

Extraction and Modification of Chitosan for Water Purification

Group 1-39

Abstract

The utilization of Chitosan and its modified forms has been researched and applied globally, with the most prominent usage being in removing highly toxic heavy metals from water sources. Discovery of novel organic sources of chitosan and different methods of modification have also increased interest in this field, coppering to many modification methods and their uses. This study aims to examine the viability of extraction and modification of chitosan for water purification. Extraction of chitin was achieved using *Lates calcarifer* scales, and was further converted to chitosan. FTIR spectroscopy tests were carried out after each step to ascertain desired product was obtained. Chitosan was modified with Aldehydes and Acid with Acetic Anhydride. The modified chitosan and unmodified chitosan were used in heavy metal adsorption tests. The effectiveness of different modified versions and unmodified chitosan was compared and the results were conclusive. Chitosan modified with aldehyde proved to have the greatest heavy metal ion sorption capabilities for Zn^{2+} ions at 100% while that modified with ethanoic acid and acetic anhydride had the greatest sorption capability for Cu^{2+} ions at 100%.

1. Introduction

Water is an essential and valuable resource for human development, but in recent years industrialisation, the advancement of technologies in agriculture and an increase in urbanisation has led to a decrease in clean and safe-to-drink water sources. Varying forms of pollutants, such as pharmaceuticals, pesticides, dyes and heavy metals have led to contamination of water sources around the globe (Ali, et al., 2018; Basheer, 2019). Heavy metals are one of the most toxic pollutants and are also non-biodegradable, making them the most hazardous out of all the pollutants. It is hence a priority to remove and eliminate them from contaminated wastewater before discharging to the environment (Mojiri, Ziyang, Tajuddin, Farraji & Alifar, 2016). Adsorption is often the most effective and economically viable way to remove non-degradable pollutants from water, especially heavy metals, as stated by Ali, Khan and Asim, 2011.

Chitosan is a linear polysaccharide composed of randomly distributed β -(1-4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit) can polymerase by means of a cross-link formation in the presence of anions and polyanions.

In the last decade, we have seen scientific discoveries of many novel organic sources of chitosan. Most involve the extraction and modification of chitin. Organic sources of chitosan include prawn shells, which Antonio *et. al.*, 2017 achieved using two 10-minute bleaching steps with ethanol after demineralization and deproteinization processes. Extraction of chitin from fish scales, on the other hand, was accomplished by Muslim, Rahman, Begum, & Rahman, 2013, using sodium hydroxide to deacetylate chitin which was already demineralized and deproteinized. Other sources include crab shells (Pambudi, et al., 2018), which need to be bleached with Hydrogen Peroxide before magnetic stirring with chitosan gel.

However, the yield of chitin to be modified into chitosan varies by source, with crab shells having the lowest yield at 10.60-12.73% (Pandharipande & Bhagat, 2016), prawn shells had a higher yield at 14.72%±0.57% (Varun, et al., 2017), whilst the scales of *Labeo rohita* had the highest yield, which is 22.36% (Muslim et al., 2013).

Chitosan has useful properties such as hydrophilicity, biodegradability and presence of highly reactive amino (-NH₂) and hydroxyl (OH⁻) groups in it, it has proven to be effective in the removal of heavy metals from polluted waters (Ahmad, Ahmed, Swami, Ikram, 2015).

Chitosan's metal-binding efficiency could be further increased through chemical modification with the cross-linking reagents including ethylene glycondiglycidyl ether, formaldehyde, glyoxal, epichlorohydrin, glutaraldehyde, and isocyanates. (Ahmad et al., 2015) The modification of chitosan to generate new biofunctional compounds has been of great interest because the modification would not alter the fundamental structure of the chitosan (Rajasree & Rahate, 2013)

