

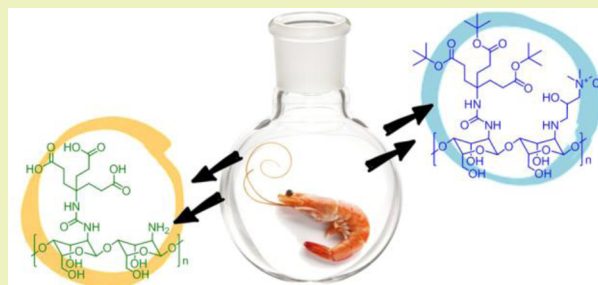
Synthesis and Characterization of Water-Soluble Dendronized Chitosan Using Newkome-Type Dendrons

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ABSTRACT: With increasing emphasis on environmental care and use of renewable resources, simple and effective dendronization processes were performed to obtain chitosan derivatives. We present different alternatives for the modification of chitosan: dendronization with a biocompatible dendron; a quaternization process with an ammonium salt, which confers a permanent positive charge to the polymer; and a combination of methodologies allowing amphiphilic polymers. This process confers unique multivalent properties to the native chitosan, and further modifications reach multivalent water-soluble polymers. Hence, the most important highlight of this work is the use of renewable natural polymers and the different methodologies applied to nontoxic solvents with low temperatures. The process described in this paper represents a sustainable alternative for obtaining a functionalized material with properties suitable for application in different areas, such as biomedicine or environmental remediation.

KEYWORDS: Chitosan, Dendrons, Dendronized polymer, Atom economy, Biopolymer



INTRODUCTION

Polymer functionalization with dendritic structures or a dendronization process is a novel, useful, and interesting path to synthesize highly functionalized and unconventional products. These dendritic moieties, such as dendrimers and dendrons, are highly defined macromolecules, characterized by their precisely branched tree-like architecture with a combination of high end-group functionality with globular or semi-globular shapes in the case of dendrons and compact molecular structure. The use of dendrons implies extending the dendritic concept to the polymer chain, yielding well-defined and highly branched structures attached to the base polymer.¹

Dendritic effect refers to functional group multiplicity, steric shielding or encapsulation effect, and changes in the nature of the internal microenvironment.² In this sense, dendronized polymers are key components with great potential, as they can be efficiently tailored to operate in a wide variety of architectures and with different functionalities, having a great influence on its final properties and applications. This kind of polymer may form interesting materials due to their simple and less time-consuming synthesis methodologies. Macromolecules with high molecular weight can be achieved in fewer steps than the conventional synthesis of dendrimers. However, research on functional dendritic polymers is still a new area, thus their physical–chemical properties and applications have rarely been reported.

Surface groups of dendrons or dendrimers can be used to couple different types of drugs, dyes, or targeting moieties, and also as ligands, as catalyst sites, or with other functionalities easily accessible to the environment.³

These materials have a vast potential in drug delivery systems, directly supplying medication to targeted human organs.^{4,5}

Modification of chitosan is routinely reported these days, but there are not many references about the dendronization process. Also, the mentioned works describe multistep synthesis pathways, usually involving activators, catalysts, and other reagents in order to obtain the dendronized polymer.^{6,7}

In particular, chitosan is a polysaccharide composed of D-glucosamine repeating units, bearing positive charges, with unique physicochemical and biological applications. Chitosan and its derivatives have received increasing attention as biomedical polysaccharides due to the biocompatibility, biodegradability, and nontoxicity of these compounds. However, the low charge density at neutral and basic pH leads to low solubility, aggregation, and poor stability of chitosan-based formulations.^{8–10}

This biopolymer exhibits three attractive reactive sites enabling modification, including a primary amine and two hydroxyl groups per glucosidic unit. The modification of chitosan using cationic moieties has been performed by quaternization or grafting of small molecules or polymer chains onto the chitosan backbone. However, modifications do not imply a change in the fundamental properties of chitosan, but does imply the introduction of new properties, such as being mucoadhesive and permeability enhancing.¹¹

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It is interesting to note that cationic polymers like chitosan, showing amine functions, can be protonated to form electrostatic complexes with anionic biomolecules, such as nucleic acids and proteins.¹² In this sense, they can be excellent candidates for gene or drug delivery, tissue engineering, and therapeutic applications.³

Furthermore, in recent years, chemical production has increased its efforts into environmental protection; consequently, the negative impact of ecological deterioration has been declining gradually. “Greener” materials are more widely used, and chemical research pays more attention to the development of green processes. In this sense, studies on natural polymers such as chitosan, which are considered as waste in some industrial processes, play a central role at this moment.

