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Trimethylchitosan hydrochloride obtained from lobster carapace chitin on a bench scale

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ABSTRACT

Starting from the chitin obtained from the lobster carapace, chitosan was obtained. This polymer, due to its high biodegradability, biocompatibility and mucoadhesivity, has a wide use in the pharmaceutical and food industry. Currently, it has great importance in the development of biomaterials for the regeneration of bone and cartilaginous tissues. However, its applications are limited by the insolubility at pH greater than 6. To increase its solubility, different methodologies have been reported. The intensive methylation of the chitosan has been one of the methodologies that have been studied in the last years, generating the N,N,N-trimethylchitosan. This molecule has the characteristic of having permanent positive charges in the chain as a consequence of the quaternization of the amino groups present in the structure of chitosan. Previous studies showed the influence of the agitation rate and the reaction time on the methylation process of the chitosan, using as an alkylating agent the dimethyl sulfate. This allowed to establish a technological process to transform the chitosan. The aim of this work was to obtain N,N,N-trimethylchitosan chloride from chitosan obtained chitin from lobster carapace bench scale. Infrared spectroscopy, Energy dispersive X-ray spectrometry, Nuclear Magnetic Resonance and Intrinsic viscosity were used to characterize the product obtained. The results of the analysis by Infrared spectroscopy, Energy dispersive X-ray spectrometry and Nuclear Magnetic Resonance showed that the methylation process of the chitosan proposed in this work was effective to obtain the desired product. The degree of quaternization, degree of dimethylation and degree of acetylation were 49.2%, 57.5% and 13.3%, respectively. While the value of the intrinsic viscosity [η] of the sample was 78.5 cm3/g. The results corroborate the possibility of modifying chitosan by applying the methodology proposed in this work.

Keywords: *Chitosan, N,N,N-trimethylchitosan hydrochloride, Chitosan trimethylation process, Degree of quaternization.*

1. INTRODUCTION

Chitosan is a derivative of chitin, a natural polymer present in the exoskeleton of crustaceans such as crabs and lobsters. Hydrolysis in basic medium of chitin causes deacetylation with formation of chitosan. This polymer has a high biodegradability, biocompatibility and mucoadhesiveness, making it a raw material of interest for the pharmaceutical and food industry. Depending on the process of obtaining, the molecular mass of the polymer of chitosan can vary, as well as the degrees of deacetylation or free amino groups, which determine the quality and later use. However, despite the advantages presented by these biopolymers, their applications are affected by the insolubility of these components at a higher pH 6 [1-9].

Chitosan is a polymer composed of monosaccharides linked by glycosidic bonds [10, 11], with three types of reactive functional groups. An amino or acetamido group and two hydroxyl groups in carbons C-2, C-3 and C-6, respectively. These characteristics determine their chemical and biological properties. The main reactions involve the primary amino group, a powerful nucleophile that can undergo N acylation, quaternization, reductive amination and Schiff reactions among others, allowing introducing different functional groups in the molecule and therefore modifying their properties [10-16].

Various alternatives to increase the solubility of chitosan have been studied. One of the main routes studied has been the intensive methylation of the amino groups present in the molecule [6].

Britto *et al*, [17] established a methodology in which dimethyl sulfate is used as an alkylating agent, which is less toxic and less expensive than the other agents described in the literature. Furthermore, when evaluating the influence of temperature on the synthesis they confirm that the reaction yield decreases as the temperature increases. On the other hand, Rodriguez-Chanfrau *et al*, [18] recently published a study on the influence of the stirring rate and reaction time in the synthesis process described by Britto *et al*, [17]. A significant influence of the agitation rate was observed.

The aim of this work was to obtain N,N,N-trimethylchitosan chloride from chitosan obtained chitin from lobster carapace bench scale.

2. MATERIALS AND METHODS

Chitosan supplied by the Centro de Investigación y Desarrollo de Medicamentos (CIDEM, Habana, Cuba) was used.

Synthesis Method.

Chitosan trimethylation process was performed at bank scale (2 L) according to the methodology established by Rodriguez-Chanfrau *et al*, [18]. A glass reactor (stirred tank type) of 5 L capacity with a marine propeller agitator and gas extraction and heating system was used during the synthesis process. The stirring speed was 300 rpm and the agitation time was 2 h.

