

CHAPTER V

CONCLUSIONS AND RECOMMENDATIONS

Water-soluble chitosan nanosphere was successfully prepared by functionalizing with mPEG and CA in water-ethanol system. When chitosan was conjugated with CA in the first step, the intermediate compound was difficult to dissolve in water and this obstructed the following step of introducing mPEG onto chitosan chains. There, one pot reaction which the introduction of both CA and mPEG was carried out in a single step was found to be successful. The molecular weight of chitosan and of mPEG and the mole ratio of chitosan:mPEG:CA were varied to observe the water solubility and degree of substitution of the product. Chitosan with Mw 48000 Dalton and 1:0.5:0.5 mole ratio of chitosan:mPEG (Mw 5000):CA were optimal conditions to achieve water solubility with the degree of substitution 31.81 and 21.21%, of mPEG and of CA respectively, as quantified by ¹H NMR. The derivative shows nanospherical shape with the size of 40 nm. After the incorporation of a model allergen, the house dust mite, the nanospherical was found to be ~200 nm, as observed by TEM. The dynamic light scattering indicated the size of the sample in the aqueous solution before and after allergen incorporation were 92 and 196 nm, respectively. The *in vitro* immune response test, preliminarily investigated by LTT, showed that CS-mPEG-CA incorporated with allergen tends to down regulate the cell response to allergen when compared to CS-mPEG-CA and allergen alone. The recommendations for the related future work are, (i) to clarify how the dust mite is incorporated in the nanosphere, (ii) to identify the quality of subcutaneous permeation of the product, and (iii) to investigate the *in vitro* immune response including the cytokine response to show the potential use as adjuvant.