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MEASUREMENT OF PHOSPHOLIPID MONOLAYER SURFACE POTENTIALS AT A HYDROCARBON-ELECTROLYTE INTERFACE

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Summary

The phospholipid monolayer spread at a hydrocarbon-electrolyte interface can be used as a model system for the plasma membrane and its properties and structure probed by measurements of surface pressure and surface potential. To facilitate such studies, (i) the theory of the vibrating plate (Kelvin) method of measuring surface potentials is reëxamined and a new interpretation given for the potentials measured and (ii) a new apparatus for performing these measurements is described. The theory and apparatus are illustrated by measurements on films of distearoyl phosphatidylcholine at the interface between 2,2,4-trimethylpentane (isooctane) and 0.1 M NaCl.

Introduction

It is virtually impossible to study the surface electrical properties of biological membranes in vivo with control over such variables as phospholipid composition, surface pressure, system temperature, or the like. For this reason, three model system have come into general use: the bimolecular lipid membrane [1]; the spherical, bilayer-bounded, approx 25 nM diameter liposome [2]; and the planar, monomolecular phospholipid surface film [3,4]. Planar monolayers in turn normally fall into either of two categories: those at the air-water interface

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and those at the hydrocarbon-water interface, the latter being preferable because they reduce domination of the pressure-area behavior by the hydrocarbon side chains. This paper describes the design and application of a novel apparatus for studying the surface potential of phospholipid films at a hydrocarbon-aqueous electrolyte interface.

In studies with surface films, there are two methods of determining the surface potential in use [5]. By far the more common is that of an ionizing electrode placed above the interface. Radiation from the source strongly ionizes the medium between the electrode and the aqueous substrate and yields the equivalent circuit shown in Fig. 1A. Surface potential is determined by tuning the compensation potential V_c until a null of i is achieved:

$$V_s = -V_c + IR_I \tag{1}$$

where the IR_I term is invariably neglected. The less common is the vibrating plate method, introduced by Pellat and by Kelvin [6] and subsequently given significant modification by Zisman [7]. In this the interface is made one plate of a capacitor while the second (vibrating) plate is positioned above the interface. An idealized equivalent circuit is shown in Fig. 1B. A simple application

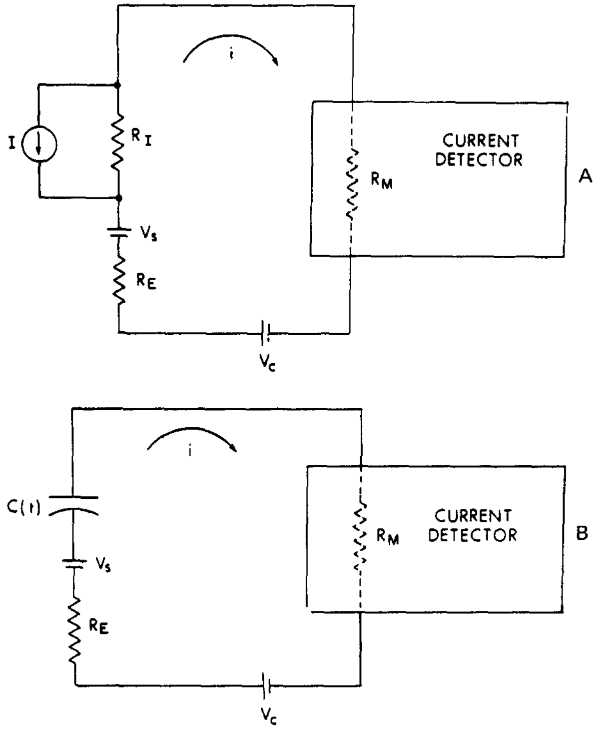

Fig. 1. Equivalent circuits for surface potential measurement. (A) Ionizing electrode: The current generator I represents the charge carried by the particles emitted by the source, R_I is the gap resistance during irradiation, V_s is the monolayer surface potential, R_E is the usually small resistance between the monolayer and the current detector, and V_c is a 'backing' voltage which is taken to include not only an ideal variable direct-current voltage source but also all interfacial or junctional potentials in the circuit except that at the monolayer; the current detector has an input resistance R_M and must be extremely sensitive to measure accurately the small current i which passes through it. (B) Vibrating plate: Here $C(t)$ is an ideal varying capacitance and the other symbols are as in A.