In this work, we report simple and efficient alternatives for the modification of chitosan: dendronization with a biocompatible dendron;¹³ a quaternization process with an ammonium salt, such as glycidyl trimethylammonium chloride (GTMAC), which confers a permanent positive charge to the polymer; and a combination of both methodologies to form a amphiphilic polymer.

Some simple parameters were calculated in order to evaluate the greenness of the first synthetic step to yield dendronized chitosan.

A Newkome-type dendron and GTMAC were proven as not toxic agents. Also, GTMAC has been used to modify acrylic-grafted chitin to yield materials promoting wound healing.¹⁴

One of our previous works has focused on the functionalization of chitosan in different fields of application. For instance, we have developed dendronized chitosan-based films with ideal properties as wound dressings that are able to maintain a moist environment at the wound interface and act as a barrier to microorganisms, removing excess exudates.¹⁵

EXPERIMENTAL DETAILS

Chemicals. The following chemicals were used as purchased: chitosan low molecular weight (Ch) 85% deacetylated, Aldrich; Weisocyanate (Weis) [ditert-butyl-4-isocyanate-4-[2-(tert-butoxycarbonyl)ethyl]-1,7-heptanedecarboxylate], 98%, Frontier Scientific; glycidyl trimethylammonium chloride (GTMAC) 90%, Aldrich; dibutyltin dilaurate (DBT) 95%, Aldrich; formic acid 85%, Biopack; acetone, Sintorgan; and ethanol, Sintorgan. Dimethylformamide (DMF), Sintorgan, was purified through vacuum distillation and dried under molecular sieves.

Equipment. Fourier transform infrared (FT-IR) spectra were obtained on a Nicolet Avatar 360 FT-IR spectrometer on KBr discs. ¹H nuclear magnetic resonance (NMR) spectra were obtained in D₂O or D₂O/DCl using a Bruker Avance II 400 MHz NMR ultra shield spectrometer. High-resolution ¹³C solid-state NMR spectra were recorded using a CP-MAS pulse sequence (cross-polarization and magic angle spinning) with proton decoupling during acquisition, using a 7T Bruker Avance II-300 spectrometer equipped with a 4 mm MAS probe (Laboratorio Nacional de Investigación y Servicios de Resonancia Magnética en Sólidos, Universidad Nacional de Córdoba). Glycine was used as an external reference for the ¹³C spectra and to set the Hartmann–Hahn matching condition in the cross-polarization experiments. Automatic potentiometric titrations were performed in a Metrohm 905 Titrando with a dosing unit. Differential scanning calorimetry (DSC) measurements were taken using a DSC 2010 differential scanning calorimeter, TA Instruments, (Chemistry Department, Universidad Nacional de Río Cuarto) on samples with a mass of about 5 mg, at a heating rate of 10 °C min⁻¹ and at a testing temperature ranging from 40 to 360 °C.

METHODS

Synthesis of Dendronized Chitosan. Chitosan:Weisocyanate (1:1), (2:1), and (3.5:1) molar ratios (Ch-Weis 1, Ch-Weis 2, and Ch-Weis 3, respectively named) were evaluated in order to perform the synthesis, following the same procedure in all the cases. Table 1 shows the details of each reagent and their ratios used for all the syntheses.

Table 1. Synthesis of Ch-Weis^a

| sample | amount of reagents and solvent used | | | [Ch:Weis] molar ratio ^c | yield (%) | D% (%) |
|-----------|-------------------------------------|-----------|-----------------------|------------------------------------|-----------|--------|
| | Ch (mg) | Weis (mg) | DMF ^b (mL) | | | |
| Ch-Weis 1 | 200 | 527 | 7 [35] | 1:1 | 88 | 11.2 |
| Ch-Weis 2 | 200 | 265 | 5 [25] | 2:1 | 93 | 19.9 |
| Ch-Weis 3 | 500 | 370 | 10 [20] | 3.5:1 | 75 | 11.8 |

^aMolar composition represents the relation between mol of Ch* and mol of dendron used in the reaction mixture. The dedronization degree (D%) was determined by potentiometric titration as the percentage of amino groups of Ch that reacted with Weis. ^bValue [] indicates the amount of solvent (mL) per gram of Ch used in the reaction. ^cMW of Ch was calculated using the following estimation according to 85% deacetylation percent calculated by ¹H NMR: [85%(MW_{D-glucosamine}) + [15%(MW_{D-acetyl glucosamine})].

