THERMAL ANALYSIS OF AMPHIPHILIC QUATERNARY AMMONIUM CHITOSAN DERIVATIVES

Rafael de Oliveira Pedro, Carla C. Schmitt, Éder T. G. Cavalheiro, Miguel G. Neumann *

¹ Instituto de Química de São Carlos, Universidade de São Paulo, Caixa Postal 970, 13560-970, São Carlos SP, Brazil;

*neumann@iqsc.usp.br; mgneumannbr@yahoo.com.br

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Abstract: *The thermal behaviour of amphiphilic systems involving (5-pentyl)trimethylammonium and dodecyl aldehyde-modified chitosans was studied. These amphiphilic chitosan derivatives were synthesized and characterized by ¹HNMR and ATR-FTIR spectroscopies. Thermogravimetry (TGA) and differential scanning calorimetry (DSC) were used to determine their properties. It was found that the modification of the chitosan structure affects directly its thermal properties. The temperature at which water is released from the compounds showed that when adding the hydrophilic substituent water originally bound to an -NH₂ group will bind to a less stronger >NH group requiring lower temperatures to be released. The addition of the hydrophobic C12 substituent confers a more micelle-like structure to the compound, leaving some water molecules within the core of the micelle, making its release more difficult. The decomposition peak observed around 300 °C, corresponding to the decomposition of the chitosan rings, is split in two peaks when adding the substituents, indicating the influence of the substituents on the ring decomposition reactions.*

Keywords: chitosan, amphiphilic derivatives, thermal stability.

Introduction

Chitin is a polysaccharide abundantly found in nature, mostly in the exoskeletons of crustaceans, molluscs and insects, and can be easily converted into chitosan by alkaline N-deacetylation process. Chitin and chitosan are composed of 2-acetamide-2-deoxy-D-glucopyranose and 2-amino-2-deoxy-D-glucopyranose units linked by glycosidic $\beta(1 \rightarrow 4)$ bonds, in variable proportions. Chitin is predominantly composed of acetamide groups whereas chitosan has predominantly amino groups on the C-2 position of the D-glucosamine repeating unit [1]. The ratio of the N-deacetylated units (amino) and acetylated units (N-acetyl) is known as degree of

deacetylation (DD, %) and significantly influences important properties of these polymers, especially chemical, physical and biological characteristics, including biocompatibility [2], biodegradability [3], antibacterial, antiviral and antifungal activity [4,5]. The D-glucosamine group has a pK_a of about 6.2–7.0, therefore, in acidic conditions the amine groups will be positively charged so that chitosan will be water-soluble. This reduces uses of this polymer in neutral pH conditions [6].

In order to improve the properties of chitosan, both amino and hydroxyl groups present in chitosan backbone can be chemically modified in nucleophilic substitution reactions such as

N-alkylation, N-carboxylation [7], crosslinking [8], N-quaternization [9], N-succinylation [10] and N-phthaloylation [11]. Increasing the positive charge density of chitosan backbone by N-quaternization results in a higher solubility of the polymer at higher pH [12]. Amphiphilic derivatives can be obtained by introduction of hydrophobic and hydrophilic groups in the polymer backbone, resulting in self-assembled nanoparticles with a core-shell in aqueous solution. In this sense, amphiphilic chitosan could be used as drug delivery system, as the solubility of hydrophobic molecules is increased, as well as the drug-loading efficiency. Recent studies reported on the use of several amphiphilic chitosans as drug carriers [7,13-17].

Thermoanalytical techniques such as thermogravimetry (TGA) and differential scanning calorimetry (DSC) can be useful to monitor physical and chemical changes in both natural and grafted chitosan [18]. Modification of the chemical structure and composition of chitosan can yield specifics and reproducible patterns in the thermoanalytical curves which can be useful to understand the behaviour of the samples [6,19,20], as well as to characterize them. It is possible to correlate the thermal data for the samples with those obtained by other techniques such as NMR and FTIR [21].

In this paper, an amphiphilic co-polymer was synthesized by grafting (5-bromopentyl)trimethylammonium bromide (BPTA) and dodecyl aldehyde (DDA) at different ratios to the chitosan (CH) backbone. The thermal degradation of the amphiphilic derivatives was investigated using thermogravimetry (TGA) and differential scanning calorimetry (DSC) in order to characterize the effect of the modifications in their thermal behavior and stabilities.

Materials and methods

Materials

Chitosan powder (low molecular weight), deacetylation degree (DD) $\geq 80\%$, 5-Bromopentyl-trimethylammonium bromide (BPTA), dodecyl aldehyde (DDA), sodium hydroxide, sodium acetate, and acetic acid were used as received. All chemicals were from Sigma Aldrich Chemical Co., Germany.

