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Characteristics and properties of carboxymethylchitosan

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ABSTRACT

Four samples of *N*,*O*-carboxymethylchitosan ($0.5 < \overline{DS} < 1.5$) were prepared by reacting chitosan ($\overline{DA} = 24\%$) with monochloroacetic acid in the presence of excess sodium hydroxide. The carboxymethylchitosan samples were soluble in a wider range of pH as compared to the parent chitosan and the X-ray diffraction showed that they adopt a less ordered arrangement. The carboxymethylation of chitosan decreased the thermal stability of the polymer as evaluated by thermogravimetry but no clear dependence of the activation energy on the average degree of substitution of carboxymethylchitosan was identified. However, the values of activation energy of carboxymethylchitosan issued from the isothermal study depended on the degree of conversion, suggesting the occurrence of a complex set of simultaneous reactions.

1. Introduction

Chitin, a linear homopolysaccharide composed of 2-acetamide-2-deoxy-D-glucopyranose units linked by β(1 → 4) bonds, occurs abundantly in the biomass (Abram & Higuera, 2004; Mathur & Narang, 1990; Roberts, 1992). The refuses of the sea-food industry which are rich in chitin, mainly the shells of crustaceans, are the raw materials for the production of this polymer (Abdou, Nagy, & Elsabee, 2008; Abram & Higuera, 2004; Percot, Viton, & Domard, 2003). Chitosan, a copolymer of 2-amino-2-deoxy-D-glucopyranose and 2-acetamide-2-deoxy-D-glucopyranose units, is the main derivative of chitin. It is a constituent of the cell wall of some fungi but the partial deacetylation of chitin is the main route used for its preparation in industry as well as in research laboratories (Lamarque, Cretene, Viton, & Domard, 2005). Chitin and chitosan are biocompatible, biodegradable and atoxic polymers which exhibit the ability to interact with metal ions, dyes, proteins, nucleic acids, lipids, herbicides, pesticides and humic acids (Chao, Shyu, Lin, & Mi, 2004; Zhao et al., 2008). They also exhibit antimicrobial activity and can be used as films and coatings to inhibit the growth of fungi and bacteria during the storage of fruits and vegetables.

Chitin is insoluble in most common solvents but chitosan is soluble in moderately acid aqueous media due to the protonation of its amino groups when $pH < pK_o \cong 6.5$. The poor solubility of chitin, restricted to systems containing toxic organic solvents, severely limits its applications. The solubility of chitosan is limited to moderately acid aqueous media and it also represents a serious drawback to many of its potential applications. Thus, the chemical modifications of chitin/chitosan are generally designed to improve the polymer processability as well as to modify some of its properties such as solubility, antimicrobial activity and the ability to interact with other substances.

Carboxymethylchitosan is soluble in a wide range of pH and its properties, including the antimicrobial activity, film-forming ability and capacity to interact with different substances, can be explored in some applications where chitosan has been limitedly applied due to its restricted solubility (Liu, Guan, Yang, Li, & Yao, 2001). Therefore, potential applications of carboxymethylchitosan include the medical and pharmaceutical areas – mainly for the controlled release of drugs (Guo & Gao, 2007), for the elaboration of orthopaedic devices and to avoid pos-chirurgical tissue adhesion (Zhao, Kato, Fukumoto, & Nakamae, 2001) – and for the treatment of industrial effluents (Rhazi et al., 2002).

According to the literature (Chen & Park, 2003; Chen, Du, Wu, & Xiao, 2002; Le Dung, Milas, & Desbrières, 1994) the choice of the appropriate reaction conditions and reagents allows the preparation of *N*-, *O*- or *N*,*O*-carboxymethylchitosan. When the carboxymetylation is carried out by reacting chitosan with mono-chloroacetic acid in 2-propanol/aqueous sodium hydroxide, *O*-substitution is favoured if the reaction is carried out at room temperature but *N*-substitution predominates by raising the reaction temperature (Tokura, Nishi, Tsutsumi, & Somorin, 1983).

As the polymer processing frequently involves its melting and extrusion, the studies on its thermal properties and stability are very important to support the technological applications of the polymer. Chitin and chitosan as well as their derivatives have good film-forming properties and they can be processed as membranes, solutions, gels and nanoparticles. Some studies on the thermal

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degradation of chitin (Alonso, Peniche-Covas, & Nieto, 1983), chitosan (Holme, Foros, Pettersen, Dornish, & Smidsød, 2001; Peniche-Covas, Argüelles-Monal, & Román, 1993) and derivatives (Britto & Campana-Filho, 2004; Qu, Wirsén, & Albertsson, 2000; Tirkistani, 1998) show that the introduction of substituents on the amino groups of chitosan decreases its thermal stability. However, there are very few results reporting on the effect of the carboxymethylation on the thermal stability of carboxymethylchitosan (Miranda et al., 2006).

In this work, the preparation and characterization of carboxymethylchitosan is described. The effects of the reaction conditions, mainly the reaction time and the excess of monochloroacetic acid and sodium hydroxide, on the chemoselectivity and the spectral characteristics of this chitosan derivative as well as on its solubility and capacity to adsorb water are discussed. The thermal degradation of chitosan and sodium carboxymethylchitosan in nitrogen atmosphere is studied by thermogravimetry under dynamic and isothermal conditions and the dependence of the degradation kinetics on the average degree of carboxymethylation is studied.

