CHAPTER VI

CONCLUSIONS

In this study, the potential application of chitosan microparticles as an efficient protein and vaccine delivery system was explored. The microparticles made of different molecular weight and sources of chitosan were prepared by spray drying technique. Size of the microparticles obtained was ranged between 3.760-7.096 μm. with positive surface charge. Either increasing molecular weight or solution concentration of chitosan resulted in larger microparticles, while spray drying at higher temperature and/or slow spray rate yielded smaller particles.

Bovine serum albumin, as a model protein, was subsequently incorporated into chitosan microparticles. When the protein was incorporated, the microparticles became slightly larger than the corresponding blank microparticles, with a little reduced zeta potential. Integrity and structural conformation of entrapped protein could be retained, when the protein was loaded at 5% w/w or higher, while they were totally lost, if the protein was loaded at 1% w/w.

The protein-loaded microparticles were modified by co-spray drying with the acceptable pharmaceutical excipients, *i.e.* gelatin, poloxamer 407, and Eudragit E, and/or crosslinking with tripolyphosphate anions. While co-spray drying with the excipients did not affect size of the particles significantly, it affected the surface properties in various ways, depending on the properties of excipient. Due to cationic nature, gelatin obviously imparted higher zeta potential to the particles. In contrast, incorporation of poloxamer 407 and Eudragit E did not influence the particle surface charge apparently. The accumulation of protein at the particle surface was disturbed by addition of the excipients. Particularly, when poloxamer 407 was included, the deposition of protein at the microparticle surface totally disappeared. The incorporated excipients mostly induced faster and/or higher amount of released protein. Ionic crosslinking of the particles caused an obvious reduction of drug release.

Poly(lactic-co-glycolic acid) and poly(α-butyl cyanoacrylate) micro-/nanoparticles were compared with chitosan microparticles. The resultant micro-/nanoparticles were much smaller than the chitosan microparticles. The poly(lactic-co-glycolic acid) microparticles were relatively neutral, while the poly(α-butyl cyanoacrylate) nanoparticles exposed either positive or negative surface charge, depending on the preparation condition.

All micro-/nanoparticles were subjected to cytotoxicity test and cellular uptake study with dendritic cells and macrophages. The chitosan, gelatin/chitosan, poloxamer 407/chitosan and poly(lactic-co-glycolic acid) microparticles were relatively non-toxic to both cells, while the Eudragit E/chitosan and poly(α-butyl cyanoacrylate) micro-/nanoparticles were quite toxic. The chitosan and chitosan composite microparticles were efficiently taken up by both cells, whereas the poly(lactic-co-glycolic acid) and poly(butyl cyanoacrylate) micro-/nanoparticles were less efficiently endocytosed. It was noticed that both types of cells owned different particle uptake behaviors.

The Japanese Encephalitis antigen was finally incorporated into the selected microparticles and administered subcutaneously in mice, compared with the commercial vaccine. The chitosan of both low and high molecular weight and the gelatin/chitosan microparticles elicited a comparable immune response to the commercial preparation. However, at week 12 after the first immunization, the serum IgG titer, induced by the low molecular weight chitosan particles, was slightly increasing, while that of the others started to decline. While the commercial vaccine constantly induced both T_H1- and T_H2-type immune response, the microparticulate formulations tended to induce predominantly the T_H2-type response. Unfortunately, a specific conclusion about the relationship between the *in vitro* interaction of the particles with the antigen presenting cells and the *in vivo* immune response could not be drawn in this investigation.

Conclusively, it was evident that the chitosan microparticles of low molecular weight presented a potential application as an efficient vaccine delivery system. However, some adjustments such as incorporation of other vaccine adjuvants might be needed in order to achieve stronger immune response.