In all cases, Ch was dispersed in anhydrous DMF, and then Weis dissolved in anhydrous DMF was added to the reaction flask. DBT (2 mol % to Weis) was added as a catalyst. The reaction mixture was kept at 85 °C for 8 h under a nitrogen atmosphere with stirring. After the reaction time, the mixture of the reaction was filtered and washed with acetone. The product was dried at 40 °C to yield a pale tan powder.

Hydrolyzed Ch-Weis (Ch-WeisCOOH). A total of 200 mg of previously synthesized Ch-Weis 3 was dissolved in 15 mL of formic acid (85% V/V). The reaction mixture was kept for 48 h at 45 °C. Afterward, the excess of formic acid and the side product of the hydrolysis were eliminated under vacuum to constant mass. The final product was obtained as a film; it was then frozen and crushed to yield a tan powder (quantitative yield).

Quaternized Dendronized Chitosan (Ch-Weis-GTMAC). A total of 155 mg of Ch-Weis 3 previously synthesized was dispersed in 4 mL of a water:DMF (10:3) solvent mixture with a stirrer. GTMAC (900 μL; 6.03 mmol) was added in three portions (300 μL each) to the reaction mixture at 2 h intervals. After reaction for 7 h at 85 °C, the clear and yellowish reaction solution was poured into cold acetone (10 mL) and kept at -4 °C overnight. Then, the mixture was centrifuged, and the product was redissolved in 1 mL of water (yield 88%).

The solution was precipitated in 10 mL of acetone:ethanol (4:1) and centrifuged to recover the solid. The redissolution in water and precipitation were repeated three times. Finally, the product was dissolved in water (2 mL), and after lyophilization, a pale tan solid product was obtained.

Dendronization Degree Estimation. Different Ch-Weis obtained samples were studied in order to evaluate the dendronization degree (%D) by potentiometric titration of free amino groups in the chitosan backbone. For all experiments, 50 mg of the dendronized chitosan was dispersed in 10 mL of HCl 0.05 M and stirred for 48 h. The resulting acid solution was titrated with NaOH 0.05M using a saturated calomel electrode as the reference electrode.

Green Metrics. Some simple parameters were calculated in order to evaluate the global greenness of chemical reactions used in this work. Using numerical data from the experiment and yields, mass intensity (MI), atom utilization (AU), and atom economy (AE) were calculated following the work of Ribeiro et al.¹⁶

RESULTS AND DISCUSSION

In order to improve the chitosan properties for applications in various fields, the natural polymer was combined with dendritic structures.

Dendronized chitosan was prepared by reaction between native chitosan and a Newkome-type dendron (Weisocyanate)¹⁷ mediated by dibutyltin dilaurate as the catalyst.^{18–20}

Different ratios of polymer:dendron were evaluated, and the amount of solvent was also varied in order to perform the synthesis technique. Table 1 shows different compositions and designations of the products obtained. Yield was determined as the perceptual relation between mol of synthesized product and mol of Ch employed.

As shown in Table 1, the dendronization reaction could be reached in different conditions in a highly effective and reproducible way.

FT-IR was used to confirm the dendron coupling. For the three dendronized products obtained, spectra were identically the same. For these reasons and due to the use of Ch-Weis 3 in the subsequent reactions, these spectra are shown as representative for the following comparison.

Figure 1 shows the FT-IR spectra of Weis, Ch, and Ch-Weis 3. According to the spectra, a new absorption peak at about

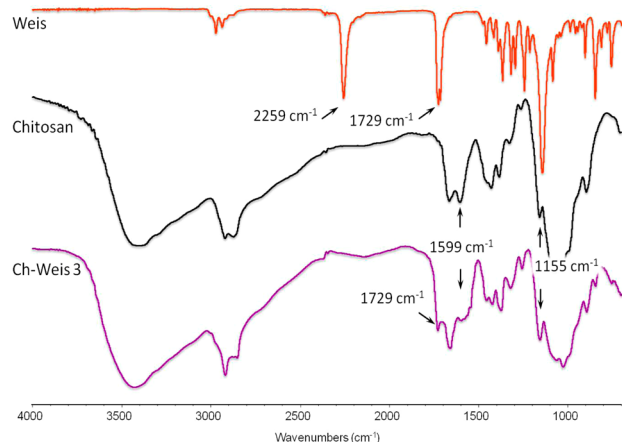


Figure 1. Infrared spectra of Weis (orange), Ch (black), and Ch-Weis 3 (purple).

