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Statistical approach to the spectroscopic determination of the deacetylation degree of chitins and chitosans

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ABSTRACT

The objective of the study is to elucidate the effect of reaction temperature and time during heterogeneous alkali reaction on degree of deacetylation (DD) of chitin. Two different analytical methods namely 1st derivative ultraviolet and Fourier transform infrared spectroscopies have been adopted to determine and compare the DD values of the prepared chitin derivates. Statistical analysis indicates that the most significant variable is the temperature followed by the time with slight interdependence between the two. By applying the developed statistical model, using temperature and time as the process variables, chitosan with 80% DD can be obtained in 9 h using 50% (v/v) NaOH and a reaction temperature of 100 °C. Thermal analysis both in air and nitrogen showed that thermodegradation occurs in two steps in the 200–600 °C range. The 1st derivative ultraviolet method has been advocated as a suitable method for the assessment of chitin derivates.

1. Introduction

Chitin a natural polysaccharide extractable from crustaceans and insects is constituted by repeating monomeric units of acetylamino-β-glucopyranose interconnected in 1,4 position by acetal bonds. Chitosan, its N-deacetylated derivative formed by the action of concentrated alkali and high temperature treatment, has received much attention because of its numerous and wide variety of applications ranging from biomedical uses to waste-water treatment and to uses in the fibre industry (Kurita, 2006). The properties of chitin and chitosan depend considerably on the degree of deacetylation (DD), a parameter defined as the mole fraction of deacetylated units in the polymer chain (Rinaudo, Milas, & Ledung, 1993; Wei, Bo, Li, & Wen, 1991). Therefore, the determination of DD has been one of the interesting parameters of study for the routine analyses performed for quality control on chitin and chitosan preparations.

For determination of deacetylation degree, several analytical methods have been employed. Infrared spectroscopy (Domszy & Roberts, 1985; Sannan, Kurita, Ogura, & Iwakura, 1978) and UV-spectrophotometry (Muzzarelli & Rocchetti, 1985) as analytical tools offers advantage over other traditional techniques, since some of the methods are either too tedious, expensive or destructive to the sample.

FTIR spectroscopy is a quick technique for a qualitative evaluation of the DD through the determination of absorption ratios. The use of IR for this purpose requires the construction of a calibration line using ratios of absorbances of a probe band, relative to a reference band. Moreover, FTIR analysis is attractive due to its non-destructive character, fastness, sensitivity and suitability for both soluble and non-soluble samples. Among the solution methods, 1st-derivative UV spectrophotometry draws attention owing to its simplicity and effectiveness in providing accurate results for highly deacetylated chitin. It was conceived by Muzzarelli and Rocchetti (1985) and relies on simple reagents and instrumentation. In addition, the results obtained from this method are reasonably independent of protein contamination. Chitin and chitosan have characteristic degradation temperatures. Alonso, Peniche-Covas, and Nieto (1983) established the possibility to determine the acetylation degree by the use of empirical correlations based on the weight losses associated with the main decomposition peaks. A similar approach has been adopted to investigate if there was any relationship between the weight loss of the sample and its DD.

There are several steps in the manufacturing process of chitosan that depend on the starting material used to extract chitin, shrimps, prawns and crabs wastes are the principal sources of chitin. The last step related to the chitin deacetylation is the focus of the present work. This step can follow a homogeneous or heterogeneous alkali method. In the first one, temperatures are lower than that of heterogeneous deacetylation but requiring long times as for example days. However, the conditions for a heterogeneous deacetylation of chitin generally uses aqueous NaOH solution at concentration

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Table 1
Settings for the 2^2 design with center point.

Variable	Levels[a]		
	−1	0	+1
Time (h)	1	2	3
Temperature (°C)	90	110	130

[a] −1 and +1 are low and high levels and 0 is center point.

range of 40–50 wt%, temperature range between 100 and 150 °C and relatively short reaction times (Kurita, 2006).

The aim of the present study is a statistical approach for the optimization of the chitin deacetylation reaction variables using heterogeneous method.

2. Experimental

2.1. Materials

Chitin from shrimp shells, practical grade powder, was purchased from Sigma (C-7170) [1398-61-4]. Chitosan low molecular weight and DD = 75–85%, was purchased from Aldrich (448869) [9012-76-4]. D-(+)-Glucosamine hydrochloride (GlcN), purity 99%, crystalline (G4875) [66-84-2], N-acetyl-D-glucosamine (GlcNAc), purity 99%, (A8625) [7512-17-6], and potassium bromide (KBr), FTIR grade (221864) [7758-02-3], were purchased from Sigma. Sodium hydroxide pellets and acetic acid were obtained from Carlo Erba and were analytical grades.