Deacetylation of Chitosan.

Deacetylated chitosan was obtained via deacetylation of a commercial chitosan sample, following a known procedure [22]. Briefly, chitosan (8.0 g, 4.96×10^{-2} mole of monosaccharide units) was dissolved in aqueous acetic acid solution (400 mL, 2 wt %). After complete solubilization, this solution was added drop-wise to aqueous NaOH (200 mL, 50 wt %) at room temperature and refluxed in nitrogen atmosphere at $105\text{ }^{\circ}\text{C}$ for 1.5 h. Afterwards it was poured in preheated water (4 L) at $60\text{ }^{\circ}\text{C}$. The decanted precipitate was washed five times with water and separated by filtration. The solid obtained was dissolved in acetic acid solution (200 mL, 2 wt %) and the procedure was repeated to achieve a higher deacetylation degree. After purification by dialysis against water for 5 days, the polymer was isolated by lyophilization (yield 7.2 g, 90%). The DD of the *N*-deacetylated products calculated from the integrals of the peaks of the ^1H NMR spectra corresponding to the three protons of the *N*-acetyl group at 2.3 ppm and the peak due to the C_2 proton at 5.3 ppm are shown in Table 1 [21].

Syntheses

PTA-modified chitosan. Grafted chitosan was prepared in aqueous NaOH solutions as described earlier [12]. Briefly, previously deacetylated chitosan (6.0 g, 3.72×10^{-2} mole) was added to 20 mL of NaOH 0.1 mol L^{-1} in a round-bottomed reaction flask equipped with a magnetic stirrer. BPTA (8.0 g, 2.77×10^{-2} mole) was subsequently added and stirring was continued for 72 h at $55\text{ }^{\circ}\text{C}$. The mixture was then purified by dialysis (membrane of MWCO

12,000 g mol⁻¹) against water. Afterwards, the product was isolated by and characterized previously by ¹H NMR and ATR-FTIR [21].

Amphiphilic Chitosans. The syntheses of the amphiphilic chitosan derivatives were performed as previously described [21,23]. Briefly, quaternized chitosan was dissolved in acetic acid and added drop-wise to ethanol to obtain a clear solution. The pH of the reaction mixture was adjusted to 5.0 by adding aqueous NaOH (1.0 mol L⁻¹). Afterwards, dodecyl aldehyde (DDA) was added to this solution and stirred for 1 h. Then, the solution was allowed to react for 24 hours with sodium cyanoborohydride. The product was purified by dialysis (membrane of MWCO 12,000 g mol⁻¹), isolated by lyophilization and characterized by ¹H-NMR, and ATR-FTIR.

Characterization methods

Thermogravimetry (TGA). The TGA curves were obtained on a thermogravimetric module SDT Q600 (TA Instruments, USA). The heating rate used was 10 °C min⁻¹ under dynamic nitrogen atmosphere (100 ml min⁻¹), using samples in the 2–5 mg range and temperatures between 25 and 1000 °C, in α -alumina sample holders. TGA curves were used to verify the thermal decomposition profile of the samples. DTG curves were used to accurately determine the temperature corresponding to the release of the moisture contents, the decomposition of the organic matter and the remaining residue.

Differential Scanning Calorimetry (DSC). The differential scanning calorimetry curves (DSC) were recorded on a Q-10 module (TA Instruments, USA). These curves were taken under dynamic nitrogen atmosphere (flow 50 ml min⁻¹) using samples of around 5.0±0.1 mg at a heating rate of 10 °C min⁻¹ under rom pressure. The modulus was calibrated for

heating and temperature with metallic indium (99.9%), according to the manufacturer's instructions.

Results and discussion

The analysed compounds (Figure 1) were deacetylated chitosan, DD ~97% (**II**), the (pentyl)trimethylammonium bromide derivative (CHP₄₀) containing PTA groups (**III**) and the amphiphilic derivatives containing the hydrophilic group PTA (40%) and 5 and 33% of hydrophobic dodecyl groups (CHP₄₀D₅, **IV**) and (CHP₄₀D₃₃, **V**).

