# CHITOSAN-NANOPARTICLE PREPARATION BY POLYELECTROLYTE COMPLEXATION

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## Introduction

To date a widely used method to produce chitosan nanoparticles relies on polyelectrolyte complexation: it requires, besides chitosan, only a polyanionic polymer. No auxiliary molecules such as catalysts or initiators are needed and the reaction is generally performed in aqueous solution. Tripolyphosphate (TPP) is the most widely used polyanion, for the preparation of chitosan nanoparticles [1]. In addition Dextran sulfate[2], carboxymethyl cellulose[3], heparin[4] and DNA[5] have been utilized as polyanion to prepare chitosan nanoparticles. Fucoidan is a negatively charged polysaccharide naturally occurring in many species of brown algae and many marine invertebrates. By virtue of its dynamic biological properties Fucoidan has been widely reported for its pharmacological activities. Fucoidan can increase the potency of nanoparticle for the delivery of anticoagulant agents on the site of application, due to its antithrombotic activity[6]. However literature on its use as drug delivery systems like micro or nanoparticles is lacking. In this paper we report a novel polyelectrolyte complexation between chitosan and Fucoidan to prepare chitosan nanaoparticles.

## Materials and method

*Preparation of Chitosan-Fucoidan complex Nanoparticles*

0.1% w/v chitosan solution was prepared by dissolving 100mg of chitosan (deacetylation degree 75-85%, viscosity 0.5% in 5% acetic acid 5-20cps, case # 0321-6250 Showa chemicals Japan) in 0.2% w/v acetic acid (Merck, Germany), and making the final volume to 100ml with 0.2%w/v acetic acid. 0.1% w/v Fucoidan solution was prepared by dissolving 100mg Fucoidan from *Fucus vesiculosus* (Sigma-Aldrich, case# F5631-1G) in deionized water, and making the final volume to 100ml. Chitosan-Fucoidan complex dispersion was prepared by mixing negatively charged Fucoidan and positively charged chitosan by dropping method; 5ml of chitosan solution (0.1%w/v in 0.2%acetic acid w/v) was taken in a beaker. 0.25ml, 0.5ml, 1ml, 2ml, 3ml, 4ml or 5 ml of fucoidan solution (0.1%w/v) was added drop wise into the chitosan solution under continuous stirring, to achieve Chitosan-Fucoidan ratio 1:0.05, 1:0.1, 1:0.2, 1:0.4, 1:0.6, 1:0.8 or 1:1 respectively. After 30min of stirring the dispersion was centrifuged at 1300g for 15 min. the pellets if any were redispersed in 3 ml water and transferred to empty glass tubes and frozen before freeze drying. To study the effect of pH of the chitosan solution, the pH of 5ml chitosan (0.1%w/v in 0.2%w/v acetic acid) was adjusted to 2, 3, 5, or 6 with 1N NaOH or HCl (DC chemical Korea), as per case. 0.25ml, 0.5ml, 1ml, 2ml, 3ml, 4ml and 5 ml of Fucoidan solution (0.1%w/v) was added drop wise into the chitosan solution under continuous stirring, to achieve chitosan to Fucoidan ratio of 1:0.05, 1:0.1, 1:0.2, 1:0.4, 1:0.6, 1:0.8 and 1:1 respectively.
The turbidity of the suspension was measured by uv-vis(UV-1601 PC Shimadzu) spectrophotometer, the yield of the dried mass was calculated gravimetrically, and the size and morphology of nanoparticles were examined by scanning electron microscopy(Hitachi, S-4300).

## Results and discussion

When Fucoidan was dropped into chitosan solution, the inter- and intra-molecular electrostatic attractions occurred between anionic sulfate groups from Fucoidan and

cationic amino groups of chitosan. These attractions could make the macromolecular chains of chitosan and Fucoidan curl up, which leads to an insoluble Chitosan-Fucoidan complex formation. The yield and condition of Chitosan-Fucoidan complex formation is shown in Table 1.