2.2. Chitin deacetylation

A screening design of experiment (DEX) for chitin deacetylation process was carried out following a factorial 2^2 with center point. Tables 1 and 2 present levels of reaction time and temperature variables and sample codes for the corresponding processing conditions, respectively.

Alkali concentration and liquor amount were maintained constant at 50% (w/v) and 50 mL of NaOH solution to 1 g of chitin, respectively. The reaction was performed under nitrogen atmosphere at constant magnetic stirring. After completion of the required time, the suspension was filtered off, washed with water to neutral pH and oven dried at 60 °C overnight.

The obtained chitosan was dissolved at 1% (w/v) in 0.1 M aqueous acetic acid solution. This solution when added into NaOH solution (50%, w/v) led to the formation of a precipitate and the insoluble material was removed by filtration. To the clear filtrate was added again NaOH solution up to pH around 8. The formed white gel was filtered and thoroughly rinsed with distilled water, until neutral pH. Purified chitosan was then freeze-dried, grounded to powder and oven dried at 60 °C overnight.

2.3. Characterization

FTIR measurements were carried out by a Perkin-Elmer Spectrum One spectrophotometer. Sample absorbance spectrum was taken as an average of 32 scans with 2 cm^{-1} resolution in the frequency range 4000–400 cm^{-1}. Prior to analysis, samples and KBr were dried at 60 °C for 2 h at reduced pressure. Sample/KBr disc was prepared with 3 mg/200 mg ratio.

Table 2
Sample codes and processing conditions.

Sample code	Temperature (°C)	Time (h)
CS9-1	90	1
CS13-1	130	1
CS9-3	90	3
CS13-3	130	3
CS11-2	110	2

TGA evaluations were performed using a Mettler TA 4000 System instrument consisting of TGA-50 furnace with a M3 microbalance, and Star software. Samples of *ca.* 7 mg were scanned at 10 °C/min from 25 to 900 °C, under 300 mL/min flow rate of both nitrogen and air.

Ultraviolet spectra were recorded in the range 200–230 nm using a Jasco V-530 UV/V spectrophotometer. A calibration curve was performed by means of standard solutions of GlcN and GlcNAc. A solution of acetic acid 0.01 M was used as blank. GlcN and GlcNAc were dissolved in acetic acid 0.01 M in the range of 0.08–0.2 M and 0.01–0.2 mM, respectively. Accurately weighed (10 mg) chitosan samples were dissolved in 2 mL of acetic acid 0.1 M and diluted 10-fold with distilled water to obtain a final acetic acid concentration of 0.01 M. Chitosan was not dissolved directly in acetic acid 0.01 M since it would be difficult to get a complete dissolution of the sample in a reasonable short time.

3. Results and discussion

The obtained deacetylated chitin was a white powder. The methods to assess the DD can be essentially confined in three groups: (1) spectroscopic; (2) conventional; (3) destructive (Kasaai, 2009). In the present study the values of deacetylation obtained by two spectroscopic (FTIR and UV) and one destructive (TGA) methods by using a statistical analysis approach will be compared.

3.1. FTIR spectroscopy

FTIR technique has specifically proved to be useful for the analysis of chitin due to its limited solubility in most of the solvents. Nevertheless, FTIR needs a calibration versus an absolute technique like nuclear magnetic resonance (NMR), which permits a direct determination of chitin deacetylation. A big effort has been devoted to identify the right combination of bands and respective baselines, which led to a large number of proposed methods found in the literature. In fact, several methods were attempted to determine the degree of acetylation (DA) by FTIR (Baxter, Dillon, Taylor, & Roberts, 1992; Brugnerotto et al., 2001; Domszy & Roberts, 1985; Duarte, Ferreira, Marvao, & Rocha, 2002; Kasaai, 2008, 2009; Khan, Peh, & Ch'ng, 2002). DA represents the molar fraction of the GlcNAc units within the polymer.

Sample analysis was carried out in random way as well as the preparation of the three replicates performed for each one. This strategy was used to obtain a better estimation of the error of analysis.

As previously described, the method to determine DA using FTIR needs the construction of a calibration curve. However, it was decided to use the calibration curve described in literature considering that the statistical analysis of the chitin deacetylation reaction is the focus of the work. Table 3 presents some calibration curves with the respective absorption ratios in literature.

Fig. 1 shows FTIR spectra in transmittance of chitin, its partially deacetylated derivatives at the lower and higher levels (see Table 2) and chitosan. The band at around 1320 cm^{-1} is similar for chitin and its derivatives CS9-1 and CS13-3. This band is assigned to the C–N stretching of the N-acetylglucosamine. This result suggests that the process variables ranges do not affect deacetylated chitin levels in a significant way. Based on this observation, the calibration curve (2) in Table 3 was selected to evaluate the level of chitin deacetylation as a function of process variables. Fig. 2 shows FTIR spectrum of chitin with the insert indicating the baselines for the probe (BL1) and internal reference (BL2) absorptions that are in the ranges 1348–1280 cm^{-1} and 1500–1404 cm^{-1}, respectively. The internal

Table 3
Calibration curves from absorption ratios versus standard DA values.

