

New chitosan-imine derivatives: from green chemistry to removal of heavy metals from water

Nuevos derivados imino-quitosano: desde la química verde a la remoción de metales pesados en aguas

Vivian Lizeth Triana-Guzmán¹, Yury Ruiz-Cruz¹, Elkin Libardo Romero-Peñaloza¹, Héctor Fabio Zuluaga-Corrales¹, Manuel Noé Chaur-Valencia^{1*}

¹Departamento de Química, Universidad del Valle. Calle 13 # 100-00. A.A. 25360. Cali, Colombia.

ARTICLE INFO:

Received: September 04, 2017

Accepted: August 14, 2018

AVAILABLE ONLINE:

November 15, 2018

KEYWORDS:

Biopolymers, chitosan, green chemistry, deacetylation degree, water treatment

Biopolímeros, quitosano, química verde, grado de desacetilación, tratamiento de aguas

ABSTRACT: Three novel imine-chitosan derivatives were synthesized by condensation of the amino terminals of chitosan and 4-bromomethyl-2-hydroxybenzaldehyde **A**₁, 4-formyl-2-hydroxybenzoic acid **A**₂, and (E)-6-((2-(pyridin-2-yl)hydrazono)methyl)picolinaldehyde **A**₃. Noteworthy, is the aqueous synthesis of imine-chitosan compounds using relatively mild conditions (70 °C) in a green chemistry fashion. The new compounds were characterized by ¹H-NMR, FT-IR, elemental analysis, thermal gravimetric analysis (TGA), and differential scanning calorimetry (DSC). A solubility study at different pH values was performed for the three compounds, obtaining very different behaviors when compared to that of the pristine chitosan. Finally, by means of atomic absorption the ability to remove heavy metal ions, such as Pb(II) and Hg(II), was investigated for the imine-chitosan derivatives, which showed a high removal percentage at basic pH (between 8-10) but low removal percentage at pH lower than 5. Additionally, the nature of the substituent determines the solubility of the resulting adducts thus widening the potential applications of chitosan derivatives.

RESUMEN: Se sintetizaron tres nuevos derivados imino-quitosano por condensación entre los grupos amino terminales del quitosano y 4-bromometil-2-hidroxibenzaldehído **A**₁, Ácido 4-formil-2-hidroxibenzoico **A**₂, y (E)-6-((2-(piridin-2-il)hidrazono)metil)picolinaldehído **A**₃. Es de destacar en este trabajo que las reacciones fueron llevadas a cabo en medio acuoso empleando condiciones suaves de reacción (70°C). Los nuevos compuestos fueron caracterizados por RMN-¹H, FT-IR, análisis elemental, análisis termo gravimétrico (TGA) y calorimetría diferencial de barrido (DSC). Se realizó un estudio de solubilidad a diferentes valores de pH para los tres compuestos, estableciendo comportamientos diferentes a los obtenidos con el quitosano extraído. Por último, mediante medidas de absorción atómica, se investigó la capacidad de los derivados imino-quitosano en la remoción de iones de metales pesados en agua, tales como Pb(II) y Hg(II), el cual mostró un alto porcentaje de remoción a pH básicos pero uno a pH por debajo de 5. Adicionalmente la naturaleza del sustituyente determinó la solubilidad de los aductos resultantes ampliando así las potenciales aplicaciones de estos derivados de quitosano.

1. Introduction

Chitosan (poli-β-(1,4)-N-acetil-D-glucosamida) is a natural biopolymer which has received great attention due to its wide range of applications such as removal of metal ions in contaminated water, dyes removal from water [1-8] and its versatility to form different types of materials such

as chitosan conjugated DNA nanoparticles, graft copolymerized chitosan, biomaterials and composite scaffolds among others [9-16]. However, the insolubility of this polysaccharide in regular organic solvents and water has encouraged researchers to change its structure to improve its potential use in different fields of interest [17-19]. One of the modifications carried out consists in the reaction between the amino groups of chitosan and aldehyde derivatives to obtain imines by condensation reactions. Such as reactions are interesting not only because they improve the solubility of chitosan by

* Corresponding author: Manuel Noé Chaur Valencia

E-mail: manuel.chaur@correounivalle.edu.co

ISSN 0120-6230

e-ISSN 2422-2844



disrupting the intermolecular hydrogen bonds [19, 20], but also because a versatile group such as an imine provides this polymer with new potential applications [21]. Even though obtaining imine-chitosan derivatives could be challenging due to the acidic aqueous media necessary to dissolve the polymer (which also should not allow the equilibrium to be displaced towards the imine products), several simple and mild aqueous methodologies have been reported allowing this type of reactions to be classified into the green chemistry field [22–24]. Chitosan, in different presentations, has already been tested in metal sorption studies with the purpose of being subsequently used in wastewater treatment with remarkable results [25–29]. Many of these studies have reported that the main center of metal ion interaction are the $-NH_2$ and $-OH$ terminals of chitosan [30–32]; however, different functionalized chitosan species have shown to be very convenient since they stabilize the polymer in acid media making it insoluble and therefore allowing post sorption separation to be easier as they add new groups that can interact with different metals of interest [33–36]. It is worth mentioning that some of these modifications reduce the sorption efficiency because of the loss of $-NH_2$ or $-OH$ groups [37, 38].

On the other hand, compounds such as hydrazones are of interest in supramolecular chemistry since appropriately substituted hydrazones are able of acting as ligands in coordination chemistry [39, 40], finding use as molecular switches [41–46], metallo-assemblies [47–49], among others [50, 51].

Herein we report a water-based synthesis of three novel imine-chitosan derivatives. Such compounds exhibited solubility in different range of pH depending on the type of functionalization. Our results represent a new class of chitosan derivatives that can be used for different applications, for instance, coordination with metal ions for wastewater treatment among others.

2. Experimental

2.1 Materials and equipment

Most reagents were purchased from Sigma Aldrich and used without any further treatment. Glacial acetic acid, methanol and chloroform were purchased from Baker and used without further purification. Mycelia derived from citric acid production were provided by SUCROAL S.A. (Cali, Colombia).

FT-IR spectra were recorded in a Thermo Nicolet 6700 spectrophotometer equipped with an ATR-FTIR accessory. 1H -NMR spectra (in deuterated chloroform and dimethyl sulfoxide) were carried out on a Bruker UltraShield 400

MHz spectrometer. Turbidimetry studies were performed on a UV-vis UV-1700 PharmaSpec spectrophotometer while elemental analysis for C, H and N were carried out with a Thermo FLASH EA 1112 series CHN analyzer. The TGA and DSC studies were performed on a simultaneous Mettler Toledo thermal analyzer, using 5-10 mg of sample of both pristine and functionalized chitosan, varying the temperature from 25°C to 600 °C at a 10°C/min rate.

