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ADVANCED TECHNIQUES FOR PREPARATION AND CHARACTERIZATION OF SMALL UNILAMELLAR VESICLES

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Abstract

Lipid vesicles have become of considerable importance as model membranes and drug delivery systems. Recently, applications in the food industry have been suggested for microencapsulation and immobilization of enzymes.

A number of methods for the preparation and characterization of liposomes have long been available. For the production of small unilamellar vesicles we have used a microfluidization technique. Microfluidization is based on a submerged jet principle in which two fluidized streams collide at extremely high velocities in a precisely designed interaction chamber.

Advantages of this technique include the absence of organic solvents or detergents, the high lipid concentrations that can be employed and the high encapsulation efficiencies that can be achieved.

Electron photomicrography was used to characterize the liposome preparation. Laser light scattering spectrometry proved to be the most reliable method for determination of mean size and size distribution of small unilamellar vesicles.

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Introduction

Liposomes have been used for a long time for various research applications at the laboratory scale. However, up to the present, and because of the absence of a suitable technology, the production of liposomes has been limited.

This constraint seems to have been overcome by a recent microfluidization technique which will be briefly described. This method allows large-scale preparation of small lipid vesicles of uniform size distribution with satisfactory entrapment efficiency (Mayhew et al., 1984; Mayhew et al., 1985).

In addition to classical laboratory applications of liposomes as model membranes and as active substance carriers, some new uses have been reported in the food industry for immobilization of enzymes (Koide and Karel, 1987). Other fields of potential commercial interest in cosmetics, dermatology and medicine have been recently discovered (Korstvedt et al., 1984).

Materials and Methods

23 g of purified lecithin (OVOTHIN 170, Lucas Meyer. Hamburg, Germany) was dispersed in 200 ml of buffer K-phosphate solution 0.025 M at pH 6.88. The solution corresponded to about 150 µmol phospholipids per ml of solution. The dispersion was heated to 30°C and stirred for 1 hour under a light stream of nitrogen. The hydrated lipids were then passed through the Microfluidizer. Fig. 1 shows a schematic representation of the Microfluidizer M110 which was used (Microfluidics. Newton. MA. USA).

The crude suspension of phospholipids was placed in the reservoir and the air regulator adjusted to the selected operating pressure (860 bars). With such a setting, when the air valve is open, the liquid dispersion flows through a filter (5 µm) into the interaction chamber where it is separated into two streams which interact at extremely high velocities in

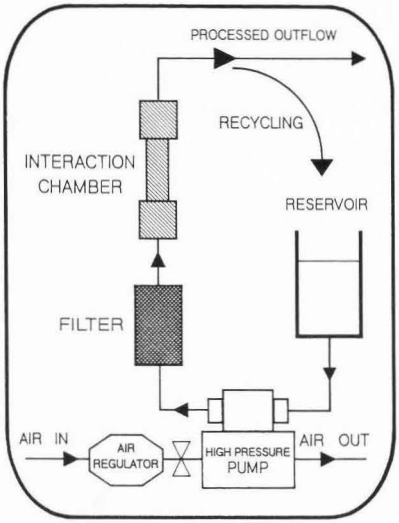

Fig. 1. Schematic representation of the Microfluidizer M110.

dimensionally defined microchannels. The suspension can be recycled through the machine and, in this eventuality, the suspension must be cooled because of the temperature increase in the interaction chamber at high operating pressure. Flow rates in the order of 100 ml/min were employed, and volumes of 200 ml were processed.

A Malvern Photo Correlation Spectrometer (Malvern Instruments) was used to measure the mean size and the size distribution of the liposomes by light scattering. The spectrometer was equipped with a 64-log-channels Malvern Autocorrelator 7032 and a Spectra-Physics 15 mW He-Ne laser (wavelength 632.8 nm).

Freeze-fracture electron microscope photographs of liposomes were obtained by use of a Cryofract (Reichert-Jung, France) and an electron microscope EM 300 (Philips, Holland).

Characterization of Liposomes

Vesicle formation can be modified by varying the pressure or the number of passes through the interaction chamber. Fig. 2 shows the reduction of the mean size of liposomes during successive passes through the Microfluidizer.

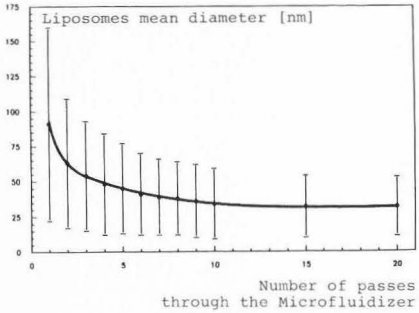

Fig. 2. Size reduction of the liposomes after successive passes through the Microfluidizer.

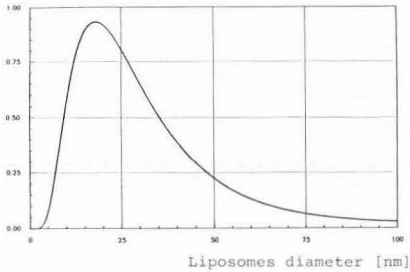

Fig. 3. Liposome size distribution.