DA calibration curve	Method for DA standard	Ref.
(1) $DA = (A_{1655}/A_{3450}) \times 155$	Titration	Baxter et al. (1992)
(2) $(A_{1320}/A_{1420}) = 0.3822 + 0.03133 \times DA$	^{1}H NMR, ^{13}C NMR	Brugnerotto et al. (2001)
(3) $(A_{1320}/A_{3450}) = 0.03146 + 0.00226 \times DA$	^{1}H NMR, ^{13}C NMR	Brugnerotto et al. (2001)
(4) $(A_{1560}/A_{2875}) = 0.2 + 0.0125 \times DA$	Elemental analysis	Kasaai (2008)

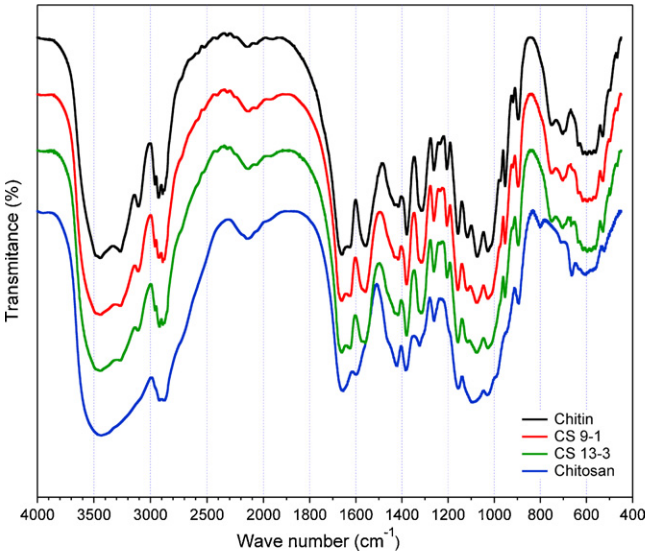

Fig. 1. FTIR spectra of chitin and its derivatives.

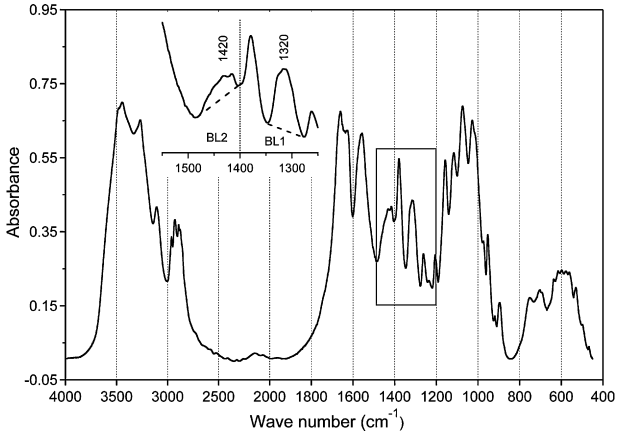

Fig. 2. FTIR spectrum of chitin.

reference absorption centered at 1420 cm^{-1} corresponds to –CH$_2$ bending. Using this equation the values of DD (1-DA) for commercial chitin and for chitosan were 0 ± 5.2% and 90%, respectively. The experiment with commercial chitosan was not replicated. However, considering that the errors of the FTIR measurements were around 5% (see Table 4), it can be confirmed that the equation proposed by Brugnerotto et al. (2001) is a reasonable approach to assess the deacetylation reaction of chitin.

Table 4
Chitin derivates deacetylation degree (DD) as a function of process variables using FTIR.

Sample	DD (%)	Error (%)
CS9-1	16.0	4.1
CS9-3	19.8	4.3
CS11-2	24.5	5.8
CS13-1	22.7	3.9
CS13-3	34.5	6.8

Table 4 records the mean values of DD of chitin as a function of process variables (Table 2). The error was calculated using Student's t-test at 95% of confidence ($t_{2;0.05} = 4.303$) as indicated by Eq. (1).

$$\text{error} = \frac{t_{\upsilon;\alpha/2} \times s}{\sqrt{n}} \tag{1}$$

where $t_{\upsilon;\alpha/2}$ is Student's t at υ freedom degree, $\alpha/2$ significance level, s is the standard deviation and n is the number of observations.

