# CHAPTER V

# CONCLUSIONS

The chitosan-pDNA nanoparticles were prepared by using a complex coacervation method. Three variables were selected; medium of chitosan, medium of pDNA and N/P ratio, to study the influence of these variables on physical properties and transfection efficiency of the nanoparticles. The particle size of the nanoparticles sharply decreased with increasing N/P ratio and stable to constant value of 102-278 nm with PI in the range of 0.20-0.51. The particle size of CSA nanoparticles became stable at N/P ratio lower than the others because acetic acid had the lowest hindrance effect compared to lactic acid and glycolic acid. Complex coacervation method was a method of spontaneous phase separation and the coacervation agent was used to increase the phase separation. Therefore, the nanoparticles formulated with pDNA in water (DNA W nanoparticles), did not use coacervating agent, and had the smallest particle size. The nanoparticles formulated with pDNA in sodium sulfate (DNA S nanoparticles) had particle size larger than the nanoparticles formulated with pDNA in sodium chloride (DNA C nanoparticles) because sodium sulfate has two sodium ions, whereas sodium chloride has only one sodium ion. Moreover, sulfate ion from sodium sulfate has hydrating power more than chloride ion from sodium chloride.

The zeta potential of the chitosan-pDNA nanoparticles increased from negative charge to positive charge with increasing N/P ratio and finally increased to constant value in the range of +7.70 to +33.53 mV. The particle size of CSA nanoparticles became stable at N/P ratio lower than the others with the same reason of the particle size. The surface charge of the DNA S nanoparticles was remarkably low compared to the others because two valences of sodium sulfate has potency to shielded the positive surface charge of the nanoparticles more than the others.

TEM images of the nanoparticles formulated with chitosan in acetic acid and pDNA in sodium sulfate at N/P ratio of 5:1 (5:1 CSA/DNA S nanoparticles), 2:1 CSA/DNA S, 2:1 CSA/DNA C and 2:1 CSA/DNA W nanoparticles, as representatives of all formulations showed

that most nanoparticles had spherical shape, while a few particles had rod shape. Aggregation of a few particles was observed in all represented formulations.

The electrophoretic mobility of 0.5:1, 1:1, 2:1, 3:1, 5:1 and 7:1 CSA/DNA S nanoparticles showed that the formation of nanoparticles was occurred at all N/P ratio.

The nanoparticles formulated at N/P ratio of 2:1 and 3:1, 5:1 and 7:1 CSA/DNA S nanoparticles were selected to study the protection effect, the *in vitro* transfection efficiency and the cell viability. The electrophoretic mobility showed that all selected formulations could render protection to pDNA.

The *in vitro* transfection efficiency study indicated that 5:1 CSA/DNA S nanoparticles exhibited the highest transfection efficiency and had some signal of fluorescence under fluorescence from HeLa cells, whereas these of other formulations were very low. The transfection efficiency of all selected formulation was higher than that of naked pDNA. The DNA W nanoparticles exhibited the most transfection efficiency followed by that of DNA C nanoparticles and DNA S nanoparticles, respectively. This was attributed to the anion from medium of pDNA hindered the attachment of the nanoparticles to HeLa cells. It seemed that the transfection efficiency had a tendency to increase with the increasing N/P ratio. Increasing N/P ratio was followed by the increase of chitosan concentration in the nanoparticles. Higher amounts of chitosan in the nanoparticles led to a higher osmotic pressure in the endosome and induced endosomal escape of the nanoparticles.

Cell viability study demonstrated chitosan in the concentration range which covered the concentration of chitosan used in the transfection efficiency study, media of selected nanoparticles and the nanoparticles of selected formulation did not affect the viability of HeLa cells. In contrast with the positive control which was toxic to HeLa cells.

This study suggested that chitosan could act as a vector of efficient and safe gene delivery. All experimental variables used in this study; medium of chitosan, medium of pDNA and N/P ratio, had an influence on physical properties and transfection efficiency of chitosan-

pDNA nanoparticles. To improve the transfection efficiency of chitosan-pDNA nanoparticles, other formulation variables such as pH of final product, should be further investigated concomitant with the synthesis of novel chitosan derivatives.