Stability of chitosan nanoparticles

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INTRODUCTION AND OBJECTIVES

Nanoparticles are used in many areas of research and applications; moreover their usability in medicine increases rapidly (Huang 2002). For the preparation of nanoparticles aimed to biomedical applications, non toxic material with controlled degradation and not activating immune reactions is required (Huang 2004). Chitosan is one of the polymers with an important role in medical applications based on nanoparticles (Sanyakamdhorn 2013).

This contribution is focused on preparation and stability of chitosan nanoparticles. Chitosan is non toxic, biodegradable and biocompatible polysaccharide consisting of ß-(1-4)-D-glucosamine and *N*-acetyl-D-glucosamine units (Riva 2011). Thanks to the presence of functional group (*e.g.* C3-amino group, primary alcohol), further chemical modifications of chitosan are accessible.

Nanoparticles stability is crucial for their medical applications. Recently, Jonassen and co-workers emphasized that chitosan nanoparticles can exhibit time instability in different physiologically relevant environments (Jonassen 2012). This findings corresponds with our experience. Unfortunately, in general these issues are not covered in details in the relevant literature in conjunction with the chitosan nanoparticles.

Chitosan nanoparticles can be prepared by different methods, which are predominantly represented by (i) pH-controlled precipitation, and (ii) ionic crosslinking with sodium tripolyphosphate (TPP). The main goal of this study was the comparison of these two principles from the point of view of stability of chitosan nanoparticles in different storage solutions. Stability of prepared nanoparticles was studied in (i) distilled water, (ii) PBS at pH 6.8, (iii) PBS at pH 7.4, and (iv) FBS-DMEM.

MATERIALS AND METHODS

Materials

Chitosan (Fluka No. 50494, DD = 76-79%, MW = 450 – 630 kDa; both values determined in our laboratory), sodium tripolyphosphate (TPP, Sigma Aldrich), phosphate buffer (PBS, Sigma Aldrich), Dulbecco's modified eagle media (DMEM), fetal bovine serum (FBS).

Preparation of chitosan nanoparticles

Chitosan nanoparticles were prepared in concentration of 0.5% w/v by two methods (Figure 1). The first one is based on the pH-controlled precipitation (the "pH" chitosan nanoparticles) and the second one on the ionic crosslinking with TPP (the "TPP" chitosan nanoparticles). Both methods result in the phase separation of chitosan in the form of nanoparticles.

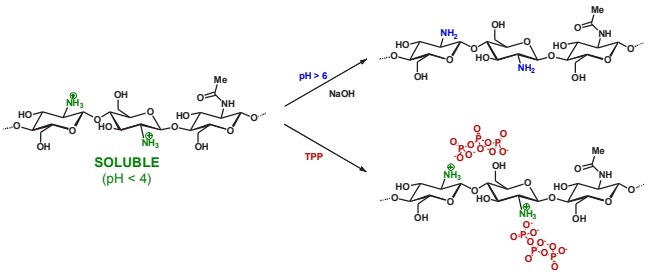

Figure 1: Preparation of chitosan nanoparticles

Stability of chitosan nanoparticles

Dispersion of chitosan nanoparticles was 10-times diluted in four different storage solvents: distilled water, PBS at pH 6.8, PBS at pH 7.4, FBS-DMEM. The stability of nanoparticles was determined by dynamic light scattering (DLS, Malvern, Zetasizer Nano ZS). The measurements were terminated if the visible aggregation and/or sedimentation of nanoparticles occurred.

RESULTS AND DISCUSSION

Characterisation and stability of chitosan nanoparticles

The size of prepared chitosan nanoparticles was around 1 µm independently of employed method. Stability of "pH" chitosan nanoparticles is shown in Figure 2. Size distribution of nanoparticles was not uniform during the stability study. No data shown for a particular storage solvent means the gross aggregation and sedimentation of nanoparticles.

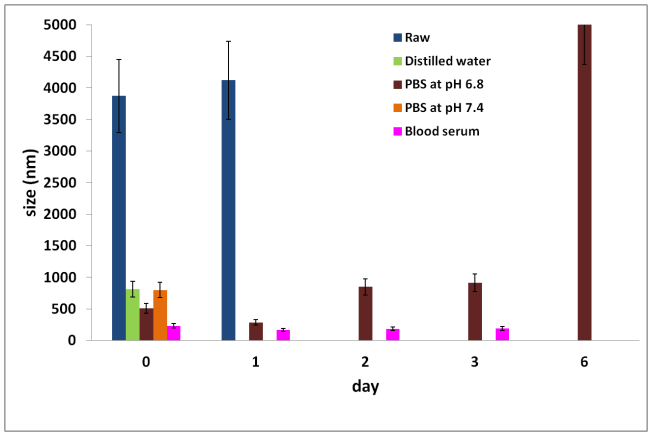

Figure 2: Stability of "pH" chitosan nanoparticles

The "pH" chitosan nanoparticles exhibit the highest stability in PBS at pH 6.8, where they were stable for 6 days. On contrary, the lowest stability was observed in case of distilled water and PBS at pH 7.4, where sedimentation of nanoparticles occurred within 24 h.

Size distribution of chitosan nanoparticles prepared by ionic cross-linking with TPP is summarized in Figure 3. The "TPP" chitosan nanoparticles have the highest stability in distilled water, where sedimentation started after 21 days after preparation. On the other hand, the lowest stability of nanoparticles was observed in FBS-DMEM (4 days). However, in all cases "TPP" nanoparticles are more stable than the "pH" ones. Considerable difference can be seen in stability of chitosan nanoparticles in PBS at pH 7.4. In this storage solution, the "TPP" nanoparticles were stable for 10 days in comparison with "pH" nanoparticles were sedimentation was observed within 24 h.

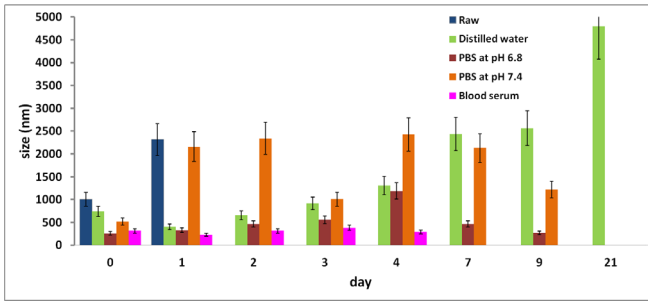

Figure 3: Stability of "TPP" chitosan nanoparticles

CONCLUSIONS

The goal of this work was preparation and stability study of chitosan nanoparticles. Nanoparticles were prepared by two different methods and their stability in various storage solutions was monitored. Stability of nanoparticles in each solution was notable affected by the method of preparation. From the point of view of nanoparticles stability, the ionic cross-linking with TPP is the more appropriate method of preparation. The "TPP" nanoparticles are stable in physiologically relevant environments (PBS, FSB-DMEM) in the range of a couple of days. In the future, we aim to employ these nanoparticles as the anticancer drug carriers.

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ACKNOWLEDGMENTS

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