Factorial designed experiments can be appropriately analysed using the DEX interaction effect plot (Fig. 3). This plot showing mean values for the levels of each factor and that of interaction between them will indicate the significance on changing from one level to another as well as the interdependence of the factors.

This plot shows that the most significant variable is the temperature followed by the time. The interaction between temperature and time was apparently not significant in the range studied. All factors indicate that higher chitin deacetylation, using 50% aqueous NaOH, can be obtained for temperatures higher than 130 °C and/or at reaction times higher than 3 h.

The analysis of variance (ANOVA) is summarized in Table 5 including as source of variation the "pure quadratic" to check for pure quadratic curvature effect. This check was performed with the center points of the 2^2 factorial design. The cut-off value of F-test at 95% of confidence with 1 and 10 freedom degrees ($F_{0.05;1;10}$) is 4.96. The F_0 values for the main effects and interaction are higher than that of cut-off and it can be concluded that they are statistically significant. On the other hand, pure quadratic effect is not significant and a first order model can be used adequately to fit results. The ANOVA confirmed the observations of DEX plot including that there is some interdependence between temperature and reaction time although small for the range of variables studied.

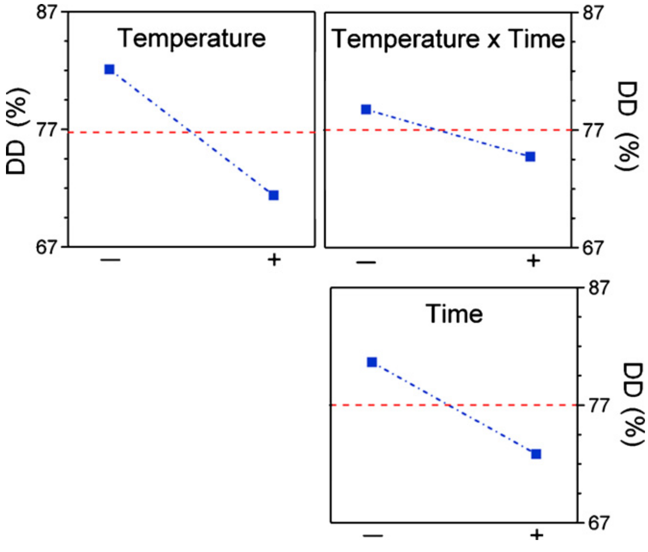

Fig. 3. DEX interaction effect plot for DD values.

Table 5
ANOVA for factorial model.

Source of variation	Sum of squares	Degrees of freedom	Mean square	F_0
A (Temperature)	343.68	1	343.68	79.74
B (Time)	184.55	1	184.55	42.82
AB	48.24	1	48.24	11.19
Pure quadratic	3.20	1	3.20	0.74
Error	43.12	10	4.31	
Total	622.79	14		
Model $R^2 = 0.926$	576.47	3	192.16	44.58

Considering the above remarks, the regression model for the chitin deacetylation process is

$$\hat{y} = 76.8 - 5.3x_1 - 3.9x_2 - 2x_1x_2 \tag{2}$$

The relationship between coded and natural variables is

$$x_1 = \frac{\text{Temp} - (\text{Temp}_{\text{low}} + \text{Temp}_{\text{high}})/2}{(\text{Temp}_{\text{high}} - \text{Temp}_{\text{low}})/2} \tag{3}$$

$$x_2 = \frac{\text{Tempo} - (\text{Tempo}_{\text{low}} + \text{Tempo}_{\text{high}})/2}{(\text{Tempo}_{\text{high}} - \text{Tempo}_{\text{low}})/2} \tag{4}$$

Consequently, the fitted model of Eq. (2) with the natural variables is

$$\text{D}\hat{\text{D}} = 118.64 - 0.22T - 6.34t - 0.02Tt \tag{5}$$

where T and t are temperature and time variables.

The ANOVA for the model is included in Table 5. The cut-off value of $F_{0.05;3;10}$ is 3.71 that is lower than that found for the model. This means that at least one coefficient of the model is different from zero. Beside, below the ANOVA of model, is reported statistics to check adequacy of fit of the model. This statistic represents the fraction of the variation about the mean that can be explained by the fitted model. So, R^2 value suggests that the model can explain about 93% of deacetylation variability.

By applying the developed statistical model, using temperature and time as the process variables, chitosan with 80% DD can be obtained in 9 h using 50% (v/v) NaOH and a reaction temperature of 100 °C.

3.2. TGA

The TGA method for chitin and chitin derivates has been used principally on thermodegradation kinetic studies (Peniche-Covas, Arguelles-Monal, & San Roman, 1993). However, a simple way to determine chitin deacetylation was proposed by Alonso et al. (1983). They take the weight loss in air atmosphere that corresponded to the maximum of DTG peak of chitin and chitosan degradation traces and correlated with deacetylation of chitin standards. They demonstrated that the empirical correlation obtained could be a fast way to control deacetylation of chitin.

