CHAPTER V

CONCLUSIONS

In this study, Caco-2 cells were characterized and used as a model to study the feasibility of using liposomes to enhance delivery of hydrophilic substances and P-glycoprotein (P-gp) substrates into and through epithelial cells. Calcein was used as a model hydrophilic substance and rhodamine 123 as a model of P-gp substrates.

Caco-2 cells cultivated under the conditions described in this study gave tight monolayers that were appropriate for the purposes of the study. The uptake studies show that accumulation of calcein in Caco-2 cells was higher from liposomes than from solution in all cases. Accumulation of calcein in Caco-2 cells from liposomes was influenced by the concentration of liposomes. Calcein uptake as a function of calcein concentration was linear with solution, whereas the saturation profile was seen with liposomes. This result is consistent with the proposed endocytotic mechanism of liposome uptake by Caco-2 cells. Flow cytometry was successfully used to support this finding. Positively charged and neutral liposomes were taken up by Caco-2 cells more efficiently than negatively charged liposomes. However, the transport studies show that though liposomes could increase the uptake of hydrophilic materials into Caco-2 cells, transcytosis did not occur efficiently enough to be of any use in enhancing transport of hydrophilic substances across the cells.

On the other hand, when rhodamine 123 was used as a model for P-gp substrates, the results of the study suggest liposomes could enhance both the cellular uptake of the fluorescent dye and the transport of the dye across Caco-2 monolayers, most likely by bypassing the activity of P-glycoprotein. Soybean phosphatidylcholine, the structural lipid used in this study, did not interfere with rhodamine 123 interaction with P-gp on the apical membrane of Caco-2 cells. On the contrary, blank liposomes might interfere with the interaction between verapamil, a specific P-gp inhibitor, and P-gp since the inhibitory effect of verapamil on P-gp was less than expected in the presence of blank liposomes. The uptake of rhodamine 123-loaded neutral liposomes into Caco-2 cells was significantly higher than the negatively charged and positively charged liposomes.

Rhodamine 123 uptake from the latter two types of liposomes was significantly less than that from the solution. In addition, comparison of the apparent permeability coefficients of rhodamine 123 from liposomes and from solution demonstrated that neutral liposomes could facilitate transport of rhodamine 123 across Caco-2 monolayers.

Thus, the overall results indicate that liposomes should be useful in enhancing cellular uptake of both hydrophilic substances and P-gp substrates and in facilitating transport of P-gp substrates, which are usually hydrophobic molecules, across Caco-2 monolayers. Physicochemical properties of liposomes such as surface charge as well as the properties of the substances contained in liposomes affected the efficiency of cellular uptake by Caco-2 cells. However, other cell types as well as other formulation factors, such as type of phospholipid and other additives, should be further explored to gain more insights in liposome-cell interaction in order to develop proper formulations to enhance delivery of such substances.