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A NOVEL METHOD FOR THE IN-SITU PREPARATION OF LIPOSOMES USING AN AEROSOL DELIVERY SYSTEM

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The use of liposomes for the delivery of active compounds has, in the past, suffered from major problems associated with long term storage of liposomes.

A novel system has been developed for in situ production of liposomes. liposomes are produced in aerosol form only when required thereby eliminating stability problems and providing a convenient dosage form particularly for direct administration to the respiratory system.

The apparatus basically consists of a two compartment aerosol delivery system. The first (organic) compartment comprises an aerosol bottle fitted with a metering valve and containing a mixture of propellant and a solution of phospholipid in organic solvent (typically ethyl alcohol). The second (aqueous) compartment contains an aqueous phase held under pressure by propellant and is fitted with a metering valve/mixing chamber. The valve arrangement is such that actuation of the system releases a metered dose of organic phase into the mixing chamber where it is intimately mixed, under pressure, with a metered dose of the aqueous phase. The resulting emulsion is ejected to the atmosphere as an aerosol. Active compounds can be incorporated in the liposomes by dissolving them in the organic or aqueous phases, depending on solubilities.

The liposomes produced using egg lecithin have been examined by electron microscopy. Liposome size distribution was determined in more than 20 preparations and the mean geometric diameter and size range (95% confidence limits) calculated for each population. A typical mean value obtained was 75nm with a range of 25 - 250nm. Subjective examination of more than 50 electron micrographs revealed the majority of liposomes to have between 2 and 5 bilayers with a maximum range of between 1 and 10 bilayers.

A range of active compounds has been included in the system and shown to be incorporated in the liposomes. For example, a system was manufactured with an organic phase comprising a mixture of a solution (3ml) of stanozolol (4mg/ml) and egg lecithin (300mg/ml) in ethyl alcohol and 4.5g dichlorodifluoromethane. The aqueous phase consisted of 40ml purified water containing 5% v/v ethyl alcohol. The valve arrangement was such that each actuation of the system simultaneously delivered 50ul organic phase and 100ul aqueous phase. Condensed aerosol was chromatographed on Sepharose 4B and subsequent analysis of the eluate fractions revealed that, of the 85ug stanozolol delivered by each actuation, more than 70% was associated with liposomes. Similarly, bitolterol mesylate (200ug per actuation) was more than 80% incorporated in negatively charged (dicetyl phosphate containing) liposomes.

Using ^{14}C-inulin as a marker, the internal aqueous volume of neutral and negatively charged liposomes was determined to be 2.8 and 5.2 ul/mg lecithin respectively. These figures are comparable with the entrapment volume of MLV (1 - 5 ul/mg) prepared by standard techniques (Szoka et al.1981).

This technique of holding the organic and aqueous phases separately has resulted in a stable system which produces liposomes instantly but only when required. Liposome stability problems are thus eliminated.

Szoka, F, et al. (1981) in Liposomes : From physical structure to therapeutic applications. Ed C.G. Knight, Elsevier North-Holland Biomedical press.