2.2 Extraction of chitosan

The extraction process was based on a previous study in our research group using the same source of chitosan [52]. *Aspergillus niger mycelium* (100 g) was washed three times with distilled water and dried at 60 °C during 12 hours. Then it was subjected to basic hydrolysis during 4 hours with a NaOH solution (40% w/v) at 100 °C. The obtained beige solid was washed with distilled water until obtaining pH of 7-8. Then, three liters of distilled water were poured over the solid and glacial acetic acid was slowly added while stirring to reach a pH of 3.8. The obtained suspension was then passed through white cambric fabric filter and the filtrate was driven to a 9-10 pH value with addition of a 30% NaOH solution. The beige precipitate was purified twice by dissolution in acetic acid media (pH 3.8) and precipitating with NaOH. Yield: 5.5 %. Elemental analysis: C: 33.52%; N: 6.25%; H: 6.49%.

2.3 Characterization of chitosan

Measure of molecular weight

The molecular weight of chitosan was calculated by gel permeation chromatography (GPC) and intrinsic viscosity measurements. HPLC chromatography and Shodex SB-805 HQ, Shodex SB-806 HQ columns were used for GPC. The calibration curve was prepared with standard pullulan solutions with molecular weight between 1300-22000 g/mol in 0.15 M $NaNO_3$ and 0.5 M $HCOOH$ with a flow of 1 mL/min. Intrinsic viscosity measurements were performed in a Ubbelohde No OC-200 viscometer using the Huggins and Mark-Houwink-Sakurada equations. The viscometer constant, $3.14 \times 10^{-7} cm^4/s^2$, was determined with a 0.2 M NaCl/0.1 M HCl solution.

Characterization of chitosan: Degree of deacetylation (DD) by potentiometric titration

Chitosan (100 mg) was dissolved in a 0.3M HCl solution and titrated with standardized NaOH solution. pH measurements were recorded using a Schottgerate CG832 pH-meter. A curve with two inflexion points was obtained by using the first derivative criteria over the original curve. The amount of acid consumed between these points is the one necessary to neutralize the amino groups in the polymer [53, 54].

¹H-NMR characterization

¹H-NMR spectra of chitosan and its derivatives were obtained, using DMSO-*d*₆/CF₃COOD and D₂O/CF₃COOD (3:1). The NMR experiments were conducted at 45 °C. NMR signals were assigned according with reported data [55–57]. The degree of deacetylation (DD) was calculated using ¹H-NMR by following the indications on reference [44]. Likewise, the DD was determined by CHN elemental analysis as described by reference [58].

2.4 Synthesis of benzaldehyde derivatives

4-(bromomethyl)-2-hydroxybenzaldehyde, A₁

The salicylaldehyde derivative was synthesized by modification of a reported methodology [59]. Salicylaldehyde (610 mg, 5 mmol) was added stepwise during 30 minutes to a paraformaldehyde solution (500 mg) in concentrated HBr (2 mL). The solution was stirred at room temperature overnight. The obtained precipitate was washed with cold water (5 x 3 mL), filtered and dried to generate a white solid. Yield: 85%. ¹H-NMR (400 MHz, CDCl₃) δ (ppm): 11.10 (s, 1H, OH), 9.92 (s, 1H, CHO), 7.63 – 7.58 (mult. 2H, H-3 and H-4), 7.02 (d, 1H, H-6), 4.53 (s, 2H, CH₂). ¹³C-NMR (100 MHz, CDCl₃) δ (ppm): 196.09, 161.61, 137.74, 133.94, 129.53, 120.44, 118.46, and 32.35.

4-formyl-3-hydroxybenzoic acid, A₂

4-formyl-3-hydroxybenzoic acid was synthesized according to a reported methodology [60]. NaOH (20 g, 0.5 mol) were dissolved in 40 mL of water; 4-hydroxybenzoic acid (10 g, 72.4 mmol) in 10 mL of MeOH was added. Then, 20 mL of chloroform were added dropwise at 60 °C; this temperature was maintained during 10 hours with stirring. The resulting solution was cooled with ice and filtered to obtain a yellow solid. Then, the solid was dissolved in a concentrated ammonium hydroxide solution and precipitated with a 15% CuSO₄ solution, yielding a green solid. This solid was dissolved in hot sulfuric acid and then cooled to obtain a yellow solid. Yield: 60%. ¹H-NMR (400 MHz, DMSO-*d*₆) δ (ppm): 12.8 (s, 1H, COOH), 11.52 (s, 1H, OH), 10.31 (s, 1H, CHO), 8.24 (d, 1H, *J* = 2.3, H-6), 8.04 (dd, 1H, *J* = 8.7, H-2), 7.08 (d, 1H, *J* = 8.8, H-5). ¹³C-NMR (100 MHz, DMSO-*d*₆) δ (ppm): 191.08, 166.87, 164.52, 137.28, 131.21, 122.52, 122.42, and 117.99.

(E)-6-((2-(pyridin-2-yl)hydrazono)methyl)picolinaldehyde, A₃

This compound was prepared following a previous methodology reported by our group [49]. 2,6-pyridinedimethanol (200 mg, 1.43 mmol) was dissolved at 40 °C in CHCl₃ and the minimum possible amount of MeOH. Then, activated MnO₂ (625 mg, 7.19 mmol) was

added to the reaction, which was followed by TLC and stopped when dialdehyde started to appear on top of it. The monoaldehyde obtained was purified by column with a 95:5 CHCl₃/MeOH solution getting a 42% yield. The monoaldehyde was condensed with 2-hydrazinopyridine which was added in a 1:1 ratio in CHCl₃ until a pale yellow precipitate was formed; this precipitate was washed with cold ethyl ether until no 2-hydrazinopyridine was observed in TLC revealed with iodide powder, giving the solid in 85% yield. This hydrazone was dissolved in CHCl₃ to be finally oxidized with 4 equivalents of activated MnO₂, purified by column using CHCl₃/MeOH (9:1) as eluent, and obtained a yellow solid. Yield 51%. ¹H-NMR (400 MHz, DMSO-*d*₆) δ (ppm): 11.36 (s, 1H, N-H), 9.97 (s, 1H, CHO), 8.24 (d, *J* = 8.0 Hz, 1H, H-5'), 8.16-8.14 (m, 2H, Himin and H-6), 8.03 (t, *J* = 7.8 Hz, 1H, H-4'), 7.82 (d, *J* = 7.5 Hz, 1H, H-3'), 7.69 (d, *J* = 8.7, 1H, H-4), 7.34 (d, *J* = 8.4 Hz, 1H, H-3), 6.86 – 6.81 (m, 1H, H-5). ¹³C-NMR (100 MHz, DMSO-*d*₆) δ ppm: 193.9, 156.9, 155.6, 152.5, 148.3, 138.6, 138.4, 138.3, 123.7, 121.3, 116.1, 107.2.

2.5 Synthesis of imine-chitosan derivatives, QA₁ - QA₃

The imine-chitosan derivatives were prepared by the solid-state methodology previously reported [61–63]. Chitosan (200 mg) was added to a 1 mL solution of 2.0% of acetic acid in methanol, then it was added dropwise an aqueous saturated solution of A₁-A₃. Then, the resulting mixture was heated at 70 °C. Reactions were monitored via ATR-FTIR by periodically evaporating the water of aliquots. After reaching equilibrium, the reaction mixture was cooled and the solvent was evaporated slowly. The resulting solids were washed with water, methanol, chloroform, hexanes, and ethyl ether until TLC did not show any non-reacting aldehyde derivatives. Finally, the solids were vacuum dried for 24 h.

