THE INCORPORATION AND RELEASE OF A DIBASIC DRUG FROM LIPOSOMES

R.S. Chawla, I.W. Kellaway, C. Marriott, and J. Stevens*
Pharmaceutics Research Unit, Department of Pharmacy, University of Nottingham,
*The Boots Company Ltd., Nottingham

The development of the liposome as a drug delivery system for a range of pharmacologically active agents has recently been the subject of many research publications. The ability to entrap and retain charged structures is generally poor, with leakage of ions occuring over relatively short periods. This work examines the incorporation and retention by liposomes of a dibasic drug, chloroquine with a pKa_1 of 8.1 and a pKa_2 of 10.2 at 30°, over the pH range of 7 to 12. Adjustment of pH was by the addition of sodium hydroxide solution and the pH was monitored throughout the study. All systems were maintained at 25°C.

The liposomes were prepared by dissolving the chloroquine base and the phospholipid in ethanol and evaporating the solvent on a rotary vacuum evaporator to produce a thin film of mixed lipid and drug. The aqueous phase was added to give a final concentration of 800 $\mu g\ ml^{-1}$ of phospholipid and 20 $\mu g\ ml^{-1}$ of chloroquine and the flask rotated until the film was dispersed to form liposomes.

Assessment of the drug incorporated into the liposomes was achieved by centrifugation of the sol at 60 000 g and assaying the supernatant spectrofluorimetrically (excitation 335 nm; emission 400 nm). Calibration curves were constructed for each pH studied as the relative fluoresence was pH dependent. All liposomes preparations were examined for chloroquine crystals by fluoresence microscopy. The rate of drug incorporation into the unsonicated liposomes was measured over 36 h and equilibrium was established in 28 h. Release rates were determined over 24 h by challenging the equilibrated systems by diluting 1 part with 4 parts of solvent, and taking measurements every 3 h.

The full pH profile was determined using egg phosphatidylcholine (EPC) liposomes. Maximum incorporation was observed in the pH 9-10 region (≈90%) and a decrease was observed as the pH was increased to 12 (≈10%). It is not clear why at pH 9 to 10 optimal uptake and retention of chloroquine by EPC should occur as the 4'-diethylamino group will be predominantly ionized whereas the 4-methylbutylamino group will be predominantly uncharged. The percentage retained by the liposomes as a function of pH produced curves of a similar shape over the 24 h period and >50% of the chloroquine remained associated with the liposomes in the pH range 9-10 at the end of the experiment.

Liposomes prepared from the synthetic phospholipids dimyristoyl - (DMPC), dipalmitoyl - (DPPC), and distearyl - (DSPC), phosphatidylcholine were examined at pH 8.0. DPPC and DSPC liposomes were found to incorporate and retain chloroquine more effectively than DMPC. At 25° DMPC is above the phase transition temp (tc) and hence the bilayers will be in a more fluid state than the corresponding DPPC & DSPC for which tc >25°.

When cholesterol (400 $\mu g\ ml^{-1}$) was incorporated with EPC (800 $\mu g\ ml^{-1}$) at pH 8.0 an increase in chloroquine uptake and retention was observed, although if expressed in terms of chloroquine uptake per unit mass of lipid the percentage incorporated is less than in the absence of cholesterol. These results would therefore support the concept of incorporating charged molecules into liposomes as a possible means of modifying delivery to the target area.