Characterization of the Sample.

Determination of the yield, viscosity and solubility. The yield was determined by the following expression:

$$\text{Yield (\%)} = \frac{M}{Mo} \times 100$$

Where M is the mass in grams of TMQ and Mo is the mass in grams of chitosan.

Viscosity was determined according to the methodology described by de la Paz *et al*, [19]. A rotary viscometer (Visco STAR PLUS, Spain) was used using the L1 spindle, at a speed of 200 rpm at 25°C. A 1% w/v TMQ solution was prepared in sodium chloride solution (0.2 mol / L). The determinations were made five times.

Solubility was determined according to the methodology described by Jia *et al*, [20]. 0,4 g of TMQ sample was transferred to a test tube and 2,0 mL of deionized water was added slowly. It was observed if a total dissolution of the sample occurs. If not, 2.0 mL more are added slowly and so until the total dissolution. The solubility in water was expressed in g/mL.

FTIR spectroscopy. FTIR spectra of the bacterial cellulose samples were measured on a FTIR - VERTEX 70/ BRUKER spectrometer (Germany). 64 cumulative scans were taken, with a resolution of 4 cm^{-1}, in the frequency range of 4000 to 400 cm^{-1}, in transmission mode.

Energy dispersive X-ray spectrometry (EDS). The determination of the elemental chemical composition of the samples studied was made by means of scanning electron microscopy (FEG-MEV; JEOL 7500F) associated with electron dispersive spectroscopy (EDS) (Thermo Scientific Ultratry). The EDS counting time (Live time) was 100 s per analysis.

Determination of the degree of quaternization (DQ), degree of methylation (DM) and degree of acetylation (DA). The ^{1}H and ^{13}C NMR spectra were acquired using a Bruker/Avance DPX-250F (Germany) device configured with a 5 mm QNP-250 probe. For this analysis, samples were dissolved in deuterated chloroform ($CDCl_3$) at a concentration of 10 g/L at a temperature of 80 °C to ensure maximum resolution during the analysis. The parameters for the acquisition of the NMR spectra were obtained at a frequency of 250.13 MHz, with 32 sweeps, 8k of data acquisition, a spectral width of 1500 Hz, and a pulse of 7 μs (90°). The spectra were processed with the software MestReNova version 6.0.2-5475 with a Fourier transform modulated by an exponential function (LB 0.3Hz). Chemical shifts were recorded on the δ scale (ppm). The signal of Trimethylsilane (TMS) was used as a reference.

DQ, DM and DA applying the methodology described by Kotze *et al*, [21] were determined.

Determination of intrinsic viscosity. Capillary viscometer type Ubbelhode No. 2121R (Bioblock scientific SP), equipped with a thermostatic bath controlled by a water recirculator (Haake, Germany) at 25 ° C was used. An M 5 measuring system with an NV std concentric cylinder sensor, in a speed gradient range between 0 and 500 s^{-1}, at a temperature of 20 ± 0.1ºC was used. The samples were prepared using 0.1 mol / L sodium chloride as solvent, as reported by Britto *et al*, [17]. The initial concentration of the polymer was 9.95 g/L, with four dilutions prepared (7,88; 6,30; 4,33 and 3,19 g/L). A solution of 4% TMQ in 0.1 mol / L sodium chloride solution was prepared, using 9 mL as the test portion. To determine the viscosimetric parameters, the decay time of each of the polymer dilutions (n = 5) was measured. The analysis was performed in triplicate. The intrinsic viscosity was determined using the Huggins equation. The average viscosity molecular weight was calculated [19, 22, 23].

3. RESULTS

A yield of 99.1% was obtained. While the viscosity was 10.3 ± 0.0514 cps and the solubility of 0.0931 g/mL. These results were similar to those obtained in previous works and published by Rodriguez Chanfrau *et al*, [18].

Figure 1 shows the FTIR spectrum of the samples of chitosan and TMQ. The spectrum corresponding to the chitosan sample (a) shows bands at 3371 cm^{-1} and 3292 cm^{-1} representative of the presence of NH and CH groups, respectively. The band that appears at 1649 cm^{-1} shows the presence of amino groups, while in the area between 1500 cm^{-1} and 1000 cm^{-1} there are bands that correspond to vibrations of pyranosic groups (1064 cm^{-1}) and vibrations of C-O-C groups (1026 cm^{-1}), all characteristics of chitosan. These results are similar to those reported by other authors for chitosan samples obtained from chitin from lobster carapace [2, 5].