1729 cm^{-1} can be observed in the spectrum of Ch-Weis 3. This signal can be assigned to the C=O stretching of dendritic esters or urea bond formation due to overlapping of vibrations. Also, the characteristic peak ascribed to the free amino deformation vibration (1599 cm^{-1}) weakened in the Ch-Weis 3 spectrum, and instead, the characteristic peak of primary alcohol of Ch positioned at 1159 cm^{-1} practically did not change, demonstrating the incorporation of the dendron to the biopolymer and that it mainly reached via the reaction of the free amino groups. Complete removal of nonreacted reagents was also evidenced in all cases.²¹

All the dendronized products obtained at this point have good dendronization degrees. The isocyanate focal point of the dendron can either react with the amino or hydroxyl groups of Ch to form a urethane or urea bond, respectively. However, due to evidence presented by FT-IR supporting the much higher reactivity of the amino group than the hydroxyl group reported for this type of reaction,^{22,23} the supposition of controlled modification of amino is valid.

Furthermore, semiquantitative high resolution ^{13}C solid-state NMR spectra were recorded using a CP-MAS pulse sequence (Figure 2). The relative intensity between the signal at 28 ppm (CH₃ of *t*-butyl dendron group) with respect to signals between 100 and 110 ppm (anomeric carbon of glucopyranose

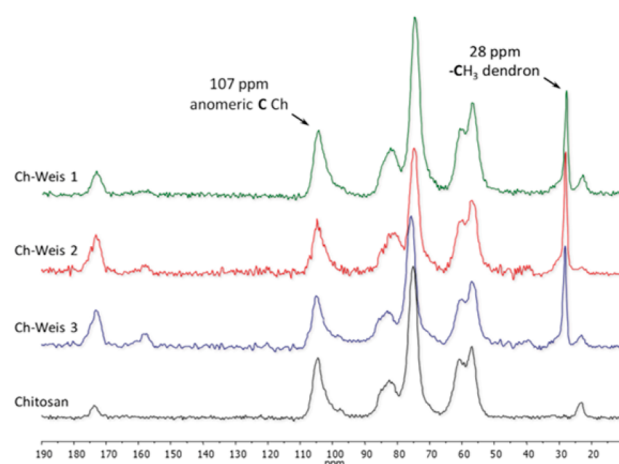


Figure 2. Ch, Ch-Weis 1, Ch-Weis 2, and Ch-Weis 3 CP-MAS spectra.

ring of Ch) was compared for the different spectra. According to these results, Ch-Weis 2 reached a higher dendronization degree, while for Ch-Weis 1 and Ch-Weis 3, this percentage was similar.

Due to the semiquantitative CP-MAS methodology employed, free amino groups could be only quantified by potentiometric titration before and after dendronization (Table 1), reaching almost 20% as the maximum value of the dendronization degree for Ch-Weis 2. The results obtained by ^{13}C solid-state NMR and potentiometric titration allowed for reaching a similar conclusion, showing the same tendency, giving another key for the dendronization degree estimation.

Note that the reaction of Ch-Weis 3, which was carried out with the least amount of solvent and low concentration of dendron, is a very effective pathway to get the desired dendronized product. All the reactions were carried out in a heterogeneous system, so the diffusion capacity of the dendron moiety through the solution and inside of the solid particle of chitosan could depend on the concentration and Ch:dendron ratio. For this reason, it is difficult to predict the dendronization degree in these kind of reactions.

These results represent an improvement in the reaction conditions, giving better dendronization degrees than ones previously reported by our research group, in order of 0.4 to 3.0%.²⁴ Greener conditions were sought in order to produce a reaction with the minimum amount of solvent and with a good atom economy.

The reaction and purification pathways were also performed in order to avoid hazardous solvents, commonly used in this type of experiments.¹⁴ In this particular case, acetone was used as solvent for purification instead chloroform, giving excellent results for that purpose.

