CHAPTER 4

CONCLUSIONS

This study has demonstrated the following summarized results:

4.1 The preparation of cationic lipids

4.1.1 The CTA can be synthesized from cholesterol and betaine hydrochloride by the DCC/DMAP coupling method with Steglich esterification, and can be purified by column chromatography.

4.1.2 The appearance of the CTA was white powder with the melting point of CTA was in the range of 138-140 ^{o} C and the percentage yield of the CTA was approximately 32.22%.

4.1.3 CTA was identified by IR, ^{1}H NMR and LC-MS, and the percentage of content of the purified CTA analyzed by HPLC was 91.30 %.

4.1.4 CTA seemed to be stable when exposed to light and high temperature.

4.1.5 A purified cationic lipid can not be obtained from the reaction of solasodine and betaine hydrochloride by the DCC/DMAP coupling method with Steglich esterification.

4.1.6 The cationic lipid, cholestranol(3β) (trimethylammonio)acetate (HCTA) was synthesized from cholestranol and betaine hydrochloride by the DCC/DMAP coupling method with Steglich esterification and purified by column chromatography, with the percentage yield 15.05 %, the melting point in the range of 128-132 ^{0}C and in white powder.

4.1.7 The HCTA was identified by TLC, melting point, IR and NMR, but it was not selected to prepare liposome formulation because the percentage yield was less than CTA.

4.2 Preparations of liposomes from the synthesized cationic lipid (CTA)

4.2.1 Seven liposomal formulations prepared by a chloroform film method with sonication. But only five liposomal formulations prepared from DPPC/Chol at 7:3, DPPC/Chol/ DDAB at 7:2:1, DPPC/Chol/ CTA at 7:2:1, DPPC/CTA/DDAB at 7:2:1, and DPPC/ CTA at 7:3 molar ratios by chloroform film method with sonication gave no sedimentation or layer separation.

4.2.2 For the five blank liposomes, the morphology indicated spherical shape investigated by the optical microscopic and TEM. The vasicular sizes of the determined by dynamic light scattering were in the range of 137-242 nm and the zeta potential values determined by dynamic light scattering were in the range of (-)18.9- (+)75.7 mV with the liposomes contained no cholesterol gave higher value (more positive) than those with cholesterol.

4.2.3 The blank liposomal formulations of DPPC/Chol/DDAB at 7:2:1 and DPPC/CTA/DDAB at 7:2:1 molar ratio showed better physical stability with no sedimentation for 3 months than other formulations.

4.3 Entrapment of insulin in the selected CTA liposomal formulation

4.3.1 The four selected liposomes composed of DPPC/Chol at 7:3, DPPC/Chol/DDAB at 7:2:1, DPPC/Chol/CTA at 7:2:1 and

DPPC/CTA/DDAB at 7:2:1 molar ratios prepared by freeze dried empty liposomes (FDEL) method were in white powder.

4.3.2 Percentages of the entrapment efficiency of human insulin in the liposomal formulations DPPC/Chol at 7:3, DPPC/Chol/ DDAB at 7:2:1, DPPC/Chol/CTA at 7:2:1, and DPPC/CTA/DDAB at 7:2:1 molar ratio were at 44.68%, 59.16%, 55.41% and 62.72%, respectively.

4.3.3 The morphology of all four liposomal formulations ehtrapped with human insulin showed the existhing of the vesicles by the optical microscopic with the vesicular size of DPPC/Chol (at 7:3 molar ratio), DPPC/Chol/ DDAB (at 7:2:1 molar ratio), DPPC/Chol/CTA (at 7:2:1molar ratio), and DPPC/CTA/DDAB (at 7:2:1 molar ratio) at 1.03$\pm$0.43 μm, 1.69$\pm$0.11 μm, 1.40$\pm$0.19 μm and 2.26$\pm$0.87 μm, respectively.

4.3.4 The zeta potential values of human insulin in the liposomal formulations DPPC/Chol (at 7:3 molar ratio), DPPC/Chol/ DDAB (at 7:2:1 molar ratio), DPPC/Chol/CTA (at 7:2:1molar ratio), and DPPC/CTA/DDAB (at 7:2:1 molar ratio) were at -8.69$\pm$1.19 mV, 41.8$\pm$1.93 mV, -6.09$\pm$0.49 mV and 47.7$\pm$1.44 mV, respectively.

4.3.5 The best blank liposomal formulation which was selected to entrap human insulin was DPPC/CTA/DDAB at 7:2:1 molar ratio because it exhibited more physical stable during 1 month than other formulation. The percentages entrapment of human insulin in this formulation was 62.72 %. The TEM of this formulation demonstrate large unilamellar shape of the vesicle. The percentage remaining of human insulin entrapped in this

liposome was higher than the unentrapped human insulin stored at 4^0C, room temperature (30 $\pm$2 ^{0}C) and 45 ^{0}C for 4 months.

In summary, CTA was synthesized from cholesterol and trimethylglycine in dichloromethane. The percentage yield of the product was 32.22 %. The best formulation composed of DPPC/CTA/DDAB at 7:2:1 molar ratio exhibited the percentages of entrapment efficiency of human insulin at 69.72 % with an average size of 2.26 μm. No sedimentation or layer separation was observed in this liposomal formulation which gave an average zeta potential of 47.7 mV. The results from this study can be applied for the further development of gene therapy and anionic drug delivery.