of Kirchhoff's voltage law reveals that tuning V_c to a null of current yields

$$V_s = -V_c \tag{2}$$

The two methods are commonly believed to give comparable results at an air-substrate interface [8]. However, a 0.1 mCi source can generate in air an R_I of 10^{10} Ω [5]; and this, by Egn. 1, implies an uncertainty of the order of 10 mV in determining V_s by the ionizing electrode technique. Further, that technique is well known to be moderately sensitive to electrode separation [9,10], whereas the vibrating plate method should be less sensitive to this variable if suitable precautions are taken [11—14]. However, the decisive factor to us was that the ionizing range of the radiation in hydrocarbon would by the Bragg-Kleeman rule [15], be only a few tens of micrometers; and the activity of the source dissipated in so small a volume, necessarily so close to the interface, would almost certainly result in the production of enough surface-active contaminants to vitiate the experiment. Moreover, an electrode-interface spacing of roughly 50 μm is so small that even a small vibration propagated across the interface would bring the monolayer into contact with the source and terminate the experiment.

An excellent experimental critique of the vibrating plate technique has been given by Surplice and D'Arcy [16]. Its use in liquid media has been discussed by Davies and Rideal [17] and by Fort and Wells [14]; and its mathematical theory has been examined, for example, by MacDonald and Edmundson [18].

Theory

In this section we shall (i) discuss the waveform of $i(t)$ and (ii) present a description of what physically is being detected when V_c is tuned to null $i(t)$.

If it is assumed that

$$C(t) = C_0 + c \cos \omega t + O(c^2) \tag{3}$$

where $c/C_0 << 1$, simple linearized network theory then implies

$$i(t) \doteq -\omega c(V_c + V_s) \sin \omega t \tag{4}$$

Thus, if the capacitance varies at a fundamental $f = \omega/2\pi$, it is sufficient to detect only the component of current at f in order to determine V_s. Moreover, the sensitivity will increase linearly in f.

Fig. 2 is an electrophysical scheme of the interfacial region. The equations for the electric scalar potential were derived from the first Maxwell equation for inhomogeneous media at low frequencies [19] and standard double layer theory [20]. The condition that vibration of the moveable plate produce no charge flow in the external circuit is mat when the electric $\vec{D}$-vector is continuous across the interface at $x = -L$; and since $\vec{D} = 0$ in a conductor, this implies $d\phi_h/dx = 0$ or $\phi(-D) = \phi_s = 0$. When $\phi_s = 0$ the several equations combine to imply

$$\phi_0 = \int_{-D}^{0} \frac{dx'}{\epsilon_i(x')} \int_{-D}^{x} \rho_i(x'')\, dx'' \tag{5}$$

