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Interaction of chitosan with natural or synthetic anionic polyelectrolytes. 1. The chitosan–carboxymethylcellulose complex

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Abstract

Chitosan, the unique cationic groups-containing polysaccharide is a natural, renewable, nontoxic and biodegradable source being considered as 'ecologically-friendly' product. It has been studied as partner in several intermacromolecular complexes (IMC) with natural or synthetic anionic polyelectrolytes. The present investigation discusses some new results on the chitosan–carboxymethylcellulose interaction studied in solution by conductometric, potentiometric and turbidimetric titration, and in the solid phase by FTIR spectra or thermogravimetric analysis. All the titration methods confirmed the formation of complexes in a stoichiometric ratio between partners. Analysis of the solid phase complex by FTIR spectra evidenced some bands characteristic of the electrostatic interaction, along with other bands, suggesting the partial recovery of the NH_2 and COOH groups. The difference between the behavior of single partner, complex and physical mixture was clearly evidenced by differential thermal analysis. The mechanism of thermal degradation in air is quite complex.

Keywords: Polysaccharides; Chitosan; Carboxymethylcellulose; Intermacromolecular complexes

1. Introduction

Chitosan, a cationic group-containing polysaccharide is obtained by deacetylation of chitin extracted from the external skeleton (shell) of marine crustaceans. Initiated as early as the thirties, the investigations devoted to chitin, chitosan and their possible applications have increased in recent years, since such polysaccharides represent a natural, renewable, nontoxic and biodegradable source, which justifies they being viewed as 'ecologically-friendly' products (Hirano, 1999; Hirano et al., 1994; Peter, 1995). The term 'chitosan' defines the chitins with a deacetylation degree exceeding 70%, whose quality and properties depend on their purity, deacetylation degree (ranging between 70 and 95%), molecular weight (between 100,000 and 1,200,000) and crystallinity (Kurita, 2001). Chitosan is insoluble in either water or organic solvents. It dissolves in hydrochloric acid and aqueous organic acids such as formic, acetic, oxalic, and lactic acids. The extent of solubility depends on the concentration and on the type of acid. The solubility decreases with increasing concentration of acid, and aqueous solutions of some acids such as phosphoric, sulfuric, citric, and sebacic are not good solvents (Gross, Konrad, & Mager, 1983). Chitosan possesses therapeutical activity, being hemostatic, hypocholesteremic, bacteriostatic and having antitumor properties (Li, Dunn, Grandmaison, & Goosen, 1992). Among its applications, mention should be made of chitosan employed as a drug support or for cell cultures. The importance of the recent studies on chitosan is evidenced by the higher frequency of review articles or chapters of books (Hirano, 1999; Hirano et al., 1994; Peter, 1995) devoted to it.

The intermacromolecular complexes (IMC) are obtained by the interaction of macromolecules carrying ionized/ionizable groups of opposite sign. The electrostatic forces may be completed by hydrogen bonds, van der Waals forces, hydrophobic bonds. IMC represent successful candidates for controlled drug-release systems, protein separation, membranes, and skin substitutes. As an unique natural cationic polymer, chitosan has been studied as partner of several IMC with natural or synthetic anionic polyelectrolytes, such as: chondroitin sulfate

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(Denuziere, Ferrier, Damour, & Domard, 1998), poly(acrylic acid) (Peniche & Argüelles-Monal, 2001; Pérez-Gramatges, Argüeles-Monal, & Peniche-Covas, 1996; Peniche et al., 1999; Wang, Li, Lu, & Zhong, 1996), poly(galacturonic acid) (Argüelles-Monal, Cabrera, Peniche, & Rinaudo, 2000; Peniche & Argüelles-Monal, 2001), sodium alginate, k-carageenan (Peniche & Argüelles-Monal, 2001). Chitosan's complexation with carboxymethylated polysaccharides, such as carboxymethylcellulose or carboxymethyldextran has been also studied (Argüelles-Monal, Gárciga, & Peniche-Covas, 1990; Fukuda, 1980; Fukuda & Kikuchi, 1978, 1979; Argüelles-Monal & Peniche, 1988; Peniche & Argüelles-Monal, 2001).