2. Experimental

2.1. Purification of chitosan

Commercial chitosan (Fluka/Biochemica, from crab shells, medium molecular weight), named as Sample Q, was dissolved in dilute acetic acid (1%) to result in a clear solution ($C_p \approx 3$ g/L) which was filtered through mixed cellulose esters membrane (Millipore, pore size 5 μm). The precipitation of the polymer was provoked by neutralization with concentrated ammonium hydroxide. After extensive washing with distilled water and drying at room temperature, the purified chitosan was milled in a domestic blender and the fraction composed by particles having an average diameter lower than 125 μm was then used to carry out the carboxymethylation reactions.

2.2. Carboxymethylation reactions

The procedure described for the carboxymethylation of cellulose (Caraschi & Campana-Filho, 1999) was used aiming the preparation of *O*-carboxymethylchitosan. Thus, 3 g of purified chitosan were dispersed in 65 ml of 2-propanol and after 20 min of magnetic stirring at room temperature, 20.4 g of aqueous NaOH (40%) and 14.4 g of monochloroacetic acid/2-propanol solution (1:1 w/w) were added to the suspension. The reaction proceeded for the desired time at room temperature and the solid product was then filtered, suspended in 150 ml of methanol and neutralized with glacial acetic acid. The product was extensively washed with 80% ethanol and dried at room temperature. Four carboxymethylchitosan samples were prepared by employing different reaction times (7 h and 10 h) and molar ratios chitosan/monochloroacetic acid (1:4.3 or 1:8.6). The carboxymethylchitosans named as samples QC_7 and QC_{10}resulted by employing the molar ratio 1:4.3 and by carrying out the carboxymethylation reaction for 7 and 10 h, respectively. The samples QC_{7E} and QC_{10E} were obtained when the molar ratio 1:8.6 was employed and the reaction proceeded for 7 and 10 h, respectively.

For the purification of these derivatives, 1.5 g of the sample was dissolved in 1.5 L of aqueous 0.1 M NaCl. The resulting solution was filtered and the carboxymethylchitosan was precipitated upon addition of absolute ethanol. Following, the carboxymethylchitosan was sequentially washed with ethanol/water mixtures of increasing ethanol content (75%, 80%, 90%), with absolute ethanol and then it was dried at room temperature. This procedure resulted in purified sodium carboxymethylchitosan samples.

2.3. Analytical methods

The occurrence of carboxymethylation and the presence of characteristic functional groups of chitosan (Q) and carboxymethylchitosan (QC) were confirmed by infrared and NMR spectroscopy. The average degrees of acetylation ($\overline{DA}$) and of substitution ($\overline{DS}$) were determined from spectral data and by titrimetry. X-ray diffraction was employed to monitor morphological changes due to carboxymethylation. The solubility of the samples as a function of the solution's pH and the capacity of the polymers to adsorb water were determined by UV/vis spectroscopy and thermogravimetry, respectively. The thermal stability and the kinetics of thermal degradation of the samples were also studied by thermogravimetry.

2.3.1. Infrared spectroscopy

The infrared spectra were registered from 48 scannings at a resolution of 4 cm^{-1} by using a FTIR BOMEM MB102 spectrophotometer. Films of chitosan, sodium carboxymethylchitosan and acid carboxymethylchitosan were prepared to acquire the infrared spectra. Thus, 0.025 g of the purified chitosan was suspended in 10 mL of aqueous 1% acetic acid and the suspension was maintained under continuous magnetic stirring for 24 h. The resulting solution was poured in a Petri plate and the set was kept at room temperature to allow the evaporation of the solvent. The film of chitosan was detached, neutralized with aqueous 1 M NH_4OH, abundantly washed with water and dried at room temperature. The same procedure was used to prepare the film of sodium carboxymethylchitosan, but deionised water instead of aqueous 1% acetic acid was used as the solvent in this case and the resulting film was neither neutralized nor washed.

The average degrees of substitution ($\overline{DS}$) were determined from the infrared spectra acquired with the films of carboxymethylchitosans in acid form. Thus, sodium carboxymethylchitosan was dissolved in deionized water; the resulting solution was exhaustively dialysed against 0.1 M aqueous HCl and then poured in a Petri plate. The solvent was allowed to evaporate resulting in a film of carboxymethylchitosan in the acid form. According to the literature (Nahalka, Nahalkova, Gemeiner, & Blanarik, 1998), the $\overline{DS}$ of carboxymethylchitosan was determined from the intensities of the bands at 1411 and 1321 cm^{-1} which are attributed to the asymmetric stretching of COO and to the amide III band, respectively.

2.3.2. NMR Spectroscopy

The NMR spectra of chitosan and carboxymethylchitosans were acquired at 80 °C by using a 200 MHz spectrometer (Bruker AC200). The ^1H and ^{13}C NMR spectra were acquired from polymer solutions at C_p = 10 and 20 g/L, respectively. Both polymers were dissolved in D_2O/HCl (100/1 v/v) for acquiring the ^1H NMR spectra but solutions of carboxymethylchitosan in D_2O were used for acquiring the ^{13}C NMR spectra. The ^1H NMR spectrum of chitosan was used for the determination of the average degree of acetylation, $\overline{DA}$, of the polymer according to the literature (Signini & Campana-Filho, 1999).

2.3.3. X-ray diffraction

The X-ray diffraction analysis were carried out in a Universal Diffractometer Model URD6 (Carl Zeiss-Jena) employing CuKα radiation (λ = 1.54Å). The samples were previously grounded and dried; the power and current for acquisition of the spectra were set at 50 kV and 100 mA.

2.3.4. UV/vis spectroscopy

The solubility of chitosan and derivatives in aqueous medium as a function of the pH was estimated by measuring the transmit-

tance of the polymer's solutions as reported in the literature (Chen & Park, 2003; Sashiwa & Shigemasa, 1999). Thus, the samples were dissolved at a concentration C_p = 1.5 g/L and the pH of the resulting solutions were adjusted by adding dilute HCl (or NaOH) aqueous solution. The spectra were acquired in the range 200 < λ < 800 nm and a given sample was considered insoluble when the transmittance of its solution at λ = 450 nm was lower than 0.85.

