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MEASUREMENTS OF THE ELASTICITY OF MONOLAYERS CONSISTING OF LIPIDS FROM NERVE MEMBRANES

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For measurement of viscoelastic properties of monolayer-covered interfaces a longitudinal wave is generated in the plane of the interface, using a horizontal oscillating barrier. The wave propagation depends on the values of the viscoelastic parameters of the monolayer. The technique is applied here to study the surface elasticity of layers consisting of lipids extracted from nerve membranes. It is concluded that mechanical disturbances are propagated as longitudinal waves. The possibility that longitudinal waves occur in nerve membranes and the role they might play in the transmission of information in biological membranes is discussed.

Introduction

The possibility of determining the viscoelastic properties of monolayer-covered air/water interfaces, using the longitudinal surface wave technique, has been described by Lucassen and Van den Tempel [1,2].

The present paper deals with application of that technique to the study of monolayers consisting of lipids extracted from nerve membranes. It is suggested [1,3] that longitudinal waves might play a role in the transmission of signals along nerve axons.

The reported measurements deal with harmonic longitudinal surface waves with frequencies in the range of the repetition frequency of action potential in nerves (up to 500 Hz). For the experiments, the arrangement described by Lucassen [1] had to be modified to account for waves reflected by the measuring probe.

Theory

A local disturbance on a monolayer-covered surface causes variation in the surface tension and concentration, which will propagate along the surface as a longitudinal wave. The wave characteristics i.e., wave number, κ, and damping coefficient, β, depend on the viscoelastic properties of the monolayer. For measurements in the frequency range up to 500 Hz, the disturbance is generated by an oscillating barrier (active barrier) driven by a loudspeaker. The wave is detected by a plate (passive barrier), touching the surface at zero contact angle positioned parallel to the active barrier at distance L (see Fig. 1). At the passive barrier, the 'incident' surface tension wave is partly reflected, leading to a force F_d

$$F_d = b(\sigma_i + \sigma_r - \sigma_t) \quad (1)$$

where b is the width of the passive barrier, σ_r the reflected and σ_t the transmitted surface-tension wave. (For convenience we write σ_n instead of

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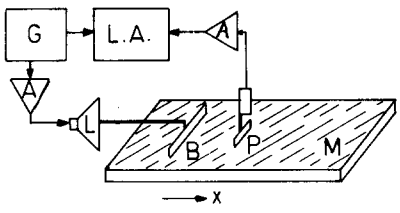

Fig. 1. Experimental arrangement M is the monolayer covered surface, B the active barrier, P the passive barrier with transducer. G is the wave generator, A are amplifiers, LA the lock-in amplifier and L the loudspeaker driving the active barrier.

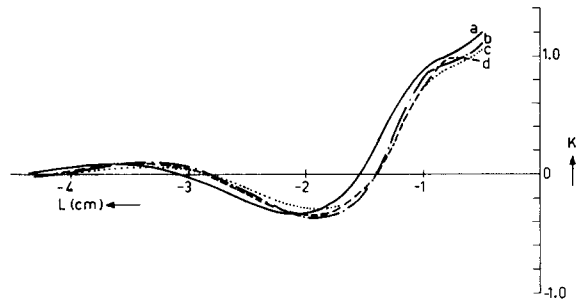

Fig. 2. K as a function of the distance between the active and passive barrier (L) as measured by the lock-in amplifier (at constant phase angle). Curve d is a measured curve obtained from a cholesterol monolayer (frequency 400 Hz). The other curves are calculated (Eqn. 6) with $\kappa = 2.30$ cm^{-1}, $\beta = 0.93$ cm^{-1} for curve b (best fit); $\kappa = 2.1$ cm^{-1}, $\beta = 0.93$ cm^{-1} for curve a and $\kappa = 2.30$ cm^{-1}, $\beta = 1.03$ cm^{-1} for curve c (K in arbitrary units).

$\hat{\sigma}_n \exp(\beta x) \exp\ i(\kappa x - \omega t + \phi_n)$, where $\hat{\sigma}_n$ is the amplitude, ϕ_n the phase of the wave, ω the angular frequency; the x-axis is perpendicular to the barrier, see Fig. 1).

The passive barrier has a mass of m g and is connected to a transducer, compliance Q; its equation of motion is given by

$$b(\sigma_i + \sigma_r - \sigma_t) - Qu = m\frac{\delta^2 u}{\delta t^2} \tag{2}$$

where u is the displacement of this barrier.