In our contribution some new results on the chitosan–carboxymethylcellulose interaction are presented. The study of this interaction was performed in solution, by conductometric, potentiometric, and turbidimetric titration, backed up by solid phase analysis methods.

2. Materials and methods

2.1. Polymers

Carboxymethylcellulose in the form of sodium salt (NaCMC) was provided by Austranal, Austria. The substitution degree (SD) of carboxymethylcellulose, determined by the sulfate method, was 0.8 (Hoeye, 1977). Chitosan (Chi) was provided by Vanson Co., Canada[1]. The characteristics of two polymers (manufacturer data) are listed in Table 1, while their chemical structure is presented in Scheme 1. The chitosan with a residual acetylation degree (DA) of 28% was insoluble in water. In our experiments, chitosan has been dissolved in water by adding of a stoichiometric amount of hydrochloric acid in order to prepare the hydrochloride salt (ChCl).

The behavior of each partner was verified by potentiometric and conductometric titration with low molecular base or acid, respectively. The titration curves of NaCMC are presented in this chapter (Fig. 1), while the titration curves of ChCl will be presented together with the curves corresponding to the complex formation. In Fig. 1 a and b are presented the potentiometric and conductometric titration curves of NaCMC with a 0.1 N HCl solution. No agreement is to be observed between the two methods. The equivalence volume determined from the conductometric curves correspond to the calculated one, according to the substitution degree. On the potentiometric curves one may notice one inflection point at $\sim$0.5 and another, less obvious one, at 1.4, neither of them corresponding to the value calculated from the SD. The two points may be attributable to some fractions of carboxylic groups accessible in a different manner to the titrating agent, as a result of the polysaccharide chain's rigid conformation. Similar results were obtained on synthetic carboxylic polyelectrolytes (Chitanu et al., 1999). The addition of low molecular salt does not change significantly the titration curve (data not shown).

[1] By courtesy of Prof. Severian Dumitriu, Sherbrooke University, Canada

Table 1
The physico-chemical characteristics of the polymer partners

Polymer	Loss on drying (%)	Acetylation degree (%)	Substitution (degree)	Molecular weight (g/mol)
NaCMC	15.8	–	0.8	550,000
Chi	4.5	20.8	–	415,000

2.2. Methods

Potentiometric titration was made with an all-purpose titrator 716 DMS Titrino, from Metrohm, Switzerland, equipped with a dosing unit, a cell with thermostatted jacket and a 6,0218,010 combined glass electrode with a built-in temperature sensor. Titration was performed by the classical technique of addition in equal increments of the titrating agent (*M*onotonic *E*nd-point *T*itration-*MET*).

Conductometric titration was made using a Radiometer Copenhagen (Denmark) conductivity meter CMD 210 equipped with a CDC 865 cell.

Turbidimetric titration involves measurements of the intensity decrease of a light flow passing through a solution containing solid particles, (IMC particles). Turbidity measurements were made with a Brinkmann PC 900 (SUA) turbidimeter equipped with a 20-23-634-5 type fiber optic cell. The device indicates the value of transmitted light (transmittance), while system's turbidity, expressed in arbitrary units, is obtained as a difference ($t\% = 100 - T\%$).

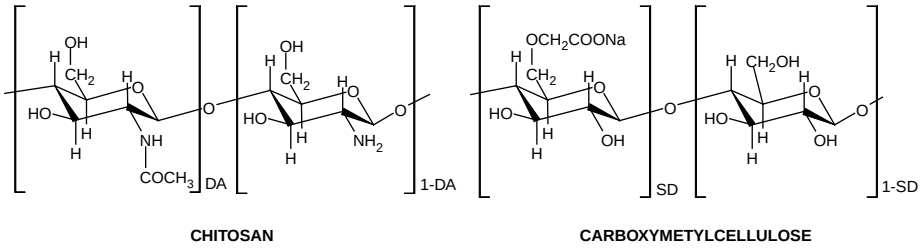
The IR Spectra have been recorded on a spectrophotometer, with the Fourier transformation element, FT-IR BOMEM MB 104, in the KBr tablet.