On the other hand, the spectrum corresponding to the sample of TMQ (b) shows bands at 3426 cm^{-1} (presence of OH groups), band at 1385 cm^{-1} corresponding to the C-H vibration of the methyl groups (not present in the spectrum of the starting chitosan) and a group of bands between 1150 cm^{-1} and 1000 cm^{-1} which are assigned to vibrations (streching) C-C and C-O. The band due to the angular deformation of the N-H in amino groups assigned near 1577 cm^{-1} se reduce, being overlaid by the signal at 1614 cm^{-1}. A small band at 1271 cm^{-1} appears, corresponding to the presence of S-O groups [24], a small trace remaining from the product synthesis process. These results were similar to the results reported by Britto *et al*, [17].

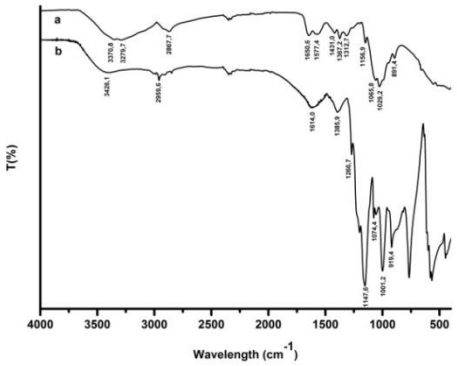

Figure 1. FTIR spectrum of the samples of chitosan (a) and TMQ (b).

SEM-EDS analysis showed the presence of chlorine in the samples, which corroborates the formation of TMQ during the synthesis process (Figure 2). It is also observed the presence of sodium and sulfur, both impurities that were left of the process. Similar results were reported by Campana-Filho *et al*, [25] confirming that the synthesis process occurred under the conditions studied.

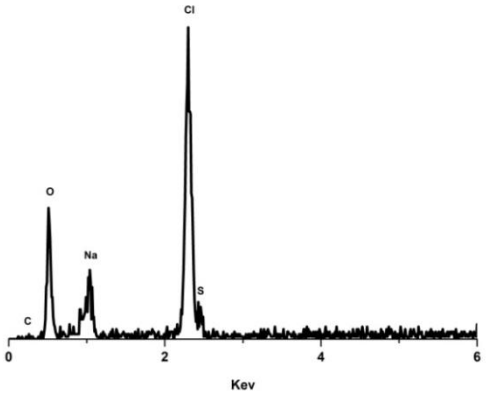

Figure 2. Results of the SEM-EDS analysis.

In the ^{1}H-NMR spectrum (Figure 3), two signals that show the methylation process is observed. One signal with chemical displacement (δ) = 3.53 ppm that integrates for 3H, was assigned to the methoxylate of position C_3 ($N(CH_3)_3$). This signal is directly related to the ocurrence of quartenization, corresponding to the hydrogen of a methyl group bonded to quaternary nitrogen [17].

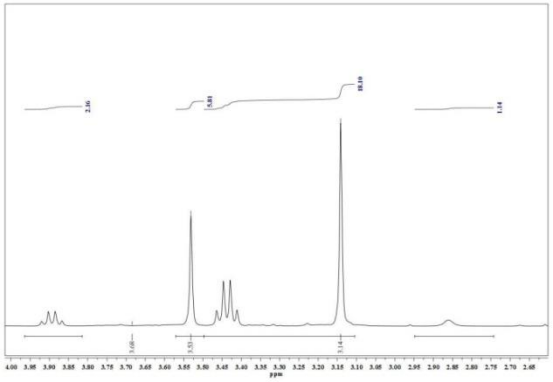

Figure 3. ^{1}H-NMR spectrum.

Another signal with chemical shift (δ) = 3.14 ppm, assigned to methyl groups bound to nitrogen (($N(CH_3)_2$) was observed. We also observe another group of signals between 3.8 < δ < 4.0 that correspond to the rest of the protons present in the molecule.