## Conclusion

Novel chitosan-Fucoidan nanoparticulate dispersion was successfully prepared with polyelectrolyte complexation method. Most of the studied Chitosan-Fucoidan mass ratios yielded turbid suspension at all the studied chitosan solution pH condition except mass ratio of 1:0.6, 1:0.8 and 1:1 at pH 6, which led to visible aggregates in the suspension. The prepared Chitosan-Fucoidan complex nanoparticles were observed by field emission scanning electron microscope (Hitachi, S-4300) and the mean size of prepared nano-particles located in matrix was measured as less than 100nm. The nanopartices tended to grow as the pH of chitosan increased and the mass ratio of chitosan and Fucoidan decreased, which led to the formation of the aggregated nanoparticles.

Table.1. Yield and conditions of Chitosan-Fucoidan complex particles formation at various pH and chitosan-Fucoidan ratio

| Chit-Fuco mass ratio | pH of Chit 2 | 3 | 3.69 (untreated) | 5 | 6 |
|---|---|---|---|---|---|
| 1 : 0.05 | 11.4±2.7 | 15.2 ±5.4 | 16.2 ±4.0 | 15.2 ±2.7 | 17.1 ±2.7 |
| 1 : 0.1 | 14.5 ±5.1 | 18.2 ±5.1 | 19.1 ±3.9 | 10.9 ±2.6 | 21.8 ±2.6 |
| 1 : 0.2 | 15.0 ±2.4 | 18.3 ±4.7 | 17.5 ±3.5 | 25.0 ±9.4 | 25.0 ±7.1 |
| 1 : 0.4 | 15.7 ±2.0 | 18.6 ±2.0 | 20.7 ±1.0 | 23.6±13.1 | 33.6±1.1 |
| 1 : 0.6 | 18.1 ±0.9 | 18.1 ±0.9 | 33.8 ±1.8 | 34.4 ±0.9 | 48.8 ±7.1(x) |
| 1 : 0.8 | 18.3 ±2.4 | 21.7 ±0.8 | 35.0 ±2.4 | 35.6 ±1.6 | 57.2 ±3.9(x) |
| 1 : 1 | 22.5 ±2.1 | 25.5 ±2.1 | 35.0 ±4.2 | 36.0 ±2.8 | 66.5 ±0.7(x) |

NOTE: Chit = chitosan, Fuco = Fucoidan, (x)=aggregation

At pH 5 a Chitosan-Fucoidan mass ratio of 1:1 led to a highly turbid suspension, having the highest absorbance value (i.e., 0.138), which had no visible aggregates and yielded 36.0% of the dried mass. It was found that the Chitosan-Fucoidan complex formation is so highly dependent on the pH of chitosan solution that the studied chitosan solution pH condition of 6 led to visible aggregates in the suspension at the Chitosan-Fucoidan mass ratio of 1:0.6, 1:0.8 and 1:1. Even though the effect of Chitosan-Fucoidan mass ratio is not much pronounced in terms of Chitosan-Fucoidan complex formation, it is so pronounced in terms of yield. Since the color of Fucoidan is yellowish brown, therefore the turbid suspensions obtained had a yellowish appearance. The yellowish pellets after centrifugation could be easily redispersed into a turbid suspension lacking visible agglomerates. The prepared Chitosan-Fucoidan complex nanoparticles were observed by field emission scanning electron microscope (Hitachi, S-4300). The mean size of prepared nano-particles located in matrix was measured as less than 100nm and the nanopartices tended to grow as the pH of chitosan increased and the mass ratio of chitosan and Fucoidan decreased, which led to the formation of the aggregated nanoparticles.

## References


1. Gan Q, Wang T, Cochrane C, McCarron P, Colloid and surfaces B: Biointerfaces 44 (2005) 65-73.
2. Chen Y, Mohanraj VJ, & Parkin J E, Letters in Peptide Science, 10 (2003) 621~29.
3. Ichikawa S, Iwamoto S, Watanabe J, Biosci. Biotechnol. Biochem 69(9) (2005) 1637-1642.
4.Zonghua Liu, Yanpeng Jiao, Fanna Liu, Ziyong Zhang, J Biomed Mater Res A. 2007 Jun 8, 10.1002/jbm.a.31407 DOI.
5. Mao H Q, Roy K, Troung-Le V L, Janes K A, Lin K Y, Wang Y, August J T and Leong K W, J. Control Release 70 (2001) 399–421.
6. Mourao P A S and Pereira M S. Trends Cardiovasc. Med., 9 (1999) 225-232.