Thermogravimetric analysis was performed with a Paulik–Paulik–Erdey type thermobalance MOM-Budapest, Hungary, under the following conditions: $m = 46 \pm 1$ mg, reference material Al_2O_3, platinum crucible, heating rate 10 °C/min, in air, under static conditions, temperature interval 10–900 °C. Processing of the thermogravimetric data was based on the Coats–Redfern (C–R) integral (Coats & Redfern, 1964) and, respectively Freeman–Caroll differential method (F–C) (Freeman & Carroll, 1958).

3. Results and discussion

The electrostatic interaction between chitosan hydrochloride (ChCl) and NaCMC may be developed according to Scheme 2.

This was followed by potentiometric and conductometric titration (electrochemical methods). The systems



Scheme 1. Chemical structure of chitosan and carboxymethylcellulose.

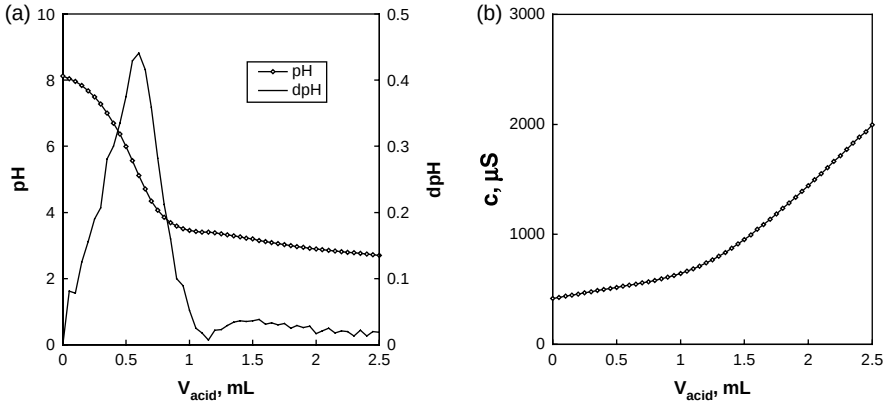
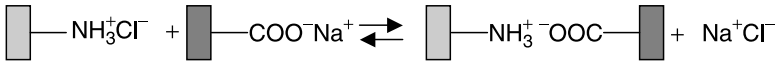


Fig. 1. Potentiometric (a) and conductometric (b) titration of NaCMC with HCl ($C_{NaCMC}=5\times10^{-3}$ eq/L).

global behavior may be followed by turbidimetric titration, while the difference between the two methods may be providing information on the interaction through weak forces. The electrochemical methods were used also in the characterization of individual partners and complex formation. Thus, Fig. 2 shows ChCl's behavior by potentiometric titration with 0.1 N NaOH solution (curve 1) respectively, with NaCMC (curve 2). On the titration curve of the ChCl with NaOH, it can be noticed a slight inflection point, which can be attributed to a low HCl excess during hydrochloride's preparation, and a marked inflection point, visible on the derived curve, as well, corresponding to the titration of hydrochloride amino groups of ChCl. The titration curve 2 shows the titration of a ChCl solution ($C_{ChCl}=5\times10^{-4}$ eq/L) with a NaCMC solution ($C_{NaCMC}=5\times10^{-3}$ eq/L). In order to reduce the effects of system's dilution, concentration of the titrating partner's solution was an order of magnitude higher that that of the titrated partner. The pH value was represented as a function of the molar (equivalent) ratio between the partners, calculated from the added volumes and concentrations. From the potentiometric curve 2 one observes that the interaction between partners takes place in a stoichiometric ratio of 1:1 as represented in Scheme 2. The difference between the titration curves with NaOH and NaCMC could be explained by the dissociation constants of the macromolecular partners. Chitosan behaves as a moderately basic cationic polyelectrolyte, the value of pK_b being 6.3 (Peter, 1995) while the pK_a value of CMC is as low as 3.0 (Dautzenberg et al., 1994). Correspondingly the conjugated base of CMC behaves as a quite weak base and the variation of pH by titration is only 2.5 units compared to about 6 units in the case of titration with NaOH.

In Fig. 3 are presented the conductometric titration of ChCl with a 0.1 N NaOH solution (curve 1) and with NaCMC (curve 2).