Fig. 4 shows typical DTG and TG (insert) traces for chitin and its derivates in air atmosphere and TG data are reported in Table 6.

As the time for a TG analysis is time-consuming, only the center point of the 2^2 factorial design was replicated three times to allow the evaluation of the experimental error in addition to checking the presence of curvature in the fitting model.

The first step in the temperature range of 30–150 °C corresponds to the equilibrium moisture of samples with that of ambient, which appears in Table 6 as volatile. The second thermo-oxidation step appeared as an overlapped peak only for the sample CS13-3 (DD = 34.5 from FTIR—see Table 4) with a maximum at 296 °C and which is slightly higher than that measured for commercial chitosan (see insert inside Fig. 4). For other chitin derivates, this step appeared as a shoulder. So, the maximum rate peak of second step around 325 °C is associated to chitin, which is also in accordance with literature (Alonso et al., 1983). The last step of thermo-oxidation in the range 350–600 °C seems to have some correlation with the capacity of the molecule to pick up moisture as indicated by the same tendency presented by the volatile value in Table 6. Peniche-Covas et al. (1993) observed that up to 430 °C both pyrolysis and thermo-oxidation TGA traces of chitosan overlap. They considered that up this temperature the decomposition is independent of atmosphere. Consequently, as this step is related to oxidation reaction of the residue it can be supposed that it depends upon the deacetylation.

Commercial chitosan, with around 85% of DD, evolved *ca.* 9% of adsorbed moisture that is only *ca.* 2.5% higher than that measured for chitin. However, the behaviour of chitin derivates does not follow the deacetylation values presented in Table 4. The first approach to explain this result can be related to the formation of some kind of intermolecular interaction that turn less accessible the hydrophilic groups for moisture adsorption. The thermo-oxidation temperature defined at 2 wt% of weight loss after that of volatile (T_d) for chitin was 243 °C suggesting to be 2 °C less stable than chitosan (DD = 85%). However, considering the replication of the experiment with the center point sample (CS11-2) and using Student's t-test at 95% of confidence (Eq. (1)), it can be said that the error of the experiment is around 3.8 °C. In conclusion, the difference observed on T_d value between chitin and chitosan is not significant. The same statement can apply for chitin derivates.

The residue at 600 °C (R_{600}) was a white powder that characterize the inorganic molecules present in the crustaceous shells. Pristine chitin left *ca.* 4% and the commercial chitosan 3%. The R_{600} values of the chitin derivatives were lower than the two limits of kind of 2-deoxy-β-D-glucopyranose materials. This is one indication that the presence of inorganic impurities depends on the purification method used. Although the results suggest that the time and temperature in NaOH concentrated solution could be factors affecting materials inorganic impurities, the error observed from CS11-2 of 1.6% weaken this possibility.

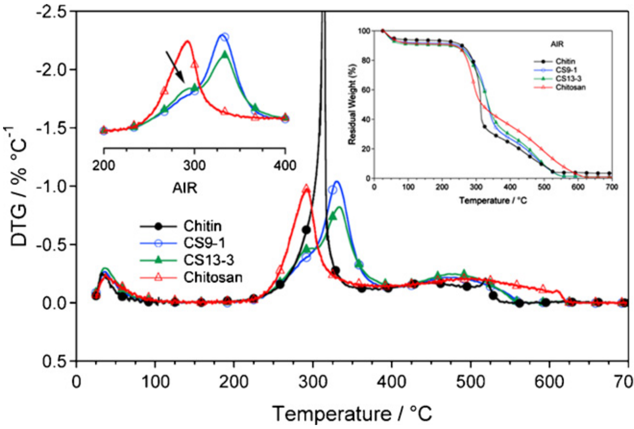

Fig. 4. Typical DTG and TG (insert) traces of chitin and its derivates in air atmosphere.

Table 6
TGA data of chitin and its partially deacetylated derivates in air atmosphere.[a]

Sample[b]	Volatile (%)	T_d (°C)	ΔM_1 (%)	T_{p1} (°C)	ΔM_2 (%)	T_{p2} (°C)	R_{600} (%)
Chitin	6.3	243	61.8	313	28.0	445	3.9
CS9-1	8.1	248	63.6	330	27.0	479	1.3
CS13-1	6.7	244	61.4	324	29.7	475	2.2
CS9-3	7.1	244	65.7	324	25.5	479	1.7
CS13-3	3.4	245	64.7	296/333	29.5	481	2.4
CS11-2	3.9	240	65.0	324	29.8	477	1.3
CS11-2	3.9	243	64.0	325	30.6	485	1.5
CS11-2	3.3	241	64.2	325	30.0	487	2.5
Chitosan	8.8	245	54.0	292	34.2	493	3.0

[a] Volatile means the weight loss up to 150 °C. T_d is the thermo-oxidation decomposition temperature defined at 2 wt% of weight loss from 150 °C; T_p is the first derivative peak; ΔM is the range of weight loss and R_{600} is the residual weight at 600 °C.

