THE PARTITIONING OF LIPOSOMES OF DEFINED SIZE AND COMPOSITION

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Liposomes are a well-established experimental system in which to model biological membranes. Studies with multilamellar vesicles (MLVs) and small unilamellar vesicles (SUVs), prepared by sonication, by Eriksson and Ålbertsson [1] and by Sharpe [2,3], have demonstrated the importance of the polar head group in determining the partitioning of liposomes.

Recently a rapid method for the production of unilamellar vesicles of defined, uniform size by an extrusion technique (VETs: Vesicles by Extrusion Techniques) has been developed in the Biochemistry Department, University of British Columbia [4].

We have compared the partitioning behaviour of VETs extruded through filters with pore sizes from 50 nm to 600 nm (VET_{50} to VET_{600}) with that of MLVs in a charge-sensitive phase system (5% Dextran T500-5% PEG 6000 (BDH) in 0.11 M sodium phosphate buffer, pH 6.8. Two different negatively charged lipsome systems were used:

i)	egg phosphatidyl glycerol (EPG)	40% w/w
	egg phosphatidyl choline (EPC)	60% w/w
ii)	egg phosphatidic acid (EPA)	40% w/w
	egg phosphatidyl choline (EPC)	60% w/w

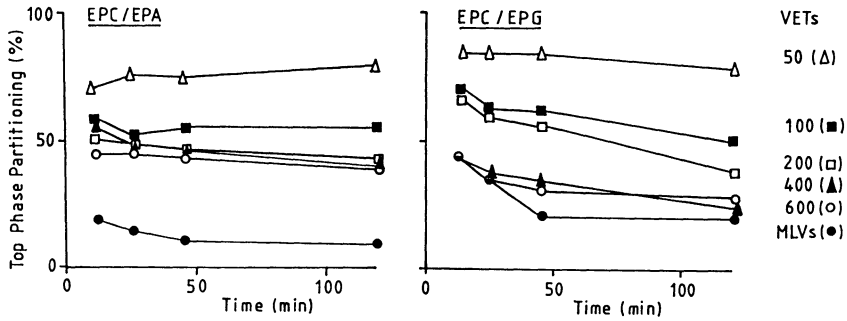

Fig. 1 *Time course of partitioning of VETs of varying sizes*

D. Fisher et al. (eds.), *Separations Using Aqueous Phase Systems*

Partitions were sensitive to the size of the vesicle preparations, top phase partitioning increasing with decreasing size.

Using preparations of a defined size (VET_{100}), the effect of the liposome composition on the partitioning in phase systems of 5% dextran-5% PEG that had low electrostatic potential difference between the bulk phases, $\Delta\psi$ (0.15 M NaCl in 0.01 M phosphate buffer), intermediate $\Delta\psi$(0.075 M NaCl in 0.068 M phosphate buffer) and high $\Delta\psi$ (0.11 M phosphate buffer) was examined.

The lipid compositions used were as follows:

neutral:	egg phosphatidyl choline (EPC)
negatively charged:	EPA-EPC (40% w/w)
	EPG-EPC (40% w/w)
	Phosphatidyl inositol (PI)-EPC (40% w/w)
	Brain ganglioside-EPC (40% w/w)
positively charged:	stearylamine-EPC (20% w/w)

Vesicles of EPC partitioned predominantly to the top phase (70%) with 15% in the bottom phase and at the interface. Partitioning was virtually independent of the $\Delta\psi$, a result consistent with the liposomes having no net charge.

Incorporation of the negatively charged lipids into the EPC vesicles rendered partitioning sensitive to $\Delta\psi$, top phase partitioning increased as $\Delta\psi$ increased. This arose from two contrasting features. Firstly, in the low $\Delta\psi$ phase incorporation of the negatively charged lipid caused increased partition to the bottom phase. This increased wetting by the lower phase has been confirmed by contact angle measurements on EPG-EPC mixtures. Secondly, as $\Delta\psi$ increased partition moved in favour of the interface and then the top phase.

The partition of the positively charged vesicles was similar to that of EPC in the low $\Delta\psi$ phase, but was very sensitive to increasing $\Delta\psi$, showing increased partition to the lower phase.

The observations indicate that liposomes will prove to be an acceptable system in which to determine the molecular features of non-charge sensitive partitioning, charge sensitive partitioning, and their inter-relationship. Furthermore, partitioning should provide useful information on surface properties of lipsomes related to their use as drug delivery vehicles.

This work is supported by a NATO grant for international collaboration in research.

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