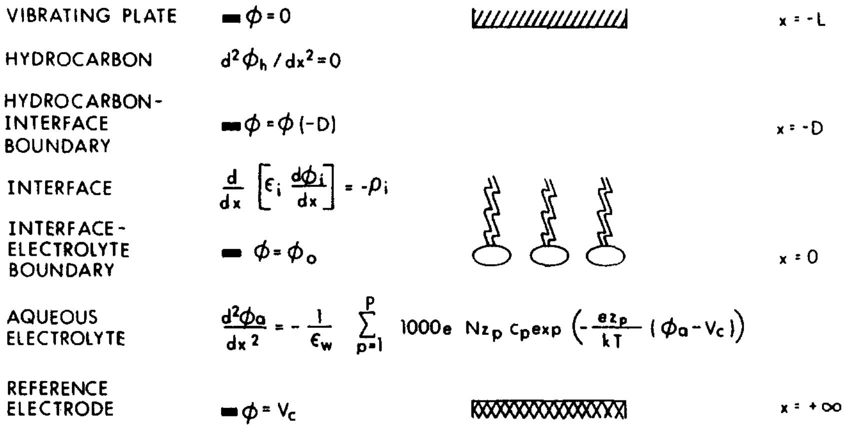

Fig. 2. Electrophysical schematic showing the potentials which exist in the interfacial region. The subscripts h, i and a stand for hydrocarbon superphase, interfacial phase, and aqueous electrolyte subphase. e is the charge on the proton (1.602 ... · 10^{-19} C), N is Avogadro's number (6.022 ... · 10^{23} particles/mol), z_p is the valence of the pth ion species and c_p (mol/l) its concentration, k is the Boltzmann constant (=1.380 ... · 10^{-23} J/K), and T (K) is the absolute temperature. ϵ(F/m) is the permittivity; and the limits of the interface are defined by $\epsilon_i \to \epsilon_h$ ($x = -D$) and $\epsilon_i \to \epsilon_w$ ($x = 0$).

and
$$\int_{-D}^{0} \rho_i(x'')\, dx'' =$$
$$\mathit{signum}(\phi_0 - V_c) \sqrt{2000kT\epsilon_w N \sum_{p=1}^{P} c_p \left[\exp\left(-\frac{ez_p}{kT}(\phi_0 - V_c)\right) - 1\right]} \qquad (6)$$

Even if V_c is assumed to be available experimentally, Eqns. 5 and 6 still involve three unknowns: ϕ_0, $\epsilon_i(x')$, and $\rho_i(x'')$. And they do not contain enough information to permit evaluation of these unknowns. They do, on the other hand, restrict them by establishing interrelationships which they must obey. Moreover, as a result of the way in which the monolayer region was defined, the validity of these equations depends only upon the validity over macroscopic areas of the interface of (a) Maxwell's equation, (b) the Poisson-Boltzmann equation, and (c) the concepts of continuum electrostatics: the formalism is thus independent of the fine structure of the monolayer.

Unfortunately, V_s cannot be determined directly because of the existence of unknown interfacial potentials at the electrode-liquid interfaces. That is, the compensation potential V_c contains, in addition to the measurable output ${}_mV_c$ of a voltage source, a steady offset V_{off}. Therefore, it is customary to determine ${}_mV_c$ both with and without a spread monolayer film and to eliminate the unknown offset by subtraction thus obtaining

$$\Delta V_s = {}_mV_c^{(nf)} - {}_mV_c^{(f)} \qquad (7)$$

where the superscript nf means 'no film spread' and the superscript f means 'film spread'.

If it is assumed (for convenience) (i) that ΔV_s is due to interfacial dipoles of surface density n (particles/m^2) and (ii) that D is made so large that the surface bears no net charge, the Eqns. 5, 6 and 7 combine to yield

$$\frac{n\Delta\mu}{\epsilon} = \Delta V_s = -\{\phi_0^{(f)} - \phi_0^{(nf)}\} \tag{8}$$

where $\Delta\mu$ (C · m) is an effective surface dipole moment and ϵ is an effective surface dielectric constant. If further it is assumed (i) that $\phi_0^{(f)}$ is due to a head-group dipole μ_{HG} in water ($\epsilon_i = \epsilon_w$) and a carboxyl linkage-associated dipole μ_{carb} in hydrocarbon ($\epsilon_i = \epsilon_h$) and (ii) that $\phi_0^{(nf)}$ is due to a bare surface-associated dipole μ_{BS} in water ($\epsilon_i = \epsilon_w$), then Eqn. 8 reduces to

$$\frac{\Delta\mu}{\epsilon} = \frac{\Delta V_s}{n} = \frac{\mu_{HG}}{\epsilon_w} + \frac{\mu_{carb}}{\epsilon_h} - \frac{\mu_{BS}}{\epsilon_w} \tag{9}$$

