CHAPTER III
AMPHIPHILIC pH-RESPONSIVE CHITOSAN NANOSPHERES

3.1 Abstract

By conjugating hydrophobic phthalic anhydride and hydrophilic α-carboxylpropyl-ω-methoxy polyethylene glycol (mPEG-COOH) onto chitosan, pH-responsive chitosan nanospheres can be obtained. The size and surface charge of nanospheres can be controlled by simply varying the pH of the solution. The pH-sensitive specific property comes from a certain amount of O-phthloyl group at hydroxyl group when the phthaloylation of chitosan was carried out in DMF condition.

Keywords: pH-responsive, chitosan, nanospheres

3.2 Introduction

Chitosan is an amino-polysaccharide of β-(1-4)-linked 2-amino-2-deoxy-D-glucose which satisfies the conditions for the use in biomaterials, especially in foods, health-care products, and pharmaceuticals due to its specific properties, i.e. biocompatibility, biodegradability, non-toxicity. Preparation of chitosan nanoparticles have been variously reported.[1] Recently, self-assembly of polymeric nanoparticles which responds external signals in terms of the changes in phase, shape, and structure has been an issue in intelligent nanohybrid materials.[2] Lee *et al.* demonstrated that specific interactions, i.e. hydrogen bonding and ionic interactions, allowed a reversible polymer/micelle nanohybrid formation under pH variable as seen in the cases of pyrene-labeled poly(ε-carpolactone)-b-poly(carboxylic acid) copolymers assembled with polyethylene glycol, poly(2-ethyl-2-oxazoline) copolymers, and poly(1-vinylpyrrollidone).[3] This reversible phenomenon depended on the shell part of the carboxylic protons which form hydrogen bonds with proton-accepting polymers.

Previously, our group succeeded in grafting of mPEG-COOH on phthaloylchitosan in DMF system.[4,5] This compound shows an inevitable white turbidity solution during dialysis against water. The systematical studies led us to the

answer that the chitosan nanospheres were in amphiphilic structure and the optimal condition gave phthalimido group substitution at amino group for ~50% as hydrophobic core and mPEG substitution at hydroxyl group for 20% as hydrophilic corona.[6]

3.3 Results and discussion

The phthaloylation was carried out in DMF, of which Kurita *et al.*[7] reported that the system initiated not only N-phthaloylation but also O-phthaloylation. This could be confirmed by FTIR spectrum at 2640 cm^{-1} for free carboxyl group belonging to O-phthaloylation. In addition, XRD pattern of phthaloylchitosan showed the broad peak from 13° to 26° 2θ which was different from that of starting chitosan with the peaks at 10°, 20°, and 22° 2θ. This is relevant to the report by Kurita *et al.*[7] showing the partial O-substitution and the bulkiness of the phthaloyl group led to the amorphous structure.

Based on the structure mentioned above, the nanospheres exhibited their sizes in the range of ~80 and ~500 nm depending on the degree of deacetylation, degree of phthaloylation, and the chain length of mPEG.[4] However, it is important to note that by simply changing the pH, the chitosan nanospheres solution perform significantly different turbidity.

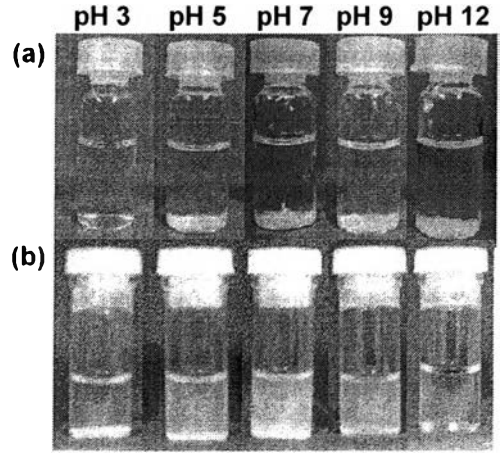


Figure 3.1 Appearances of: (a) chitosan, and (b) chitosan nanospheres in various pHs.

Chitosan is known to dissolve in acid and precipitate in basic solution as shown in Figure 3.1(a). However, our chitosan nanospheres give colloidal solution and become clear in basic condition as shown in Figure 3.1(b). In addition, we also found that the nanospheres showed the negative surface charge in water about -54 mV, which is quite high negative value. To clarify the colloidal solution and the significant negative surface charge, the chitosan nanospheres were dispersed in various buffers; citrate buffer (pH 2–6), tris buffer (pH 7–9), and KCl/ NaOH (pH 12). As mentioned above, the appearances of the solutions are totally different from those of the starting chitosan (Figure 3.1). For example, at pH 3, our chitosan nanospheres precipitate out of the solution but the starting chitosan is dissolved to give a clear solution. As the pH of the solution increased, the nanospheres maintain their dispersibility. The solution becomes more transparent as the pH increased. At pH 12, the solution is transparent which is in contrast to that of chitosan where precipitation occurs.

