difficult to predict. Preparation method has some effect: sonication (Almog et al., 1991) and other high shear processes (Lang and Vigo-Pelfrey, 1993) have been shown to increase the rate of lipid peroxidation. Contact of formulations with the metal parts of certain size reducing equipment (e.g., the Microfluidizer) may also render them more susceptible to degradation (Lang and Vigo-Pelfrey, 1993). Liposome peroxidation can lead to changes in bilayer fluidity, permeability, and encapsulation efficiency in the same way as hydrolysis; thus, it is necessary to monitor and reduce this problem.

Lipid peroxidation can be monitored by several methods. These include determination of thiobarbituric acid reactive species (i.e., malondialdehyde and other aldehyde products), changes in UV absorbance at 234 nm (arising from formation of conjugated dienes from radical catalyzed rearrangement of, for example, linoleic and linolenic acid), and other methods. Since the various methods monitor the extent of oxidation at different points in the mechanism and may actually monitor transient species, care must be taken to choose the method most indicative of stability of the formulations of interest, and if necessary, two different methods. Lang and Vigo-Pelfrey, (1993) compared several different methods for monitoring peroxidation as a quality control procedure for liposomes, and found that conjugated diene measurement at 234 nm increased continuously and correlated well with changes in fatty acid composition, whereas neither malondialdehyde formation nor lipid hydroperoxide formation (measured by an iodometric assay) showed any such correlation. In any case, such procedures should be considered as screening methods, and final conclusions on excipient stability should be based on assays of individual phospholipids by HPLC or gas chromatography methods. Cholesterol has also been shown to be susceptible to peroxidation (Lang and Vigo-Pelfrey, 1993); thus, formulations containing it should also be assayed for cholesterol as part of a stability protocol. If the formulation is found to be susceptible to peroxidation, antioxidants such as BHA, BHT, or tocopherols can be added to improve stability, or phospholipids containing only saturated phospholipids can be used. Tris buffer has been shown to retard oxidation of PC liposomes due to its ability to scavenge hydroxyl free radicals (Almog et al., 1991).

There is some evidence that the processes of hydrolysis and peroxidation are interrelated. Since the latter process generally has a lag phase, hydrolysis usually begins first. The resulting increase in bilayer fluidity and permeability due to hydrolysis may render the phospholipid tailgroups more exposed to initiators and propagators of lipid peroxidation. Thus, incorporation of 3 mol% LPC into diarachidonyl PC liposomes increased malodialdehyde production by about 25% (Montfort et al., 1987). Similarly, LPC formation has been found to be retarded in liposomes stored under nitrogen, suggesting that peroxidation renders the bilayer more susceptible to hydrolysis (Hernandez-Caselles et al., 1990). SUV liposomes have been found to undergo both hydrolysis and peroxidation more rapidly than MLV structures due to the greater curvature (Lang and Vigo-Pelfrey, 1993). This is in line with the findings that phospholipids in mixed micelles undergo peroxidation faster compared with those in bilayers (Maiorino et al., 1995). Compounds that decrease bilayer fluidity (e.g., cholesterol and to an even greater extent, ergosterol and estradiol) have been found to retard lipid peroxidation (Wiseman et al., 1990). Tocopherol appears to have a dual role in stabilizing liposomes: it serves primarily not only as an antioxidant but also stabilizes the bilayer by specific binding to phospholipid molecules (Hernandez-Caselles et al., 1990). This interaction appears to be between the hydroxyl groups of alpha-tocopherol and the fatty acyl carbonyl of phospholipids (Urano et al., 1990).

In general, liposomes cannot be frozen and thawed to overcome chemical stability problems, since this leads to increases in liposome size and decreases in encapsulation efficiency. A better approach to extend the shelf life of liposomes has been lyophilization, with reconstitution just before use. Early attempts to do this generally led to an unacceptable increase in particle size of liposomes and decrease in encapsulation efficiency, but it has been found that inclusion of a disaccharide (e.g., lactose, sucrose, or, especially, trehalose) in the formulation prevents this (Crommelin and Van Bommel, 1984; Crowe et al., 1987; Crowe and Crowe, 1992). It has been proposed that the mechanism for the stabilization is that the disaccharide can substitute for water in binding to