Thus, no choice of ϵ will yield a strictly meaningful $\Delta\mu$ and any choice $\epsilon_h \leqslant \epsilon \leqslant \epsilon_w$ will have at least some merit. For this paper we employ a defined surface dipole moment

$$\Delta\mu_s = \frac{\epsilon_w \Delta V_s}{n} \tag{10}$$

Apparatus

General description of surface film apparatus. The basic apparatus consisted of a glass interfacial trough of the type introduced by Brooks and Pethica [21] contained in a thermostatted box (±0.1°C) mounted on a massive, vibration-isolating table. Temperature of the aqueous subphase within the trough was further controlled by a stream of temperature-regulated cooling solution (±0.05°C) passed through a glass cooling coil which rested on the bottom of the trough. Surface pressure was determine to a precision of ± 10 μN/m, by the Wilhelmy plate technique in conjunction with an electromicrobalance mounted within the box. All metal surfaces within the box and the subphase cooling solution were connected to a common ground. The vibrator assembly was independently mounted outside the box and extended down into the box on a rigid post.

Adjusting and vibrating the plate. At the lower end of the rigid post, just above the interface, is mounted a spring-loaded, micrometer-adjusted gimbal (Model GM-15 Jodon Engineering, Ann Arbor, MI 48103) for attaining with ease the necessary [11] surface parallelism. Within the gimbal is mounted a simple, aluminum-cased piezoelectric crystal (Model ED-50, Jodon Engineering, Ann Arbor, MI 48103) to which the vibrating electrode is attached via a short, stiff nylon tube. Although electrostatic vibrators have been used for some time [16] and various of their advantages appreciated [22,23], it is only recently that directly piezoelectric drive has been utilized [24], and then not in the convinient form employed here.

The vibrating electrode itself is a cylindrical brass cup (24 mm deep and 35 mm in diameter) which has been heavily nickel plated. It was found that a thoroughly degreased nickel surface which had been equilibrated several days in the hydrocarbon gave stable surface potential readings and, in contrast to gold, lasted indefinitely without deterioration.

Explosion hazards. The use of hydrocarbon superphases, especially at elevated box temperatures, can lead to the production of significant quantities of flammable vapor [24—27]. Since the principal ignition hazard seemed to be electric sparks which can result if the piezoelectric crystal is overdriven [27], the crystal was never driven at more than 20% of rated voltage and its interior was continually flushed with clean dry nitrogen (approx. 1 ml/s) which then vented upward through an axial tunnel in the rigid post, thus protecting the high tension drive lines containing therein.

Electronics. The vibrator is commonly driven at ~207 Hz since this permitted satisfactory rejection of power line harmonics and since, as a result of roll-off in the detection circuitry, little increase in sensitivity if gained by going to higher frequencies; however, operation to well over 1 kHz is readily possible.

The detection circuit was based upon a voltage reference source of absolute accuracy better than ±50 μV (Model GTX 332, Julie Research, New York, NY 10023) and a stable, operational amplifier ammeter the output of which was analyzed by a lock-in amplifier. The decision to sense current with a nearly ideal ammeter, though novel when this work was begun, has since been introduced by others [29,30]; the use of a lock-in amplifier is standard. The somewhat unusual configuration in which the vibrating plate was grounded (cf. Fig. 2) was motivated by a desire to reduce stray electrostatic coupling between the plate and neighboring grounded surfaces (cf. ref. 31). In an alternative configuration, the subphase was grounded and the detection electronics connected to the vibrating cup; this arrangement has superior electrical noise characteristics. The two configurations yielded equivalent results, as was expected since any contribution to V_c which arose from capacitance variations between the moving plate and neighboring grounded conductors should be cancelled by the differencing operation leading to ΔV_s.

Materials and Methods

Synthetic distearoyl phosphatidylcholine (1,2-distearoyl-*sn*-glycero-3-phosphorylcholine) was spread from an ethanolic-2,2,4-trimethylpentane solution at the interface between 100 mM aqueous solution of NaCl and pure 2,2,4-trimethylpentane. General technique and preparation of the reagents have been described elsewhere [32,33].