2.6 Estimation of solubility at different pH values

Water solubility pH-dependence of extracted chitosan and imine-chitosan derivatives was estimated by turbidimetry following an already established methodology [56]. Therefore, solutions of the polymers were prepared in 1.0% acetic acid and a concentrated NaOH solution was added stepwise while measuring the transmittance at each previously determined pH. The λ used was 750 nm.

2.7 Heavy metals removing study. Pb(II) and Hg(II)

Standard solutions of Pb (100ppm) and Hg (1000 ppb) were prepared. These were diluted in order to obtain a calibration curve for Pb(II) (from 2-10 ppm) and for

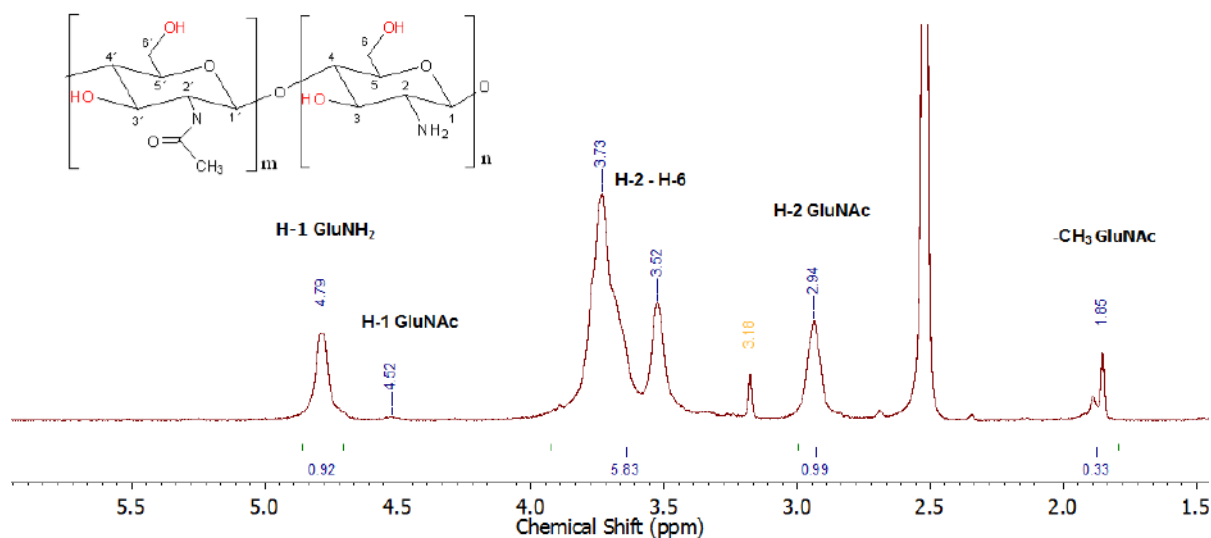


Figure 1 $^1\text{H-NMR}$ spectrum (400 MHz) of isolated chitosan in $\text{CF}_3\text{COOD/DMSO-d}_6$ (1:3) at 45°C

Hg(II) (from 6–30 ppb). Samples doped with Pb (10 ppm) were treated with 2–4 mg of the absorber (chitosan or imine-chitosane derivative) in aqueous media adjusting the pH to 4.0 with diluted acetic acid and other sample with diluted NaOH to adjust the pH to 10. The solutions were stirred during 4 hours, then filtered and the filtrate diluted to 25 mL. The same procedure was used to the samples doped with Hg . Pb and Hg were measured by atomic absorption spectroscopy with the flame technique for Pb and by hydrides generation for Hg . The amount of Pb(II) and Hg(III) absorbed in mg per mg of polymer (Q_t) was determined by Equation (1) [64]:

$$Q_t = \frac{(C_0 - C_t) V}{W} \quad (1)$$

C_0 and C_t are the initial and final concentration (mg/L), respectively. W is the amount of imine-chitosan derivative, and V is the volume of the solution.

3. Results and discussion

3.1 Extraction and characterization of chitosan

The extracted chitosan showed a viscosity average molecular weight of 6.49×10^6 g/mol. This high molecular weight should make the polymer very insoluble, but the degree of deacetylation was high enough to help chitosan protonation in many sites thus improving its solubility in acidic media. The degree of deacetylation (DD) was determined by potentiometric titration and $^1\text{H NMR}$ (see Table 1) getting similar results. The DD by $^1\text{H NMR}$ was determined by the $\text{H}_1(\text{GluNAC})\text{-H}_1(\text{GluNH}_2)$ integration set as previously proposed [56, 57]. In which, a DD of 84.74% was obtained (see supplementary information).

Assignment of the signals was performed using previous reports (See Figure 1) [57–65]. An acceptable agreement between data obtained by potentiometry and NMR was observed, selecting the DD determined by $^1\text{H-NMR}$ as the most reliable [54]. In addition, the DD can be also calculated by CHN elemental analysis, based on the methodology reported in reference [58] we obtained a DD of 87.27 % which is in good agreement with the potentiometric titration and NMR determinations.

Table 1 Degree of deacetylation of chitosan determined by potentiometric titration, $^1\text{H NMR}$ and CHN

Technique	DD [%]
Titration	88
$^1\text{H-NMR}$	85
CHN	87

3.2 Synthesis and characterization of chitosan-imine derivatives

After characterization of chitosan, the imine-chitosan derivatives were synthesized in a green chemistry fashion by the reaction between extracted chitosan and aldehyde derivatives previously synthesized (See Figure 2 and supporting information). With this purpose, the imine derivatives were prepared without the use of organic solvents, just needing a very small amount of methanol. Noteworthy, aldehyde derivatives **A**₁ and **A**₂ possess a hydroxyl group in the orto position (with respect to the aldehyde moiety). This -OH group can allow the formation of an intramolecular hydrogen bond upon imine formation and therefore stabilizing the product. Evidence for imine-bond formation (-C=N) is provided by the