Due to the high hydrophobicity reached by the peripheral *t*-butyl ester groups of the dendron, later modification of Ch-Weis was required. In this way, hydrolysis of *t*-butyl ester and the incorporation of GTMAC were carried out to yield water-soluble products. All products obtained were characterized by infrared and nuclear magnetic resonance spectroscopy, calorimetric techniques, and titration of free amino groups.

Scheme 1 shows synthetic routes employed to obtain the final products from native chitosan.

Figure 3 shows comparative spectra of Ch-WeisCOOH and Ch-Weis-GTMAC.

Scheme 1. Synthesis Routes of Modified Chitosan

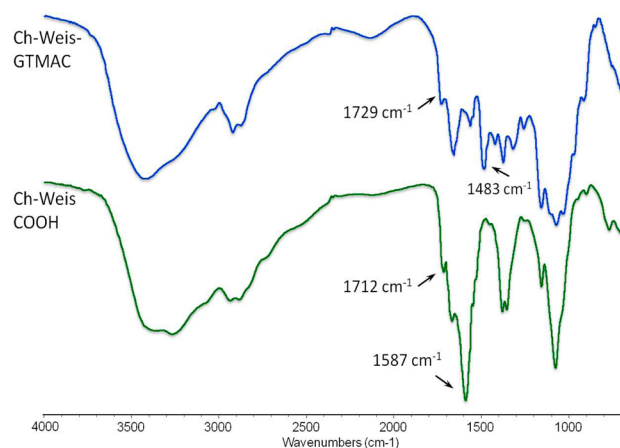
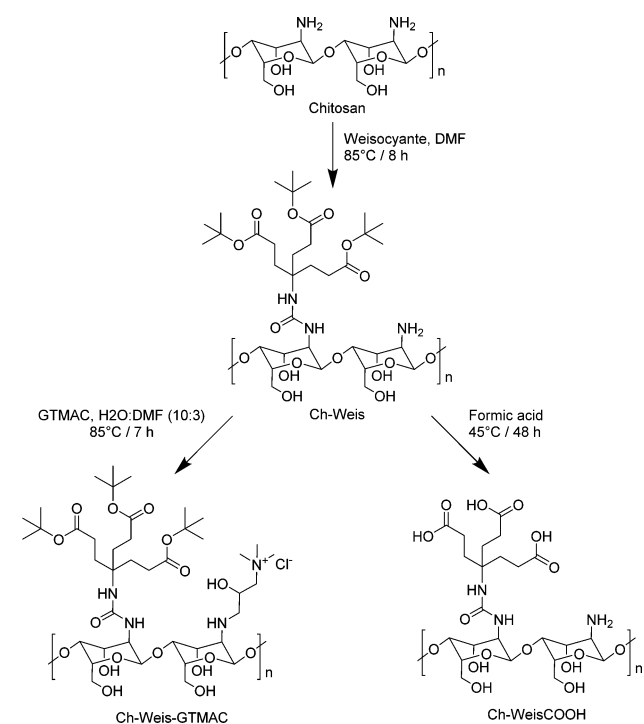


Figure 3. FT-IR spectra of Ch-Weis-GTMAC (blue) and Ch-WeisCOOH (green).

After quaternization (Ch-Weis-GTMAC), the carbonyl signal at 1729 cm⁻¹ did not change, demonstrating the stability of the dendronized chitosan under the reaction conditions. In the spectrum, there was also another new peak around 1483 cm⁻¹, which was assigned to the C–H bending introduced with the methyl groups in the quaternary ammonium salt. Also, the distinctive peak of the free amino deformation vibration of Ch (1599 cm⁻¹) disappeared, indicating complete reaction between almost 100% of these groups and GTMAC, in full concordance to the report by Lou et al.²¹

In the Ch-WeisCOOH spectrum, the carbonyl signal moves at lower wavenumbers, according to the presence of acid groups resulting from the hydrolysis of the peripheral ester groups of the dendron. The broad signal around 1587 cm⁻¹ was assigned to the asymmetric stretching of carboxylate (COO⁻) groups. After hydrolysis, *t*-butyl groups corresponding to the dendron disappear, and the spectra show several changes between 1500

and 1000 cm⁻¹. The C–O stretching vibrations of esters actually consist of two asymmetrical coupled vibrations C–(CO)–O and O–C–C. These bands appear in the region of 1300–1000 cm⁻¹.