Following spreading, the monolayer was allowed to equilibrate for at least 30 min until both Π and V_s were stable. Then it was compressed in a series of steps, enough time ($\geqslant$5 min) being allowed at each step for Π and V_s to come to equilibrium. If desired, further readings were taken during expansion steps. More monolayer solution was then spread and additional readings taken during recompression. This process was repeated until an adequate range of surface densities was achieved.

The unprocessed Π-A and ΔV_s-A curves for the several spreadings normally did not overlap exactly because of uncertainties in the concentration of phospholipid in the spreading solutions, uncertainties in the determination of film area, and the possibility (especially at higher surface concentrations) that not all of the phospholipid ended up in the monolayer. This problem was circumvented by reasoning that a particular surface concentration should, other things being equal, always yield the same Π. Therefore a Π-A isotherm for $A \geqslant 3.5 \cdot 10^{-18}$ m^2 was compared with a standard [33,34] and a multiplicative constant derived such that, the experimental areas per molecule having been multiplied by it, the experimental and standard curves overlap; the high area limit was chosen because Π-A are highly reproducible in this region and also because they relatively insensitive to the hydrocarbon used in the superphase. Second-spread areas per molecule were then multiplicatively corrected so that their Π-A isotherm overlapped the first-spread isotherm. Third-spread data were similarly corrected to overlap second-spread data and, additionally were sometimes compared with standard low area data to validate the procedure. Such overlap constants varied between 0.95 and 1.05 times the reference values. In the hands of this group and those of the group at Unilever Research Laboratories (Port Sunlight, England) this process has yielded consistent and comparable results.

Three other noteworthy modifications of experimental technique have been made. First, the temperature-regulating water which circulates through the glass coil in the subphase electrolyte proved to be a potent source of line frequency interference; to reduce this and also combat triboelectric noice it was replaced by a dilute solution of KNO_3 and earthed. Second, in addition to compressing and sucking clean the bare interface before a spread, the normally parallel moveable barriers were nudged into contact to form a thin triangular wedge of surface which was then sucked clean near the apex as it was slowly closed; this procedure resulted in better and more rapid cleaning of the interface. Third, in order to obtain constant Π readings, the hydrocarbon about the suspension of the Wilhelmy plate must be maintained at a fairly constant depth despite evaporation; depth is now regulated as needed without perturbation to the system by spreading fresh hydrocarbon slowly over the hydrocarbon-air interface using a large hypodermic syringe and a suitable needle.

For use in calculating $\Delta\mu_s$ from Eqn. 10, the relative permittivity of water was taken the data and equation of Malmberg and Maryott [35].

Results

As configured, the apparatus routinely permits resolution of the surface potential to a precision of ±20 μV. This potential is however, as a result of low pass filtering of the output of the lock-in amplifier, an average based upon the past half minute or so. And minute by minute it undergoes a slow random walk within a region roughly a millivolt wide.

Reproducibility from day to day is of the order of ±5 mV.

Fig. 3 shows the Π and ΔV_s results for a typical run as well as (i) the surface dipole moment defined by Eqn. 10, and (ii) a comparison of the Π(n) data with the two-dimensional van der Waals equation

$$\left[\Pi + \frac{\alpha}{A^2}\right] (A - A_0) = kT \tag{11}$$

where $A = 1/n$ is the area per molecule of surface film, A_0 is an excluded area, and $\alpha(<0)$ is an empirical constant. These data are included only to illustrate the technique: a full presentation of our results to date is given in the following