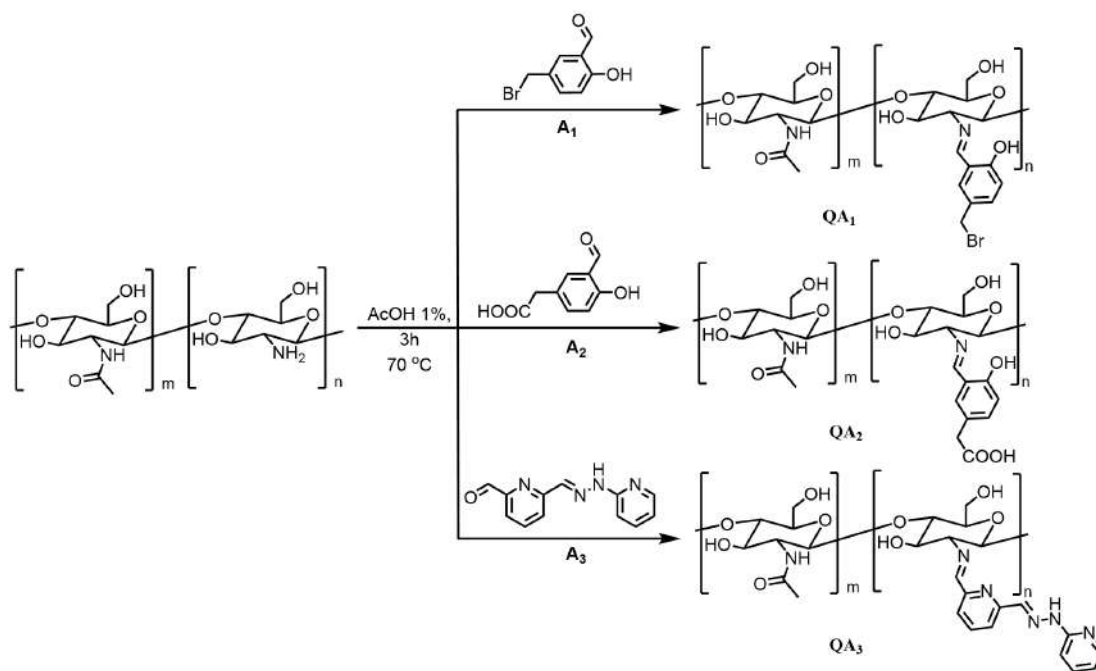
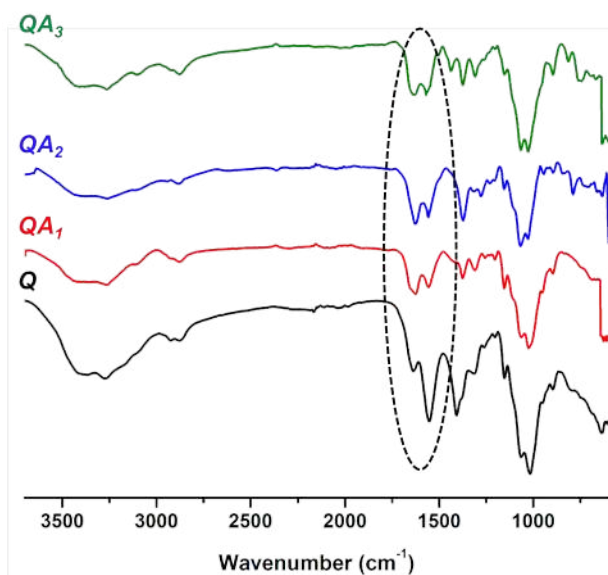


Figure 2 Synthesis of imine derivatives of chitosan

appearance of the imine vibration band between 1630 and 1640 cm^{-1} in the IR spectra for the chitosan derivatives, as well as by the decrease in intensity of the band around 1560 cm^{-1} which corresponds to the $-\text{NH}_2$ group. There is also the disappearance of the aldehyde band around 1650 cm^{-1} which supports the notion of imine-bond formation [63] (See Figure 3); however, it is necessary to be cautious with the assignment of the imine band since the amide band in chitosan is very close to this region and can be overlapped, making the assignment more difficult [61–66]. The $^1\text{H-NMR}$ experiments showed new signals in the aromatic region attributed to the molecules attached to chitosan (see supplementary information).

Thermogravimetric analysis showed that imine-chitosan derivatives are decomposed in two main steps (See Figure 4 and Table 2). The first step starts at 45 and 50 °C, usually attributed to the loss of residual or physically adsorbed water in the polymers [67]. Extracted chitosan showed two events at 80.6 °C and 281.6 °C due to the loss of water and several processes such as dehydration of the glycosidic rings, depolymerization and decomposition of the deacetylated and acetylated units, respectively [18, 68, 69]. Whereas the loss of water for the imine-derivatives occurs between 75°C and 90 °C, the temperature were extracted chitosan loses the most of its mass is higher than that for the derivatives QA₁ and QA₂, but lower than the one for QA₃. In the case of QA₁ and QA₂, both aldehyde derivatives have very polar groups, including carboxyl, amine, and alcohol terminals able to

Figure 3 ATR-FTIR of chitosan (black), QA₁ (red), QA₂ (blue) and QA₃ (green)

form hydrogen bonds and electrostatic interactions in the structure which can lead to more effective cross-linking, increasing the stability of the polymer. This effect has been previously achieved with other types of chitosan derivatives [70]. On the other hand, the lower stability for QA₃ may be due to the inserted substituents that should disorganize the crystalline structure of chitosan, achieved by hydrogen bonds formed between chains of polymer [71–74]. Additionally, the hydrazone group would not be

able to generate enough electrostatic forces to stabilize the obtained polymer.

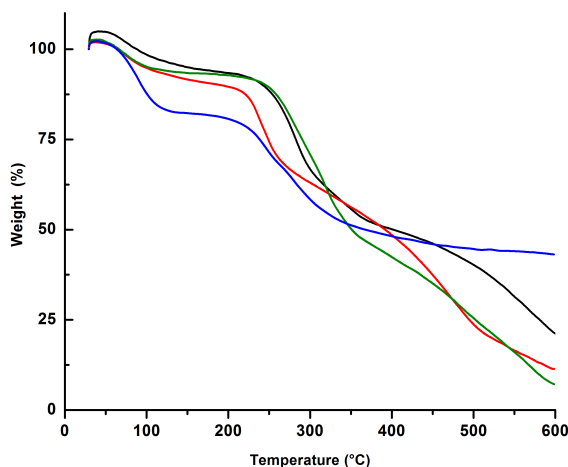


Figure 4 TGA curves of chitosan (black), QA₁ (red), QA₂ (blue), and QA₃ (green). and their corresponding first derivatives (dotted lines)

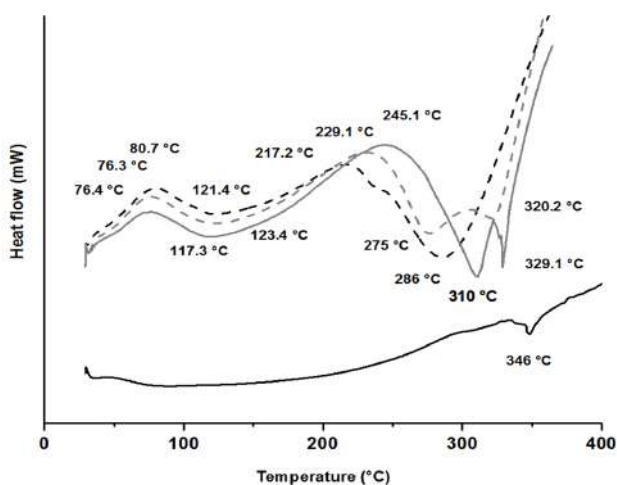


Figure 5 DSC curves for chitosan (black line), QA₁ (Black Dash), QA₂ (Gray Dash), and QA₃ (Gray line)

In the DSC curves, we found important differences when comparing endothermic peaks of chitosan and imine-derivatives (See Figure 5). Previously it has been established that chitosan has two possible polar groups that can associate with water molecules: hydroxyl and amine groups all along the polysaccharide [75]. Since hydrogen bonds, formed with amine groups are weaker than those from with hydroxyl groups, it has also been established that desorption at temperatures lower than 100 °C corresponds to water interacting