The ^{13}C-NMR spectrum showed two signals at 57.5 and 49.1 ppm corresponding to methoxy and methyls, respectively (Figure 4).

The degree of acetylation and the degree of substitution of the amino group (quaternization) are the most important characteristics of chitosan and its derivatives. Of all the techniques known for its determination, ^{1}H NMR provides the most accurate results [13].

The DQ (%), DM (%) and DA (%) were determined from the 1H-NMR spectra. The results were 49.2%, 57.5% and 13.3%, respectively. The DQ value obtained is within the mean values reported by different authors [17, 18, 21, 26].

The DM value (%) shows that the quaternization process of the amino groups does not occur completely, so there is the presence of N, N-dimethylated sites in the final product. This corresponds to the results referenced by other authors in the literature [17, 18, 26-28].

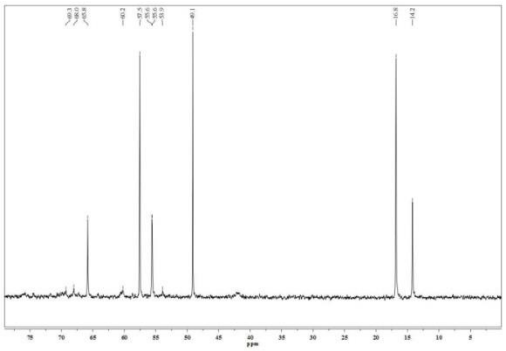

Figure 4. ^{13}C-NMR spectrum.

This result shows that the quaternization did not occur at 100%, which coincides with the results reported by other authors who have applied this synthesis variant [17, 18, 27].

This aspect is very controversial in the literature and several modifications were proposed such as making a greater number of successive alkylations in the multi-stage methods or increasing the reaction time in order to achieve a higher degree of alkylation. This last aspect, according to Torres [27], must be well studied because an increase in the reaction time by applying the Britto [17] methodology could increase the degree of trimethylation but at the cost of a parallel increase in methoxylation of the product. It is therefore undesirable to achieve a process of trimethylation in a shorter time is satisfactory.

On the other hand, Torres [27] in his work suggests that the control of methoxylation is an aspect that should be studied in greater depth, because it will not always be an undesired phenomenon. Previous work published by Rodriguez Chanfrau *et al*, [18] was shown that by increasing the speed of agitation in the synthesis process proposed by Britto *et al*, [17] an adequate trimetilation process was guaranteed, in a shorter time, obtaining a product with a low content of ortho methylation.

The values of DA (%) are considered low. This is an indication that the degree of conversion of chitin to chitosan by deacetylation was satisfactory [5].

The degradation process that occurred during the reaction was evaluated by measuring the intrinsic viscosity (η), since (η) is directly related to the average molar mass [17, 29]. The intrinsic viscosity was calculated using the Mark-Houwink-Sakurada equation.

The value of the intrinsic viscosity [η] of the sample was 78.5 cm^3/g. The average molecular weight of the viscosity of the sample calculated as Mv was approximately 33,546.8 g/mol. This results showed that the intrinsic viscosity [η] of the chitosan sample ([η] = 281.5 Paz *et al*, [5]) after the trimethylation process is reduced by approximately 50%. Britto *et al*, [17]. obtained similar results. These results demonstrate the degradation of the polymer during the quaternization reaction.

4. CONCLUSIONS

FTIR and NMR spectroscopy showed that the synthesis process used at bench scale transforms chitosan into TMQ. The values of degree of quaternization, degree of methylation and degree of acetylation are adequate, as well as the yield achieved. The result of the measurement of intrinsic viscosity confirms polymer degradation during the quaternization reaction.

In conclusion, the results showed that the established synthesis procedure modifies the chitosan from chitin obtained from lobster carapace in N,N,N-trimethylchitosan chloride.

