THE EFFECT OF CRYOPROTECTIVE ADDITIVES ON THE ZETA POTENTIAL OF LIPOSOMES

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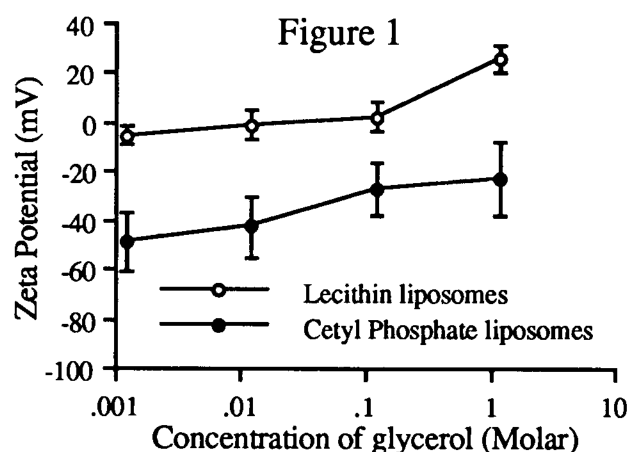
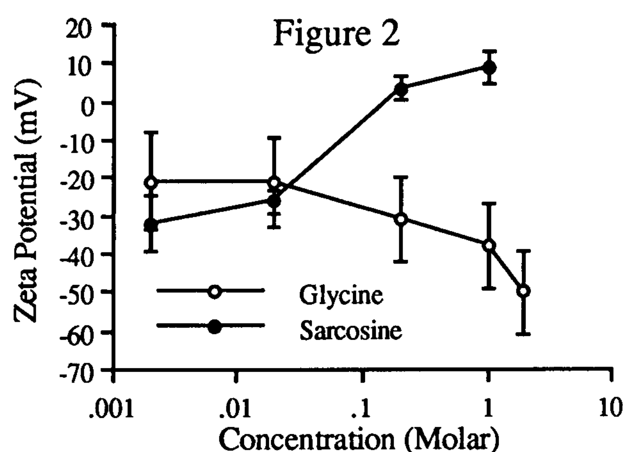
The ability of polyols and other cryoprotective additives to stabilise phospholipid vesicles during freezing and freeze–drying has been attributed to various interactions between the additive and the phospholipid headgroups (Crowe et al 1988). A number of postulates have evolved relating these interactions to changes in electrostatic and hydration repulsions between membranes (Rand & Parsegian 1986). Zeta potentials have been widely used as a means of characterising the surface potential of liposomes. The surface potential is an important parameter influencing the distribution of liposomes in vivo and moderating the rate of aggregation and fusion in vitro. This communication describes the novel use of zeta potential determination as a means of investigating the interactions of cryoprotectants with membranes.

Multilamellar liposomes were prepared from egg lecithin and egg lecithin with 10mol% dicetylphosphate by hydration of a thin film of lipid with double distilled water at 50°C. The liposome suspensions were diluted by addition of a few drops to 10mL of cryoprotectant solution. The zeta potentials of the liposomes in the various additive solutions were measured by electrophoretic light scattering using a Coulter Delsa 440 at 25°C.





The liposomes prepared from lecithin alone had a zeta potential of -21±10mV and those containing dicetylphosphate a zeta potential of -54±16mV. The effect of glycerol, after compensation for changes in viscosity, is shown in figure 1. Trehalose, glucose, inositol and DMSO appeared to have no effect on the zeta potential of the lecithin liposomes, at similar concentrations. The effects of glycine and sarcosine on lecithin liposomes are shown in Fig. 2. Glycine caused no change in the observed zeta potential of the liposomes containing dicetylphosphate. Glycinebetaine was found to reduce the zeta potential of the liposomes containing dicetylphosphate to a value comparable to that of liposomes prepared from lecithin alone, but had no effect on the zeta potential of lecithin liposomes.

Since low concentrations of glycerol reduce the zeta potential of lecithin liposomes to zero, this may explain why glycerol behaves as a fusogenic agent. The effect observed with glycerol supports the evidence of Alonso-Romanowski et al (1989) which suggests an interaction between glycerol and the phospholipid head groups. Previous work in our laboratory (unpublished) has shown that liposomes containing 10mol% dicetylphosphate to be less stable on freezing than those prepared from lecithin alone. Similarly liposomes frozen in glycine are less stable on freezing than those prepared in sarcosine or betaine. These observations may be related to the negative zeta potential of the liposomes observed for both cases. The results for glycinebetaine, sarcosine and glycine would suggest that these compounds interact with the phospholipid membrane surfaces in different ways. As the sugars do not appear to modify the zeta potential of the liposomes, it seems that they exert their cryoprotective effects without causing changes in the surface charge.Clearly, this technique offers an additional method for the elucidation of the mechanisms of action of cryoprotective additives.

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