. This means that higher drift velocities make flocs with closer figures. This tendency agrees with the results of a computer simulation in 2D space for diffusion limited aggregation to one particle with drift velocity[8] or diffusion limited aggregation to one line with drift velocity.[9] The results of each computer simulation suggest that the more closely packed aggregates are formed according to the increase in the drift probability toward the aggregation points. Our study shows experimentally that the anisotropic movement of particles induces more compact aggregated structures.

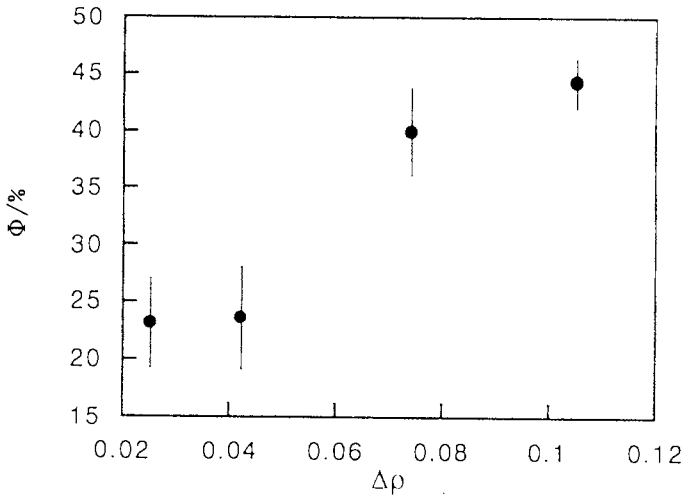

Fig. 4. Area fraction for each microscopic pattern in Fig. 2. as a function of density difference ($\Delta\rho$).
Vertical lines show standard deviations.

D_2O	0.0	0.4	0.7	1.0
$\Delta\rho$	0.025	0.042	0.074	0.105

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