Table 2 TG analysis of imine-chitosan derivatives

Compound	T (°C)	Weight loss (%)	T(°C)	Weight loss (%)
Chitosan	80.6	6.7	281.6	24.4
QA ₁	73.6	1.5	241.4	21.1
QA ₂	87.8	7.4	248.9	28.3
QA ₃	75.4	1.6	307.5	32.4

with -NH₂ groups, while the imine-derivatives showed desorption at 118 – 124 °C assigned to water bonded to -OH groups [76]. Since the formation of chitosan derivatives occurs between the carbonyl and the -NH₂ groups, there will be less free amino groups able to form hydrogen bonds with water. Decomposition signals after 250 °C agree with observations in the TG analysis, since all the chitosan derivatives showed a lower temperature of decomposition than that for the extracted chitosan due to the functionalization of chitosan with molecules possessing polar groups simultaneously increasing intermolecular forces and decreasing chain stiffness. Thus, the decomposition temperature is lower for the imine-chitosan derivatives than that of chitosan [77]. Moreover, the glass transition temperature (T_g) for all the imine-chitosan derivatives compared with the pristine chitosan suggested an increase in the flexibility due to the addition of those aldehyde derivatives to the chain of the polymer.

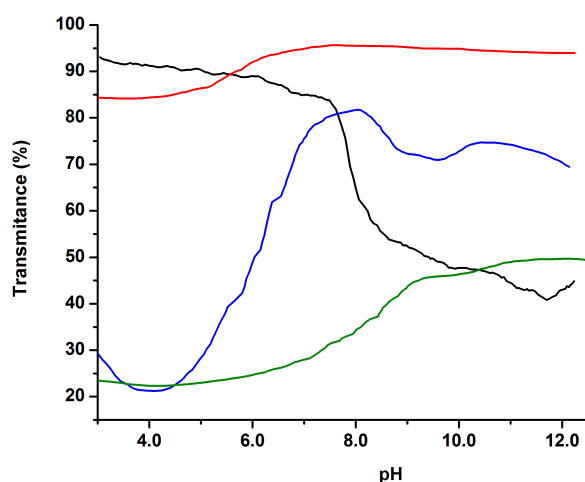
3.3 pH dependence studies of chitosan-imine derivatives

A pH-dependence study on water solubility was carried out since the presence of new functional groups should restrict or enhance solubility at different pH values. Figure 6 shows the solubility change tendencies when going from pristine chitosan to imine-derivatives. QA₁ dissolves well in most of the pH range, explained by the presence of both, non-functionalized -NH and hydroxyphenyl groups. For QA₂ and QA₃, both of which have strong acidic groups, an increase in the solubility on basic media is evident. Since one of the interests of functionalizing chitosan for wastewater applications is allowing the polymer to stabilize (stay insoluble) in a wider range of pH values [28, 76, 78, 79], the result obtained for QA₃ is quite interesting, since only at pH values higher than 9 it starts to significantly dissolve. Noteworthy, functionalization of chitosan modulates its solubility in water; therefore, QA₂ and QA₃ are potential candidates for other applications in pH's where chitosan lacks of solubility. For instance, if dealing with water waste treatment these derivatives can be used at different pH's depending on the type of waste.

Table 3 Removal results of Pb and Hg

Imine-chitosan	pH 4 for Pb ²⁺				pH 10 for Pb ²⁺			
	Weight(mg)	Abs	Ct(ppm)	Q _t (mg/g)	Weight(mg)	Abs	Ct(ppm)	Q _t (mg/g)
QA ₁	2.00	0.285	8.94	13.3	1.80	0.091	2.57	103.2
	2.50	0.271	8.48	15.2	1.80	0.094	2.67	101.8
QA ₂	2.50	0.281	8.81	11.9	1.90	0.114	3.33	87.8
	2.30	0.277	8.68	14.3	2.00	0.085	2.37	95.3
QA ₃	2.90	0.226	7.00	25.8	1.90	0.081	2.24	102.1
	2.90	0.253	7.89	18.2	1.80	0.083	2.31	106.8
Chitosan	4.10	0.080	2.21	59.6	4.00	0.300	9.41	16.2

Imine-chitosan	pH 4 for Hg ²⁺				pH 10 for Hg ²⁺			
	Weight(mg)	Abs	Ct(ppm)	Q _t (mg/g)	Weight(mg)	Abs	Ct(ppm)	Q _t (mg/g)
QA ₁	3.00	0.018	7.09	0.02	2.90	0.040	12.30	-0.02
	3.10	0.022	8.04	0.02	3.00	0.015	6.38	0.03
QA ₂	3.70	0.019	7.33	0.02	2.70	0.045	13.49	-0.03
	3.72	0.020	7.56	0.02	2.90	0.023	8.27	0.02
QA ₃	3.50	0.008	4.72	0.04	3.50	0.036	11.36	-0.01
	3.60	0.005	4.01	0.04	3.60	0.022	8.04	0.02
Chitosan	5.10	0.027	7.09	0.01	3.50	0.040	12.30	-0.02

**Figure 6** Solubility of chitosan and its derivatives. Chitosan (black line), QA₁ (Black Dash), QA₂ (Gray Dash), and QA₃ (Gray line)

3.4 Metal Removal studies of chitosan-imine derivatives

The possible use of imine-chitosan derivatives for metal removal from contaminated water was evaluated by atomic absorption spectroscopy (see experimental section). As it is shown in Table 3, the solution pH controls the ability of metal removal. To pH 10 the imine-chitosan derivatives QA₁ and QA₂ are soluble and interact with the Pb(II) ions since the carboxyl and hydroxyl groups are deprotonated, however when the pH is acid the ability to absorb decreases due to the competition for the active

sites between hydrogen ions in the media and Pb(II) ions [80]. On the other hand, the presence of hydrazoneyl groups on imine-chitosan derivatives QA₃ allows coordination of metal ions showing interesting results. In the case of Hg(II) the results established the sensibility of the method is not high enough to determine concentration ca 10 ppb (See Table 2).

As it was mentioned before, pristine chitosan exhibits a larger Pb(II) removal in acid media while imine-chitosan derivatives a superior in basic pH's. These results are of great importance since leads to the potential use of chitosan for treatment of contaminated water and therefore it deserves a further analysis as it is going on in our research group.

4. Conclusions

Functionalization of chitosan was achieved by the formation of Schiff bases between the free amine groups of the chitosan and three selected aldehydes. These compounds were characterized by ¹H-NMR, elemental analysis, potentiometric methods, FT-IR, TGA, and DSC. Furthermore, the poor solubility of chitosan in basic media was overcome by functionalization, finding higher solubility for the compound bearing a carboxylic group. Likewise, the new compounds exhibit larger capacities for the removal of Pb (II) ions in solution at basic pH's.

5. Acknowledgments

Authors are grateful to the Vicerrectoría de Investigaciones and the Centro de Excelencia en Nuevos Materiales (CENM) from the Universidad del Valle (Colombia) for the financial support of this project. The authors are also grateful with the Departamento Administrativo de Ciencia, Tecnología e Innovación (COLCIENCIAS) for their financial support. Finally, the authors acknowledge the tecnoparque SENA nodo Cali for the TGA analysis.