The conductometric curves evidence modifications of the slopes corresponding to the excess of the titrating agents. The potentiometric and conductometric titrations agree, indicating an electrostatic interaction of the partners in an approximate stoichiometric ratio of 1:1. Until the equivalence point the conductivity of the system appears constant possibly due to the fact that the number of ions which participate to the conductivity of the system is constant (according to Scheme 2), then conductivity



Scheme 2. Interaction of chitosan hydrochloride with sodium carboxymethylcellulose.

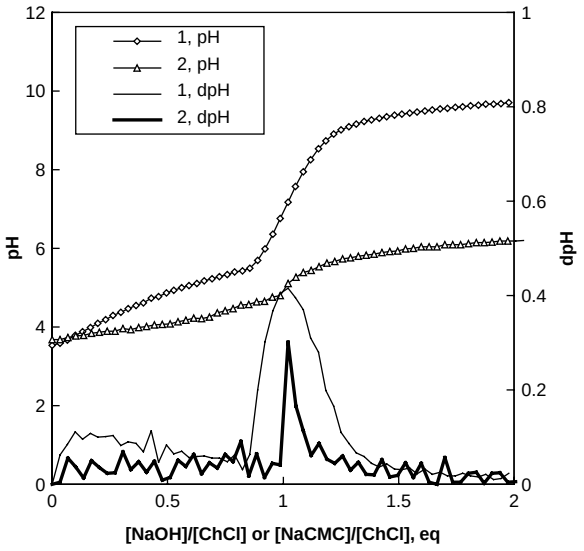


Fig. 2. Potentiometric titration of ChCl with NaOH (1) and with NaCMC (2) ($C_{ChCl}=5\times10^{-4}$ eq/L; $C_{NaCMC}=5\times10^{-3}$ eq/L; $C_{NaOH}=0.1$ N).

increases because of NaCMC excess. The behavior of this system is different from the results mentioned by other authors (Zezin et al., 1999) that have studied the interaction between weak polymeric acids (bases) and the salts of polymeric bases (acids), when a strong acid or a strong base is delivered.

The titration curve using turbidimetric measurements shown in Fig. 4 evidences a sudden increase of turbidity at [NaCMC]/[ChCl] about 1, in agreement with the electrochemical methods; this suggests that the interaction between partners involves mainly electrostatic forces. It can be also noticed that the titration curve of ChCl with NaOH evidences as well a slight turbidity at [NaOH]/[ChCl] = 1 confirming that non-ionised chitosan is not water-soluble (Li et al., 1992).

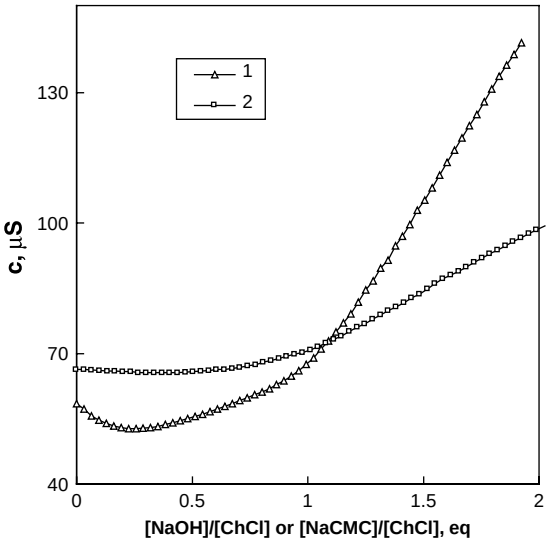


Fig. 3. Conductometric titration of ChCl with NaOH (1) and with NaCMC (2) ($C_{ChCl}=5\times10^{-4}$ eq/L; $C_{NaCMC}=5\times10^{-3}$ eq/L; $C_{NaOH}=0.1$ N).