Chitosan can also be modified with aldehydes. Sobahi et al., cross-linked chitosan with glutaraldehydes in water and stirred it for 15 minutes. The resultant was decanted and the remaining moisture was removed with a vacuum oven at 40°C for 24 hours. The uncrosslinked aldehydes had an average polymer capacity for FeCl₃ of about 16.18 mg/g and for Cu₂O₄ it was around 18.26, while the cross-linked aldehydes once again had much better adsorption capabilities. The crosslinked aldehydes had an average polymer capacity for FeCl₃ of about 15.48 mg/g and for Cu₂O₄ it was around 17.88 mg/g. (Sobahi et al., 2011)

However, the main roadblock hindering more widespread use of adsorption as a method of water purification is the lack of adequate regeneration methods for recycling spent

adsorbents, as mentioned by Vakili *et al.*, 2019. Chitosan as an adsorbent is advantageous due to its abundance, low price, antibacterial property, non-toxicity as well as biodegradability and high adsorption capability (Nie, Deng, Wang, Huang, & Yu, 2014).

This study aims to extract chitosan from *Lates calcarifer* (Asian Sea Bass) using the methods outlined by Pandharipande, Jana, & Ramteke, 2018, before modification to test the abilities of chitosan in terms of its heavy metal ion adsorption capacity.

2. Materials and Methods

2.1 Materials

Scales of *Lates calcarifer* was obtained from various supermarkets in Singapore. All other chemicals were obtained from the Biology Laboratory in the SRC.

2.2 Methods

Extraction of Chitin from Fish Scales

Fish scales (*Lates calcarifer*, 200g) were washed with tap water and then dried in an oven for overnight. Then, the scales were blended to powder before it was demineralized using 1 mol/L HCl (1:10 W:V) for 24 hours at 30°C. It was subsequently thoroughly rinsed with DI water through a cloth filter for at least 6 times. Leftover fish scales present in the filtrate were obtained through centrifugation at 7000 rpm for 7 min. After that, it was deproteinized using 1M Sodium Hydroxide solution overnight at room temperature. The resultant was rinsed several times with DI water through a cloth filter and then dried overnight before being ground into powder. Leftover chitin present in the filtrate was obtained through centrifugation at 7000 rpm for 7 min. An FTIR Spectrophotometer was used to ascertain the intermediate product is Chitin.

Extraction of Chitosan from Chitin

Chitin was deacetylated by adding 2M NaOH to each sample 1:10 (W/V) and then boiled at 80°C for 75 minutes (mins) on a hot plate. The samples were then washed continuously with deionized water until a clear solution was obtained and filtered in order to retain the solid matter, which is chitosan. Leftover chitosan present in the filtrate was obtained through centrifugation at 7000 rpm for 7 min. The samples were left uncovered and oven-dried for 24 hours before being

ground into powder. An FTIR Spectrophotometer was then used to ascertain that the product is Chitosan.

The Chemical Modification of Chitosan (Aldehyde)

Chitosan (0.5 g) was suspended in 1.0g of distilled water for 1h. A few drops of ethanoic acid were added and placed in an ultrasonic bath for a further 2h until complete solubility was obtained. Hexanal (0.5 g) was added dropwise while stirring. The mixture was stirred for a further 3 h. The material formed was separated by filtration. The obtained product was dried in vacuum at 40 °C for 24 h. FTIR was used to check for chitosan peak and aldehyde peak present in the compound.

The Chemical Modification of Chitosan (Acid and Acetic Anhydride)

Chitosan (0.5 g) was suspended in the least amount of distilled water for 1 h. Few drops of ethanoic acid was added using a dropper and sonicated for a further 2 h until homogeneity was obtained. About 2 ml of acetic anhydride was dropwise added while stirring and the reaction mixture was stirred for a further 30 min. The reaction mixture was heated for 1 h at 50 °C and then an aqueous sodium hydroxide solution was added carefully after cooling to adjust the medium at pH 8.5. The formed pale yellow material was filtered off and dried in vacuum at 40 C for 24 h. FTIR was used to check for chitosan peak and acid peak present in the compound.

