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Capillary Ripple Response of Cholesterol and Lecithin Monolayers

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Capillary ripple characteristics of monolayers of cholesterol and dipalmitoyllecithin and a 1:1 mixture of the two over air-water interface were studied. The ripple damping coefficient and the wavelength were determined as a function of the surface concentration. A stronger chain-chain interaction exists between the molcules in the pure cholesterol monolayer compared to pure lecithin as indicated by the sharp maximum in the damping coefficient curve for the former case. In the monolayer of the mixture, there is evidence of an interaction between the chains of the two substances.

The focus of this paper is the exploration of the capillary ripple response of the monolayers from lecithin, cholesterol and a 1:1 cholesterol–lecithin mixture on air-water interface. In particular, the dependence of the ripple damping coefficient and the wavelength on the surface concentration was studied. It is expected that this work would generalize into a new technique for the study of biomembrane surface properties.

The capillary ripple method allows study of the dynamic response of monolayers over the frequency range of 10 Hz to 2 kHz. In this method, ripples are generated on the air-water interface on which the appropriate monolayer is spread and the damping coefficient and the wave number are measured. The value of these parameters reflect the elastic and viscous response of the monolayer. The review article by Hansen and Ahmad[1)] summarizes several studies using the method.[2–5)] The dependence of the values of the ripple parameters on the surface tension and the surface tension gradient was shown by van den Tempel and coworkers.[6)] Hansen and Mann[7)] included additional elastic and viscosity terms in their treatment.

Experimental

The experimental set up was similar to the one used by other workers.[1)] It involved generation of ripples at the air/water interface on which a monomolecular film of interest was spread. The ripples were generated by a probe made from a stainless steel razor blade. A steel wire was soldered to the end of the blade and connected to the coil of an electromechanical transducer, essentially a modified speaker. The speaker was excited by the output from the reference channel of a precision lock-in amplifier. The frequency and the level of the input signal were monitored. For the results reported here, the input signal of 200 Hz was used. The ripple sensing probe was similar in design to the generating probe. The waves generated are reflected by the sensing probe and a stationary wave pattern is set up with the probes acting as boundaries. The sensing probe is fitted to a phonographic cartridge in the place of the usual needle. The motion of the receiving probe was converted into an electrical signal which was fed into the lock-in amplifier, where only that part of the signal was amplified which had the same frequency as the reference signal used to drive the generating probe.

The precise determination of the ripple parameters by the method described by Mann and Ahmad[8)] is a tedius procedure and is impractical when several measurements have to be made. Instead, a variation of the method was followed in the present set of experiments. The probe output voltage, a quantity assumed proportional to the wave amplitude, is given by Eq. 2 of Mann and Ahmad.[8)] This quantity is an oscillatory function of the distance between the probes. The maxima in the voltage-distance curve $\overline{V}(L)$, are given by

$$\overline{V}(L) = \frac{V_0\{1-\exp(\beta)\}}{1-\exp\{-(2\alpha L+\beta)\}}\exp(-\alpha L), \tag{1}$$

where V_0 is the output voltage, when the probe separation, L, is zero. α is the ripple damping coefficient and β is the reflection coefficient at the boundaries. If α is of the order of 0.4 cm^{-1}, L is kept around 4 cm or more and the reflection coefficient, β, is of the order of 1, then

$$1-\exp\{-(2\alpha L+\beta)\} \approx 1.$$

The upper limit to the error introduced by this approximation is 2%. Thus Eq. 1 can be rewritten as

$$\overline{V}(L) = C\exp(-\alpha L). \tag{2}$$

If for pure water, the distance between the probes is fixed such that they are perfectly in phase at a desired frequency, the output voltage is given by Eq. 2. If the monolayer is now spread on the surface, the wave number will be different from the case of the pure water and the two probes may not be in phase. If the probe separation is adjusted slightly to bring back the probes in phase, Eq. 2 can be applied. The maximum adjustment needed will not be more than one half of a wavelength. The maximum error introduced by this change of the probe separation is about 2%. If for the monolayer covered surface, output voltage is $\overline{V}'(L)$, then

$$\overline{V}'(L) = C\exp(-\alpha' L). \tag{3}$$

From Eq. 2 and 3 $\ln\dfrac{\overline{V}'(L)}{\overline{V}(L)} = L(\alpha—\alpha')$.

