Note that this equation may also be used to predict loading of lipophilic active ingredients in a liposome vehicle.

For water-soluble compounds, inactivation can occur if sufficient preservative of interest is entrapped and has such a low bilayer permeability that, after the initial microbial challenge, it is unavailable for subsequent challenges. If such is determined to be true, addition of the preservative should be delayed until liposome formation is completed.

E. Stability

Formulation stability can be subdivided into chemical, physical, and liposome-specific characteristics.

1. Chemical

For chemical stability of phospholipid-based liposomes, lipid peroxidation and fatty acyl–ester hydrolysis are the primary concerns. Phospholipid peroxidation is accelerated by oxygen, transition metal ions, elevated temperature, and light. It is usually controlled by the addition of chelating agents to bind iron salts (27), antioxidants such as α-tocopherol (28), by using phospholipid that has saturated or mono-unsaturated fatty acids, or combinations. Cholesterol, another primary component of liposome formulations, is minimally affected by oxidation in liposomes because the phosphatidylcholines are very effective inhibitors (29).

Fatty acid ester hydrolysis is a time-, temperature-, and pH-dependent process. Hydrolysis results in the formation of lysophosphatidylcholine and free fatty acid. Depending on the active ingredient, changes in liposome size, efflux rate, percutaneous absorption, and in vivo performance may be affected. Frokjer et al. (30) have published a very excellent, systematic study of the chemical, physical, and release rate stability of phosphatidylcholine–cholesterol liposomes. The pseudo–first-order rate constants for phosphatidylcholine hydrolysis at 70°C showed a pH minimum at 6.5. Only 6% hydrolysis of distearylphosphatidylcholine to lysophosphatidylcholine was observed after 300 days at pH 6.5 and 25°C. Thus, ester hydrolysis can be minimized by formulating in the vicinity of pH 6.5.

2. Physical

Changes in the bulk physical properties of a liposome preparation usually reflect changes in vesicle aggregation or increasing liposome size.

Gross aggregation of liposomes can be observed macroscopically as "creaming" or flocculation of the originally homogeneous disper-