Heavy Metal Ion Adsorbent Test

Batch experiments were carried out at room temperature ($25\pm 2^\circ\text{C}$) in stoppered conical flasks by shaking a fixed mass of 0.1g of modified chitosan adsorbents or pure chitosan, with 20mL of Zn^{2+} (or Cu^{2+}) solution at 250 rpm until equilibrium was reached. The initial, intermediate and final concentration of Zn^{2+} (or Cu^{2+}) was determined by a colourimeter.

3. Results and Discussion

3.1 FTIR Spectroscopy Tests

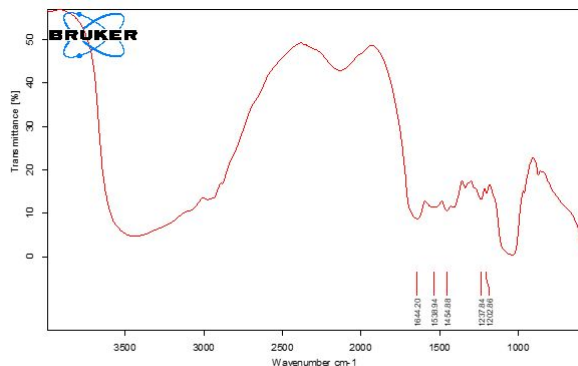


Figure 3.1a

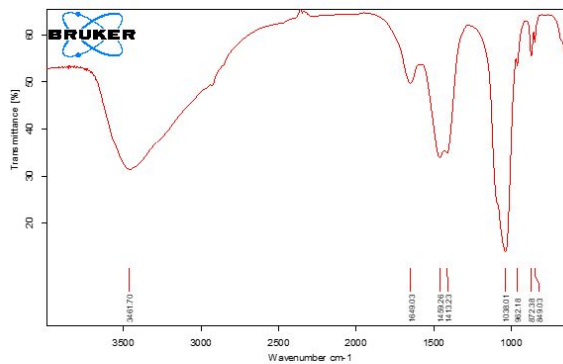


Figure 3.1b

Figure 3.1a is the FTIR Spectroscopy graph to ascertain that chitin was obtained from fish scales. The presence of a peak at $3650 - 3200 \text{ cm}^{-1}$ indicates the presence of -OH functional group, whereas peaks at $2960 - 2850 \text{ cm}^{-1}$ indicate the presence of sp^3 Carbon-Hydrogen bonds (Get In The Zone - The basics of Reading Infrared Spectrometry Graphs, n.d.). A peak at around 1600 cm^{-1} indicates the presence of the acetamide functional group (Acetamide, n.d.). Together, these indicate that the substance extracted is indeed chitin (Chitin, n.d.).

Figure 3.1b is the FTIR Spectroscopy graph to ascertain that chitin was successfully modified into chitosan. The presence of a peak at $3650 - 3200 \text{ cm}^{-1}$ indicates the presence of -OH functional group (Get In The Zone - The basics of Reading Infrared Spectrometry Graphs, n.d.), while a peak at about 1650 cm^{-1} indicates the presence of a primary amide (Jabs, n.d.). The removal of amide II and amide III during deacetylation were also reflected in the absence of peaks at around 1556 cm^{-1} and 1315 cm^{-1} . Thus, the compound obtained was chitosan.