If we assume that there is no mass transport between the surface and the underlying (bulk) fluid (no diffusion of monolayer molecules), mass conservation yields

$$V_i + V_r = V_t \tag{3}$$

where V_i is the incident, V_t the transmitted and V_r the reflected surface velocity wave. We define the complex quantity K

$$K = \frac{V_{pd}}{V_{ab}} \tag{4}$$

where V_{pb} and V_{ab} are the complex velocities of the passive and active barrier, respectively. For convenience we choose $x = 0$ at the position of the passive barrier, $x = -L$ at the position of the active barrier. Eqn. 4 can now be rewritten as

$$K = \frac{(V_t)_{x=o}}{(V_i + V_r)_{x=-L}} \tag{5}$$

The quantity K is measured using a lock-in amplifier. As reference signal we use V_{ab}, the measuring signal coming from the transducer measuring $(V_t)_{x=0}$. K can be calculated from Eqn. 2, 3, 5 using standard procedures concerning standing waves (analogous to the theory of the sound transmission through different media [4]). We obtain

$$K = \frac{P + iR}{E + iF}$$

with

$$P = \frac{-2b\sqrt{\frac{\eta\rho\omega}{2}}\,(\kappa + \beta)}{\omega m - Q/\omega} \tag{6}$$

$$R = \frac{-2b\sqrt{\frac{\eta\rho\omega}{2}}\,(\kappa - \beta)}{\omega m - Q/\omega}$$

$$E = ((P+1)\cos\kappa L - R\sin\kappa L)e^{\beta L} - (\cos\kappa L)e^{-\beta L}$$

$$F = ((P+1)\sin\kappa L + R\cos\kappa L)e^{\beta L} + (\sin\kappa L)e^{-\beta L}$$

where ρ is the density, η the viscosity of bulk fluid.

We learn from Eqn. 6 that K depends only on the parameters κ and β. By fitting the theoretical curve through the measured values of K, values of β and κ can be obtained. In Fig. 2 a theoretical curve is compared with a measured curve for one of our measurements. Curve b ($\kappa = 2.30$ cm^{-1},

$\beta = 0.93\ \mathrm{cm}^{-1}$) represents the best fit.

In the same figure, calculated curves are given with different κ and β values, from which we conclude that the experimental error in both the κ and β values thus obtained is 5%. In turn, using the dispersion equation [2]:

$$i(\epsilon_r + i\omega\eta_m)(\kappa - i\beta)^2 = (\eta\omega^3\rho)^{1/2}\exp(i^{\pi}/4) \quad (7)$$

surface elasticity ϵ_r and surface viscosity η_m can be evaluated. (Eqn. 7 holds for elastic layers at liquid/air interfaces. In case of an elastic bilayer separating two water phases, the right-hand side of this equation has to be multiplied by 2 (Ref. 2).)

Experiments

A Langmuir through, $50 \times 20 \times 1.5$ cm, was used. The active barrier (stainless steel, waxed with paraffin, 12 cm width) was driven by the coil of a standard loudspeaker. The loudspeaker was connected with an a.c. supply (wave generator and power amplifier). Frequencies up to 500 Hz were used; the amplitude of the barrier was calibrated using an accelerometer; amplitudes between 5 and 20 μm were applied. The receiving probe consisted of the passive barrier (stainless steel, waxed with paraffin, 5 cm in width, mass 0.2 g) glued onto the needle of a standard electrodynamic pick-up element (Shure M75 compliance approx. $40 \cdot 10^{-3}$ $\mathrm{m \cdot N^{-1}}$.) The receiving probe was mounted on a carriage moving in the x direction with a constant speed of $3 \cdot 10^{-4}$ $\mathrm{m \cdot s^{-1}}$. A continuous recording of K as a function of the position was thus obtained. Fig. 1 shows a schematic arrangement. In addition to monolayers of the nerve lipid extract which contains no cholesterol, monolayers of nerve lipid and cholesterol (60:40 wt.%) were studied. The average surface pressure during the experiments was chosen to be $40 \cdot 10^{-3}$ $\mathrm{N \cdot m^{-1}}$, except in the case of cholesterol, which cannot retain this pressure, for which we chose $35 \cdot 10^{-3}$ $\mathrm{N \cdot m^{-1}}$. To compare our results with those of other authors, measurements on phosphatidylcholine monolayers are reported (dipalmitoylphosphatidylcholine).

Cholesterol and phosphatidylcholine monolayers were treated as described by Snik et al. [5]. The nerve lipid extract was obtained from Sigma (bovine brain extract, Folch Fraction V). To spread this extract on the bulk fluid (water) we used chloroform (pro analysi, Merck). The water was distilled three times. All glass apparatus and the trough were cleaned with chromic acid. All experiments were performed at 21 ± 0.5°C.

