Exploring Polymer-lipid Complexes for Potential Applications in Drug Delivery and Antimicrobial Systems

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SMART SUMMER MATERIALS RESEARCH @ TULANE

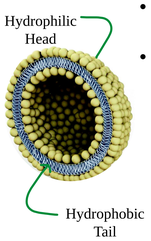
Introduction

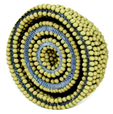
Liposomes

The flexible structure and self-assembly properties of liposomes have made them a major technology in the biomedical field. These spherical vesicles have been greatly investigated as drug delivery devices as well as models for the cell membrane.

Building them up

- Can be single or multilayered.
- Made up of polar head and hydrophobic tails.

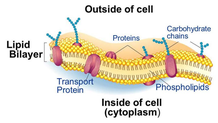




Breaking them down

Breaking down liposomes is a beneficial technique in the pharmaceutical industry.

- Learn to kill undesired cells, such as cancer cells that possess a similar bilayer.
- Build multilayered liposomes from pieces allowing for the optimization of drug transfer.

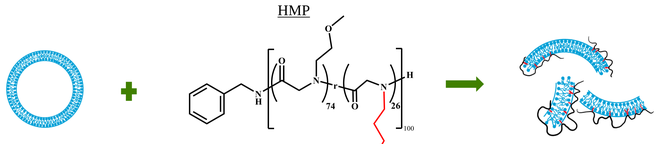


1. Bozzuto, G.; Molinari, A., **2015**.; 2. Mohammadi, A.; Jafari, S. M.; Mahoonak, A. S.; Ghorbani, M. **2020**.; 3. Escalante-Martinez, J. E.; Morales-Mendoza, L. J.; et al. **2018**.; 4. Zhang, Y.; Xuan, S.; Owoseni, O.; Omarova, M.; Li, X.; Saito, M. E.; He, J.; McPherson, G. L.; Raghavan, S. R.; Zhang, D.; John, V. T. **2017**.

Previous Work with Hydrophobically Modified Polypeptoids

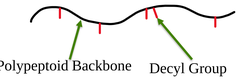
Prior research has been done studying the development of multilaminar vesicles from liposome fragments. These pieces have been produced using hydrophobically modified polypeptoids (HMP). Nonpolar decyl groups that are attached along the polypeptoid backbone embed themselves into the bilayer, disrupting the structure of the liposome.

HMP



Cryo-TEM of 100 nm Liposomes

Cryo-TEM of Liposome Pieces (10-15 nm)

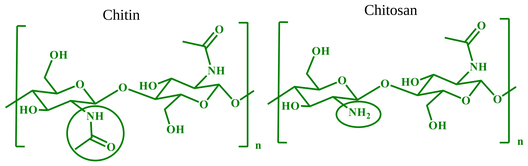


5. Zhang, Y.; Xuan, S.; Owoseni, O.; Omarova, M.; Li, X.; Saito, M. E.; He, J.; McPherson, G. L.; Raghavan, S. R.; Zhang, D.; John, V. T. **2017**.

Using Chitosan to Break Liposomes

Chitosan

Chitosan is a polysaccharide that is derived from the deacetylation of chitin, a polymer found in many insects, squid, and crustaceans such as shrimp, crab, and lobster. The crystalline structure of chitosan makes it insoluble in water. However, it has greatly been used in the biomedical field, such as in hydrogel formation.



Why Chitosan?

- More accessible
- Easier to manufacture/already assembled

6. Champagne, L. Louisiana State University and Agricultural and Mechanical College, Louisiana, **2008**.

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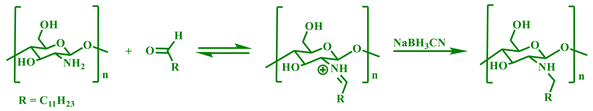
Objective-Synthesizing Hydrophobically Modified Chitosan

Chitosan was hydrophobically modified with dodecyl alkyl chains, allowing the polymer to plant itself into the bilayer of the liposomes, like the polypeptoids. At high concentrations, it is hypothesized that the chains will disrupt the structure of the liposomes and break them into pieces. Modifications were made at various percentages, meaning out of the total monomer units in the sample, only 5, 10, or 25% of them were modified. Different molecular weights of chitosan were also tested: 50-190 kDa and 15 kDa.