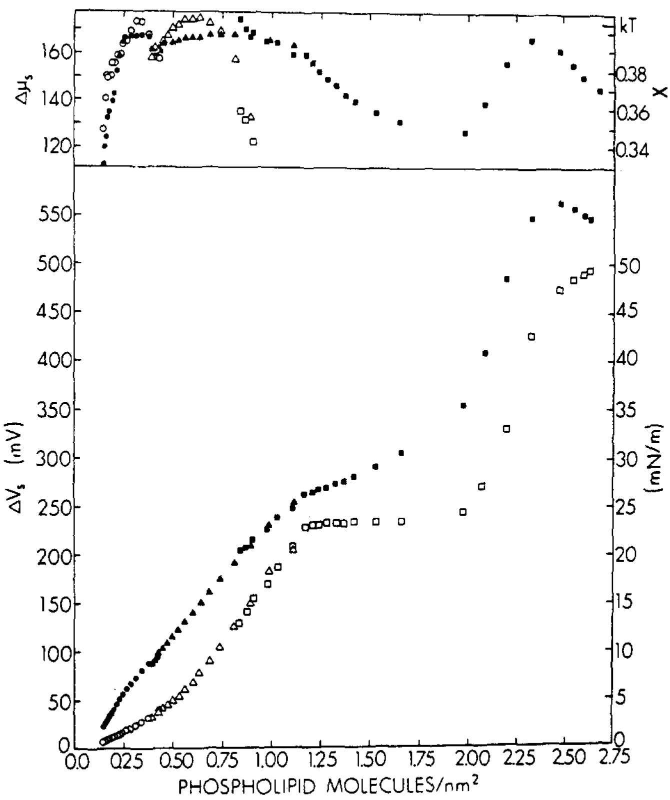

Fig. 3. Variations with film density of surface pressure and potential at 20° C. Distearoyl phosphatidylcholine at the interface between 0.1 M NaCl and 2,2,4-trimethylpentane. Solid symbols are for surface potential and hollow symbols are for surface pressure. Circles (●,○) denote the first spread, triangles (▲,△) the second, and squares (■,□) the third. The inset at the top shows dipole moments (solid symbols) as calculated from Eqn. 10 and a test of the two-dimensional van der Waals equation (Eqn. 11) (hollow symbols) using $\alpha = -70 \cdot 10^{-40}$ N · m^3 and $A_0 = 75 \cdot 10^{-20}$ m^2; the units of $\Delta\mu_s$ are C · m × 10^{30} and those of the variable X ($= (\Pi + \alpha/A^2)(A - A_0)$) are J × 10^{20}.

paper [36], where the variations of $\Delta\mu_s$ and the inadequacy of Eqn. 11 will be discussed in greater detail.

Discussion

We shall defer extended discussion of the experimental data to the following paper [36] and here confine ourselves (i) to an examination of the unexpectedly large size of $\Delta\mu_s$ ($\sim 150 \cdot 10^{-30}$ C · m; 1 Debye unit = 3.335... · 10^{-30} C · m) and (ii) to a brief comment on surface potential measurements in general.

In common with most other investigators of uncharged monolayers [5,34, 37] we observe positive surface potentials. However, the values of $\Delta\mu_s$ computed from them using Eqn. 10 are rather larger than commonly quoted and correspond roughly to two elementary charges separated by a nanometer. Since the phosphate and choline moieties of the headgroup are only about 0.4 nm apart, moments of this magnitude seem at first glance to be counterintuitive. The most reasonable explanation would seem to follow from Eqns. 9 and 10 and the essentially arbitrary decision to utilize ϵ_w in Eqn. 10. Had ϵ_h been used instead, surface dipole moments of the order of $4 \cdot 10^{-30}$ C · m would have been obtained; these are more in keeping with the accepted values for organic acids [38] but lie well below the $60 \cdot 10^{-30}$ C · m expected from the head group. Since, by Eqn. 9, $\Delta\mu_s$ should also include a term μ_{BS} which presumably involves both ϵ_w and the dipole moment of water itself, we opted for ϵ_w in Eqn. 10. Conversion to an ϵ_h basis can be accomplished using the dielectric constant data of Smyth and Stoops [39] for 2,2,4-trimethylpentane to obtain ϵ_h/ϵ_w ratios.

The accuracy (and convenience) obtainable with the apparatus is more than sufficient for the stabilities of ΔV_s which were observed: attempts to improve the apparatus should be forgone in favor of attempts to improve the stability of ΔV_s. We conclude with Surplice and D'Arcy [16] that 'at the present time the precision of the Kelvin method is limited much more severely by the nature of the . . . surface(s) than by the effects of (other miscellaneous experimental problems)'.

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