Results and Discussion

Fig. 3 shows resulting κ values as a function of frequency for several monolayers. The β/κ ratio proved to be 0.41 throughout, indicating that no surface viscosity occurs [1]; in other words, $\eta_m = 0$. In Fig. 3, a comparison is shown between the measured points and the drawn lines representing the theory of Eqn. 7. For these lines, values of ϵ_r have been used which are listed in Table I. The standard deviation given in this table result from the experimental error of ϵ_r, being 10%. For comparison, results of other authors are also listed in this table.

We conclude that the results are in satisfactory agreement. (Pasechnik and Servuss obtained their values from experiments on bilayers, using totally different measuring techniques.) From our results we can calculate the velocity of a longitudinal wave in a nerve lipid extract-cholesterol bilayer, frequency 500 Hz, to be 7.5 $\mathrm{m \cdot s^{-1}}$. This value lies within the range of velocities of signal transmission in nerves (4–20 $\mathrm{m \cdot s^{-1}}$ [6]). Additionally, we can calculate that the distance such a wave can travel before it is damped 3 dB is $5 \cdot 10^{-3}$ m. From

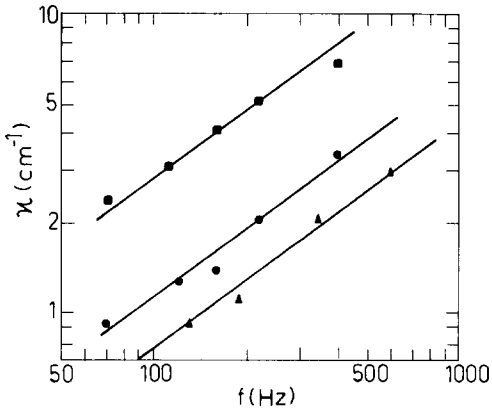

Fig. 3. Wave number (κ) as a function of frequency obtained from cholesterol (▲), nerve lipid extract (■) and lipid extract-cholesterol (●) monolayers. The lines drawn have been calculated using Eqn. 7 with ϵ_r values as listed in Table I.

TABLE I

SURFACE ELASTICITY OF MONOLAYERS CONSISTING OF CHOLESTEROL, PHOSPHATIDYLCHOLINE, A NERVE MEMBRANE LIPID EXTRACT , AND A MIXTURE OF CHOLESTEROL AND NERVE LIPID EXTRACT (40 : 60 WT.%).

For comparison, surface elasticities obtained by other authors are also listed (surface elasticity and standard deviation in $N \cdot m^{-1}$).

Component	Present results	Lucassen [1]	Pasechnik et al. [7]	Servuss et al. [8]
Cholesterol	2.5 ±0.4	1.8		
Phosphatidyl choline	0.40 ±0.05	0.15	0.3	0.6±0.1
Nerve lipid extract	0.17 ±0.03			
Lipid extract-cholesterol	1.0 ±0.1			

the literature we learn that a mechanical disturbance of a nerve membrane can be caused by applied pressure (in mechanoreceptors [9]), binding of an ion by an ion channel [12] or variation of the electric field across the membrane [10]. The present results indicate that such disturbance could well be propagated through the membrane as longitudinal waves. Such propagated disturbances might influence ion channels [9–11]. Our result may be seen as extra support for the suggestion that longitudinal waves play a part in the transmission of information in biological membranes [1,3].

Recently, Tasaki et al. [13] reported on mechanical changes associated with propagated action potentials in isolated nerve fibres in salt solutions. In order to relate their observations to longitudinal waves in the membrane, a theoretical relationship is developed (see Appendix). The theory given in the Appendix uses a cylindrical surface in order to simulate nerve fibres, for the propagation of longitudinal waves. It is shown that volumetric changes and a tension wave in the fibre are expected whenever longitudinal waves occur in the membrane. Both phenomena were observed by Tasaki [13]. Further research on this subject is in progress.

Appendix

To cope with longitudinal waves in cylindrical surfaces we shall first consider the hydrodynamics of these waves in planar surfaces. The equation for the force balance for a surface element under strain (in the x- direction), according to Crone [3] is:

$$\frac{\delta\sigma}{\delta x} = \eta_1 \left(\frac{\delta V}{\delta z} \right)_{z=-0} + \eta_2 \left(\frac{\delta V}{\delta z} \right)_{z=+0} \tag{A-1}$$

where η_1 is the viscosity of the medium below, η_2 the viscosity of the medium above the surface layer (for the definition of x and z see Fig. 1). The conservation of mass for the surface is

$$b \frac{\delta V}{\delta x} c = -\frac{\delta b}{\delta t} c = -c \frac{\delta b}{\delta t} - b \frac{\delta c}{\delta t} \tag{A-2}$$

where c is the concentration of molecules in the surface, b the width of the surface, which is taken as a function of time. Using the definition of surface elasticity [2]

$$\epsilon = \frac{1}{c} \frac{\delta\sigma}{\delta 1/c} \tag{A-3}$$

Eqn. A-2 can be rewritten as

$$b \frac{\delta V}{\delta x} = -\frac{\delta b}{\delta t} + \frac{b}{\epsilon} \frac{\delta\sigma}{\delta t} \tag{A-4}$$