5. REFERENCES

1. Escobar, S.D.M.; Ossa, O.C.P.; Quintana, M.A.; Ospina, W.A. Optimización de un protocolo de extracción de quitina y quitosano desde caparazones de crustáceos. *Scientia et technica* **2013**, *18*, 260-266, http://dx.doi.org/10.22517/23447214.7555.
2. Cervera, M.F.; Heinämäki, J.; Räsänen, M.; Maunu, S.L.; Karjalainen, M.; Acosta, O.N.; Colarte, A.I.; Yliruusi, J. Solid-state characterization of chitosans derived from lobster chitin. *Carbohydrate Polymers*, **2004**, *58*, 401-408, https://doi.org/10.1016/j.carbpol.2004.08.017.
3. Paulino, A.T.; Simionato, J.I.; Garcia, J.C.; Nozaki, J. Characterization of chitosan and chitin produced from silkworm crysalides. *Carbohydrate Polymers*, **2006**, *64*, 98-103, http://dx.doi.org/10.1016/j.carbpol.2005.10.032.
4. Yamaguchi, I.; Itoh, S.; Suzuki, M.; Sakane, M.; Osaka, A.; Tanaka, J. The chitosan prepared from crab tendon I: the characterization and the mechanical properties. *Biomaterials*, **2003**, *24*, 2031-2036, http://dx.doi.org/10.1016/S0142-9612(02)00633-6.
5. de la Paz, N.; Fernández, M.; López, O.D.; Nogueira, A.; García, C.M.; Pérez, D.; Tobella, J.; Montes,Y.; Díaz, D. Optimización del proceso de obtención de quitosano derivada de la quitina de langosta. *Revista Iberoamericana de polímeros*, **2012**, *13*, 103-116
6. Rodríguez, C.J.E.; Rodríguez, R.Z. Aspectos generales relacionados con la síntesis del N,N,N–Cloruro de trimetilquitosano. *Revista Iberoamericana de Polímeros*, **2017**, *18*, 90-97.
7. Mosafer, J.; Sabbaghi, A.H.; Badiee, A.; Dehghan, S.; Tafagho, M. Preparation, characterization and in vivo evaluation of alginate-coated chitosan and trimethylchitosan nanoparticles loaded with PR8 influenza virus for nasal immunization. *Asian Journal of Pharmaceutical Sciences*, **2019**, 14: 216-221, https://doi.org/10.1016/j.ajps.2018.04.005
8. Soubaneh, Y.D.; Ouellet, S.; Dion, C.; Gagnon, J. Formation of highly quaternized *N*,*N*,*N*-trimethylchitosan: a chemoselective methodology in aqueous media. Pure and Applied Chemistry 2019, 91, 489–496, https://doi.org/10.1515/pac-2018-0924.
9. Soares, S.F.; Fernandes, T.; Trindade, T.; Daniel-da-Silva, A.L. Trimethyl Chitosan/Siloxane-Hybrid Coated Fe_3O_4Nanoparticles for the Uptake of Sulfamethoxazole from Water. *Molecules* **2019**, *24*, 1958-1976, https://doi.org/10.3390/molecules24101958.
10. Felipe, L.O.; Rabello, L.A.; Júnior, E.N.O.; Santos, I.J.B. Quitosana: da Química Básica à Bioengenharia. *Quím. nova esc*. **2017**; 39(4): 312-320
11. Bautista-Baños, S.; Ventura-Aguilar, R. I.; Correa-Pacheco, Z.; Corona-Rangel, M.L. Chitosan: a versatile antimicrobial polysaccharide for fruit and vegetables in postharvest– a review. *Revista Chapingo Serie Horticultura*. **2017**; 23(2), 103-121. **doi:** http://dx.doi.org/10.5154/r.rchsh.2016.11.030.
12. Rinaudo, M. Chitin and chitosan: properties and applications. *Progress in polymer science* **2006**, *31*, 603-632, http://dx.doi.org/10.1016/j.progpolymsci.2006.06.001.
13. Dutta, P.K.; Ravikumar, M.N.V.; Dutta, J. Chitin and chitosan for versatile applications. *Journal of Macromolecular Science, Part C: Polymer Reviews* **2002**, *42*, 307-354, http://dx.doi.org/10.1081/MC-120006451.
14. Kulkarni-Abhijeet, D.; Patel-Harun, M.; Surana-Sanjay, J.; Vanjari-Yogesh, H.; Belgamwar-Veena, S.; Pardeshi-Chandrakantsing, V. **N,N,N**-Trimethyl chitosan: An advanced polymer with myriad of opportunities in nanomedicine. *Carbohydrate polymers.* **2017**; 157: 875-902, https://doi.org/10.1016/j.carbpol.2016.10.041.