Conjointly with the size reduction, a progressive decrease of the spread of the size distribution which corresponds - in Fig. 2 - to the contraction of the bars magnitude as a function of the number of passes, was observed.

Increasing the number of microfluidization cycles beyond 20-25 did not result in any further reduction in liposome size.

Fig. 3 shows a typical liposome size distribution obtained after the 20th pass in the Microfluidizer. This distribution was obtained from light scattering data. The mean size of the particles was 31 nm and about 86% of the liposomes population was situated in the range 5 nm - 50 nm.

A common example of electron micrographs of liposomes produced in this way is shown in Fig. 4. These images illustrate the smooth spherical shape of the liposomes obtained by microfluidization, and the trapped volume compartment. The smooth fracture faces are representative

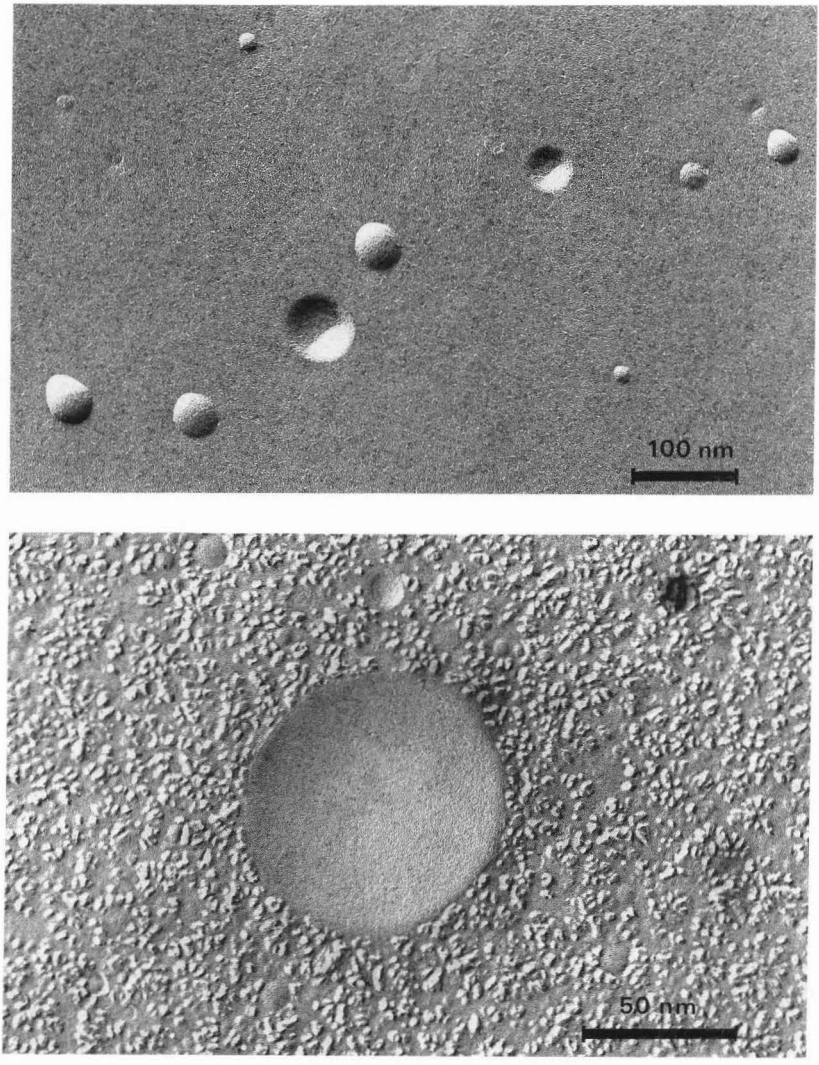

Fig. 4. Freeze-fracture electron micrographs.

of the entire field, and the absence of multistep surfaces unambiguously demonstrates the unilamellar nature of the liposomes. Moreover, the vesicles are seen to be fairly homogeneous in size. This confirms the results obtained by light scattering. The few larger vesicles, and also the material which does not produce vesicles, can easily be removed by centrifugation or by gel chromatography.

Discussion

Microfluidization provides a practical and convenient means of preparing research or commercial quantities of small unilamellar vesicles. According to our own experience, the best characterized unilamellar liposomes were obtained by microfluidization of aqueous suspensions of egg phospholipids. As confirmed by light scattering measurements, the main advantages of the use of Microfluidizer technique are the uniformity of the size distribution and that the liposomes formed are smaller than the smallest unilamellar vesicles prepared by more conventional means such as sonication.

The composition of lipids may have great importance on the size of the unilamellar vesicles that can be produced (Mayhew et al., 1984). However, the Microfluidizer can operate at considerably higher lipid concentrations than are possible with other techniques.

The Microfluidizer process does not involve the use of organic solvents and the liposomes can be prepared by a continuous process rather than a batch process normally required for other preparations.

Electron microscope pictures of the vesicles obtained by microfluidization show smooth spherical shapes and give clear evidence of the unilamellarity of the liposomes.

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Editor's Note: All of the reviewer's concerns were appropriately addressed by text changes, hence there is no Discussion with Reviewers.