Figures 4 and 5 show ¹H NMR spectra of both products, Ch-WeisCOOH and Ch-Weis-GTMAC, respectively. Both spectra

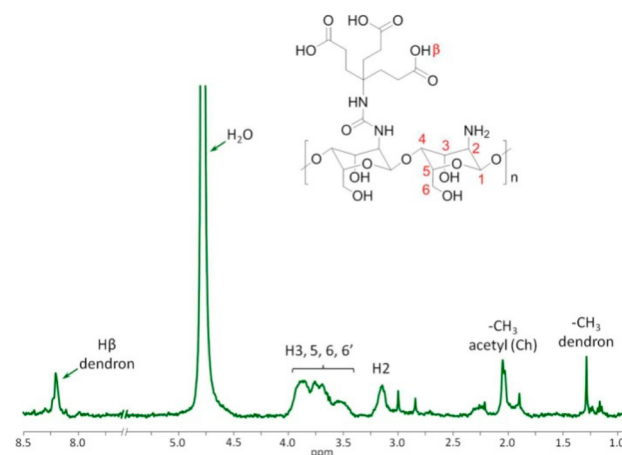


Figure 4. Ch-WeisCOOH ¹H NMR spectrum.

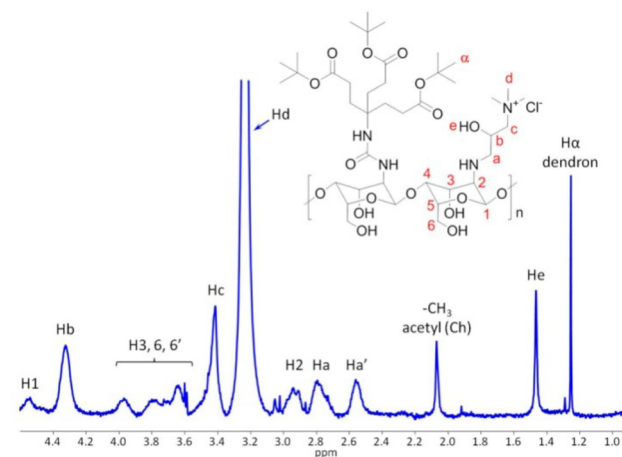


Figure 5. Ch-Weis-GTMAC ¹H NMR spectrum.

show broad characteristic signals of Ch in the range between 2 and 5 ppm.²⁵ In the spectrum corresponding to the hydrolyzed product, the presence of a new signal at 8.20 ppm corresponding to the acid proton (–COOH) of the dendron is observed. This spectrum also reveals a small peak at 1.28 ppm, which was assigned to the methyl groups (–CH₃) of the nonhydrolyzed esters of the dendron (incomplete hydrolysis process).^{22,21,26}

At the Ch-Weis-GTMAC spectrum, characteristic signals of Ch mentioned above was also observed. The appearance of a very strong signal at 3.23 ppm was attributed to the methyl groups of the quaternary ammonium salt. The signal at 1.28 ppm corresponding to the methyl groups of the dendron indicates the presence of the dendritic structure in the polymer.¹⁶

According to the spectroscopic evidence, different products were obtained. The reaction between Ch and Weis was effective (Ch-Weis), and subsequent hydrolysis to yield Ch-WeisCOOH

and the quaternarization with GTMAC to give Ch-Weis-GTMAC were carried out successfully.

It is important to note that Ch-WeisCOOH and Ch-Weis-GTMAC showed good solubility in water solutions. Indeed, Ch-Weis-GTMAC kept an amphiphilic structure capable of being dispersed in aqueous solutions.

Thermal behavior of the samples was analyzed by differential scanning calorimetry (DSC). Ch and the modified products (Ch-Weis, Ch-WeisCOOH, and Ch-Weis-GTMAC) have polar groups; thus, water is adsorbed onto the polymers while hydrogen bonds are formed. A weak exothermic peak in DSC at around 100 °C corresponds to the loss of adsorbed water in all the polymers analyzed. In all the samples, the thermo-oxidative degradation is observed at around 310 °C, although a difference in the position of the exothermic peak of modified chitosan was found. These peaks have been reported as degradations of Ch backbone. In the Ch-WeisCOOH sample, decarboxylation of acids was observed to be around 250 °C.

