CHAPTER VI

CONCLUSION

The fruit pulp of tamarind contains naturally occurring AHAs especially tartaric acid. In this study, tamarind fruit pulp's crude extract, containing tartaric acid in an amount of 20-30%w/w was obtained from lyophilization process. Since the high potential to lead the irritation occurs on skin and its hydrophilic property causes the difficulty to penetrate to skin by lipophilic pathway. Liposome is interesting system to deliver the tamarind fruit pulp's AHAs to skin.

Reverse phase evaporation method was used to prepare liposomes. This method are wildly use for application which require high encapsulation of water soluble agent.

The optimum process for liposomes preparation developed from this study were by dissolved 2:1 lipoid®/cholesterol molar ratio in 1:1 v/v chloroform/diethyl ether, adding tamarind's extract solution which had 2% w/v of tartaric acid and sonicating for 7-20 minutes to form W/O emulsion. This emulsion was then evaporated for 1 hr to get rid of organic solvent and receive a gel film form then shake for 1 minute to obtained liposomes suspension. The optical micrograph indicated the well-form bilayer vesicle to improve the successful of this preparation method.

The effect of O:W ratio on entrapment efficiency of liposomes was studied. The higher entrapment of tartaric acid in liposomes (41.39%) was achieved by mixing 5:1 volume oil/water ratio. Furthermore, the effect of lipoid® and cholesterol molar ratio on entrapment efficiency of liposomes was also investigated, 51.13% entrapment was obtained when using 2:1 lipoid/cholesterol molar ratio. These liposomes vesicle had size in range of 300-500 nm. After extrusion by an extruder with pore size of 100 nm, the narrow size of liposomes about 100-200 nm was obtained.

Moreover, the developed system was modified to improve the strenght of liposomes by coating liposomes with chitosan. The method was performed by adding

liposomes suspension into chitosan solution pH 5 while stirring. The obtained results from TEM micrograph indicated the clear different appearance of uncoated and coated liposomes. We studied on 3 parameters that might affected the coating properties of chitosan, including source of chitosan, concentration and amount of chitosan solution. The results obtained showed that coating liposomes with chitosan did not affect the encapsulation efficiency of liposomes but increasing liposomes sizes from 100-200 nm to 200-300 nm. Besides, source of chitosan did not influence on the coating efficiency of chitosan. However, the important parameter tends to be chitosan concentration. Zeta potential of vesicle and coating efficiency also increased when the concentration of chitosan was increased from 0.1 to 1% w/v.

The release profile of the developed system was well fit to Higuchi's model. The result from in vitro release study indicated that, the tartaric acid released from liposomes was prolonged. Moreover, the higher amount of tartaric acid remained in chitosan coated liposomes (54.67%), comparing to that remained in liposomes (29.04%), after *in vitro* study could be assumed that, coating liposomes with chitosan increasing the rigidity of liposomes's shell; that improves the stability of developed system even in storage or topical application.

In vitro model of human skin cells used in this study were keratinocyte and melanocyte cells. The effects of tamarind fruit pulp's extract in free form and in loaded system were investigated on HaCaT human keratinocyte proliferation and melanogenesis inhibitory of MML-1 human melanocyte.

Although some publishes reported the potential of lactic and glycolic acid on keartinocyte proliferation, no publish has reported the potential of tamarind's AHAs on keratinocyte proliferation. The results obtained in this study clearly indicated the potential of tamarind fruit pulp's AHAs on the kearatinocyte proliferation, the highest increased 2-fold of cell viability comparing as control obtained from cells treated with 1000 ug tartaric acid loaded in liposomes formulation. Since the significantly different results obtained from cell treated with the crude extract. We suggest that the acidity and hydrophilic molecule of tamarind's AHAs caused the difficulty to be uptake into cell. In contrast, the

amphiphatic molecule and the main compound in liposomes, phospholipids, can promote the ability to uptake the active compound into cells.

There has been evidence that, AHAs can suppress melanin formation by directly inhibiting tyrosinase activity and accelerating epidermal turnover which improves the appearance of hyperpigmentation. Such evidence confirmed in this study. Moreover, AHAs might also directly inhibiting melanin formation in melanocyte.

In this study, the result of melanogenesis inhibitory was not observed, both in the extract and positive control, kojic acid, a well known whitening agent. We assumed that the *in vitro* cell culture model, at least partially, affect on the clarification of the obtained results. Thus, the hypothesized that tamarind's AHAs do affect human pigment production should be still accounted into consideration and clarified by another model.