peroxidation by higher valence state metals, such as Fe^{3+}, via one electron transfers can lead to the formation of lipid radicals [220]. The use of azo compounds is another strategy which has been employed to investigate lipid peroxidation. Azo compounds, often referred to as azo initiators, are molecules which decompose, leading to the generation of radicals. Azo compounds vary in terms of the efficiency with which they generate radicals and their propensity to reside in the lipid bilayer or in aqueous solution [221]. Because of the difficulties that have been encountered when using traditional azo compounds (e.g., 2,2-azobis-(2-methyl-propanimidamide) dihydrochloride, AAPH; 2,2′-azobis (2,4-dimethylvaleronitrile), AMVN) to investigate lipid peroxidation, significant research has been conducted to synthesize and characterize azo compounds that reside and generate radicals in the lipid bilayer efficiently [221, 222]. Although many studies have endeavored to determine the factors that play a role in the oxidative degradation of aqueous liposomal formulations, this chapter focuses on reports which have investigated the effect of lipid saturation, inclusion of cationic or anionic lipids, and the use of antioxidants.

Aqueous Liposomal Formulations: Effect of Lipid Saturation

The effect of the particular lipids incorporated into the liposomes has been examined as well as the effect of the addition of cholesterol. Mowri et al. [223] investigated the peroxidation of liposomes composed of either 1-hexadecanoyl-2-(9Z-octadecenoyl)-sn-glycero-3-phosphocholine (POPC; 16:0, 18:1) or 1-hexadecanoyl-2-(5Z,8Z,11Z,14Z-eicosatetraenoyl)-sn-glycero-3-phosphocholine, PAPC (16:0, 20:4) in the presence of iron (II) and ascorbic acid. POPC was expected to be insensitive to peroxidation. Conversely, PAPC was expected to undergo peroxidation due to the presence of a polyunsaturated fatty acid, arachidonic acid, at the sn-2 position. The thiobarbituric acid-reactive substances (TBARS) assay, which detects secondary products such as aldehydes, was utilized along with gas chromatography (GC) to assess changes in the fatty acid content. Analysis of the PAPC samples showed an increase in peroxidation products with a concomitant decrease in arachidonic acid. In contrast, the formation of peroxidation products was not detected in POPC liposomes, and no detectable change in the fatty acid content was observed [223]. The effect of mixing PAPC with different peroxidation-insensitive lipids, POPC (16:0, 18:1) or 1,2-dioctadecanoyl-sn-glycero-3-phosphocholine (DSPC; 18:0) was also assessed. For the PAPC:POPC formulation (1:9 mol ratio) the concentration of peroxidation products detected was negligible; however, a significant concentration of peroxidation products was detected for the PAPC:DSPC (1:9 mole ratio) formulation. Analysis of the arachidonic acid fatty acid content revealed no detectable change in the arachidonic acid content for the PAPC:POPC (1:9) liposomes; however, for the PAPC:DSPC (1:9) liposomes, a significant decrease in the arachidonic acid was detected. This study was conducted at 37 °C, which is above the liquid crystalline-to-gel transition temperature for both POPC and PAPC. Mowri et al. [223] suggested that POPC and PAPC were homogeneously distributed in the bilay-