References

- [1] T. R. A. Sobahi, M. Y. Abdelaal, and M. S. I. Makki, "Chemical modification of chitosan for metal ion removal," *Arabian Journal of Chemistry*, vol. 7, no. 5, pp. 741–746, Nov. 2014.
- [2] Z. Karim, A. P. Mathew, M. Grahn, J. Mouzon, and K. Oksman, "Nanoporous membranes with cellulose nanocrystals as functional entity in chitosan: Removal of dyes from water," *Carbohydrate Polymers*, vol. 112, pp. 668–676, Nov. 2014.
- [3] T. Urugami, T. Saito, and T. Miyata, "Pervaporative dehydration characteristics of an ethanol/water azeotrope through various chitosan membranes," *Carbohydrate Polymers*, vol. 120, pp. 1–6, Apr. 2015.
- [4] W. Zhang, J. Zhang, Q. Jiang, and W. Xia, "The hypolipidemic activity of chitosan nanopowder prepared by ultrafine milling," *Carbohydrate Polymers*, vol. 95, no. 1, pp. 487–491, Jun. 2013.
- [5] P. Chantarasatoporn and *et al.*, "Water-based oligochitosan and nanowhisker chitosan as potential food preservatives for shelf-life extension of minced pork," *Food Chemistry*, vol. 159, pp. 463–470, Sep. 2014.
- [6] E. P. Minet and *et al.*, "Slow delivery of a nitrification inhibitor (dicyandiamide) to soil using a biodegradable hydrogel of chitosan," *Chemosphere*, vol. 93, no. 11, pp. 2854–2858, Nov. 2013.
- [7] J. Pérez and *et al.*, "N,O6-partially acetylated chitosan nanoparticles hydrophobically-modified for controlled release of steroids and vitamin E," *Carbohydrate Polymers*, vol. 91, no. 1, pp. 143–151, Jan. 2013.
- [8] N. Mati and *et al.*, "Chitosan as an adhesive," *European Polymer Journal*, vol. 60, pp. 198–212, Nov. 2014.
- [9] R. Jayakumar and *et al.*, "Chitosan conjugated DNA nanoparticles in gene therapy," *Carbohydrate Polymers*, vol. 79, no. 1, pp. 1–8, Jan. 2010.
- [10] R. Jayakumar, M. Prabaharan, R. L. Reis, and J. F. Mano, "Graft copolymerized chitosan—present status and applications," *Carbohydrate Polymers*, vol. 62, no. 2, pp. 142–158, Nov. 2005.
- [11] R. Jayakumar, N. Nwe, S. Tokura, and H. Tamura, "Sulfated chitin and chitosan as novel biomaterials," *International Journal of Biological Macromolecules*, vol. 40, no. 3, pp. 175–181, Feb. 2007.
- [12] Y. Wang, H. Chen, J. Wang, and L. Xing, "Preparation of active corn peptides from zein through double enzymes immobilized with calcium alginate–chitosan beads," *Process Biochemistry*, vol. 49, no. 10, pp. 1682–1690, Oct. 2014.
- [13] A. Anitha and *et al.*, "Synthesis, characterization, cytotoxicity and antibacterial studies of chitosan, O-carboxymethyl and N,O-carboxymethyl chitosan nanoparticles," *Carbohydrate Polymers*, vol. 78, no. 4, pp. 672–677, Nov. 2009.
- [14] M. Peter and *et al.*, "Novel biodegradable chitosan–gelatin/nano-bioactive glass ceramic composite scaffolds for alveolar bone tissue engineering," *Chemical Engineering Journal*, vol. 158, no. 2, pp. 353–361, Apr. 2010.
- [15] T. Jiang, R. James, S. G. Kumbar, and C. T. Laurencin, "Chitosan as a biomaterial: Structure, properties, and applications in tissue engineering and drug delivery," in *Natural and Synthetic Biomedical Polymers*, S. G. Kumbar, C. T. Laurencin, and M. Deng, Eds. Oxford, England: Elsevier, 2014, pp. 91–113.
- [16] M. Vakili and *et al.*, "Application of chitosan and its derivatives as adsorbents for dye removal from water and wastewater: A review," *Carbohydrate Polymers*, vol. 113, pp. 115–130, Nov. 2014.
- [17] H. Sashiwa and S. I. Aiba, "Chemically modified chitin and chitosan as biomaterials," *Progress in Polymer Science*, vol. 29, no. 9, pp. 887–908, Sep. 2004.
- [18] Y. Xie, X. Liu, and Q. Chen, "Synthesis and characterization of water-soluble chitosan derivate and its antibacterial activity," *Carbohydrate Polymers*, vol. 69, no. 1, pp. 142–147, May 2007.
- [19] G. Q. Ying, W. Y. Xiong, H. Wang, Y. Sun, and H. Z. Liu, "Preparation, water solubility and antioxidant activity of branched-chain chitosan derivatives," *Carbohydrate Polymers*, vol. 83, no. 4, pp. 1787–1796, Feb. 2011.
- [20] S. Nishimura, O. Kohgo, K. Kurita, and H. Kuzuhara, "Chemospecific manipulations of a rigid polysaccharide: syntheses of novel chitosan derivatives with excellent solubility in common organic solvents by regioselective chemical modifications," *Macromolecules*, vol. 24, no. 17, pp. 4745–4748, Aug. 1991.
- [21] B. Shi, Z. Shen, H. Zhang, J. Bi, and S. Dai, "Exploring N-Imidazolyl-O-Carboxymethyl chitosan for high performance gene delivery," *Macromolecules*, vol. 13, no. 1, pp. 146–153, Nov. 2011.
- [22] V. Saggiomo and U. Lüning, "On the formation of imines in water—a comparison," *Tetrahedron Letters*, vol. 50, no. 32, pp. 4663–4665, Aug. 2009.
- [23] C. Godoy, A. K. Yatsimirsky, and J. M. Lehn, "Structure-stability correlations for imine formation in aqueous solution," *Journal of Physical Organic Chemistry*, vol. 18, no. 10, pp. 979–985, Oct. 2005.
- [24] V. K. Rao and *et al.*, "Synthesis of schiff's bases in aqueous medium: a green alternative approach with effective mass yield and high reaction rates," *Green Chemistry Letters and Reviews*, vol. 3, no. 3, pp. 217–223, Oct. 2010.
- [25] V. Nair, A. Panigrahy, and R. Vinu, "Development of novel chitosan–lignin composites for adsorption of dyes and metal ions from wastewater," *Chemical Engineering Journal*, vol. 255, pp. 491–502, Oct. 2014.
- [26] T. Anitha, P. S. Kumar, K. S. Kumar, B. Ramkumar, and S. Ramalingam, "Adsorptive removal of Pb(II) ions from polluted water by newly synthesized chitosan–polyacrylonitrile blend: Equilibrium, kinetic, mechanism and thermodynamic approach," *Process Safety and Environmental Protection*, vol. 98, pp. 187–197, Nov. 2015.
- [27] V. Mohanasrinivasan and *et al.*, "Studies on heavy metal removal efficiency and antibacterial activity of chitosan prepared from shrimp shell waste," *3 Biotech*, vol. 4, no. 2, pp. 167–175, Apr. 2014.
- [28] A. Maleki, E. Pajootan, and B. Hayati, "Ethyl acrylate grafted chitosan for heavy metal removal from wastewater: Equilibrium, kinetic and thermodynamic studies," *Journal of the Taiwan Institute of Chemical Engineers*, vol. 51, pp. 127–134, Jun. 2015.
- [29] Y. Xu and *et al.*, "Preparation and characterization of carboxyl-functionalized chitosan magnetic microspheres and submicrospheres for Pb²⁺ removal," *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, vol. 482, pp. 353–364, Oct. 2015.
- [30] I. M. N. Vold, K. M. Vårum, E. Guibal, and O. Smidsrød, "Binding of ions to chitosan—selectivity studies," *Carbohydrate Polymers*, vol. 54, no. 4, pp. 471–477, Dec. 2003.
- [31] E. Guibal, "Interactions of metal ions with chitosan-based sorbents: a review," *Separation and Purification Technology*, vol. 38, no. 1, pp. 43–74, Jul. 2004.
- [32] W. S. Wan, L. C. Teong, and M. A. K. M. Hanafiah, "Adsorption of dyes and heavy metal ions by chitosan composites: A review," *Carbohydrate Polymers*, vol. 83, no. 4, pp. 1446–1456, Feb. 2011.
- [33] M. Ruiz, A. M. Sastre, and E. Guibal, "Palladium sorption on glutaraldehyde-crosslinked chitosan," *Reactive and Functional Polymers*, vol. 45, no. 3, pp. 155–173, Oct. 2000.
- [34] W. S. Wan, C. S. Endud, and R. Mayanar, "Removal of copper(II) ions from aqueous solution onto chitosan and cross-linked chitosan beads," *Reactive and Functional Polymers*, vol. 50, no. 2, pp. 181–190,