15. Gupta, M.; Gupta, V.; Gogoi, H.; Bhatnagar, R. Novel application of trimethyl chitosan as an adjuvant in vaccine delivery. *Int J Nanomedicine*. **2018**; 13: 7959-7970, https://doi.org/10.2147/IJN.S165876.
16. Kravanja, G.; Primožič, M.; Knez, Ž. Chitosan-based (Nano)materials for Novel Biomedical Applications. *Molecules*, **2019**; 24(10): 1960, https://doi.org/10.3390/molecules24101960
17. Britto, D.; Assis, O.B. A novel method for obtaining a quaternary salt of chitosan. *Carbohydrate Polymers* **2007**, *69*, 305-310, http://dx.doi.org/10.1016/j.carbpol.2006.10.007.
18. Rodríguez, C.J.E.; Rodríguez, R.Z. Influence of Stirring Rate and Reaction Time on the Chitosan Trimethylation Process. *The Pharmaceutical and Chemical Journal* **2018**, *5*, 84-91.
19. de la Paz, N.; Pérez, D.; Fernández, M.; López, O.D.; Nogueira, A.; Rapado, M.; Altanés, S.; García, C. Evaluación viscosimétrica del quitosano derivado de la quitina de la langosta. *Rev Iberoam Polim.* **2013**, *14*, 84-91
20. Jia, Z.; Shen, D.; Xu, W. Synthesis and antibacterial activities of quaternary ammonium salt of chitosan. *Carbohydrate Research* **2001**, *333*, 1-6, http://dx.doi.org/10.1016/S0008-6215(01)00112-4.
21. Kotzé, A.F.; Lueßen, H.L.; de Leeuw, B.J.; Verhoef, J.C.; Junginger, H.E. Comparison of the effect of different chitosan salts and N-trimethyl chitosan chloride on the permeability of intestinal epithelial cells (Caco-2). *Journal of controlled release* **1998**, *51*, 35-46, https://doi.org/10.1016/S0168-3659(97)00154-5.
22. Paredes-Zaldivar, M.; Altanés-Valentín, S.; Rapado-Paneque, M. Caracterizacion viscosimetrica de quitosana radiodegradada. Revista CENIC Ciencias Químicas. **2005**, *36* (Numero Especial).
23. International Organization for Standarization. Plastics: Determination of the viscosity of polymers in dilute solution using capillary viscometers. Genove. 1998; pp. 1-16.
24. Pereira, L.; Sousa, A.; Coelho, H.; Amado, A.; Ribeiro-Claro, P. Use of FTIR, FT-Raman and ^{13}C-NMR spectroscopy for identification of some seaweed phycocolloids. *Biomolecular Engineering*. **2003**, *20*, 223-228, http://dx.doi.org/10.1016/S1389-0344(03)00058-3.
25. Campana-Filho, S.P.; Britto, D. Estudo das interações entre o complexo polieletrolítico trimetilquitosana/carboximetilcelulose e cu+2, ácido húmico e atrazina em solução aquosa. *Quim Nova*. **2009**, *32*, 1461-1466, http://dx.doi.org/10.1590/S0100-40422009000600019.

26. Mourya, V.K.; Inamdar, N.N. Trimethyl chitosan and its applications in drug delivery. *J Mater Sci*: *Mater Med*. **2009**, *20*, 1057–1079, https://doi.org/10.1007/s10856-008-3659-z.
27. Torres-Vergara, P. Evaluación de la influencia de derivados de quitosano sobre la permeabilidad intestinal de capreomicina sulfato en modelos "in vivo" e "in vitro". Tesis para optar titulo de Quimico Farmaceutico.Universidad de Concepción. Chile. **2006**
28. Curti, E.; Britto, D.; Campana-Filho, S. Methylation of chitosan with iodomethane: Effect of reaction conditions on chemoselectivity and degree of substitution. *Macromolecular Bioscience* **2003**, *3*. 571–576, https://doi.org/10.1002/mabi.200300030.
29. Florence, A.; Attwood, D. *Polymers and Macromolecules. Physicochemical Principles of Pharmacy*, 4ta edition.; Grayslake: Pharmaceutical Press, UK, 2006; pp. 274-327.

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