In the case of the experimental apparatus, a liquid/air interface is involved; therefore $\eta_2 = 0$, it has a constant width; therefore b is constant. Then equation A-4 becomes

$$\frac{\delta V}{\delta x} = \frac{1}{\epsilon} \frac{\delta\sigma}{\delta t} \tag{A-5}$$

Combining this equation with A-1 gives the equation of motion for our experimental arrangement:

$$\frac{\delta^2 V}{\delta x^2} = \frac{\delta}{\delta t} \eta_1 \left(\frac{\delta V}{\delta z} \right)_{z=0} \tag{A-6}$$

(Substituting the wave solution in this equation, according to Crone [3], produces the dispersion equation: Eqn. 7 in the main text.)

Dealing with cylindrical surfaces instead of planar surfaces, the penetration depth δ_p of the wave in the media surrounding the layer is of great importance. In formulae:

$$\delta_p = \sqrt{\frac{2\eta}{\omega\rho}} \tag{A-7}$$

For the order of magnitude: for a wave with frequency of 500 Hz, δ_p has a value of about 20 μm.

When the radius R_c of the cylinder is much larger than δ_p, the surface is assumed to be planar and the equations given above are applicable. When $R_c \ll \delta_p$, the water inside the cylinder is assumed to move with an uniform velocity, equal to the surface velocity $V(x, t)$. Conservation of mass for a cylinder element is:

$$S\frac{\delta V}{\delta x} = -\frac{\delta S}{\delta t} \tag{A-8}$$

where S is the surface area of the cross-section of the cylinder. S is related to b:

$$S = \frac{1}{4\pi} b^2 \tag{A-9}$$

Eqn. A-8 predicts swelling and shrinking of the cylinder when the surface propagates a longitudinal wave: b depends upon time. Then combining equations A-8 and A-9 produces

$$\frac{\delta V}{\delta x} = -\frac{2}{b}\frac{\delta b}{\delta t} \tag{A-10}$$

Eqn. A-10 combined with Eqn. A-4 gives:

$$\frac{\delta V}{\delta x} = \frac{2}{\epsilon}\frac{\delta \sigma}{\delta t} \tag{A-11}$$

Eqn. A-11 is similar to Eqn. A-5 if the value of ϵ is halved in Eqn. A-5. In conclusion, under the circumstances given, the equations that apply to the behaviour of longitudinal waves in planar surfaces are very similar to those that apply to cylindrical surfaces.

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References

1 Lucassen, J. (1968) Trans. Faraday Soc. 64, 2221–2235
2 Lucassen, J. and Van den Tempel, M. (1972) J. Colloid Interface Sci. 41, 491–498
3 Crone, A.H.M., Snik, A.F.M., Poulis, J.A., Kruger, A.J. and Van den Tempel, M. (1980) J. Colloid Interface Sci. 74, 1–7
4 Stumpf, F.B. (1980) Analytical Acoustics, p. 111, Ann Arbor Science Publishers, Ann Arbor, MI
5 Snik, A.F.M., Kruger, A.J. and Joos, P. (1978) J. Colloid Interface Sci. 66, 435–439
6 Ruch, T.C. and Patton, H.D. (1965) Physiology and Biophysics, p. 48, W.B. Saunders, Philadelphia. PA
7 Pasechnik, V.I. and Kuznetsova, Y.S. (1977) Biofizika 22, 941–942
8 Survuss, R.M., Harbich, W. and Helfrich, W. (1976) Biochim. Biophys. Acta 436, 900–903
9 Pasechnik, V.I. (1977) Biofizika 22, 1024–1029
10 Cotterill, R.M.J. (1980) Phys. Scr. 22, 188–192
11 Haines, T.H. (1979) J. Theor. Biol. 80, 307–323
12 Koeppe, R.E., Berg, J.M., Hodgson, K.O. and Stryer, L. (1979) Nature 279, 723–725
13 Tasaki, I. and Iwasa, K. (1980) Biochem. Biophys. Res. Commun. 94, 716–720