[b] See Table 2 for code definition.

Table 7
TGA data of chitin and its partially deacetylated derivates in nitrogen atmosphere.[a]

Sample[b]	Volatile (%)	T_d (°C)	ΔM_1 (%)	T_{p1} (°C)	ΔM_2 (%)	T_{p2} (°C)	ΔM_3 (%)	R_{600} (%)
Chitin	6.0	257	–	–	73.0	371	4.8	16.2
CS9-1	7.7	271	15.7	296	50.7	389	5.5	20.4
CS13-1	5.8	268	19.1	297	49.5	391	5.4	20.2
CS9-3	6.4	270	16.5	297	53.6	390	4.4	19.1
CS13-3	5.1	270	18.5	298	42.7	395	6.4	24.3
CS11-2	5.5	275	17.9	299	50.9	393	5.4	20.3
Chitosan	10.3	270	57.7	302	–	–	–	32.0

[a] Volatile means the weight loss up to 150 °C. T_d is the pyrolysis decomposition temperature defined at 2 wt% of weight loss from 150 °C; T_p is the first derivative peak; ΔM is the range of weight loss and R_{600} is the residual weight at 600 °C.

[b] See Table 2 for code definition.

Typical traces of DTG and TG (insert) of chitin and its derivates under nitrogen atmosphere are presented in Fig. 5. DTG traces (Fig. 5) show clearly that pyrolysis occurs in two steps in the 200–600 °C range. The first weight loss up to 150 °C is related to moisture volatilization as previously verified for samples decomposed in air atmosphere. Unlike the DTG profile in air atmosphere, the second step of chitin derivates in the 200–330 °C range overlaps the third step assigned to chitin but it is still possible to take both maximum peak temperatures (T_p). Table 7 reports pyrolysis data.

3.3. UV spectrophotometry

The method of the first derivative UV spectrophotometry is one of the most used for determination of the DD (Muzzarelli & Rocchetti, 1985; Tan, Khor, Tan, & Wong, 1998). It is the simplest and the most convenient among all the presently available methods. In a recent article, the method of UV first derivative has been proposed as a straightforward method for the determination of the degree of acetylation (DA). The authors have proposed a formula to calculate the DA of chitosan directly from the mass concentration of the chitosan solution (da Silva, Mano, & Reis, 2008). Our approach has been similar in calculating the DD of the prepared chitin derivates.

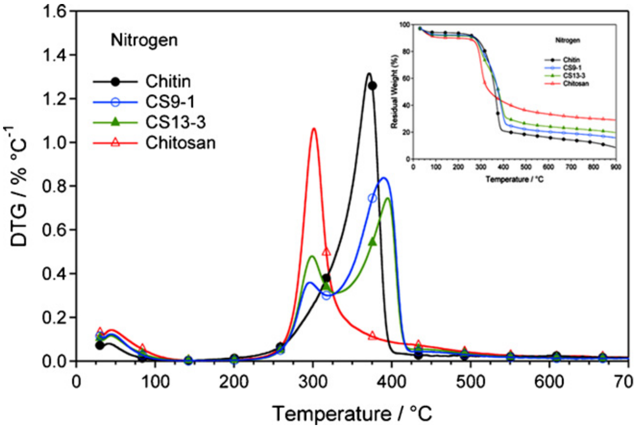

Fig. 5. Typical DTG and TG (insert) traces of chitin and its derivates in nitrogen atmosphere.

The two model substances, GlcNAc and GlcN, are UV chromophoric groups, which contribute in a simple addictive way to the total absorbance of the material at a defined wavelength. Based on this evidence Liu, Wei, Yao, and Jiang (2006) derived a linear relationship between absorbance and the total molar concentration of the monomers permitting DA determination.

The high absorbance of the acetic acid at the working concentration disturbs the determination of both GlcNAc and GlcN residues when using the zero order UV spectra. The 1st derivative spectra of the acetic acid solutions share a common point at around 202 nm for concentrations from 0.005 up to 0.03 M, designated as the zero crossing point by Muzzarelli and Rocchetti (1985). Consequently, the determination of the amine and N-acetyl containing concentration should be relatively insensitive to fluctuations in the acetic acid concentration.