2.3.5. Thermogravimetry

Thermogravimetric analysis was carried out to determine the capacity of the polymers to adsorb water in atmosphere of controlled humidity and to study the kinetics of thermal degradation. Thus, the samples were stored during 15 days in an ambient of controlled humidity to attain the equilibrium before being analysed by thermogravimetry. Closed glass recipients containing phosphorus pentoxide and saturated with water vapour constituted the ambients of 0% and 100% relative humidity, respectively. The samples (≅3.0–4.0 mg) were analysed in a TGA50 termogravimetric analyzer (Shimadzu) under synthetic air flow (ϕ = 50 mL/min) and heating rate 10 °C/min.

The experiments aiming to study the thermal degradation of chitosan and sodium carboxymethylchitosan were carried out in dynamic as well as in isothermal conditions. In both set of experiments, the samples were initially heated at a rate of 10 °C/min from 25 to 110 °C and then maintained at this temperature during 15 min for evaporation of the water. In the dynamic experiments the samples were subsequently heated at 10 °C/min to 800 °C while the loss of weight was monitored. In the isothermal experiments, following the elimination of water the samples were then heated at 10 °C/min to the desired temperature and the loss of weight was monitored as a function of the reaction time. The zero time for the isothermal experiments was taken in the moment at which the temperature stabilized.

2.3.6. Titrations

The aqueous solutions of chitosan and carboximethylchitosan ($C_p \cong 1$ g/L in both cases) were titrated with standardized aqueous 0.1 M NaOH to determine the average degrees of acetylation ($\overline{DA}$) and of substitution ($\overline{DS}$) of the polymers. These solutions were contained in a glass cell maintained at 25± 0.4 °C and the titrations were carried out under nitrogen bubbling. The solution of NaOH was added by using an automatic burette (Schott Titronic Universal) and the conductivity and the pH of the polymer's solution were simultaneously measured after each addition. The Handylab LF1 conductivimeter and the CG 843 P pH meter, both from Schott-Geräte, were used in these experiments.

3. Results and discussions

3.1. Characterization of chitosan and carboxymethylchitosan

In the following, chitosan and carboxymethylchitosan will be generally referred as Q and QC, respectively.

Although the literature reports that the carboxymethylation of chitosan occurs selectively according to the reaction conditions, our experience show that a structurally complex product is generally obtained when ordinary conditions are used. Thus, when chitosan reacts with monochloroacetic acid in 2-propanol/aqueous sodium hydroxide at room temperature, *N*,*O*-QC is formed. It is attributed to the fact that the hydroxyl groups bonded to the carbon atoms 3- and 6- of the glucopyranose unit as well as the amino group bonded to the carbon atom 2 are the sites available for carboxymethylation. Indeed, a *N*,*N*-disubstituted derivative may be obtained depending on the reaction conditions. Additionally, it should be noted that the carboxymethylation reaction is seldom complete, and thus some hydroxyl and amino groups remain unsubstituted. Finally, one must consider that if the chitosan is not completely deacetylated there are also the 2-acetamide-2-deoxy-D-glucopyranose units coming from the partial deacetylation of the parent chitin. From these considerations one concludes that at least 12 different units should occur in the chains of QC (Abreu & Campana-Filho, 2005). However, although the complete characterization of this derivative of chitosan may be difficult due to its structural complexity, the main structural changes due to the carboxymethylation of chitosan were confirmed by the spectroscopic techniques used in this work.

The main bands observed in the infrared spectrum of chitosan were: (i) a strong and broad band due to the axial stretching of O—H and N—H bond centred at 3400 cm^{-1}; (ii) a band centred at 2890 cm^{-1} corresponding to the axial stretching of C—H bonds; (iii) a band centred at 1659 cm^{-1} which is attributed to the axial stretching of C=O bonds of the acetamide groups, named as amide I band; (iv) a band at 1590 cm^{-1} due to the angular deformation of the N—H bonds of the amino groups; (v) a band at 1378 cm^{-1} due to the symmetric angular deformation of CH_3; (vi) the amide III band at 1316 cm^{-1}; (vii) the band corresponding to the polysaccharide skeleton, including the vibrations of the glycoside bonds, C—O and C—O—C stretching, in the range 1153–897 cm^{-1}.

The carboxymethylation provoked structural changes which were clearly identified by comparing the infrared spectra of chitosan and sodium carboxymethylchitosan. Thus, the broader band centred at 3400 cm^{-1}revealed the more hydrophilic character of carboxymethylchitosan as compared to the parent chitosan while the occurrence of an intense band in 1588 cm^{-1} and a moderate band at 1411 cm^{-1}, which were attributed to the symmetric and asymmetric axial deformations of COO, respectively, confirmed the introduction of the carboxymethyl groups (Zhao, Wang, & Wang, 2002).

In the ^{1}H NMR spectrum of chitosan the signal centred at $\delta \cong 2.00$ ppm was attributed to the hydrogen atoms of the methyl moieties pertaining to the acetamide groups. The signal observed between 3.10 and 2.90 ppm corresponds to the hydrogen bonded to the carbon atom C2 of the glucosamine ring, while the signals between 3.30 and 4.00 ppm correspond to the hydrogen atoms bonded to carbons C3, C4, C5 and C6 of the glucopyranose. The hydrogen bonded to carbon C1 gives rise to the signals in the range 4.40 < δ < 5.00. From this spectral analysis and taking into account the intensity of the signals due to the hydrogen bond to C2 and the hydrogen atoms of the methyl moieties pertaining to the acetamide groups of chitosan, the average degree of acetylation was determined as $\overline{DA} = 24.3\%$.