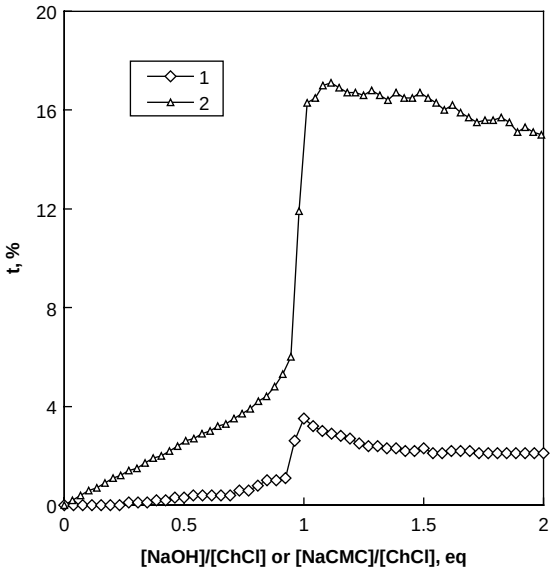


Fig. 4. Turbidimetric titration of ChCl with NaOH (1) and with NaCMC (2) ($C_{ChCl}=5\times10^{-4}$ eq/L; $C_{NaCMC}=5\times10^{-3}$ eq/L; $C_{NaOH}=0.1$ N).

The Ch–CMC complex, prepared through partners' interaction has been recovered by filtration, dried at 40 °C, under low pressure, and characterized by FTIR spectra and thermogravimetric analysis. The spectra of ChCl, NaCMC, 1:1 ChCl:NaCMC mixture and Ch–CMC complex are presented in Fig. 5. In the ChCl spectrum, the already mentioned characteristic bands (Denuziere et al., 1998) may be observed at: 1635 cm^{-1} (amide I), 1523 cm^{-1} (NH_2), 1379 cm^{-1} (amide II). The absorption bands at 1157, 1072 and 1033 cm^{-1} are characteristic of the polysaccharide skeleton. The absorption band at 2054 cm^{-1} is characteristic for the $NH_3^+Cl^-$. The FTIR spectrum of NaCMC evidences, too, bands at 1419 and 1618 cm^{-1} corresponding to the symmetrical and asymmetrical stretching vibrations and, respectively, to the carboxylate groups. The Ch–CMC complex shows a characteristic spectrum, different from those of the polymer partners. Besides, the bands corresponding to the polysaccharide skeletons, characteristic bands/peaks may be observed at 1589 and 1755 cm^{-1}, which can be attributed to the NH_3^+ and COOH groups, respectively. The presence of the band at 1589 cm^{-1} is expected, since the intermacromolecular complex formation occurs through the electrostatic interaction of the cationic groups from ChCl with the anionic ones from NaCMC. The band at 1755 cm^{-1} suggests, nevertheless, that the formation reaction presented in Scheme 2 is reversible, so that the reaction given in Scheme 3 becomes also possible.

In this case, one may assume that the complex stability is maintained by hydrogen bonds.

The interaction between ChCl and NaCMC according to the Scheme 3 is confirmed also by the thermal analysis. Additionally the thermal analysis can give useful information for the further application of the complex. In the

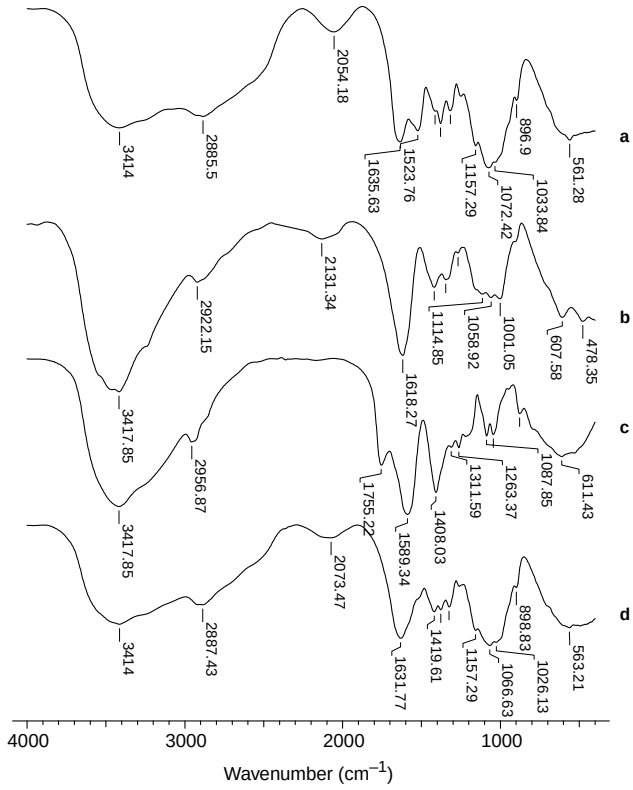
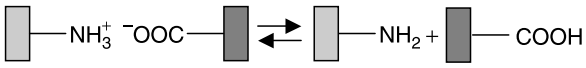