Green Metrics. In order to compare the different syntheses employed to obtain Ch-Weis, following the work of Ribeiro et al.,¹⁶ several quantitative mass metrics were calculated: E-factor, mass intensity (MI), atom utilization (AU), and atom economy (AE).

These parameters were calculated considering a typical reaction between reactants A and B to yield product P; green metrics were defined as follows in which m_p is the mass of the product, m_A and m_B are the masses of the stoichiometric reagents, m_{aux} is the mass of auxiliary reagents, such as solvents and catalytic reagents, and MW_A , MW_B , and MW_P are the molecular weights of stoichiometric reagents and product.

E-factor. The E-factor is the ratio of the total waste mass to the mass of the product.

$$E = (m_A + m_B + m_{aux} - m_p)/m_p$$

Mass Intensity (MI). Mass intensity is the ratio of the total mass of the reagents to the mass of the product.

$$MI = (m_A + m_B + m_{aux})/m_p$$

Atom Utilization (AU). Atom utilization is the percentage of the mass of the product related to the mass of all the substances produced in the chemical reaction.

$$AU = 100(m_p/(m_A + m_B))$$

Atom Economy (AE). Atom economy is the percentage of the mass of the atoms of stoichiometric reagents that are incorporated in the final product (molecular weight of the product) related to the mass of the total atoms of stoichiometric reagents (sum of the molecular weights of stoichiometric reagents). It was assumed that there were no losses in the process and that all stoichiometric reagents have been converted to product and byproducts.

$$AE = 100(MW_p/(MW_A + MW_B))$$

For calculations of metrics mentioned above, Ch, Weis, DMF, and dibutyltin dilaurate were considered as reactants and auxiliary reagents, while Ch-Weis was considered as a product. For the different Ch-Weis obtained, molecular weights were calculated using the following estimation, according to the 85% deacetylation percent calculated by ¹H NMR and the dendronization percentage (%D) calculated before

$$\begin{aligned} MW_{Ch-Weis} = & [15\%(MW_{D-acetyl\ glucosamine})] \\ & + [D\%(MW_{dendronized\ D-glucosamine})] \\ & + [(85\% - \%D)(MW_{D-glucosamine})] \end{aligned}$$

Table 2 shows the results of these metrics applied to different synthesis routes to obtain Ch-Weis.

Table 2. Green Metrics Applied to Ch-Weis Synthesis

| | E-factor | MI | AU (%) | AE (%) |
|-----------|----------|-------|--------|--------|
| Ch-Weis 1 | 83.25 | 84.25 | 31.33 | 31.42 |
| Ch-Weis 2 | 58.95 | 59.95 | 61.16 | 61.22 |
| Ch-Weis 3 | 67.94 | 68.94 | 56.77 | 56.61 |

E-factor and MI evaluate the mass of waste, which is an important factor for loss of greenness. The results show that the reaction using 2 equiv of Ch with 1 equiv of the dendron to yield Ch-Weis 2 seems to be the greener experiment (lowest E-factor and higher AU). This result is evident if we consider that a higher dendronization percentage was obtained in this case.

In the hydrolysis and quaternization processes shown above, Ch-Weis 3 was used due to its good atom economy and also because the dendronization degree reached in this case (less than in Ch-Weis 2) leads to more reactive free amino groups for the reaction with GTMAC.

CONCLUSIONS

Two different modified biopolymers (Ch-WeisCOOH and Ch-Weis-GTMAC) were obtained in this work in order to overcome the poor water solubility of the native polymer. The precursor of named products (Ch-Weis) was prepared with a very effective and reproducible pathway. Further modification was employed to yield the desired water-soluble dendronized chitosan. In those simple processes, diverse groups could be attached to the polymer with ionizable character or permanent charge to improve the solubility of the final products.

It is a potential method to combine Ch with dendritic structures and obtain interesting materials with considerable importance in many fields because of their biocompatibility, biodegradability, and functionality.

We demonstrated a simple and green method for the preparation of dendronized soluble chitosan by reaction in aqueous solutions.

It should be noted in scientific research that there is a need for the production and design of green chemical materials that use natural resources and waste materials in pollution-free technologies and also to reduce environmental hazards.

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Notes

The authors declare no competing financial interest.

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