The structural modifications introduced by the carboxymethylation were observed by comparing the ^{1}H NMR spectra of chitosan and carboxymethylchitosan. Indeed, it was observed that this latter spectrum is considerably different and more complex than that of the parent chitosan. The occurrence of *N*-carboxymethylation was evidenced by the signals in the range 3.1–3.4 ppm in the spectrum of QC, assigned to mono and disubstitution of the amino groups (Muzzarelli, Ilari, & Petraluro, 1994). However, it is not possible to determine if the carboxymethylation occurred in all the amino groups because the signal of the hydrogen bonded to C2 overlaps to the signals assigned to the *N*-carboxymethyl sites.

Further evidences on the structural changes due to the carboxymethylation of chitosan were observed by comparing the ^{13}C NMR spectra of chitosan and carboxymethylchitosan. In the spectrum of chitosan the signals observed at 177.9 and at 25 ppm were assigned to the carbonyl carbon of $COCH_3$ and the methyl carbon (CH_3), respectively. The signal at 101.3 ppm was assigned to carbon C1 of chitosan and those signals at 59.6, 73.1, 81.1, 78.6 and 64 ppm were assigned to carbons C2, C3, C4, C5 and C6, respectively. In the spectrum of carboxymethylchitosan, the signals due to C1 and C1″ carbons are shifted from 101.3 ppm (signal assigned

to C1 carbon in chitosan) to 105.9 ppm because of the electron-withdrawing effect of the carboxymethyl substituents. Since several different units occur in the structure of carboxymethylchitosan, the splitting of the signals present in the spectrum of chitosan were observed in the spectrum of carboxymethylchitosan. Thus, the signals at 60.1 ppm (C2 + C2″), 73.8 ppm (C3), 73.2 ppm (C3″), 82.2 ppm (C4 + C4″), 78.2 ppm (C5 + C5″) and 63.9 ppm (C6 + C6″) are split and they are shifted compared with those detected in the spectrum of the parent chitosan. The signals due to C3 and C6 are also split in the spectrum of CMCh, probably as consequence of the carboxymethylation of the hydroxyl groups bonded to C3 and C6. According to the literature (Zhao et al., 2002), the signal observed at 180.7 ppm is assigned to the carbonyl carbons of carboxymethyl groups while that one detected at 177.9 ppm corresponds to the carbonyl carbon of —$COCH_3$ of the parent chitosan. The methylene groups (—CH_2—) gave rise to the signals at 53 and 57.4 ppm, respectively, however, none signal was detected at 53 ppm in the spectrum of the QC and the weak signal at 58.4 ppm can be probably assigned to the methylene (—CH_2—) bonded to the amino group(—NH). These features were taken as evidence that the carboxymethylation occurred at the hydroxyl as well as at the amino groups of chitosan.

3.2. Average degrees of acetylation and carboxymethylation

The titrimetric analyses were carried out to determine the average contents of acetamide and carboxymethyl groups in the chains of chitosan and carboxymethylchitosan (Table 1). A good agreement was found for the results of the titrations monitored by conductimetry and pHmetry, both of them allowing the determination of the average degree of acetylation of chitosan as $\overline{DA} \cong 24\%$, in good agreement with the value determined by ^{1}H NMR spectroscopy. The data in Table 1 also showed that the $\overline{DA}$ values of the carboxymethychitosans were higher than that of the parent chitosan, confirming the occurrence of *N*-carboxymethylation, as pointed out by ^{1}H and ^{13}C NMR spectroscopy.

The average degrees of substitution ($\overline{DS}$) were determined from the titrimetric analyses and infrared spectra. The $\overline{DS}$ of samples QC_7 and QC_{10} were quite similar (Table 1), showing that when the reaction proceeded for 10 h the carboxymethylation efficiency has not significantly changed. This probably occurred because the monochloroacetic acid concentration decreased as a consequence of its reaction with sodium hydroxide, present in very large excess. However, a more substituted sample (QC_{10E}) was obtained when the reaction was carried out for 10 h in the presence of a higher excess of monochloroacetic acid.

3.3. X-ray diffraction

The comparison of the X-ray patterns (Fig. 1) of chitosan (Sample Q) and sodium carboxymethylchitosan (Sample QC_7) revealed that the carboxymethylation of chitosan provoked important changes in the arrangement of the polymer chains in the solid state. Indeed, the spectrum of the Sample QC_7 is representative of the whole set of QC samples prepared is this work, exhibiting poorly defined and much less intense peaks as compared to chitosan. This is attributed to the presence of the carboxymethyl moieties which substitute the hydrogen atoms of the hydroxyl and amino groups of chitosan. Thus, as the carboxymethyl groups are much more voluminous than the hydrogen atoms, an important excluded volume effect occurs and a polyelectrolyte effect must also be considered due to the presence of charged groups in the chains of sodium carboxymethylchitosan. The carboxymethylation of chitosan also affected the establishment of hydrogen bonds involving its hydroxyl and amino groups, these interactions being responsible for the adoption of a more ordered arrangement by the parent chitosan. Therefore, although the spectra of chitosan and sodium carboxymethylchitosan have not been quantitatively treated to determine the degree of order, it is assumed that the sodium carboxymethylchitosan samples adopt a less ordered arrangement as compared to chitosan.