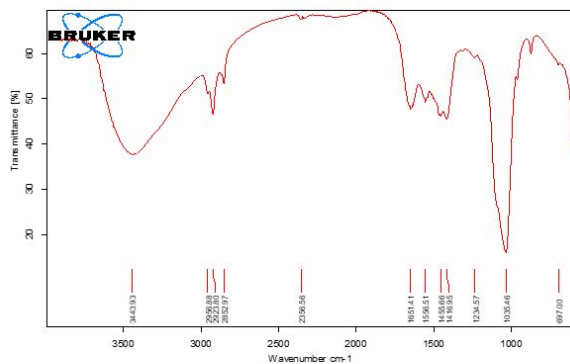


Figure 3.1c

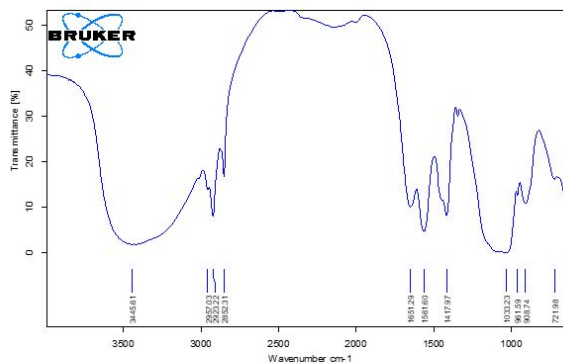


Figure 3.1d

Figure 3.1c and d are the FTIR Spectroscopy graphs of aldehyde modified chitosan and acetic anhydride modified chitosan respectively. The presence of a peak at 2700 cm^{-1} indicates the presence of the aldehyde functional group for hexanal-modified chitosan, while the presence of the carboxylic acid peak at $1690\text{--}1760\text{ cm}^{-1}$ shows that chitosan was successfully modified with acetic anhydride.

3.2 Heavy Metal Ion Sorption Test

All 3 chitosan adsorbents proved to have high heavy metal ion sorption capabilities, with the acid and acetic anhydride-modified chitosan adsorbent having the greatest capability for copper (II) ions at 100% and the aldehyde-modified chitosan adsorbent having the greatest capability for zinc ions at 100%.

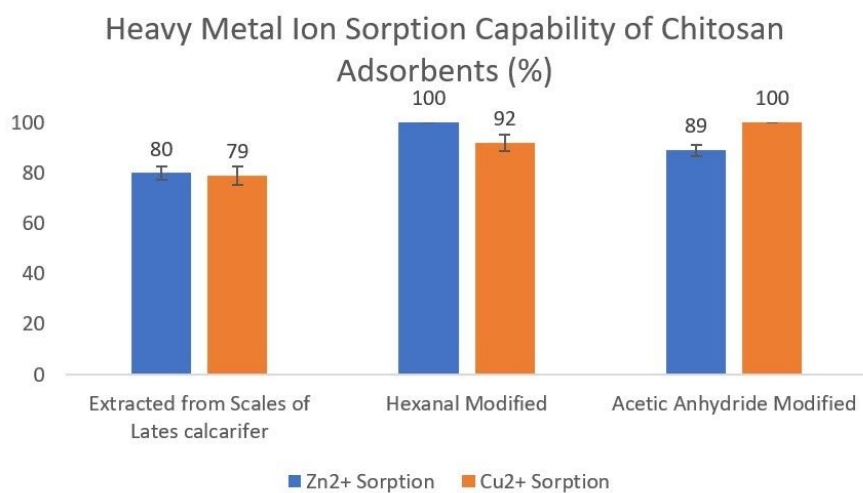


Figure 3.2

3.3 Data Analysis

The Kruskal-Wallis test was carried out to determine significant differences in the heavy metal ion sorption and antibacterial properties between the different chitosan adsorbents.

Heavy Metal Ion Sorption Between Chitosan Adsorbents			
Chitosan Adsorbent	Pure Chitosan	Aldehyde-Modified Chitosan	Acid & Acetic Anhydride-Modified Chitosan
p-value (Zinc (II) Ion Sorption)	0.009	0.009	0.009
p-value (Copper (II) Ion Sorption)	0.009	0.009	0.009

Figure 3.3

From the p-values obtained, it can be concluded that the results obtained by both modified chitosan adsorbents are significantly different from that of pure chitosan adsorbents. This is true for antibacterial properties, copper (II) ion sorption and zinc (II) ion sorption.

4. Conclusion

Based on the FTIR spectroscopy tests, chitin was successfully extracted from *Lates calcarifer* scales, and was then successfully converted into chitosan. Chitosan was also successfully modified using the aldehyde hexanal and an acetic anhydride-ethanoic acid combination. Based on heavy metal ion sorption tests carried out using Cu^{2+} and Zn^{2+} ions, the sorption capability of modified variants of chitosan proved significantly different from pure chitosan, with aldehyde-modified chitosan showing the greatest Zn^{2+} sorption capability, and acetic anhydride-modified chitosan showing the greatest Cu^{2+} sorption capability.