Individual calibration curves for both GlcNAc and GlcN were drawn through a linear regression between the concentration and the 1st derivative UV signal arising from each one (Figs. 6 and 7, respectively). This was deduced from the Beer's lamberts law for diluted solutions, which correlates the concentration (C) with the absorbance (A), for a given wavelength (λ) as shown in Eqs. (6)–(9). Each spectrum shown in Figs. 6 and 7 represents the average of two independent data sets.

$$A(\lambda) = \varepsilon(\lambda) l_{\mathrm{UV}} C \qquad (6)$$

where ε is the molar absorption and l_{UV} is the optical length that in the present work is 1 cm. Since both l and C are independent on the wavelength:

$$\frac{dA}{d\lambda} = \frac{d\varepsilon}{d\lambda} lC = \varepsilon'(\lambda) l_{\mathrm{UV}} C \qquad (7)$$

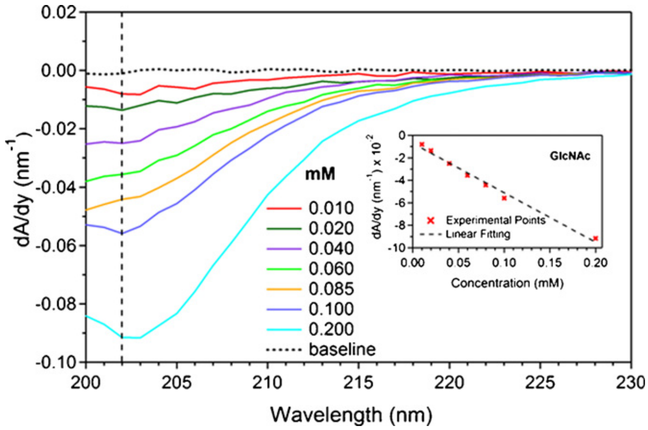

Fig. 6. First derivative UV spectra of GlcNAc at λ = 202 nm and respective calibration curve (insert).

It is known that the acetic acid gives also a signal at λ = 202 nm, thus Eq. (7) should be corrected as shown below:

$$A - A_{\mathrm{AcOH}} = \varepsilon l_{\mathrm{UV}} C \tag{8}$$

$$\frac{dA}{d\lambda} - \left(\frac{dA}{d\lambda}\right)_{\mathrm{AcOH}} = \varepsilon' l_{\mathrm{UV}} C \tag{9}$$

Denoting the 1st derivative of the GlcNAc and GlcN molar absorptivities as ε_a and ε_g, respectively, the linear regression of the experimental data gives:

$$\varepsilon'_a l_{\mathrm{UV}} = -459.5\,\mathrm{M}^{-1} \quad \text{and} \quad \varepsilon'_g l_{\mathrm{UV}} = -3.33\,\mathrm{M}^{-1}$$

The method for the determination of DA by means of 1st derivative UV is based on the assumption that the molar absorptions of both GlcN (ε_g) and GlcNAc (ε_a) chromophoric groups does change when they are covalently bound through β-(1→4) glycosidic linkages (da Silva et al., 2008). Consequently, the monosaccharides contribute in an additive way to the total absorbance, which in the presence of acetic acid can be expressed as:

$$A = \varepsilon_a l_{\mathrm{UV}} C_a + \varepsilon_g l_{\mathrm{UV}} C_g + \varepsilon_{\mathrm{AcOH}} l_{\mathrm{UV}} C_{\mathrm{AcOH}} \tag{10}$$

where the concentrations (C_i) are in mol/L.

Since the optical path length (l) and the concentration (C_i) are independent of the wavelength, differentiating Eq. (10) gives,

$$\frac{dA}{d\lambda} - \left(\frac{dA}{d\lambda}\right)_{\mathrm{AcOH}} = \varepsilon'_a l_{\mathrm{UV}} C_a + \varepsilon'_g l_{\mathrm{UV}} C_g \tag{11}$$

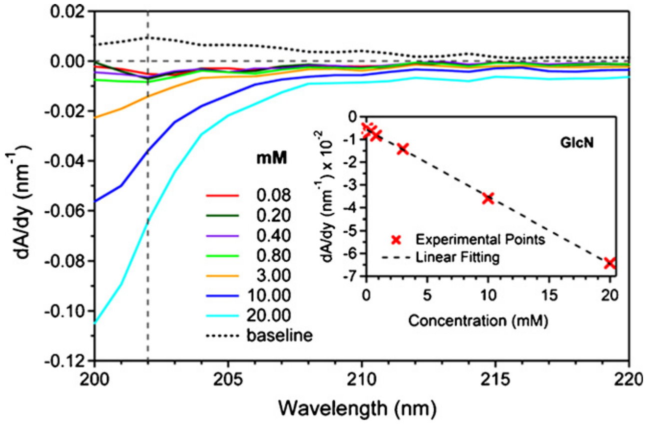

Fig. 7. First derivative UV spectra of GlcN at λ = 202 nm and respective calibration curve (insert).

