

THE STUDY OF MEMBRANE INTERACTIONS BY DIELECTRIC SPECTROSCOPY

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Phospholipid vesicles exhibit a radio frequency dielectric dispersion which has been explained in terms of the diffuse thermal rotation of the zwitterionic head groups (Pottel et al 1985). The dispersion has also been attributed to a Maxwell-Wagner charging of the membrane (Schwan et al 1970). It was the aim of this study to show that the dispersion is significantly dependent on the environment of the head group region, and therefore dielectric spectroscopy may be used as a probe to monitor surface membrane interactions.

Large multilamellar lecithin vesicles (LMV) were prepared from grade 2B egg lecithin (Lipid Products) in either 0.1mM metal chloride or 0.1mM tetra-alkylammonium bromide (Lloyd et al 1988). The frequency dependent real permittivity of a 400 μ l sample was then measured between 1-100MHz (23°C) using a Hewlett Packard HP4194 Impedance analyser and probe. The dispersion was characterised by non-linear regression of the real permittivity data, providing values for the step height ($\Delta\epsilon$) and dispersion frequency (f_0). The effect of the above salts on f_0 and $\Delta\epsilon$ are discussed below.

LMV gave a dielectric dispersion centred around 30MHz, in agreement with Pottel et al (1985) and Schwan et al (1970). A plot of $\Delta\epsilon$ as a function of lecithin concentration was linear and passed through the origin (correlation coefficient 0.9996). Also, a linear relationship between f_0 and $\Delta\epsilon$ is observed for the inorganic cations, with decreasing values for both parameters in the order shown (table). Additions to the system of tetramethylammonium bromide (TMAB) or the corresponding ethyl and pentyl salts (TEAB and TPAB respectively) effects changes to both f_0 and $\Delta\epsilon$ which are inversely related to the N-alkyl group chain length, but unlike the inorganic ions show no linearity between f_0 and $\Delta\epsilon$ (table).

Table. Effect of 0.1mM additive on the dispersion parameters

Additive	$\Delta\epsilon$	f_0	Additive	$\Delta\epsilon$	f_0
MgCl ₂	1.60	31.84	TMAB	1.08	26.96
NaCl	1.75	32.37	TEAB	1.18	27.81
KCl	2.47	35.49	TPAB	2.37	32.23
LiCl	2.60	35.70			
CsCl	2.70	36.12			

The linear relationship between f_0 and $\Delta\epsilon$ for the inorganic cations suggests that variations in f_0 and $\Delta\epsilon$ reflect different binding energies of these cations to a specific site on the membrane. Magnesium exhibits the smallest values for f_0 and $\Delta\epsilon$, due to its divalency and consequent strong association with the membrane. The rank order obtained for the alkali metal ions corresponds to that obtained for the binding energies to fixed anionic sites separated by approximately 0.4nm (Eisenmann 1961). This distance is comparable to the intramolecular spacing of phosphatidylcholine molecules in the gel state. These observations may therefore provide information on the packing of the head groups in lecithin bilayers. The lack of linearity between f_0 and $\Delta\epsilon$ for the organic cations was rationalised by considering the specificity of these molecules for different parts of the membrane. TPAB will partition towards the hydrophobic core of the membrane and will therefore have less influence on the head group region. In contrast, TMAB and TEAB both exert a marked effect on the dielectric properties, and therefore must significantly affect the properties of the membrane surface.

In conclusion, the above data strengthen the hypothesis that the radio frequency dielectric properties of lecithin liposomes are primarily dependent on the interfacial properties of the thin layers incorporating and in close proximity to the head groups. The technique is therefore of use in studying membrane surface interactions.

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