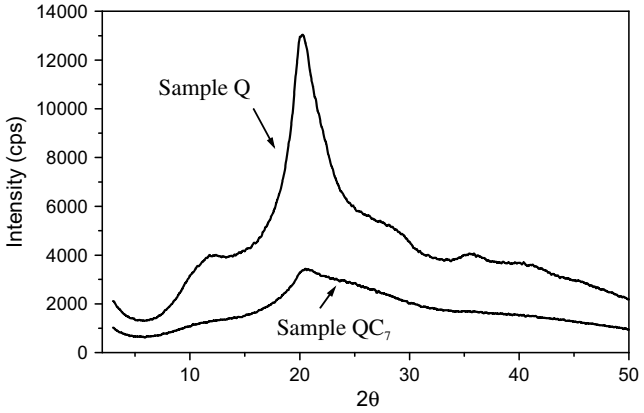

Fig. 1. X-ray diffraction patterns of chitosan (Sample Q) and sodium carboxymethylchitosan (Sample QC_7).

3.4. Adsorption of water and solubility

The TG analyses revealed the higher capacity of QC to adsorb water as compared to the parent chitosan. Indeed, treating the DTG curves (data not shown) resulted in the data shown in Table 2 which confirm that the weight loss in the range 25–120°C is much more important for QC than for chitosan, regardless of the previous storage of the samples be carried out at RH = 0% or 100%. Additionally, the temperature corresponding to the maximum loss of weight in that range of temperature was higher in the case of QC as compared to chitosan. Both facts are attributed

Table 1
Values of $\overline{DS}$ and $\overline{DA}$ of chitosan (Sample Q) and carboxymethylchitosans (QC) determined by titrimetry and infrared spectroscopy

Sample	$\overline{DA}$ (%)[a]	$\overline{DA}$ (%)[b]	$\overline{DS}$[a]	$\overline{DS}$[b]	$\overline{DS}$[c]
Q	24.0	24.6	–	–	–
QC_7	48.3	52.6	0.60	0.77	0.79
QC_{10}	46.8	44.8	0.52	0.56	0.49
QC_{7E}	49.7	50.5	0.80	0.71	0.81
QC_{10E}	44.4	46.0	1.44	1.36	1.35

[a] Values determined from the titrations monitored by conductivity measurements.
[b] Values determined from the titrations monitored by pH measurements.
[c] Values determined from the infrared spectra.

Table 2
Content of moisture (%) and temperature of maximum loss of weight (T_{max}) for chitosan and sodium carboxymethylchitosan

Sample	$\overline{DS}$[a]	Moisture (%) (RH = 100%)[b]	Moisture (%) (RH = 0%)[b]	T_{max} (°C)
Q	–	26.60	2.30	42.80
QC_{10}	0.52	68.10	4.70	69.70
QC_7	0.72	68.30	4.40	77.10
QC_{7E}	0.77	69.80	5.20	76.20
QC_{10E}	1.38	70.90	5.20	75.90

[a] Averaged from the data shown in Table 1.
[b] The values of RH stand for the relative humidity of the ambient where the samples were stored before the analyses.

to the higher hydrophilicity of QC and to the better accessibility of the water molecules to the hydrophilic sites of this polymer as compared to chitosan. The first statement is justified by the hydrophilic character of the negatively charged carboxymethyl groups and the second one is supported by the X-ray diffraction analyses which evidenced the less ordered arrangement of QC.

The study on the dependence of the solubility of Q and QC samples on the pH revealed that the parent chitosan was soluble at pH $\leqslant$ 7.2, in agreement with the literature (Chen & Park, 2003; Sashiwa & Shigemasa, 1999), while the solubility of QC depended on its $\overline{DS}$. The different solubilities of Q and QC may be understood on the basis of their chemical structures, mainly the acid/base properties of its ionic groups, as discussed below.

The protonation of the amino groups of chitosan results in the quaternization of its nitrogen atoms and consequently it introduces positive charges on the polymer chains. Thus, chitosan is a cationic polyelectrolyte in aqueous solution if enough acid is available for the protonation of its amino groups but as the polymer is predominantly uncharged when pH > 7.2 it is insoluble in alkaline media. Also, it must be added that although the solubility of the polymer was not quantitatively determined, the transmittance of the solution of chitosan was slightly smaller than 85% for pH 6.6 and 7.2, which can be taken as an evidence of its partial solubility in these conditions. On the other hand, the samples of sodium carboxymethylchitosan exhibited a better solubility as compared to chitosan as they were soluble even at pH > 8.0. This may attributed to the presence of the carboxymethyl groups which contributed to impart an anionic polyelectrolyte character to the polymer when pH > 4.0 due to the dissociation of these groups. However, the results also revealed that the less substituted QC samples (samples QC_7 and QC_{10}) were insoluble when 2.5 < pH <7.2 while the more substituted samples were insoluble in the ranges 1.4 < pH < 6.6 (Sample QC_{7E}) and 1.4 < pH < 5.6 (Sample QC_{10E}). These data showed that the prevalence of positive or negative charges along the polymer chains at each pH, and then the solubility of the polymer, is determined by the balance involving the protonation of the amino groups and the dissociation of the carboxymethyl groups. Thus, the negative charges predominated if the medium was moderately acidic to neutral and alkaline while the positive charges were predominant in acid medium. Apparently, the main factor determining the solubility of the polymer at a given pH is the excess of positive or negative charges, the insolubility of the polymer resulting of an insufficient excess of charge. Accordingly, the more substituted QC samples exhibited a wider range of solubility, and independently of the value of $\overline{DS}$ the carboxymethylchitosan samples were more soluble than the parent chitosan.