Thus knowing L and the damping coefficient for pure water, α', the damping coefficient for the monolayer can be calculated. The procedure is repeated at various film pressures. The other important parameter of the capillary ripple experiment, the wavelength, can be measured in a separate experiment by observing the voltage change as the probe separation is increased in small steps, and measuring the distance between the probe positions corresponding to successive maxima and minima. The wave number, k, can then be calculated by the relation

$$k = \frac{2\pi}{\lambda};\ \lambda \text{ being the wavelength.}$$

Along with the ripple measurements, simultaneous measurements of the film pressure were made with a balance similar to the one described by Mann and Hansen.[9)] The film balance system involved a Teflon trough milled out of a single Teflon slab, and a movable surface piston or "barrier". Near one and of the trough was a torsion balance.

A Teflon float was coupled to the torsion wire by the help of a vertical bar. A horizontal bar was fixed to the torsion wire, and was coupled to the core of a 0.084 cm travel linear variable differential transformer (LVDT). The LVDT output as monitored on a strip chart recorder was calibrated in terms of the force acting on one cm of the float.

The reproducibility of the values of the damping coefficient and the wave number is 4% or better. The film balance could detect a change in surface pressure of 5×10^{-5} N m^{-1}.

Solutions of known concentration of synthetic β,γ-dipalmitoyl-DL-α-lecithin, cholesterol and an equimolar mixture of the two were made in chloroform. The lecithin used was supplied by Nutritional Biochemical Corporation, Cleveland, Ohio, U.S.A. and cholesterol by General Biochem. Lab., Chagrin Falls, Ohio, U.S.A. The concentration of the solute was such that approximately 200 microliters of the solution spread on the trough gave a sub-monomolecular layer suitable for compression to full coverage. Line distilled water distilled from the alkaline $KMnO_4$ solution and redistilled from a quartz still was used as the substrate. The monolayer was spread from a microsyringe drop by drop. The surface piston was then moved in steps to decrease the area available to the monolayer. After each change of the barrier position, the surface was allowed to come to equilibrium, indicated by a constant film pressure. The ripple measurements were then made. A series of measurements was made until the monolayer collapsed as indicated by leveling off of the surface pressure.

From the measured values of α and k, dimensionless parameters $Y_1\left(=\frac{\rho\omega^2}{\gamma k^3}\right)$ and $Y_2\left(=\frac{\alpha}{k}\right)$ were obtained. Here ρ is the density of water, γ the surface tension calculated from the film pressure, and ω is the angular frequency given by $\omega=2\pi\nu$; ν being the frequency of the probe vibration.

Results and Discussion

Figure 1 shows the damping coefficient curves. The curve for the mixture is intermediate in character between the curves for the pure compounds. All the damping coefficient curves show a maximum which is sharp in the case of cholesterol and a plateau rather than a distinct maximum for lecithin.

The mixed film also shows a relatively sharp maximum.

The damping coefficient and the sharpness of its maximum can be related to the chain-chain interaction in the molecules forming the monolayer. The effect of the surface diffusion on the wave damping has been calculated from the hydrodynamic theory.[1] This theory predicts a maximum in the damping coefficient as the surface elastic modulus increases. This has been verified experimentally for various soluble and insoluble film-forming substances.

Physically we can interpret the results as follows. As the wave passes, the surface is alternately compressed and expanded. Because of the surface elasticity in the presence of the surfactant molecules, the waves are damped. The damping is caused by the surface tension gradients set up by the stretching and contraction of the surface as the wave passes. If there is appreciable surface to bulk or surface to surface diffusion of the surfactant molecules in one wave period, these surface tension gradients are reduced and the damping is reduced. For insoluble monolayer like cholesterol and lecithin, the bulk to surface diffusion is irrelevant, only the surface to surface diffusion is important. If the chain-chain interaction in the monolayer is strong, this diffusion is low, resulting in a high damping coefficient.

From the results it is obvious that the chain-chain interaction in cholesterol monolayer is stronger than in the lecithin film. The relatively sharp maximum in the curve for the mixture indicates that there is appreciable interaction between the molecules of the mixture. This evidence favors the suggestion by Demel and Joos[10] that at low surface pressures, there is an interaction between the chains of the two substances in a monolayer.

