Synthesis and Characterization of Chitosan Nanoparticles for Oral Delivery

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Introduction and Objective

Chitosan nanoparticles (CS-NPs) are promising vehicles for oral drug delivery, offering a means to increase the bioavailability of therapeutic agents which may otherwise be limited by their low intestinal absorption and instability in the gastrointestinal (GI) tract. (Chen et al., 2013) Chitosan (CS), a polysaccharide derived from the deacetylation of chitin, is known for its biocompatibility, nonreactivity, and mucoadhesivity. In nanoparticle form, CS's properties only improve, allowing for greater interaction with the surrounding microbial environment. (Landriscina et al., 2015)

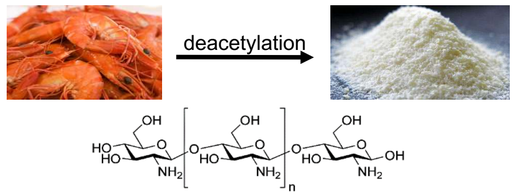


Figure 1. Derivation and chemical structure of chitosan

Chitosan's concentration, molecular weight, and deacetylation degree have been shown to affect CS-NP synthesis. This summer, we sought to:

1. Observe the effects of varying the molecular weight and concentration of CS on CS-NP size
2. Calculate the encapsulation efficiency of metformin (a drug shown to slow cyst formation in polycystic kidney disease) when encapsulated within 2mg/mL low molecular weight CS-NPs

Impact of Professor's Research

The Chung Lab uses nanomedicine and tissue engineering to address clinical limitations, aiming to design self-assembling, peptide amphiphile micelle nanoparticles to target diseases such as atherosclerosis and autosomal dominant polycystic kidney disease (ADPKD), and also to serve as contrast agents to enhance imaging techniques such as magnetic resonance imaging (MRI) and positron emission tomography (PET). Other goals include combining stem cell technology with citric acid-based scaffolds for regenerative purposes.

Figure 2 (right). Concept to application. (Source: Chung Laboratory)

Techniques and Skills Learned

CS-NP synthesis via ionic gelation
Method based around the electrostatic interactions between CS's positively charged amine groups and tripolyphosphate (TPP) anions, commonly used as a cross-linking agent due to its nontoxic nature. (Mohammadpourdougnighi, 2009)

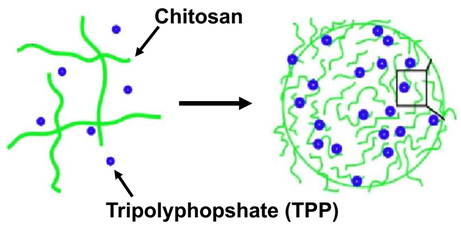


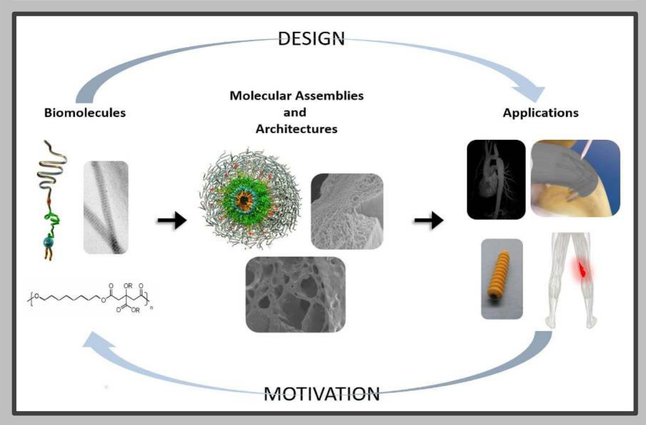
Figure 3. Formation of CS-NPs via ionic gelation (adapted from Chávez, 2011)

Dynamic light scattering (DLS)
A technique used to characterize the size distribution of particles suspended in solution.

Microplate reader
A machine that can read samples in a 96-well plate to measure absorbance, fluorescence, luminescence, etc. Gained valuable troubleshooting skills while working with this instrument.

NanoDrop
Wrote procedure for the NanoDrop, a UV-Vis spectrophotometer that can read 1-2μL samples (ex: DNA, RNA, protein).

Figure 4. NanoDrop One (source: Labtech)



Methods and Results

Effects of varying the molecular weight of chitosan on chitosan nanoparticles

1. Synthesized 2mg/mL chitosan nanoparticles via ionic gelation using both low and medium molecular weight chitosan, observed chitosan nanoparticles using TEM. (see Fig. 5)

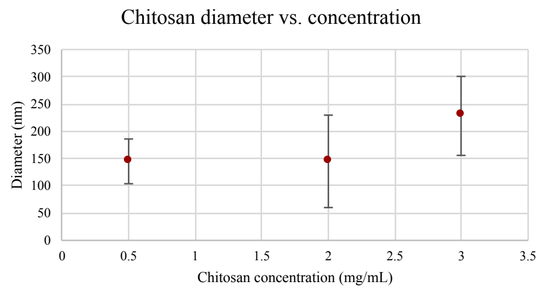
2. Measured size distribution of chitosan nanoparticles using DLS, averaged results from each run to find average diameter.

CS molecular weight (Da)	Average diameter (nm)
Low (50k-190k)	231.2
Medium (190k-310k)	383.45

Effects of varying the concentration of chitosan on chitosan nanoparticles

1. Synthesized 0.5mg/mL, 2mg/mL, and 3mg/mL chitosan nanoparticles using low molecular weight chitosan.

2. Measured size distribution of chitosan nanoparticles using DLS, averaged results from each run to find average diameter.



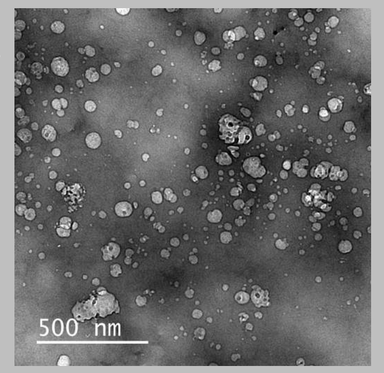
CS concentration (mg/mL)	Average diameter (nm)
0.5	146.04
2	145.51
3	229.03

Calculating encapsulation efficiency of metformin in 2mg/mL low molecular weight chitosan nanoparticles

$$\text{Encaps. efficiency} = \frac{\text{total mass} - \text{free mass}}{\text{total mass}}$$

Data is still in the process of being collected, but we expect metformin's encapsulation efficiency to be around 67% (the encapsulation efficiency previously found from encapsulating fluorophores within CS-NPs).

Figure 5. TEM image of low molecular weight 2mg/mL chitosan nanoparticles (Source: Jonathan Wang)



Next Steps for You OR Advice for Future SHINE Students

This experience has further affirmed my interests in STEM and my aspirations to pursue engineering and conduct research in college.

My advice for future SHINE students:

- Don't be afraid to ask questions
- Collaborate with and talk to people! (whether that be your SHINE cohort or others in your lab, there's always something that can be learned from others)

Acknowledgements

A huge thank you to Dr. Chung for opening up her lab to me and giving me college advice, my wonderful PhD mentor Jonathan for teaching me so much, and everyone in the Chung Lab for being really awesome people and helping me out whenever I had questions (special shout-out to Chris, Shivani, and Trip). I would also like to thank Dr. Katie Mills, the SHINE coordinators, and my SURE mentor Josh Posen for their guidance.