### pH-Gradient Method ("Remote Loading")

The pH-gradient method has been designed to improve the encapsulation efficiency of ionizable amphoteric compounds (Mayer et al., 1993; Cullis et al., 1997). In this method, liposomes with or without the drug are first prepared by the techniques described earlier. The transmembrane pH-gradient of the liposomes is then generated either by adding an alkalinizing agent to increase the pH of the external medium to a desired level or by exchanging the extravesicular media with a buffer using gel exclusion chromatography or dialysis techniques. The drugs will be redistributed across the lipid bilayers in response to the pH-gradient, and can potentially achieve entrapment efficiencies of almost 100%. One advantage of this method is its high efficiency to encapsulate lipophilic amines such as doxorubicin and dopamine. DOXIL (Table 14.1) is prepared by this method. Another advantage is that this technique allows the drug to be encapsulated just before use, which eliminates the stability problems associated with the postencapsulation processing and long-term storage. Any lipids or mixture of lipids can be used as long as they form liposomes capable of maintaining a stable transmembrane pH-gradient. However, this technique is not applicable to nonionizable drugs.

### Gas-Bubbling Method

The gas-bubbling method is a simple method developed by Talsma et al. (1994). In this method, a stream of gas is introduced into the bottom of a bottle that contains an aqueous dispersion of lipid particles. After bubbling for a sufficient time, liposomes are formed in the reservoir. To reduce the processing time, the size of the lipid particles is usually decreased (e.g., by micronization), and the temperature of the bubbling process is controlled above the gel/liquid phase transition temperature of the lipids. This method is a simple and inexpensive single-step process using no organic solvents or strong mechanical forces; the bubbling gas can be recycled and reused in the production. The drawbacks of this method are (1) the production time is long; (2) the particle size tends to be large and heterogeneous; (3) heating the system above the gel/liquid transition could induce the degradation of the drugs; (4) scale-up is difficult; and (5) encapsulation of water-insoluble drugs is inefficient, as they must be solubilized in the system before liposome formulation. If liposome preparation by the gas-bubbling method is followed by one of the size reduction methods discussed earlier, the size heterogeneity will be diminished.

## Aseptic Filtration for Parenteral Formulations

Aseptic filtration is necessary for parenteral formulations. Because both lipids and the structure of liposomes are unstable at high temperatures, conventional terminal steam sterilization is not suitable for liposome formulations. Thus, the membrane aseptic filtration is the most reliable method for sterilizing liposome formulations. Since the possibility exists for the membrane being defective, it is advisable to test the integrity of the assembled unit by carrying out a *bubble-point* test. This test relies on the fact that after a membrane has been wetted, the surface tension between water and air is such that air cannot be passed through the membrane until sufficient pressure has been reached to overcome that surface tension. The critical pressure at which air will pass (i.e., the point at which a bubble will appear) is directly related to the size of the pores. For a filtration unit to be effective, the size of the pore should be within the limit.

Membrane filters used for sterile filtration are usually the *tortuous pore* type membrane, which consists of fibers crisscrossed over each other to give a matrix in which channels are formed from the random spaces winding in and out between the fibers, tracing a tortuous path from one side of the membrane to the other. The average diameter of these channels is controlled by the density of the fibers in the matrix. Because of the convoluted nature of the channels, microorganisms and liposomes that are larger than the channel diameter (usually 0.22 μm) are retained when passing through such a membrane, and thus the filter clogs up easily.