CHAPTER V

CONCLUSION

The transmembrane pH gradients process was versatile technique to encapsulate drugs in liposomes. Several features of this process were practicularly desirable for pharmaceutical applications. First, the ability to entrap approximately 100 % of the drug in preformed liposomes enabled the formulation to be constituted just prior to use. Second, the pH gradient imparted excellent drug retention properties to the liposomal systems subsequent to drug uptake. Third, drug/lipid ratios far greater than those previouly obtainable could be achieved, thus reducing production costs associated with lipid components.

The use of transmembrane pH gradients to encapsulate certain amphipathic drugs resolved many problem associated with passive trapping techniques. The importance of parameters such as ΔpH (pH_{in} and pH_{out}), entrapped buffering capacity, drug to lipid ratio and lipid composition were involved the uptake process. Lipophilic drug that contain titratable, amine moieties was permeable bilayer membranes in the neutral (uncharged) form faster than in the positive charged form. The mechanism of drug uptake induced by pH gradients was probably similar to the ΔpH-dependent transmembrane redistribution of other weak bases, where the unprotonated (neutral) species diffused across the membrane and accumulated in the vesicle interior until $[AH^+]_i/[AH^+]_o = [H^+]_i/[H^+]_o$ according to classical Henderson-Hasselbach relationships, where AH^+ indicated the protonated form of drug. The neutral species of lipophilic, amine drugs (propranolol) could equilibrate

across membranes; hence, drug molecules on both side of the membrane would be in equilibrium between protonated and neutral forms on the basis of the pK_a of the drug and the pH of the bulk media. The protons were consumed as the neutral drug species equilibrated with the charged form upon exposure to the acidic vesicle interior, resulting in a net depletion of the entrapped proton pool. Failure to ensure a sufficient intravesicular buffering capacity could, therefore, lead to a collapse of the transmembrane pH gradient and a preparation with inferior encapsulation and drug retention properties.

Alterations in the lipid composition of liposomes using in pH gradient-mediated drug encapsulation might be made on the basis of biological response requirements or pharmaceutical issues such as chemical and physical stability. These changes often involved cholesterol content or phospholipid acyl chains that affected the rates of drug uptake and drug retention. The general trends could be identified from these research. First, increasing the acyl chain length of phospholipid component (which increased membrane rigidity depended on experimental temperature) increased the drug amount of entrapped. Second, increased drug-to-lipid ratios and trapping efficiencies were observed for higher interior buffering capacities. Third, inclusion of negatively charged amphiphiles in the membrane resulted in increased entrapped propranolol/lipid ratio; however, more rapid released of entrapped drug was also noticed, comparing with EPC:cholesterol liposomes (2:1, in mole). Fourth, incorporating cholesterol and hydroxyl cholesterol analogues increased the ability of the liposomes to retain entrapped propranolol in some period of times. Since release rate of entrapped drug was very slow at low temperature; thus, liposome preparation should be kept at low temperature prior to use.

This pH gradient-dependent encapsulation technique was extremely versatile, and well characterized liposomal drug preparations could be

generated to exhibit a wide range of properties such as vesicle size, lipid composition, drug-to-lipid ratio and drug release kinetics. The manipulations of the physical characteristics of liposome, prepared by pH gradient method would likely lead to formations with improved therapeutic activity.