3.5. Kinetics of the thermal degradation of chitosan and carboxymethylchitosan

The kinetic analysis of a thermal degradation process shall begin by expressing the reaction rate by a general equation such as:

$$\frac{d\alpha}{dt} = k(T)f(\alpha) \tag{1}$$

where t represents the time, α is the extent of reaction, T is the temperature, $k(T)$ is the temperature-dependent rate constant and $f(\alpha)$ is a temperature-independent function that represents the reaction model. The rate constant $k(T)$ is given by the Arrhenius equation:

$$k(T) = A\exp\left(-\frac{E_a}{RT}\right) \tag{2}$$

where A is the pre-exponential or frequency factor and E_a is the activation energy. Thus, Eq. (1) may be rewritten as:

$$\frac{d\alpha}{dt} = A\exp\left(-\frac{E_a}{RT}\right)f(\alpha) \tag{3}$$

The integration of Eq. (3) as proposed in the literature (MacCallum, 1989 Vyazovkin & Wight, 1998) allows the determination of E_a by using:

$$\frac{E_a}{RT} + \ln[g(\alpha)] - \ln A = \ln t \tag{4}$$

where $g(\alpha)$ results from the integration of $f(\alpha)$.

Thus, several isothermal experiments may be carried out and the logarithm of the time taken to reach a fixed extent of conversion, α, plotted against the reciprocal of the temperature of the experiment will yield the straight line whose slope is E_a/R.

If the temperature is changed with the time (ϕ = dT/dt) as occurs in non-isothermal experiments, the Eq. (3) assumes the form:

$$\frac{d\alpha}{dT} = \frac{A}{\phi}\exp\left(-\frac{E_a}{RT}\right)f(\alpha) \tag{5}$$

Among the approximate solutions for the Eq. (5) which are found in the literature, that one proposed by Broido (1969) is frequently used in the studies of the thermal degradation of chitosan and other polymers. This approach assumes $f(\alpha) = (1-\alpha)$ and the following expression as a solution for the Eq. (5):

$$\ln\left[\ln\left(\frac{1}{1-\alpha}\right)\right] = \frac{-E_a}{RT} + \ln\left[\left(\frac{R}{E_a}\right)\left(\frac{A}{\phi}\right)T_m^2\right] \tag{6}$$

where T_m is defined as the temperature of the maximum reaction rate.

The activation energy is determined from the slope of the straight line which results by plotting ln[ln[1/(1-α)]] versus 1/T while the frequency factor, A, is determined from the intercept of the curve at the y-axis.

A single experiment is enough to determine the kinetic parameters E_a and A but the validity of this approach is object of strong criticism (Vyazovkin & Wight, 1998), the results issued from the isothermal method being pointed out as the most coherent ones.

The isoconvertional method (Ozawa, 1970), which seems to be better founded from a physical chemical point of view, adopts the Doyle approximation (Doyle, 1962) and is expressed by:

$$\log\phi = \log\frac{AE_a}{g(\alpha)R} - 2.315 - \frac{0.4567E_a}{RT} \tag{7}$$

Therefore, if a set of experiments is run at different heating rates, ϕ, then the value of E_a can be obtained from the plot of log ϕ against 1/T for a fixed degree of conversion, the frequency factor, A, being determined by:

$$A = \frac{\phi E}{RT_m^2}\exp\left(\frac{E_a}{RT_m}\right) \tag{8}$$

where T_m is the temperature of the maximum reaction rate.

The method proposed by Kissinger (1957) relies on experiments carried out at different heating rates, ϕ, and is expressed by:

$$\ln\frac{\phi}{T_m^2} = \left\{\ln\frac{AR}{E_a} + \ln\left[n(1-\alpha_p)^{n-1}\right]\right\} - \frac{E_a}{RT_m} \tag{9}$$

where α_p is the maximum conversion and n is the reaction order. Thus, the plot of ln ϕ/T_m^2 versus $1/T_m$ will yield the straight line whose slope is E_a/R.

The kinetics of the thermal degradation of the parent chitosan and carboxymethylchitosans was investigated by carrying out dynamic and isothermal experiments and the results of this study are discussed in the following.

3.5.1. Non-isothermal studies on the thermal degradation of chitosan and carboxymethylchitosan

The TG curves of chitosan and carboxymethylchitosans (data not shown) were acquired in nitrogen atmosphere from room temperature to 800 °C. These curves exhibited similar profiles and from them we determined the temperatures for the occurrence of the main thermal events and the corresponding losses of weight (Table 3).

The first thermal event occurred in the temperature range 25–110 °C and it was attributed to the evaporation of water. The weight losses attained 11–15% and the carboxymethylchitosans, for being more hydrophilic due to presence of the carboxymethyl groups and for adopting a less ordered arrangement, presented the higher weight losses in this interval of temperature.

The thermal degradation of the polymers presented a second stage which involves the loss of substituent groups as volatile fragments, the complete thermal degradation occurring in the third stage and corresponding to the rupture of the pyranose rings, depolymerization and pyrolitic reactions.

The focus of this study was centred in the second degradation stage, which occurred in the range 200–450 °C for chitosan and 190–400 °C for the carboxymethylchitosans, to determine the effect of substituents on the degradation kinetics. The data in Table 3 show that the introduction of carboxymethyl groups in the chitosan chain decreased the thermal stability of the polymer as the temperature corresponding to the onset of this degradation step was shifted to lower values. The same effect affected the maximum and final degradation temperatures, the higher the average degree of substitution the lower the temperatures. The loss of weight attained 40% and 43% for chitosan and the less substituted derivative (Sample QC_{10}), respectively, but it decreased to ≅34% in the case of the more substituted carboxymethylchitosans. The decrease in the thermal stability of the carboxymethylchitosan as compared to the parent chitosan may be attributed to the substitution reaction, an effect already described in the literature concerning other chitosan derivatives (Britto & Campana-Filho, 2004; Holme et al., 2001; Peniche-Covas et al., 1993; Tirkistani, 1998). An effect due to the occurrence of depolymerization and the destruction of crystalline domains during the derivatization of chitosan has also been reported (Alonso et al., 1983) but it was not observed in this study. Similar results were reported for the first stage of the thermal degradation of chitosan while *N*-carboxymethylchitosan showed a different pattern (Miranda et al., 2006), however, the effect of the degree of substitution on thermal stability was not investigated.