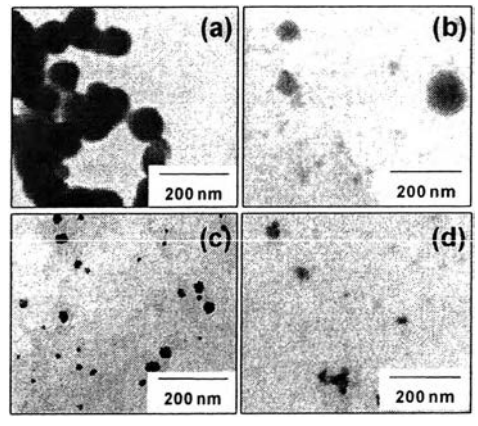


Figure 3.2 TEM micrographs of nanospheres in pH; (a) 3, (b) 5, (c) 7, and (d) 12.

As the solutions changed from turbid to transparent, this brought the question about the relationship between the turbidity and the nanosphere size. The solution was left overnight before taking the particles out to observe by TEM. Figure 3.2(a) shows the nanospheres with some aggregation in acidic buffer. It is clear that the spherical shape is maintained. The mean individual particle diameter obtained from acidic solution (pH 3) is about 300–400 nm. At pH 5, the solution is homogeneously

turbid and the spheres taken from the solution give the mean diameter about 200–300 nm. At pH 7, the nanosphere diameters are about 40–50 nm (Figure 3.2(c)). When the pH was increased to 12, the size of the nanospheres significantly changes to be as small as 20–30 nm. This level of nanosphere size supports the transparent appearance in Figure 3.1(b) at pH 12. By simply varying pH, the solution appearances as well as chitosan sizes changed drastically. This implied an effective pH-controlled self-assembly of amphiphilic chitosan.

Sun *et al.*[8] reported a self-assemble poly(ethylene glycol)-poly(L-lactide)-poly-(L-glutamic acid) which has a pendant carboxyl group on each LGA unit. These micelles changed to rod-like structure when the pH changed from 3.9 to 3.2. The morphological change was based on the acidic form promoting adhesive collision of micelles. In our case, the pH sensitive group might also be the carboxyl groups which were obtained from O-phthaloylation. In acid conditions, the carboxyl group formed hydrogen bond resulting in precipitation out of the solution. As the pH was increased to basic condition, the carboxyl group tended to be in anionic form resulting in a repulsive effect among chitosan chains.[9] This brought the decrease in nanosphere size.

Another question to us is about the surface charge which should also be involved with the nanosphere size and turbidity. Yu *et al.* reported the surface charge of chitosan at pH 3 about ~+60 mV.[10] The charge gradually decreased with an increase of pH until the net charge was zero at pH 8. Then, the surface charge of chitosan was -5 mV at pH 12. In our case, the surface charges of the nanospheres in various pHs were evaluated by a Zetasizer Nano ZS (Figure 3.3(a)). At pH 2, the surface charge of the nanospheres is +20 mV and changes to -6 mV at pH 3. The negative charge is much more significant as pH increased and reaches -27 mV at pH 8, and becomes -38 mV at pH 12. The changing of surface charge supports the existence of carboxyl group as anionic corona.