- Jan. 2002.
- [35] A. Ramesh, H. Hasegawa, W. Sugimoto, T. Maki, and K. Ueda, "Adsorption of gold(III), platinum(IV) and palladium(III) onto glycine modified crosslinked chitosan resin," *Bioresource Technology*, vol. 99, no. 9, pp. 3801–3809, Jun. 2008.
- [36] L. Zhou, Y. Wang, Z. Liu, and Q. Huang, "Characteristics of equilibrium, kinetics studies for adsorption of Hg(II), Cu(II), and Ni(II) ions by thiourea-modified magnetic chitosan microspheres," *Journal of Hazardous Materials*, vol. 161, no. 2-3, pp. 995–1002, Jan. 2009.
- [37] L. Martinez and *et al.*, "Cross-linking of chitosan and chitosan/poly(ethylene oxide) beads: A theoretical treatment," *European Journal of Pharmaceutics and Biopharmaceutics*, vol. 67, no. 2, pp. 339–348, Sep. 2007.
- [38] N. Li and R. Bai, "Development of chitosan-based granular adsorbents for enhanced and selective adsorption performance in heavy metal removal," *Water Science and Technology*, vol. 54, no. 10, pp. 103–105, 2006.
- [39] X. Su and I. Aprahamian, "Hydrazone-based switches, metallo-assemblies and sensors," *Chemical Society Reviews*, vol. 43, no. 6, pp. 1963–1981, Jan. 2014.
- [40] L. A. Tatum, X. Su, and I. Aprahamian, "Simple hydrazone building blocks for complicated functional materials," *Accounts of Chemical Research*, vol. 47, no. 7, pp. 2141–2149, Apr. 2014.
- [41] J. M. Lehn, "Conjecture: imines as unidirectional photodriven molecular motors-motional and constitutional dynamic devices," *Chemistry: A European Journal*, vol. 12, no. 23, pp. 5910–5915, Aug. 2006.
- [42] M. N. Chaur, D. Collado, and J. M. Lehn, "Configurational and constitutional information storage: Multiple dynamics in systems based on pyridyl and acyl hydrazones," *Chemistry: A European Journal*, vol. 17, no. 1, pp. 248–258, Jan. 2011.
- [43] E. L. Romero, R. F. D. Vries, F. Zuluaga, and M. N. Chaur, "Multiple dynamics of hydrazone based compounds," *Journal of the Brazilian Chemical Society*, vol. 26, no. 6, pp. 1265–1273, 2015.
- [44] X. Su, T. F. Robbins, and I. Aprahamian, "Switching through coordination-coupled proton transfer," *Angewandte Chemie International Edition*, vol. 50, no. 8, pp. 1841–1844, Feb. 2011.
- [45] S. M. Landge and *et al.*, "Isomerization mechanism in hydrazone-based rotary switches: Lateral shift, rotation, or tautomerization?" *Journal of the American Chemical Society*, vol. 133, no. 25, pp. 9812–9823, May 2011.
- [46] M. L. Croteau, X. Su, D. E. Wilcox, and I. Aprahamian, "Metal coordination and isomerization of a hydrazone switch," *ChemPlusChem*, vol. 79, no. 8, pp. 1214–1224, Aug. 2014.
- [47] C. C. Carmona, I. Y. Váquiro, L. M. Jaramillo, J. M. Lehn, and M. N. Chaur, "Grid-type complexes of m^{2+} (M = Co, Ni, and Zn) with highly soluble bis(hydrazone)thiopyrimidine-based ligands: Spectroscopy and electrochemical properties," *Inorganica Chimica Acta*, vol. 468, pp. 131–139, Nov. 2017.
- [48] S. Ulrich, E. Buhler, and J. M. Lehn, "Reversible constitutional switching between macrocycles and polymers induced by shape change in a dynamic covalent system," *New Journal of Chemistry*, vol. 33, no. 2, pp. 271–292, Jan. 2009.
- [49] E. L. Romero and *et al.*, "New pyrazolino and pyrrolidino[60]fullerenes: the introduction of the hydrazone moiety for the formation of metal complexes," *Journal of Physical Organic Chemistry*, vol. 30, no. 2, p. e3601, Feb. 2017.
- [50] M. A. Gordillo, M. Soto, G. Gutiérrez, R. F. D'Vries, and M. N. Chaur, "Theoretical and experimental comparative study of a derivative from 2-pyridinecarboxaldehyde which exhibits configurational dynamics," *Journal of Molecular Structure*, vol. 1119, pp. 286–295, Sep. 2016.
- [51] M. A. Fernandez, J. C. Barona, D. Polo, and M. N. Chaur, "Photochemical and electrochemical studies on lanthanide complexes of 6-(hydroxymethyl)pyridine-2-carboxaldehyde[2-methyl-pyrimidine-4,6-diy]bis-hydrazone," *Revista Colombiana de Química*, vol. 43, no. 1, pp. 5–11, Jan. 2014.
- [52] G. Muñoz, C. Valencia, N. Valderruten, E. Ruiz, and F. Zuluaga, "Extraction of chitosan from *Aspergillus niger* mycelium and synthesis of hydrogels for controlled release of betahistine," *Reactive and Functional Polymers*, vol. 91-92, pp. 1–10, Jun. 2015.
- [53] L. G. Parada, G. D. Crespín, R. Miranda, and I. Katime, "Caracterización de quitosano por viscosimetría capilar y valoración potenciométrica," *Revista Iberoamericana de Polímeros*, vol. 5, no. 1, pp. 1–6, Mar. 2004.
- [54] N. Balázs and P. Sipos, "Limitations of pH-potentiometric titration for the determination of the degree of deacetylation of chitosan," *Carbohydrate Research*, vol. 342, no. 1, pp. 124–130, Jan. 2007.
- [55] Z. M. dos Santos, A. L. P. F. Caroni, M. R. Pereira, D. R. da Silva, and J. L. C. Fonseca, "Determination of deacetylation degree of chitosan: a comparison between conductometric titration and CHN elemental analysis," *Carbohydrate Research*, vol. 344, no. 18, pp. 2591–2595, Dec. 2009.
- [56] A. Tolaimate and *et al.*, "On the influence of deacetylation process on the physicochemical characteristics of chitosan from squid chitin," *Polymer*, vol. 41, no. 7, pp. 2463–2469, Mar. 2000.
- [57] N. Kubota, N. Tatsumoto, T. Sano, and K. Toya, "A simple preparation of half n-acetylated chitosan highly soluble in water and aqueous organic solvents," *Carbohydrate Research*, vol. 324, no. 4, pp. 268–274, Mar. 2000.
- [58] A. Hirai, H. Odani, and A. Nakajima, "Determination of degree of deacetylation of chitosan by ^1H NMR spectroscopy," *Polymer Bulletin*, vol. 26, no. 1, pp. 87–94, Jul. 1991.
- [59] S. S. Razi, R. Ali, P. Srivastava, and A. Misra, "A selective quinoline-derived fluorescent chemodosimeter to detect cyanide in aqueous medium," *Tetrahedron Letters*, vol. 55, no. 5, pp. 1052–1056, Jan. 2014.
- [60] Z. Zhang and *et al.*, "A novel dinuclear schiff-base copper(III) complex modified electrode for ascorbic acid catalytic oxidation and determination," *Dalton Transactions*, vol. 41, no. 4, pp. 1252–1258, 2012.
- [61] L. Marin and *et al.*, "Imino-chitosan biopolymeric films. obtaining, self-assembling, surface and antimicrobial properties," *Carbohydrate Polymers*, vol. 117, pp. 762–770, Mar. 2015.
- [62] L. Marin and *et al.*, "Antifungal vanillin-imino-chitosan biodynamic films," *Journal of Materials Chemistry B*, vol. 1, no. 27, pp. 3353–3358, May 2013.
- [63] L. Marin, B. Simionescu, and M. Barboiu, "Imino-chitosan biodynamicers," *Chemical Communications*, vol. 48, no. 70, pp. 8778–8780, Jul. 2012.
- [64] A. T. Paulino and *et al.*, "Effect of magnetite on the adsorption behavior of Pb(II), Cd(II), and Cu(II) in chitosan-based hydrogels," *Desalination*, vol. 275, no. 1-3, pp. 187–196, Jul. 2011.
- [65] M. X. Weinhold and *et al.*, "Strategy to improve the characterization of chitosan for sustainable biomedical applications: SAR guided multi-dimensional analysis," *Green Chemistry*, vol. 11, no. 4, pp. 498–509, Feb. 2009.
- [66] J. Brugnerotto and *et al.*, "An infrared investigation in relation with chitin and chitosan characterization," *Polymer*, vol. 421, no. 8, pp. 3569–3580, Apr. 2001.
- [67] M. Jiang and *et al.*, "Preparation and characterization of water-soluble chitosan derivative by michael addition reaction," *International Journal of Biological Macromolecules*, vol. 47, no. 5, pp. 696–699, Dec. 2010.
- [68] M. Bengisu and E. Yilmaz, "Oxidation and pyrolysis of chitosan as a route for carbon fiber derivation," *Carbohydrate Polymers*, vol. 50, no. 2, pp. 165–175, Nov. 2002.
- [69] T. Wanjun, W. Cunxin, and C. Donghua, "Kinetic studies on the pyrolysis of chitin and chitosan," *Polymer Degradation and Stability*, vol. 87, no. 3, pp. 389–394, Mar. 2005.
- [70] A. Pawlak and M. Mucha, "Thermogravimetric and FTIR studies of chitosan blends," *Thermochimica Acta*, vol. 396, no. 1-2, pp. 153–166, Feb. 2003.
- [71] Z. H. Zhang, Z. Han, X. A. Zeng, X. Y. Xiong, and Y. J. Liu, "Enhancing mechanical properties of chitosan films via modification with vanillin," *International Journal of Biological Macromolecules*, vol. 81, pp. 638–643, Nov. 2015.
- [72] L. E. Abugoch, C. Tapia, M. C. Villamán, M. Yazdani, and M. Díaz, "Characterization of quinoa protein-chitosan blend edible films," *Food Hydrocolloids*, vol. 25, no. 5, pp. 879–886, Jul. 2011.