Characterization of the amphiphilic derivatives

Deacetylation, quaternization and substitution degrees were calculated from the ¹H NMR spectra using the methods described formerly [12]. The degrees of deacetylation (DD) were calculated from the ¹H NMR spectra using the areas at δ 1.73 ppm (corresponding to acetamidomethyl protons) and the doublet at δ 5.20 ppm (corresponding to resonance of the anomeric proton doublet – H₂). The degree of quaternization (DQ) of the chitosan derivative obtained by the reaction with BPTA was calculated, using the singlet at δ 3.41 ppm and the signal at δ 1.77 ppm, which correspond to the resonance of the trimethylammonium protons of the -CH₂CH₂CH₂CH₂CH₂N⁺(CH₃)₃ substituent (Fig. 1) and the resonance of the methylene protons CH₂CH₂CH₂CH₂CH₂N⁺(CH₃)₃, respectively. The degree of quaternization of CHP₄₀ could be calculated as being 42.2%. The degree of substitution for the derivatives containing the dodecyl group (DDA) was calculated from areas at δ 1.56 ppm, attributed to resonance of the methyl protons. The DDA for CHP₄₀D₅ was 5.2% and for CHP₄₀D₃₃ was 33.8%. The degrees of substitution of the compounds at different sites of chitosan are shown in Table 1 [21].

Table 1. Properties and DS (degree of substitution) of the chitosan derivatives [21].

Sample	DD ^a (%)	DQ ^b (%)	DDA ^c (%)
CH	96.9	-	-
CHP ₄₀	-	42.2	-
CHP ₄₀ D ₅	-	42.2	5.2
CHP ₄₀ D ₃₃	-	42.2	33.8

^a Deacetylation degree^b Degree of hydrophilic substitution^c Degree of hydrophobic substitution

Thermal Analyses of pure chitosan and its derivatives

The TGA/DTG and DTA curves of chitosan and its derivatives in air are presented in Fig. 2. In general, all samples showed dehydration stage, followed by decomposition of the polymer and production of carbonaceous material, followed by its burning. The changes observed in the thermogravimetric profiles were attributed to the presence of the modifiers in the structures. Quantitative data are summarized in Table 2.

Fig 1 Structural formulae of the analysed samples and compounds used in its syntheses.

Fig. 2. (a) TGA/DTA (blue dash) and (b) DTG curves of chitosan and its derivatives. Sample mass = 2 - 5 mg, heating rate = 10 °C min⁻¹, open α -alumina sample holder.

The initial mass loss in TGA curves, up to ~100°C, indicates that the samples contain approximately 10 - 13% of water, involving the various types of water present in chitosan, *i.e.*, adsorbed water and/or water weakly hydrogen-bonded to the polymers [19]. The presence of the substituents decreases the weight proportion of water in the polymer, so that the total of released water will be less when adding hydrophilic and hydrophobic substituents to CH.

The higher temperature required to release total water also increases from CHP₄₀ to CHP₄₀D₃₃. In CH most of the water is H-bonded to the –OH and –NH₂ groups. The incorporation of PTA will transform a significant amount of –NH₂ groups into >NH groups which will reduce the strength of the H-bond, and therefore require lower temperatures for the total release of the bound water. No significant effect will be sensed by the water bound to the hydroxy groups. Thus, it could reasonably be considered that most of the water linked to external

quaternary ammonium groups will be easier to release.

The addition of the hydrophobic C12 chain will induce the formation of micelle-like structures, in which water molecules may be included, implying that the release of the bound water to internal groups will involve also a certain modification of the micellar structure, making the total release of water more difficult and requiring higher temperatures. This is consistent with the fact that for CHP₄₀D₃₃ the total release of water goes up to 166 °C, whereas for CHP₄₀D₅ 153 °C is sufficient.

A decrease of ~45% of the mass is observed in the range of 150 - 400 °C corresponding to the thermal decomposition of the polymer. The mass loss in the first thermal event for CH, CHP₄₀, CHP₄₀D₅ is quite similar, probably because the mol mass of the repeating units in CH is close to that of the modifier. However, after addition of the heavier DDA-group, the first mass loss step rises up to 55%, extending it to 400°C (Table 2).

Table 2. DTG results for the thermal decomposition of the chitosan samples.

Sample	Temperature Interval (°C)	Weight Change (%)	Temperature Interval (°C)	Weight Change [1] (%)	Temperature Interval (°C)	Peak max	Weight Change [2] (%)	Residue (%)	Ratio [1]/[2]
CH	22-193	13.3	193-393	42.6	393-622	300	43.0	1.6	0.99
CHP ₄₀	20-136	12.1	136-286	43.3	286-586	240+270	42.1	2.5	1.03
CHP ₄₀ D ₅	22-153	12.6	153-269	44.0	269-737	260+320	42.7	1.2	1.03
CHP ₄₀ D ₃₃	22-166	10.5	166-403	55.4	403-810	240+320	30.3	3.8	1.83

This fact reveals the influence of the substituent in the decomposition profile of the resulting polymer.