The application of the Broido's approach (Eq. (6)) to the experimental data concerning the study of the thermal degradation of chitosan and carboxymethylchitosan in dynamic conditions allowed the determination of the activation energy. Straight lines with different slopes can be traced depending on the temperature range considered and as a function of the sample, revealing the occurrence of distinct degradation processes and its dependence on the structural characteristics of the polymer. As already mentioned, the main degradation stage corresponded to the temperature interval in which the mass loss was more important. Thus, the apparent activation energies corresponding to this step of the thermal degradation of chitosan and carboxymethylchitosan were determined (Table 4) by applying the Broido's approach.

The activation energy for the thermal degradation of the carboxymethylchitosan samples were lower than that of the parent chitosan but no clear trend concerning its dependence on the average degree of substitution is identified. Another study on the thermal degradation of chitosan and *N*-carboymethylchitosan in dynamic conditions reported a similar value for the activation energy of the chitosan degradation at low degree of conversion, but higher values were found for the chitosan derivative (Miranda et al., 2006). From the data available, it is not possible to compare the influence of *N*- and *N*,*O*-carboxymethylation on the thermal stability of the resulting chitosan derivatives. However, as hydroxyl and amino groups are involved in the establishment of different hydrogen bonds (Focher et al., 1992), the carboxymethylation of these groups may affect the arrangement of the polymer chains in the solid state as well as the thermal behaviour of the polymers in different ways. Additionally, as the kinetics of the thermal degradation of polymers in dynamic conditions relies on complex assumptions, its validity and the results issued from its application should be considered with reserves.

Table 3
Temperatures (°C) corresponding to the onset, endset and peak of the thermal degradation of chitosan and carboxymethylchitosans (CQ) in dynamic conditions, and corresponding losses of weight (WL%)[a]

	$\overline{DS}$[b]	Temperature (°C)			WL (%)[a]
		Onset	Endset	Peak	
First stage					
Chitosan	–	25	110	47.2	11.2
QC_{10}	0.52	25	110	54.9	14.0
QC_{7}	0.72	25	110	49.9	13.6
QC_{7E}	0.77	25	110	48.1	14.4
QC_{10E}	1.38	25	110	48.3	15.0
Second stage					
Chitosan	–	200	450	317.0	43.5
QC_{10}	0.52	190	405	303.3	40.3
QC_{7}	0.72	190	401	296.7	34.7
QC_{7E}	0.77	190	375	295.5	34.1
QC_{10E}	1.38	187	365	293.3	33.8

[a] Values corresponding to two independent determinations.
[b] Values averaged from the titrations and infrared spectroscopy.

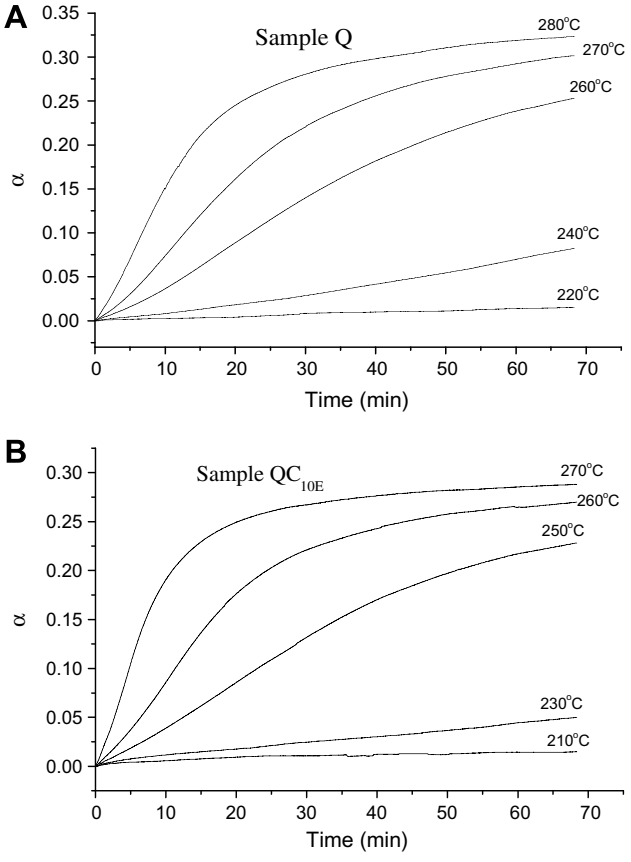

Fig. 2. Plots of degree of conversion (α) versus reaction time for the isothermal degradation of chitosan (Sample Q) and sodium carboxymethylchitosan (Sample QC_{10E}) at different temperatures.

3.5.2. Isothermal studies on the thermal degradation of chitosan and carboxymethylchitosan

The kinetics of the thermal degradation of chitosan and carboxymethylchitosan was also studied in isothermal conditions. The characteristic curves showing the dependence of the degree of conversion (α) on the reaction time (t) for the isothermal degradation of the parent chitosan (Sample Q) and carboxymethylchitosan (sample QC_{10E}) as a function of the temperature are shown in Fig. 2.

