phosphatidyl ethanolamine (DSPE), and dipalmitoyl phosphatidyl choline (DPPC), respectively (Mason and O'Leary, 1990). $T_m$ can be most easily determined by differential scanning calorimetry and other microcalorimetric methods. However, a variety of other techniques, such as X-ray diffraction, NMR spectroscopy, and fluorescence, have been used to study the transition.

If unsaturation is present in the phospholipid acyl chains, $T_m$ is generally below room temperature, and the transition is somewhat broader. For multicomponent systems, the main phase transition is considerably broadened or essentially abolished. Cholesterol is the most important molecule that moderates lipid bilayer fluidity (both in physiological membranes and in synthetic vesicles) owing to its ability to interact with the phospholipid headgroup and tailgroups by hydrogen-bonding and hydrophobic attractions, respectively. Above 20 mol% cholesterol, a unique and stable phase termed the *liquid-ordered phase* arises, characterized by a high-lateral (translational) diffusion but high conformational order (Mouritsen and Jorgensen, 1993). At higher concentrations, however, cholesterol may perturb the tight packing of the bilayer.

An understanding of phase transitions and fluidity of phospholipid membranes is important in both the manufacture and application of liposomes. The phase behavior of a liposome membrane determines such properties as permeability, fusion, aggregation, and protein binding, all of which can markedly affect the stability of liposomes as well as their behavior in biological systems. In addition, drugs can change the transition temperature. For example, cationic amphiphilic compounds were shown to depress the transition temperatures of dipalmitoyl phosphatidic acid (DPPA) liposomes, apparently because of both headgroup and tailgroup interactions (Haupft and Mohr, 1985; Borchardt et al., 1991). A neutral hydrophobic anticancer drug, teniposide, has been shown to decrease and broaden the main $T_m$ of dimyristoyl phosphatidyl choline (DMPC) and DPPC liposomes in a linear fashion from 1 to 5 mol%. The pretransition was decreased by teniposide concentrations as low as 0.1 mol% and abolished by 1 mol% drug (Wright and White, 1986).

### Conformation of Lipid Chain

In the gel phase, the bulky headgroup of PC occupies a 42 $Å^2$ area of the membrane, while the two straight chain fatty acids occupy a smaller area (~39 $Å^2$). Accordingly, the hydrocarbon chains are thought to tilt relative to the plane of the membrane at an angle of 58°, thereby filling up the extra space created by the headgroups. Chain fluidization and transition to the liquid crystalline phase eliminates some degree of the chain tilt, with the result that there is looser chain packing; consequently fluid bilayers will be thinner than the corresponding ordered ones (Cevc and Seddon, 1993).

### Phase Separation

Normally, lipid bilayer components will be randomly and uniformly distributed, even when nonphospholipid compounds such as cholesterol are introduced into the bilayer to confer desirable properties. Under some conditions, however, the unwanted phenomenon of phase separation will occur, wherein like components will segregate into zones or domains within the bilayer. This can result in bilayer leakage, aggregation of vesicles, and membrane fusion. Thus, care should be taken in devising liposome compositions and keeping the number of components widely divergent in structure at a minimum (Cevc, 1992). Like-charged lipids will generally be electrostatically repelled from one another, yielding a uniform distribution, but the presence of high concentrations of divalent salts may cause clustering of charged lipids and phase separation. Mixtures of shortand long-chain PC liposomes were shown to undergo phase separation in the gel state (Bian and Roberts, 1990).

### Membrane Permeability and Partitioning

Liposome membranes, like biological membranes, are semipermeable. They serve as a very tenuous barrier to molecules with a high solubility in both organic and aqueous media. In contrast, polar