The main reaction of structure cleavage in chitosan is the formation of a radical resulting from the cleavage of the C-NH₂ bond. The formed radicals will induce a chain reaction leading to the splitting of the glucopyranose rings and decomposition of the polymer. This process corresponds to the peaks observed at 300, 240, 270, and 240 °C for CH, CHP₄₀, CHP₄₀D₅, and CHP₄₀D₃₃, respectively. The onset of this step is strongly dependant on the nature of the substituent and extension of the modification performed on the structure. In general, substitution displaces this decomposition to lower temperatures, but increases for higher contents of DDA.

In addition, the bare CH the mass loss seems to take place as a single event. The inclusion of the hydrophilic PTA group leads to a sharp additional exothermic signal in DTA revealing a change on the decomposition mechanism when this group is present. This is corroborated by an additional sharp peak that can also be seen in the corresponding DTG curve (Fig. 2). Probably the initial fraction of the mass loss is due to the reaction in the remaining unmodified chitosan and the sharp signal corresponds to reactions of the hydrophilic PTA group or the chitosan units substituted by it. This effect of the addition of modifiers has been observed earlier by Nieto et al. [13,24] for the addition of PEO to chitosan.

When the longer dodecyl chain is incorporated to the CHP₄₀ system the mass loss observed at the beginning of the decomposition can be assigned to unsubstituted and PTA-substituted chitosan.. This decomposition is followed by a broader signal in the DTG analysis, probably representing the decomposition of the DDA containing segments, as the second signal increases as more DDA is attached to the structure, in agreement with the sharp exothermic peak in the DTA.

The DTG signals between approximately 300 and 600 °C are due to the pyrolysis of the remaining carbonaceous material resulting in vaporization and elimination of volatile products [24]. This process is similar for all the samples, although in CHP₄₀, the process seems to occur at lower temperatures possibly due to the degradation of the residual modifier.

The ratios of mass loss 1 and mass loss 2, in Table 2 reveal that the decomposition of CHP₄₀ and CHP₄₀D₅ occurs in a similar way than bare CH regarding the decomposition its first step followed by pyrolysis of the carbonaceous matter generated in this processes. On the other hand, the first step of decomposition and volatilization of the substituted portion of CHP₄₀D₃₃ clearly generates less carbonaceous matter, corroborating that the modifier is released in the first decomposition step, after dehydration.

The DSC curves of derivatives are shown in Fig. 3. The curves presented a broad endothermic peak centred around 60 °C, attributed to the loss of water adsorbed on the hydrophilic groups of the polymers. The different content of hydrophilic substituent on each derivative promotes shifting of the water loss peak. For example, CH exhibits the peak around 66 °C, whereas the more hydrophilic CHP₄₀ sample shows the peak above 70 °C. This confirms that the interaction of the polymer with water depends directly on the degree of hydrophilicity. This behaviour is similar for the amphiphilic derivatives. The introduction of the hydrophobic dodecyl groups displaces the peak towards lower temperatures,

58.5 °C for CHP₄₀D₃₃ 62.7 °C for CHP₄₀D₅, conforming that a larger amount of hydrophobic groups displaces the peak towards lower temperatures. Thus, DSC results are in agreement with those obtained by NMR [21] and TG measurements concerning the amphiphilic behaviour of the derivatives.

The unexpected peak at *c.a.* 75°C in DSC curve of the CHP₄₀ can be related to the presence of water, or even ethanol, linked to the hydrophilic portion of the PTA modifier or even to residual free BPTA. This possibility can be correlated with the sharp mass loss observed in the DTG curve (Fig. 2b).

Fig. 3. DSC curves of chitosan and its derivatives. Sample mass = 5 mg, heating rate = 10 °C min⁻¹, aluminium sample holder with a central pinhole.

Conclusions

Thermogravimetry (TGA) and differential scanning calorimetry (DSC) showed that the modification of the chitosan structure affects directly its thermal properties.

The temperature at which water is released from the compounds showed that the

substitution of the hydrophilic PTA substituent decreases the temperature necessary to release all the water. This effect is assigned to water originally bound to -NH₂ groups is converted to water bound weaker to >NH groups. The addition of the hydrophobic C12 substituent confers a more micelle-like structure to the compound, leaving some water molecules

within the core of the micelle, making its release more difficult. This effect is consisted with the previous postulation drawn from spectroscopic and imaging observations.

The decomposition peak observed around 300 °C, corresponding to the decomposition of the chitosan rings, is split in two peaks when adding the substituents, indicating the influence of the substituents on the ring decomposition reactions.

From the relationship between the mass losses of the second and third peaks it can be assumed that the mechanism by which the total decomposition of bare CH, CHP₄₀, and CHP₄₀D₅ occur are similar, whereas for CHP₄₀D₃₃ it seems to be different. This difference may be ascribed to the micelle-like structure of this compound, as suggested in a previous report.

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