Provided by Dr. Srinivasa Raghavan's Group

1) 

50-190 kDa - Various Modifications

2) 

15 kDa – 25% Modified

3) 

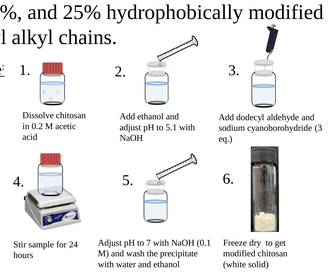
15 kDa – 25% Modified Water Soluble

7. Desbrières, J.; Martinez, C.; Rinaudo, M. **1996**.

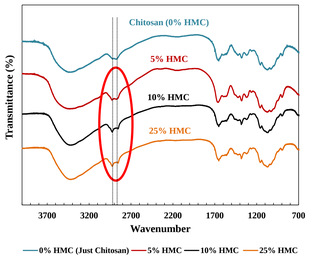
Synthesizing HMC (50-190 kDa)

Low molecular weight chitosan (50-190 kDa) was 5%, 10%, and 25% hydrophobically modified with dodecyl alkyl chains.

Procedure:



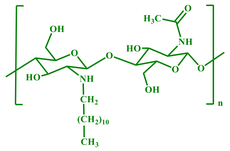
IR Data of 50kDa HMC



Sharper peak at 2923 cm^{-1} (C-H stretch) indicates addition of dodecyl alkyl chain.

8. Desbrières, J.; Martinez, C.; Rinaudo, M. **1996**.; 9. Vo, D.-T.; Whiteley, C. G.; Lee, C.-K. **2015**.

HMC (50-190 kDa) + Liposome Interactions



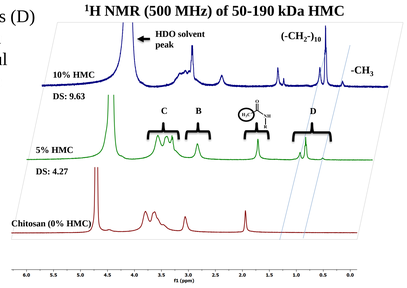
The presence of peaks (D) between 0 to 1.5 ppm indicate the successful modification with the dodecyl alkyl group.

Degree of Substitution (DS):

$$\frac{D}{B+C} = \frac{n*(DS)}{6}$$

B: H_2 peak area
C: $H_{2'}$; $H_{3,4,5,6}$ peak area
D: Substituent peak area
n: Hydrogen atom amount in substituent

^{1}H NMR (500 MHz) of 50-190 kDa HMC



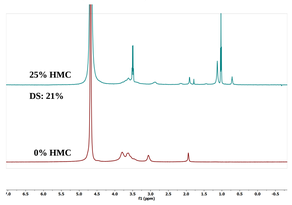
*The 25% HMC did not dissolve in the deuterated solvent

10. Nikmawahda, H.; Sugita, P.; Arifin, **2015**.

HMC (15 kDa)

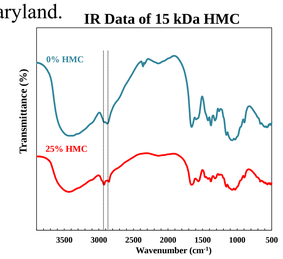
Low molecular weight chitosan (15 kDa) with 25% hydrophobic modifications was provided by Dr. Srinivasa Raghavan's Group at the University of Maryland.

^{1}H NMR (500 MHz) of 15kDa Chitosan



The appearance of the peaks between 0 to 1.5 ppm indicates the addition of the dodecyl alkyl chain.

IR Data of 15 kDa HMC

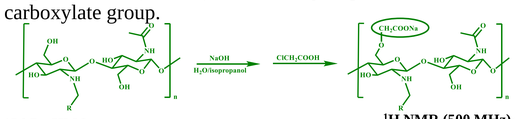


Sharper peak at 2920 cm^{-1} (C-H stretch) indicates the presence of the dodecyl alkyl chain.

