Size Reduction Using Sonication

Sonication is an effective method for reducing the liposome particle size for small batches. On the basis of the sonicator type, probe sonication and bath sonication have been developed. Difficulty in uniformly sonicating large batches of material, generation of personal hazard, risk of causing the degradation of the components by elevated temperatures, and production of the *limiting size* vesicles are shortcomings of the sonication method.

Probe sonication: This type of sonication is conducted by immersing a metal probe below the liquid surface. Because of the high input of energy in this method, there are high risks of lipid degradation resulting from the high temperatures and increased gas exchange associated with operation of the probe. It is essential, therefore, that the sonicator vessel be cooled efficiently at all times. However, it is also desirable that the lipids are sonicated above their transition temperature T_c since breaking and resealing of vesicles does not occur efficiently below this temperature. While this can be achieved very easily for egg lecithin (which has a T_c around −15°C with an ice bath), saturated lipids such as DMPC need to be maintained at room temperature or above. In these cases, a circulating water bath with a high rate of flow is essential. Owing to the high energy input of this method, the particle size of the MLV can be reduced rapidly and reproducibly. Small and homogeneously distributed SUV liposomes can be produced by this method. However, this method may introduce contamination due to metal leaching from the sonicator probes.

Bath sonication: Traditional laboratory bath sonicators normally do not impart enough energy to liposomes to reduce vesicle sizes; only *cup-horn* type sonicators (e.g., of Branson) are powerful enough for liposome preparation. This setup has the advantage of avoiding direct contact of the formulation with the probe, but is limited to small quantities (<100 mL) of material.

Size Reduction Using High-Pressure Homogenization

There are several homogenizers capable of generating sufficient high-shear forces to produce both unilamellar and MLVs with defined size distributions (Mayhew et al., 1984; Vidal-Naquet et al., 1989). For example, the Microfluidizer® (Microfluidics, Inc.) is a machine that pumps fluid at a very high pressure (up to 18,000 psi) through a membrane filter, after which it is forced along defined microchannels that direct the two streams of fluid to collide at right-angles with very high velocities in an interaction chamber, thus providing a very efficient transfer of energy (Figure 14.6). The lipids can be introduced into the fluidizer either as a suspension of large MLVs, or as slurry of lipid in an aqueous medium. In the latter case, use of organic solvents may sometimes be unnecessary. The fluid collected can be recycled through the pump and the interaction chamber until vesicles of the required dimensions are obtained.

The homogenization method of the Microfluidizer is very efficient. After a single pass, the size of nondrug loaded vesicles can be reduced to between 0.1 and 0.2 μm in diameter, the exact size distribution depending on the nature of the components of lipids and of the hydration medium. In general, the presence of negatively charged lipids tends to decrease the vesicle size, while increasing the amount of cholesterol in the formulation gives larger liposomes. The nature of the drug can also affect the final size distribution of a liposomal-drug formulation. Continuing the cycling time generally brings the size to a narrow size distribution of low value. However, in some cases (e.g., liposome-containing doxorubicin) the diameter can increase after prolonged recycling, demonstrating that the nature of the drug is very important for reduction of particle size.

Large-scale Microfluidizer homogenizers capable of producing liposomes at high rate, such as 3.8–5.7 L/min for the Microfluidizer Model 210EH, are available, meaning that relatively large volumes of liposomes can be prepared easily. A larger size unit (Microfluidizer Model 610) with a much higher rate is also available; however, the efficiency of its interaction chamber is not as high as the smaller model. The liposomes produced at low lipid concentrations usually contain very high