4.1 Future Work

Antibacterial tests can be conducted on the chitosan adsorbents, and heavy metal ion loaded chitosan adsorbents can also be regenerated. Different methods of chitosan modification can also be applied and tested using similar methods to evaluate the best overall chitosan

adsorbent in terms of heavy metal ion sorption and antibacterial properties. Once established, the selected adsorbent(s) can be tested in polluted water sources to show applicability.

5. References

- Ahmad M, Ahmed S, Swami BL & Ikram S (n.d.). *Adsorption of Heavy Metal Ions: Role of Chitosan and Cellulose For Water Treatment*. *Int J Pharmacognosy* 2015; 2(6): 280-89. doi: 10.13040/IJPSR.0975-8232.IJP.2(6).280-89
- Acetamide. (n.d.). Retrieved May 14, 2020, from: <https://pubchem.ncbi.nlm.nih.gov/compound/Acetamide>
- Ali, M. E. (2018). *Synthesis and adsorption properties of chitosan-CDTA-GO nanocomposite for removal of hexavalent chromium from aqueous solutions*. *Arabian Journal of Chemistry*, 11(7), 1107–1116. doi: 10.1016/j.arabjc.2016.09.010
- Antonino, R. S., Fook, B. R., Lima, V. A., Rached, R. Í., Lima, E. P., Lima, R. J., . . . Viníci, M. (2017, May 15). *Preparation and Characterization of Chitosan Obtained from Shells of Shrimp (Litopenaeus vannamei Boone)*. Retrieved from US National Library of Medicine: doi: 10.3390/md15050141
- Azevedo, E. P. (n.d.). Aldehyde-functionalized chitosan and cellulose: chitosan composites: application as drug carriers and vascular bypass grafts. *Iowa Research Online*, 2011.
- Bhatt, R., Sreedhar, B., & Padmaja, P. (2015). *Adsorption of chromium from aqueous solutions using crosslinked chitosan–diethylenetriaminepentaacetic acid*. *International Journal of Biological Macromolecules*, 74, 458–466. doi: 10.1016/j.ijbiomac.2014.12.041
- Bhuvaneshwari, S., Sruthi, D., Sivasubramanian, V., & Kanthimathy, K. (2012). Regeneration of chitosan after heavy metal sorption. *Journal of Scientific & Industrial Research*, 71, 266-269.
- Chitin. (n.d.). Retrieved May 14, 2020, from <https://www.sciencedirect.com/topics/food-science/chitin>
- Cui, L., Gao, S., Song, X., Huang, L., Dong, H., Liu, J., . . . Yu, S. (2018). Preparation and characterization of chitosan membranes. *RSC Advances*, 28433-28439.
- Dahmane, E. m., Taourirte, M., Eladlani, N., & Rhazi, M. (2014). Extraction and Characterization of Chitin and Chitosan from *Parapenaeus longirostris* from Moroccan Local Sources. *International Journal of Polymer Analysis and Characterization*, 342-351.
- Fan, L., Luo, C., Lv, Z., Lu, F., & Qiu, H. (2011). *Removal of Ag from water environment using a novel magnetic thiourea-chitosan imprinted Ag*. *Journal of Hazardous Materials*, 194, 193–201. doi: 10.1016/j.jhazmat.2011.07.080