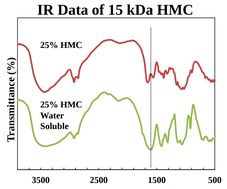
11. Vo, D.-T.; Whiteley, C. G.; Lee, C.-K. **2015**.

Water Soluble (WS) Chitosan (15 kDa)

Low molecular weight chitosan (15 kDa) with 25% hydrophobic modifications was further modified with a carboxylate group.

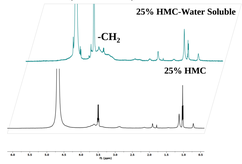


IR Data of 15 kDa HMC



Peak around 1650 cm^{-1} demonstrates the attachment of the carboxylate group.

^{1}H NMR (500 MHz) of 15 kDa Chitosan



The presence of the CH_2 peak confirms the attachment of the CH_2COONa group.

12. Vo, D.-T.; Whiteley, C. G.; Lee, C.-K. **2015**.; 13. Chen, Y.; Javvaji, V.; MacIntire, I. C.; Raghavan, S. R. **2013**.

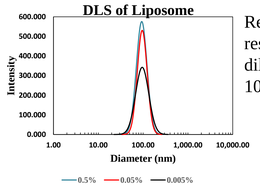
Liposome Interaction

Making 100 nm Liposomes

Procedure:

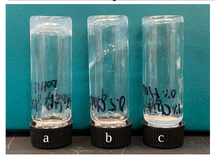
1. Measure 50 mg of PC lipids and place it in a round bottom flask with 7.5 mL of chloroform and methanol mixture (2:1)
2. Remove solvent via rotary evaporator at 100 mbar
3. Hydrate at 50 °C with DI water to get desired concentration
4. Extrude for 31 times with 100 nm membrane

DLS of Liposome



Reproducible DLS results at different dilution showing 100 nm liposomes.

Chitosan and Liposome Interaction



(a) 1% (5% HMC-50 kDa) _ 1% Liposomes
(b) 0.5% (5% HMC-50 kDa) _ 1% Liposomes
(c) 1% (0% HMC-50 kDa) _ 1% Liposomes

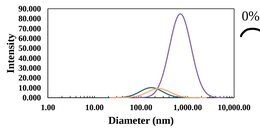
Sample (a) formed a gel. Sample (b) did not form a gel but was too viscous to analyze by TEM and DLS. Therefore, the 5% HMC – 50 kDa did not break the liposomes.

14. Zhang, Y.; Xuan, S.; Owoseni, O.; Omarova, M.; Li, X.; Saito, M. E.; He, J.; McPherson, G. L.; Raghavan, S. R.; Zhang, D.; John, V. T. **2017**.

Liposome Interaction Using DLS

Still working towards collecting reproducible DLS data showing chitosan and liposome interactions.

0% HMC - 50 kDa Dilution

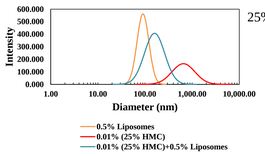


0% HMC -50 kDa

*DLS data is inconsistent

It is possible that chitosan self aggregation affects its ability to break down the liposomes. This is shown by the large intensity of the 0.1% (0% HMC) sample.

0.5% Liposomes with 25% HMC -15 kDa



25% HMC -15 kDa

The chitosan and liposome mixture increases in diameter rather than decreasing to the size of the liposome fragments (10-15 nm). Therefore, the liposomes did not break.

Discussion and Future Work

Summary:

- NMR and IR data support hydrophobic modification of the 50 kDa and 15 kDa chitosan.
- Low molecular weight chitosan (50 kDa) is impractical to work with because it either forms a gel or is difficult to dissolve.
- Current DLS data shows that the 25% HMC – 15 kDa does not break the liposomes. The increase in diameter of the chitosan and liposomes mixture suggests that the chitosan is latching onto the liposomes, but not disrupting the structure enough to break it

Next Step:

- Continue working toward collecting DLS data for the liposome and HMC interaction.
- Perform Cryo-TEM to visually see liposome and HMC interactions

Acknowledgments

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