The lower thermal stability of the carboxymethylchitosan compared to the parent chitosan is clearly seen in Fig. 2. Thus, from the curve corresponding to the isothermal degradation of carboxymethylchitosan (Sample QC_{10E}) at 260 °C one can determine that the degree of conversion at 10, 20 and 30 min attained 0.083, 0.17 and 0.22, respectively. However, in the case of the isothermal degradation of chitosan at the same temperature and for reaction times of 10, 20 and 30 min, the degree of conversion was 0.036, 0.09 and 0.14, respectively. Also, it is observed that the curve corresponding to the thermal degradation of carboxymethylchitosan at 270 °C attained a plateau for reaction time t = 40 min while in the case of chitosan it was achieved at 50 min but yet at higher temperature (T = 280 °C).

The plots of ln t versus $1/T$ as a function of the degree of conversion (α) for chitosan (Sample Q) and carboxymethylchitosan (sample QC_{10E}) allowed the determination of the activation energy for all samples (Table 5). The values of the activation energy for the isothermal degradation of chitosan are roughly constant in the range of degree of conversion $0.015 < \alpha < 0.19$, however, they increase with increasing degree of conversion in the case of carboxymethylchitosan. This is attributed to the fact that the degradation of carboxymethylchitosan is intrinsically more complex than that of chitosan due to the presence of the carboxymethyl groups. Such a hypothesis is supported by other results reported in the literature (Britto & Campana-Filho, 2004; Kittur et al., 2002; Peniche-Covas et al., 1993; Qu et al., 2000) according to which additional degradation reactions occur in the latter stages of the thermal degradation of chitosan derivatives.

The values of the activation energies for the isothermal degradation of chitosan and carboxymethylchitosans were, respectively, roughly 1.5 and 2 times higher than those determined from the dynamic experiments. Also, the results issued from the isothermal experiments showed that the E_a values for the thermal degradation of the carboxymethylchitosans were higher than that of chitosan while the dynamic experiments resulted in the contrary. These discrepancies should be attributed to the very different assumptions adopted for the treatment of the data of isothermal and dynamic degradation. In fact, the former treatment considers the effect of temperature for constant conversion, focusing on the temperature dependence of the overall reactions, while the latter one involves much more complex assumptions and the E_a values derived from this treatment should be considered with reserve.

Table 4
Activation energy (E_a) determined according to Broido's method for the thermal degradation of chitosan (Q) and carboxymethylchitosan (QC) in dynamic conditions

Sample	$\overline{DS}$[a]	E_a (kJ/mol)
Q	–	110.7 ± 1.34
QC_{10}	0.52	79.8 ± 0.14
QC_7	0.72	73.7 ± 0.35
QC_{7E}	0.77	77.3 ± 0.21
QC_{10E}	1.38	83.7 ± 1.91

[a] Values averaged from the titrations and infrared spectroscopy.

Table 5
Activation energy (E_a) determined according to MacCallum's method for the thermal degradation of chitosan (Q) and carboxymethylchitosans (QC) as a function of the degree of conversion (α)

Sample	$\overline{DS}$[a]	Activation energy (kJ/mol)			
		α = 0.015	α = 0.05	α = 0.1	α = 0.19
Q	–	142.9 ± 5.4	148.1 ± 2.0	155.1 ± 12.1	151.8 ± 9.8
QC_{10}	0.52	133.6 ± 7.4	164.5 ± 10.0	190.0 ±11.1	174.7 ± 4.4
QC_7	0.72	115.8 ± 9.4	157.9 ± 3.1	163.7 ± 12.6	170.3 ± 12.8
QC_{7E}	0.77	140.8 ± 9.7	175.0 ± 5.4	196.4 ± 5.3	196.2 ± 3.9
QC_{10E}	1.38	152.7 ± 5.6	181.3 ± 2.0	186.4 ± 3.0	185.0 ± 2.0

[a] Values averaged from the titrations and infrared spectroscopy.

4. Conclusions

The carboxymethylation of chitosan was successfully achieved and samples of different average degrees of substitution ($0.5 < \overline{DS} < 1.5$) were prepared according to the reaction conditions employed. The use of a higher excess of monochloroacetic acid and sodium hydroxide resulted in the more substituted samples but prolonging the reaction did not strongly affect the average degree of substitution. The occurrence of *N*-carboxymethylation was evidenced by ^{1}H NMR and ^{13}C NMR spectroscopy, revealing that *N*,*O*-carboxymethylchitosans were produced in the reaction conditions employed in this work, although the literature report that *O*-carboxymethylation should predominate in these conditions. The introduction of carboxymethyl groups strongly affected the packing of the polymer chains in the solid state, resulting in a less ordered arrangement as compared to chitosan. Other consequences of the carboxymethylation were the higher capacity to adsorb water and the better solubility exhibited by the carboxymethylchitosans as compared to the parent chitosan.

The isothermal and dynamic methods employed to study the thermal degradation of chitosan and *N*,*O*-carboxymethylchitosan in nitrogen atmosphere clearly showed the decrease of thermal stability provoked by the carboxymethylation reaction. However, the dependence of the activation energy on the average degree of substitution of *N*,*O*-carboxymethylchitosan was not identifiable. This is taken as evidence that other factors, such as the crystallinity of the polymers, must have an important role in the thermal degradation process of carboxymethylchitosan derivatives. The energy of activation for the thermal degradation of chitosan and *N*,*O*-carboxymethylchitosan determined from the isothermal experiments were roughly 1.5 and 2 times higher than those issued from the dynamic studies. This is attributed to the different assumptions adopted for modelling isothermal and dynamic thermal degradation reactions, the latter involving more complex assumptions which impart a great deal of scepticism to its validity.

Further work is in progress to better understand the dependence of the thermal stability and other physical chemical properties of *N*,*O*-carboxymethylchitosan on the conditions employed to carry out the carboxymethylation of chitosan.

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