- Get In The Zone - The basics of Reading Infrared Spectrometry Graphs.* (n.d.). Retrieved from pdf4pro.com:
<https://pdf4pro.com/cdn/get-in-the-zone-the-basics-of-reading-infrared-1eaf97.pdf>
- Jabs, A. (n.d.). Determination of Secondary Structure in Proteins by FTIR Spectroscopy. Retrieved May 14, 2020, from http://jenalib.leibniz-fli.de/ImgLibDoc/ftir/IMAGE_FTIR.html
- Jideowno, A., Okuo, J.M., & Okolo, P.O., (2007). *Sorption of Some Heavy Metal Ions by Chitosan and Chemically Modified Chitosan.* Trends in Applied Sciences Research, 2(3), 211–217. doi: 10.3923/tasr.2007.211.217
- Lavertu, M., Darras, V., & Buschmann, M. D. (2012). Kinetics and efficiency of chitosan reacylation. *Carbohydrate Polymers*, 1192-1198.
- Muslim, T., Rahman, M. H., Begum, H. A., & Rahman, M. A. (2013). Chitosan and Carboxymethyl Chitosan from Fish Scales of *Labeo rohita*. *Dhaka University Journal of Science*, 61.
- Ostrowska-Czubenko, J., Gierszewska, M., & Pieróg, M. (2011). State of water in noncrosslinked and crosslinked hydrogel chitosan membranes - DSC studies.
- Pambudi, G. B., Ulfin, I., Harmami, H., Suprpto, S., Kurniawan, F., & Ni'mah, Y. L. (2018). Synthesis of water-soluble chitosan from crab shells (*Scylla serrata*) waste. *AIP Conference Proceedings*, 020086.
- Pandharipande, S. L., & Bhagat, P. H. (2016, May). Synthesis of Chitin from Crab Shells and its Utilization in Preparation of Nanostructured Film. *International Journal of Science, Engineering and Technology Research (IJSETR)*, 5(5), 1378-1383.
- Pandharipande, S., Jana, R., & Ramteke, A. (2018). Synthesis and Characterization of Chitosan from Fish Scales. *International Journal of Science, Engineering and Technology Research (IJSETR)*, 287-291.
- Rahate, Rajasree, R., & K.P. (2013). AN OVERVIEW ON VARIOUS MODIFICATIONS OF CHITOSAN AND IT'S APPLICATIONS. *INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH*, 4(11), 4175-4193.
- Rajasree R and Rahate KP. (n.d.). *An overview on various modifications of Chitosan and its applications.* Int J Pharm Sci Res 2013; 4(11): 4175-93. doi: 10.13040/IJPSR.0975-8232.4(11).4175-93
- Reactivity of Aldehydes & Ketones.* (2019, June 23). Retrieved from Chemistry LibreTexts:
[https://chem.libretexts.org/Bookshelves/Organic_Chemistry/Supplemental_Modules_\(Organic_Chemistry\)/Aldehydes_and_Ketones/Reactivity_of_Aldehydes_and_Ketones](https://chem.libretexts.org/Bookshelves/Organic_Chemistry/Supplemental_Modules_(Organic_Chemistry)/Aldehydes_and_Ketones/Reactivity_of_Aldehydes_and_Ketones)

- Samoila, P. C., Humelnicu, A. C., Ignat, M. C., Cojocaru, C. C., & Harabagiu, V. C. (2019). *Chitin and Chitosan for Water Purification*. *Chitin and Chitosan*, 429–460. doi: 10.1002/9781119450467.ch17
- Sobahi, T. R., Abdelaal, M. Y., & Makki, M. S. (2014). Chemical modification of Chitosan for metal ion removal. *Arabian Journal of Chemistry*, 741-746.
- Takeshita, S., Konishi, A., Takebayashi, Y., Yoda, S., & Otake, K. (n.d.). *Aldehyde Approach to Hydrophobic Modification of Chitosan Aerogels*. Retrieved from ACS Publications.
- Varun, T. K., Senani, S., Jayapal, N., Chikkerur, J., Roy, S., Tekulapally, V. B., . . . Kumar, N. (2017, February 12). Extraction of chitosan and its oligomers from shrimp shell waste, their characterization and antimicrobial effect. *Veterinary World*. doi:10.14202/vetworld.2017.170-175
- Xu, J., Liu, Y., & Hsu, S.-h. (2019). Hydrogels Based on Schiff Base Linkages for Biomedical Applications. *Molecules*.
- Y. Nie, S. Deng, B. Wang, J. Huang, G. Yu (n.d.). *Removal of clofibric acid from aqueous solution by polyethylenimine-modified chitosan beads*. *Front. Environ. Sci. Eng.* 8 (2014) 675–682, <https://doi.org/10.1007/s11783-013-0622-0>.