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EFFICIENT ENTRAPMENT OF DRUGS IN LIPOSOMES OF REDUCED SIZE

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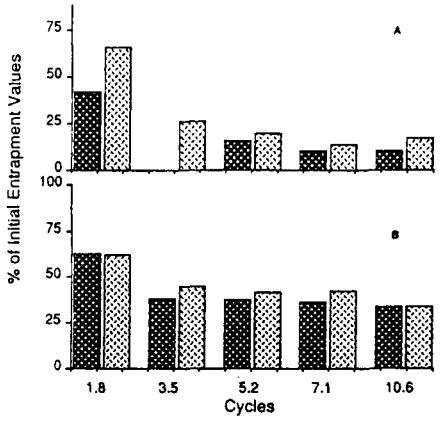
Efficient entrapment of drugs in liposomes using minimal amounts of lipids is likely to reduce both the cost of formulations and the risk of lipid-induced toxicity following injection. To this end, a method developed recently (Kirby and Gregoriadis, 1984) produces large (micrometer size range) multilamellar liposomes (dehydration-rehydration vesicles; DRV) entrapping up to 80% or more of the starting material. DRV are cleared rapidly from blood circulation to end up in the reticuloendothelial system. They are therefore unsuitable for maintaining high concentrations of solutes (eg. haemoglobin in haemosomes) within the vascular system or for ligand-mediated targeting to alternative tissues and cells (Gregoriadis, 1988). In addition, intramuscularly injected large liposomes do not reach the regional lymph nodes efficiently to deliver vaccines and other agents (Gregoriadis, 1990). Such tasks can be carried out by smaller vesicles of about 200 nm or less in diameter which, however, entrap solutes poorly unless very large amounts (eg. 300 μmoles/ml) of lipid are employed (Mayhew et al, 1984). We report here that microfluidization of DRV produces smaller liposomes (down to about 100 nm diameter) retaining much of the originally entrapped solute. DRV composed of equimolar egg phosphatidylcholine and cholesterol and containing 14C-labelled maltose or 125I-labelled tetanus toxoid were passed through a Microfluidizer 110™ for up to 10.6 full cycles. DRV were either devoid (washed) of or in mixture (unwashed) with non-entrapped material. Solute retention values (% of original entrapment values) by maltose-containing washed (dark bars) or unwashed (light bars) DRV microfluidized in the presence of water(A) or PBS (B)are shown in the Figure. Results indicate that much more maltose is retained by DRV in the presence of PBS and similar data were obtained for toxoid-containing DRV (not shown). This could be attributed to a reduction by PBS of osmotic shock expected to occur on dilution of DRV in hypotonic media. Further, there was generally more solute retained by unwashed DRV, presumably because the presence of unentrapped solute during microfluidization helps to diminish solute leakage through membrane breakages. The mean diameter of DRV measured by dynamic light scattering after a given cycle interval was smaller when microfluidization was carried out in water. Z average mean sizes (nm) were 463.5 (washed) and 473.9 (unwashed) after 1.8 cycles but were reduced to 115.0 and 116.9 respectively after 5.2 cycles. There was no substantial further reduction in sizes after 10.6 cycles. Thus, solute retention depends on the extent of microfluidization, the medium in which microfluidization is carried out and whether or not, before processing, DRV are previously separated from unentrapped solute. It would appear that the method can produce liposomes of reduced sizes (<200nm) containing much (about 50%) of the originally entrapped solute.



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