Care must be taken that the pH change is gradual enough such that partitioning of the drug into the liposome bilayer will occur rather than drug precipitation.

Another important factor is the actual location and orientation of the drug molecule within the lipid bilayer. These would be determined by the molecular size, lipophilicity, and geometry of the drug molecule. Relatively flat, amphiphilic molecules that are roughly the same size and shape as phospholipid molecules would be most favorably incorporated and oriented in parallel with the tail chains of phospholipids, analogously to cholesterol. Hydrogen-bonding and/or electrostatic attractions of the polar portions of drug with the phospholipid headgroups would render this orientation especially favorable. Flat molecules with no polar regions (e.g., aromatic compounds) can be envisioned as being sandwiched between the two tail groups of the liposomal membrane. An example of the former orientation is heme (Tipping et al., 1979; Ginsburg and Demel, 1983; Cannon et al., 1984), while tetraphenyl porphyrin and its analogs exhibit the latter orientation (Tsuschida et al., 1983; Yuasa et al., 1986).

CHARACTERIZATION OF LIPOSOMES

To ensure reproducibility of the performance of drug-loaded liposomes in vivo, one must assess the appropriate physicochemical parameters of the formulation. Some of the key parameters, along with the most commonly used techniques, are listed in Table 14.3.

Liposome size is probably the parameter that has the largest influence on the physical properties and appearance of the formulation. Dynamic and quasielastic light-scattering techniques (e.g., Malvern and Coulter Counter apparatus), including laser light scattering (e.g., Nicomp, Sympatec-Helos), are probably the most commonly used method for measuring size. It has the advantage of quickly giving quantitative results on size distribution. A related technique, forward laser light scatter by flow cytometry (fluorescence activated cell sorter), was used to characterize liposomes prepared using the Microfluidizer. The results correlated well with those of electron microscopy (Childers et al., 1989). Size exclusion chromatography such as molecular sieve chromatography,

TABLE 14.3
List of Parameters and Common Techniques for Characterizing Liposomes

Parameter	Technique
Size	Light-scattering
	Size exclusion/molecular sieve chromatography
	Electron microscopy
	Ultracentrifugation
	Ultrafiltration
Number of lamellae	NMR spectroscopy
	Small-angle X-ray scattering
Encapsulation volume	Encapsulation of water-soluble markers
Encapsulation efficiency	Size exclusion/molecular sieve chromatography
	Ultrafiltration/dialysis
	Ultracentrifugation
Bilayer fluidity	Fluorescent probes
	Spin label EPR
	NMR probes
	Calorimetry
Charge	Microelectrophoresis
	Zeta potential