SIZE AND STABILITY OF LIPOSOME SUSPENSIONS MEASURED BY PHOTON CORRELATION SPECTROSCOPY

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The size of phospholipid vesicles, liposomes, is an important parameter as regards the uptake of these potential drug carriers by cells or organs in vivo, large vesicles for example being removed from the bloodstream more readily than small vesicles by the spleen and lungs (Sharma et al 1977). We have investigated by photon correlation spectroscopy the size and stability of anionic liposomes produced by a method commonly used in studies of their biological activity. A dry lipid film composed of 29.2 mg dipalmitoyl L- α -phosphatidyl choline, 4.3 mg cholesterol and 3.1 mg dicetyl phosphate was hydrated at 45° with 4 ml phosphate buffered (pH 7.2, 6.7 mM) normal saline for two hours and the resultant milky suspension sonicated (MSE 150W sonicator, 19mm probe, 20 ml tube) at 45° under nitrogen for 20 min. Optical clarity was achieved after 5 min. 2 ml sonicated material was applied to a 1.3 x 20cm Sepharose 4B column, jacketed at 25°, and eluted with buffered normal saline, flow rate 2.5 ml/min, 0.5 ml fractions being collected. Absorbance (400nm) of each fraction was determined. One drop from each liposome fraction was added to 2.5 ml filtered (membrane, 0.22μm nominal pore size) buffered saline in cleaned light scattering cells. The cells were sealed and the decay rates of the time dependent concentration fluctuations of the dilute suspensions measured on a Malvern Instruments 48 Channel, 7023, single clipped photon correlation spectrometer (He/Cd Laser, λ = 441.6nm; temperature 25°) at a scattering angle θ of 90°. The derived z-average diffusion coefficient was used to calculate a mean hydrodynamic vesicle diameter using the Stokes-Einstein equation. The normalised variance of distribution (NVD) was also calculated. Further measurements were made at time intervals during storage of the sealed cells at 25° and 45°.

Table 1. Absorbance at 400nm (lipid concentration) and mean vesicle diameter of column liposome fractions.

Fraction No.	56	58	60	62	64	66
Elution Vol. (ml)	28	29	30	31	32	33
Absorbance (400 nm)	0.150	0.415	0.535	0.840	0.400	0.305
Mean dia. (nm)	138	109	96	91	88	86
NVD	0.169	0.102	0.083	0.049	0.046	0.050

There was marked variation of vesicle diameter through the elution profile so that pooling of these fractions would result in a polydisperse suspension which could have a significant effect on uptake and other biological effects. Careful fraction cutting would however yield a relatively mono-disperse suspension.
An increase in vesicle mean diameter was found after storage at 45° and 25°, i.e. above and below the transition temperature of the dipalmitoyl lecithin, although slight compared to that reported for pure egg lecithin vesicles (Chen et al 1976) probably because the net -ve charge on the present system due to the dicetyl phosphate restricts, by mutual repulsion, vesicle aggregation.

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Chen, F.C, Tu, S.I, Chu, B. (1976) in Colloid and Interface Science, Vol. 5, 63-76, Ed. M. Kerker, Academic Press