- [73] H. E. Salama, G. R. Saad, and M. W. Sabaa, "Synthesis, characterization and biological activity of schiff bases based on chitosan and arylpyrazole moiety," *International Journal of Biological Macromolecules*, vol. 79, pp. 996–1003, Aug. 2015.
- [74] M. A. Diab, A. Z. El-Sonbati, and D. M. D. Bader, "Thermal stability and degradation of chitosan modified by benzophenone," *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, vol. 79, no. 5, pp. 1057–1062, Sep. 2011.
- [75] D. R. Rueda, T. Secall, and R. K. Bayer, "Differences in the interaction of water with starch and chitosan films as revealed by infrared spectroscopy and differential scanning calorimetry," *Carbohydrate Polymers*, vol. 40, no. 1, pp. 49–56, Sep. 1999.
- [76] C. G. T. Neto and *et al.*, "Thermal analysis of chitosan based networks," *Carbohydrate Polymers*, vol. 62, no. 2, pp. 97–103, Nov. 2005.
- [77] L. S. Guinesi and T. Gomes, "The use of DSC curves to determine the acetylation degree of chitin/chitosan samples," *Thermochimica Acta*, vol. 444, no. 2, pp. 128–133, May 2006.
- [78] G. Crini and P. M. Badot, "Application of chitosan, a natural aminopolysaccharide, for dye removal from aqueous solutions by adsorption processes using batch studies: A review of recent literature," *Progress in Polymer Science*, vol. 33, no. 4, pp. 399–447, Apr. 2008.
- [79] M. S. Chiou, P. Y. Ho, and H. Y. Li, "Adsorption of anionic dyes in acid solutions using chemically cross-linked chitosan beads," *Dyes and Pigments*, vol. 60, no. 1, pp. 69–84, Jan. 2004.
- [80] C. Tejada and Á. Villabona and L. Garcés, "Adsorption of heavy metals in waste water using biological materials," *Tecno Lógicas*, vol. 18, no. 34, pp. 109–123, 2015.