THE INFLUENCE OF PREPARATION TEMPERATURE ON THE PROPERTIES AND STABILITY OF EGG LECITHIN LIPSOMES

R Mounajed, C J Olliff and J M Padfield*, Department of Pharmacy, Brighton Polytechnic, Brighton and *Glaxo Group Research Ltd., Ware.

It is generally recommended that, when preparing liposomes, the lipids used should be hydrated at temperatures above the gel-liquid crystalline phase transition temperature (Szoka and Papahadjopoules 1980). Egg lecithin has a phase transition temperature of -15°C (Ladbrooke & Chapman 1969) and therefore preparation at temperatures above this should produce liposomes of egg lecithin having similar properties. This communication demonstrates difference in properties when liposomes are prepared at room temperature (about 20°) and 40°.

Liposomes were prepared using egg lecithin (Lipid Products Grade I) either alone or with 10 mole % dicetylphosphate or 10 mole % stearylamine using 0.1 M tris buffer (pH 7.0) containing 0.1MKCl as the aqueous phase: multilamellar liposomes were prepared by shaking this suspension, under N_2, using a Rotamixer. Solutions of 0.1 M tris buffer containing 5% ^{W}/v potassium dichromate, 5% ^{W}/v amaranth or 2%^{W}/v dextran blue were also used for hydration of the lipid.

The following properties were examined as a function of temperature: electrophoretic mobility (E.M.) using a Rank Mk. II microelectrophoresis apparatus (4-50°), absorbance (A) at 400 nm (4-50°) and dye efflux into pH 7.0 tris buffer (4-37°). Linear relationships between E.M. or A and temperature were obtained for all liposomes (containing dicetylphosphate, stearylamine, amaranth or dichromate) prepared at 40° whereas for those prepared at room temperature, discontinuities in the profiles were seen in the 18-20° region.

Similar results were obtained for the efflux experiments, with the dichromate efflux rate, k, (approx.1.2×10^{-4} sec^{-1}) at 18-20° for liposomes prepared at room temperature, being of the order of the value of k (1.04×10^{-4} sec^{-1}) obtained at 37°.

Arrhenius plots gave activation energies of 21.5 kJ mol^{-1} for negatively charged liposomes prepared at 40°C whereas those prepared at room temperature had a value of 41.8 kJ mol^{-1} for measurements taken below 18° and 26.4 kJ mol^{-1} above 20°C. The value of k, at a specific temperature, for both dichromate and amaranth was greater by a factor of 2-3 for negatively charged liposomes prepared at room temperature than those prepared at 40° whereas the reverse occurred for dextran blue. The surface negative charge of the liposomes prepared at room temperature had no effect on k but for those prepared at 40° the value of k increased with surface charge.

Electronmicrographs of negatively stained liposomes showed that those prepared at room temperature were approximately double the size of those prepared at 40°. Also it was found that on storage for 24 hours at 18° aggregation had occurred for liposomes prepared at room temperature whereas no detectable change had taken place for those prepared at 40°.

From these results it would appear that liposomes prepared from egg lecithin at room temperature have a relatively unstable porous membrane structure which undergoes rearrangement if heated above 20°C whereas liposomes prepared at 40°C are less porous and have greater stability. It may be concluded that for the preparation of egg lecithin liposomes it is advisable to use a temperature of 40° during the hydration stage.

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Ladbrooke, B.P., Chapman, P. (1969) Chem. Phys. Lipids 3: 304-312

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