CHAPTER V

CONCLUSIONS

The influence of surfactant, DDAB and cholesterol concentrations on size, PI and zeta potential of SLN could be described by the quadratic model equations and their surface responses. The statistically significant interaction terms in the model equations suggested the main effect of these factors was involved by each other. It was found that within the experiment region, the increment of surfactant content in SLN tended to increase size, PI and reduce zeta potential. In addition, size, PI and zeta potential considerably increased with increment of DDAB content. The addition of cholesterol in SLN tended to led to size and zeta potential reduction but increased PI. For CSN, the increased chitosan:tripolyphosphate ratio led to the increment in particle size, PI and zeta potential. However, at very low chitosan:tripolyphosphate ratio, aggregation of the neutral complexes occurred causing larger particle size and PI of CSN.

The ability of SLN and CSN to form complex with pHIS-HIV-hugag was not only affected by an electrical force but also the interaction between surface of nanoparticles and pHIS-HIV-hugag. The SLN containing medium content of surfactant (5%), DDAB (0.64%) and with or without cholesterol could immobilize the pHIS-HIV-hugag under a supplied electric field at lower SLN:pDNA ratio of 1,000:1. The CSN containing high chitosan content could immobilize pHIS-HIV-hugag at much lower ratio.

The *in vitro* cytotoxicity test showed that HeLa cells tolerated in a range of 100 to 200 μg/ml of SLN in DMEM without FBS while they could survive with 100% cell viability in varying concentrations of CSN in DMEM without FBS, 100-10,000 μg/ml.

The results of transfection study at the concentration of 200 μg/ml of nanoparticles showed the potential of SLN and CSN for using as an *in vitro* pHIS-HIV-hugag transfection vector, particulary the SLN containing high content of cholesterol (0.68%) with medium content of surfactant (5%) and DDAB (0.64%). This finding suggested that the optimum formulation could promote the transfection efficiency of pHIS-HIV-hugag in HeLa cells.

The SLN-pHIS-HIV-hugag complexes elicited more gag protein-specific IgG titers in mice immunized by either intradermal injection or topical application than that of naked pHIS-HIV-hugag. The CSN-pHIS-HIV-hugag could elicit more gag protein-specific IgG titer by

intradermal injection than naked pHIS-HIV-hugag but topical CSN-pHIS-HIV-hugag complexes elicited statistically comparable immune response to topical naked pHIS-HIV-hugag immunization. The skin penetration study using confocal microscope showed that the skin penetration pathways of nanoparticles-pHIS-HIV-hugag complexes were skin furrows and pilosebaceous unit.

However, the ability of obtained nanoparticles for using as an *in vitro* and an *in vivo* transfection vector was lower than the established transfection reagent and also the DOPE-SLN, a positive control for mice immunization. Thus, improvement in SLN formulation such as using powerful helper lipid, low toxic cationic lipid possessing high potential for transfection and coating cell specific ligand on nanoparticle surface; and using modified chitosan polymer for CSN should be further investigated.