Encapsulation efficiencies for water-insoluble drugs will normally be quite high, since the amount of drug in the external aqueous phase will be limited by the solubility. In this case, the encapsulation efficiency will be related to the partition coefficient of drug between the aqueous phase and the lipid bilayers, or actually the distribution coefficient if working with an ionizable drug at a particular pH. If there is any appreciable water solubility, the partition or distribution coefficient will actually reflect a lower limit for the encapsulation efficiency, since there could be drug dissolved in the aqueous regions within the liposome. Partition coefficient is usually expressed in terms of ratio of drugs in respective volumes of lipid bilayer and buffer, in which case the former must be estimated on the basis of lipid weight and density. Austin et al. (1995) measured the distribution coefficients of four ionizable molecules into DMPC SUV liposomes as a function of pH, using an ultrafiltration method to separate free drug from liposomes. Results were consistent with the known pK_a values of the compounds, and the partition coefficient of the unionized species was generally within an order of magnitude to those from octanol/buffer partition coefficients. However, for the charged species of some compounds (especially protonated amines), the partitioning profiles in the lipid/buffer did not agree with octanol/water distribution coefficient data, in that they partitioned into the lipid bilayer while not into the octanol. This is not surprising, since charged moieties would be expected to interact favorably with the headgroups of phospholipids. Similarly, membrane partition coefficients for a series of steroids in egg PC liposomes were measured by determining the solubility in buffer and liposomes by a dialysis technique; the partition coefficient was then determined by calculating the ratio of the two solubility values times the calculated volume ratio of lipid to buffer (Heap et al., 1970). Other factors affect the distribution coefficient as well. For example, lipid/buffer distribution coefficients for a 5-lipoxygenase inhibitor in liposomes, determined by ultrafiltration, were found to be dependent on lipid composition and concentration, with 50% cholesterol increasing in the formulation; the distribution coefficient increased almost twofold (Gupta et al., 1996).

Bilayer fluidity is an important parameter that affects liposome stability, liposome behavior in vivo, and other properties. As discussed earlier, calorimetric methods (e.g., DSC and mDSC) are useful to determine actual phase transition temperatures for the gel to liquid crystalline transition. Alternatively, one can incorporate fluorescent probes into the bilayer that have different fluorescent properties depending on the molecular motion of their environment, and thus are sensitive to whether the bilayer is in the gel or liquid crystalline state. Examples of these probes are dipyrenyl PC (Vaukonen et al., 1990), diphenyl hexatriene (Diederichs et al., 1992), indolyl-labeled PC, and carbazole-labeled PC (Gardam and Silvius, 1990). Motion in the headgroup region can be monitored by dansyl PE (Diederichs et al., 1992). Similarly, NMR probes such as deuterated PC (Davis, 1983), spin label electron paramagnetic resonance (EPR) probes such as nitroxide tagged lipids (Cevc and Seddon, 1993), or FT-IR of 13C-DPPS liposomes (Huber et al., 1994) can be used to obtain the same information. The EPR method is not widely used because of the expense of the equipment, and has been supplanted by the other techniques. The experiments with probes need to be done at several temperatures spanning storage and physiologic temperatures, and the probe concentration must be kept low enough to ensure that the probe itself does not alter the fluidity.

The charge on the liposomal surface is a property that has major effects on the stability, biodistribution, and cellular uptake of liposomes, and is governed by lipid headgroup composition and by pH. It can be monitored by micro electrophoresis (i.e., capillary zone electrophoresis), or by measurement of the zeta potential (Egorova, 1994).

STABILITY OF LIPOSOMES

When evaluating the stability of liposomal formulations, there are a number of factors to consider. The physical characteristics of the liposomes (parameters listed in Table 14.3) can be altered on storage and should be monitored by the appropriate technique as a function of time of storage. In addition, chemical stability of the phospholipid and other components is also an important parameter to assess. Chemical degradation of the lipid can in turn alter the physical properties of the