Table 8
Chitin derivates deacetylation degree (DD) using UV.

Sample	DD (%)	Error (%)
Chitin	0.3	1.4
Chitosan	94.1	0.9
CS9-1	18.5	4.8
CS11-1	22.9	2.9
CS13-1	33.1	2.7

The degree of deacetylation (DD) is defined as the molar fraction of the GlcN units and can be expressed as the ratio between the GlcN and total monosaccharides concentrations (C_t):

$$\mathrm{DD} = \frac{C_g}{C_a + C_g} = \frac{C_g}{C_t} \tag{12}$$

Combining Eqs. (11) and (12) and rearranging, it is obtained:

$$\frac{1}{C_t}\left[\frac{dA}{d\lambda} - \left(\frac{dA}{d\lambda}\right)_{\mathrm{AcOH}}\right] = \varepsilon'_a l_{\mathrm{UV}} - (\varepsilon'_a l_{\mathrm{UV}} - \varepsilon'_g l_{\mathrm{UV}})\mathrm{DD} \tag{13}$$

Eq. (13) lays the foundation of the method proposed herein. It is interesting to notice that the method developed by Muzzarelli and Rocchetti (1985) is a particular case of the last equation. Since $\varepsilon_g \gg \varepsilon_a$, Eq. (13) can be simplified as follow,

$$\frac{1}{C_t}\left[\frac{dA}{d\lambda} - \left(\frac{dA}{d\lambda}\right)_{\mathrm{AcOH}}\right] \approx \varepsilon'_a l_{\mathrm{UV}} - (\varepsilon'_g l_{\mathrm{UV}})\mathrm{DD} \tag{14}$$

C_t within a chitosan sample cannot be achieved without knowing the DD. Thus, it is more convenient to express the copolymer concentration in terms of solute mass ($\bar{C}_t$) in g/L, which is defined experimentally. These two concentration values are related by the next equation:

$$\frac{\bar{C}_t}{C_t} = M_a - (M_a - M_g)\mathrm{DD} \tag{15}$$

where M_a and M_g are the molecular weights of the GlcNAc and GlcN units within the copolymer. Combining Eqs. (13) and (15), it is obtained,

$$\frac{1}{\bar{C}_t}\left[\frac{dA}{d\lambda} - \left(\frac{dA}{d\lambda}\right)_{\mathrm{AcOH}}\right] = \frac{\varepsilon'_a l_{\mathrm{UV}} - (\varepsilon'_a l_{\mathrm{UV}} - \varepsilon'_g l_{\mathrm{UV}})\mathrm{DD}}{M_a - (M_a - M_g)\mathrm{DD}} \tag{16}$$

Following a rearrangement, the DD assumes the following expression:

$$\mathrm{DD} = \left[\frac{\varepsilon'_a l_{UV} - (M_a/\bar{C}_t)((dA/d\lambda) - (dA/d\lambda)_{\mathrm{AcOH}})}{(\varepsilon'_a l_{UV} - \varepsilon'_g l_{UV}) - ((M_a - M_g)/\bar{C}_t)((dA/d\lambda) - (dA/d\lambda)_{\mathrm{AcOH}})}\right] \tag{17}$$

The molecular weight of N-acetyl and amino glucopyranoses comonomers are M_a = 203 g/mol and M_g = 161 g/mol, respectively. Therefore, Eq. (17) allows a straightforward determination of the DD. The results obtained for the different chitin derivate samples are reported in Table 8.

4. Conclusion

Temperature and time as process variables of the chitin deacetylation reaction were analysed by using a statistical approach. Taking on FTIR as one of the most traditional method to determine chitin deacetylation degree (DD), it was verified that the higher level of both variable studied (130 °C-3 h) resulted a chitin derivative with *ca.* 35% of DD. The ANOVA indicated that the most important variable is the temperature and that there is a slight interdependence between both variables in the range studied. A regression model was developed, using which the time and temperature could be calculated to obtain chitosan with the desired deacetylation degree.

The thermal behaviour of the prepared chitin derivates was analysed basically on the thermodegradation principle. Thermo-oxidation data from TGA showed that the equilibrium moisture of the chitin derivates is equivalent to that of pristine chitin whose value was around 7%.

UV spectroscopy measurements were performed by drawing a linear regression between the concentration and the 1st derivative UV signal arising from the absorption of the two UV chromophoric groups GlcNAc and GlcN. The values of DD assessed from the 1st derivative of UV absorption spectrum are in accordance with that attained by using FTIR.

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