The advantages of this method are (1) this method is a fast and simple procedure, (2) the method can be used to produce LUVs, and (3) the production can be easily scaled up. The drawbacks of this method are (1) liposomes that are heterogeneous in size may be produced; (2) removing the organic solvent from the mixture is necessary; and (3) due to the large dilution in the dynamic mixing process, the product produced is very dilute, perhaps necessitating concentration procedures.

Dialysis Method

Following its introduction in the early 1970s, the dialysis method has since developed into a useful method of preparing relatively large batches of liposomes for clinical use (Schwendener, 1986). The general procedures of this method are first, the mixture of lipids, drug, and detergent is dissolved in an organic phase and the organic solvent(s) is evaporated to form a film; next, the film is hydrated to form mixed micelles; and finally the unilamellar vesicles are produced by removing the detergent using dialysis systems. The commonly used detergents are sodium cholate, sodium deoxycholate, and some synthetic detergents such as octylglucoside. These all have reasonably high critical micelle concentrations (5–20 mM) to facilitate their removal.

The dialysis system is low cost and includes valves, pumps, capillary dialysis cartridges, and reservoirs. The number and the types of the dialysis cartridges can be varied, for example, two or more cartridges can be connected in a consecutive mode. Several types of disposable cartridges are available that differ in the total surface area available, void volume, and ultrafiltration rate.

In the dialysis method, the parameters that can be adjusted to control the size and homogeneity of the liposomes produced are (1) lipid and drug concentrations, (2) number and type of cartridges used, (3) the flow rates of the mixed micelles and of the dialysis buffer, and (4) the physicochemical properties of the detergent used. These parameters influence the kinetics of the detergent removal from the mixed micelles systems. For example, increasing lipid concentrations usually cause slower detergent removal and less homogeneous liposomes. With an increase of both the membrane surface of the dialysis cartridge and the flow rate of the dialysis buffer, the size of the liposomes can be kept small. The type of the detergent used has a more profound effect on the liposome sizes than the other factors.

The dialysis method is well suited for the encapsulation of lipophilic drugs. The liposomes prepared by this method are usually homogeneous in size with good reproducibility, and the encapsulation condition is mild compared with other methods. The drawbacks include (1) low entrapment efficiency for hydrophilic molecules, (2) the complete removal of residual detergent is impossible, (3) the procedure is lengthy, and (4) scale-up is difficult.

Freeze-Drying Method

The freeze-drying (or dehydration–rehydration) method was developed by Ohsawa et al. (1984) and Kirby and Gregoriadis (1984). This method is used to prepare MLVs with high entrapment efficiency. In this method, small *empty* unilamellar vesicles are first prepared, which are then mixed with the aqueous phase containing the molecules to be encapsulated. The mixture is freeze dried by conventional means. The liposome will be generated by adding an aqueous phase, followed by shaking, usually resulting in large MLVs as products. Alternatively, if the drug has a stability problem during the freeze-drying process, an aqueous solution containing the drug can be mixed with a prefabricated freeze-dried lipid product to produce liposomal drug.

The advantages of this method are as follows:

1. It produces liposomes with high entrapment efficiency.
2. The procedure's flexibility may aid product stability, as noted earlier.
3. Scale-up is possible.
4. The lyophile can be stored and rehydration can be done immediately before use.

The drawbacks of the method are possible particle size instability of the liposome during the freeze-drying process and the high cost of the freeze-drying process.