The capillary ripple theory[1] makes it obvious that the ripple damping coefficient is sensitive to the elastic modulus only at intermediate values of the elastic modulus *i.e.* near the damping coefficient maximum. At low or high surface elasticity values the damping coefficient is virtually independent of the surface elastic modulus. This method is therefore sensitive to the value of the surface elastic modulus and hence to any property affecting this modulus only at such intermediate values. Thus, though this study suggests that there is an appreciable molecular interaction at intermediate surface elasticity which corresponds to quite dense films for these systems, it does not rule out such interaction at lower surface pressures.

A consideration of the dimensionless group Y_1 and

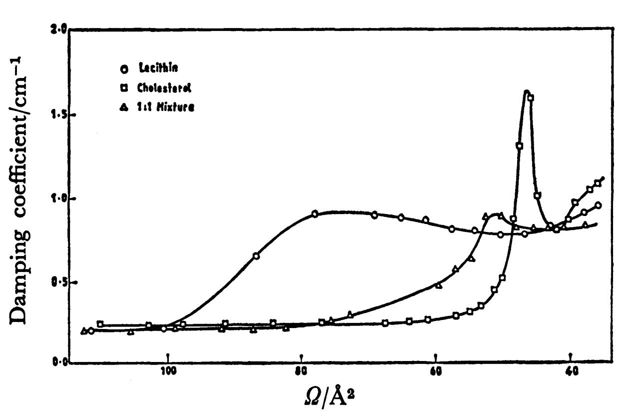

Fig. 1. Capillary ripple damping coefficient for the monolayers at various surface concentrations, frequency=200 Hz, temperature=25 °C.

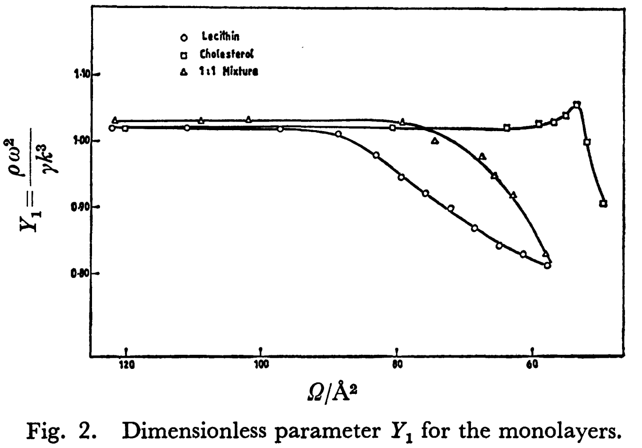

Fig. 2. Dimensionless parameter Y_1 for the monolayers.

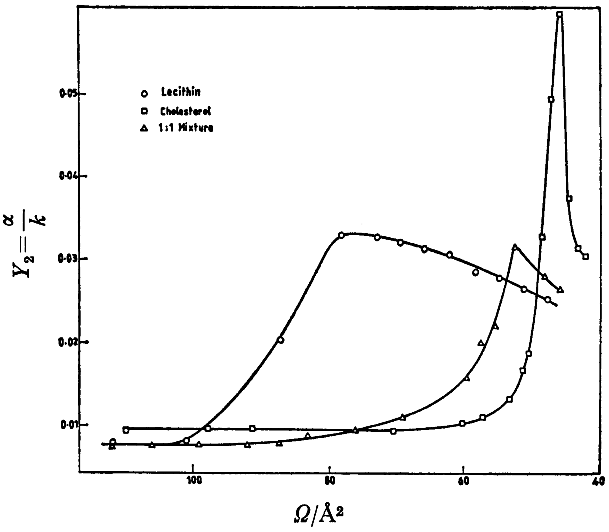

Fig. 3. Parameter Y_2 as a function of area per molecule, Ω, for the various monolayers.

Y_2 is useful when comparing monolayers formed by different substances. $Y_1=1$ is the Kelvin equation and is followed by pure water and by the monolayers in the limits of zero surface elasticity and surface viscosity. The curves in Fig. 2 follow this pattern. At high film densities the deviation from ideality sets in, this being due to compressional elastic modulus as well as surface viscosity. The different surface elastic moduli in the pure and the mixed film cause the curves to separate out at higher densities.

The amplitude of the waves in this study was small enough that the monolayer did not collapse by the passage of the waves, even for the near rigid films. This was confirmed by the stability of the values of the film pressures during the course of the ripple experiment.

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