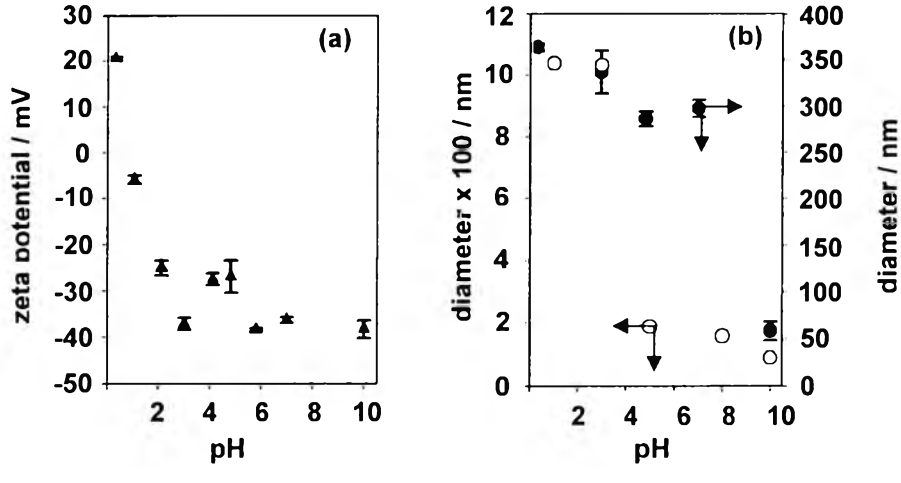
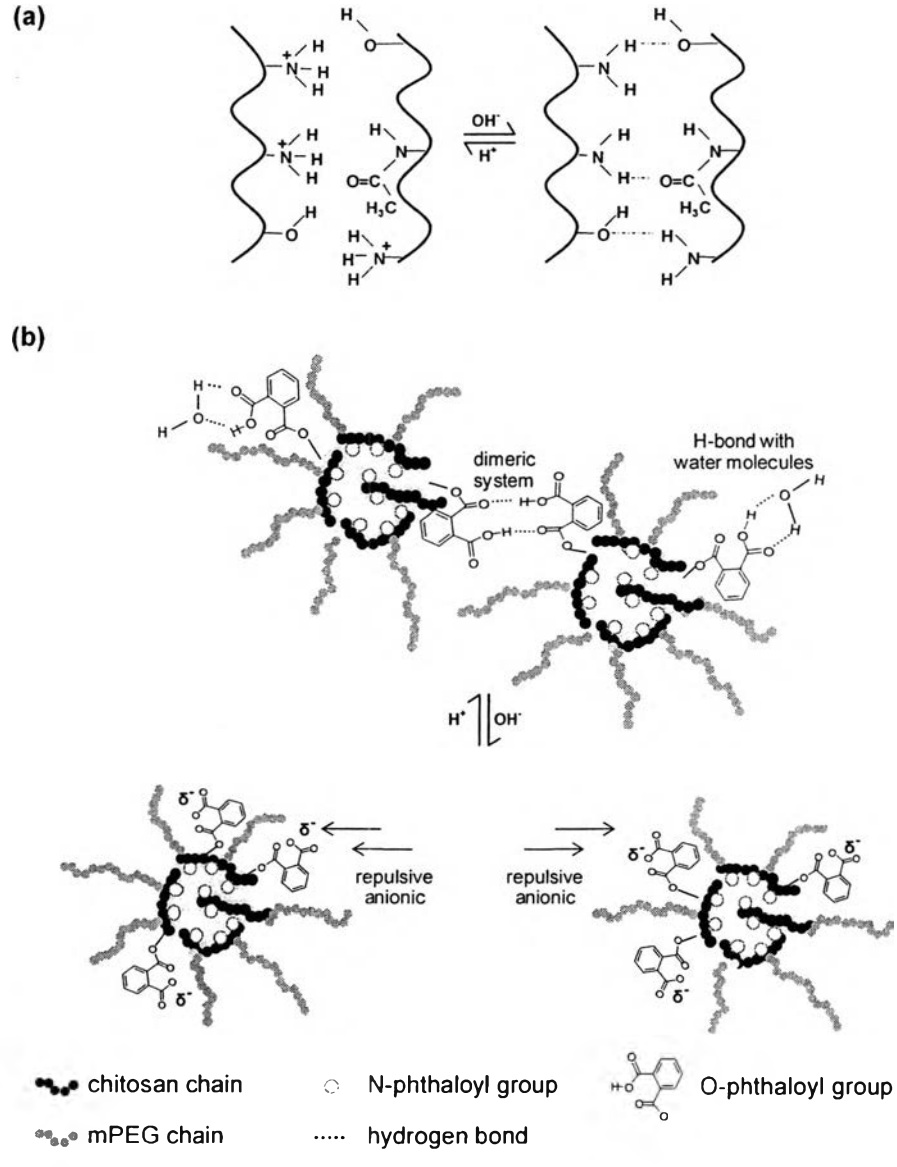


Figure 3.3 (a) Zeta potential, and (b) diameter of nanospheres measuring by DLS (●) and by TEM micrographs (○) as a function of pH.

The nanospheres were also determined by dynamic light scattering (DLS) to obtain the information about the nanospheres in solution state. Figure 3.3(b) shows a decrease in nanosphere size with an increase in pH which is similar to the results from TEM. However, it is important to note that the sizes observed by DLS are larger than the ones by TEM implying the aggregation of the nanospheres in the solution.



Scheme 3.1 Schematic of (a) chitosan chains, and (b) chitosan nanospheres in acid and base conditions

Based on the pH-responsive results as observed from (i) the turbidity, (ii) the nanosphere size and (iii) the negatively charged surface, we speculated the structure as in Scheme 3.1. In the case of chitosan (Scheme 3.1(a)), which is a cationic polymer with pKa 6.5,8 the amino group of chitosan can be protonated in acidic condition to present the positive charge. Thus, the acid condition obstructs the inter- and intra- molecular hydrogen bond resulting in a good solubility of chitosan. In contrast, in basic solution, the amino groups of chitosan strongly form hydrogen

bonds which initiate the precipitation. In the case of our chitosan nanospheres, the carboxyl groups belonging to O-phthaloylation play an important role in forming dimeric system via hydrogen bond in acid condition and being anionic carboxylate in basic condition as shown in Scheme 3.1(b).

3.4 Conclusion

In conclusion, the amphiphilic chitosan formed nanospherical structure after grafting hydrophobic and hydrophilic groups. Surprisingly, the nanospheres obtained showed a pH-responsive system to consequently give (i) a controlled colloidal-transparent solution, (ii) a changeable nanosphere size, and (iii) a variable negative surface charge. The fact that the phthaloylation in DMF brings not only N-phthaloyl group but also a certain amount of O-phthaloly group, the simple variation of pH initiated the carboxylic acid of O-phthaloyl group to play an important role in forming hydrogen bond or anionic carboxylate group. The hydrogen bond might be formed as a dimeric system or with water molecules. This led chitosan nanospheres be a self-assemble structure which can be controlled by pH. The present work is a good example to show the simple pathway to develop an external stimuli system to chitosan which never be possible in the original chitosan.

3.5 Acknowledgments

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3.6 References

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