Fig. 5. FTIR spectra of ChCl (a), NaCMC (b), complex (c) and 1:1 mixture (d).

literature we found only limited data about the thermal behavior of IMC (Argüelles-Monal, Gárciga, & Peniche-Covas, 1990). The thermograms recorded for chitosan hydrochloride, sodium carboxymethylcellulose, 1:1 complex, and 1:1 mixture, are shown in Figs. 6–8. The DTG curves (Fig. 7) evidence very clearly a different behavior for the complex compared to the individual partners and their mixture. Decomposition of the complex occurred in two stages at different temperatures compared to the mixture.

Analysis of the thermogram shows that the temperature at which the loss in weight begins is below 100 °C, when traces of solvents/water used in sample preparation are removed. The thermal decomposition takes place in two degradation stages, with different weight losses, as shown in Table 2. In the case of chitosan, the most important weight loss was noticed in the second degradation stage, at temperatures ranging between 420 and 705 °C. A common feature, namely a constant conversion degree and a strong exothermal effect (Fig. 8), characterizes this last stage of thermal degradation. Compared to ChCl, by NaCMC the most important weight loss was noticed in the first



Scheme 3. The equilibrium between the IMC and free partners.

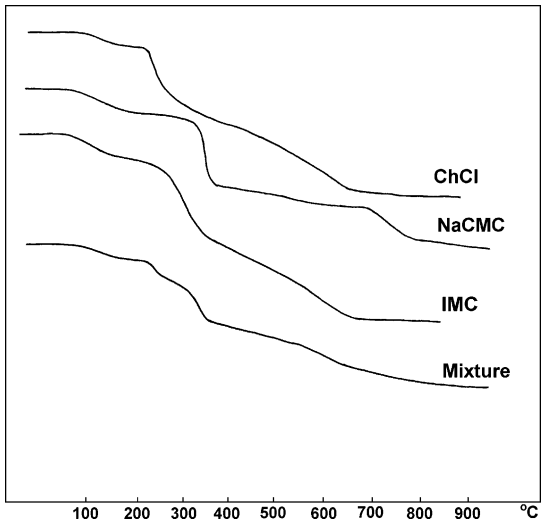


Fig. 6. TG curves of chitosan hydrochloride, sodium carboxymethylcellulose, complex and 1:1 mixture.

degradation stage at temperatures ranging between 300 and 400 °C, NaCMC being more thermal stable than ChCl.

Considering the temperature at which the thermal degradation starts as a criterion of the thermal stability, the thermal stability decreases according to the series:

NaCMC > IMC > ChCl

Evaluation of the kinetic parameters from thermogravimetric data, under dynamic conditions of temperature, is based on some specific calculation methods, namely differential and integral methods. The evaluation of the kinetic parameters was made using 'Mathcad 2000' version programs, which allowed determination of the following kinetic parameters: activation energy (E_a), reaction order (n), preexponential factor (A), the value of which are listed

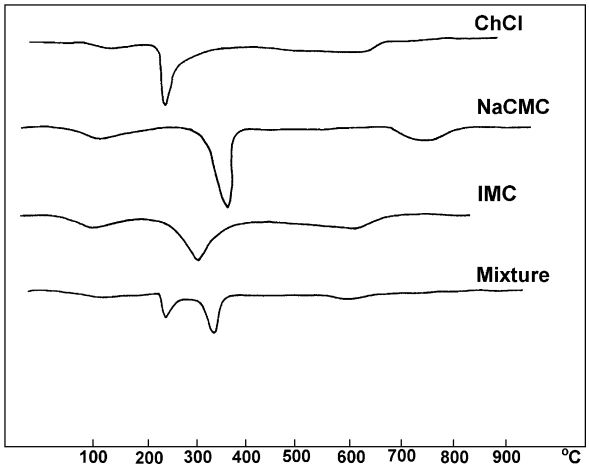


Fig. 7. DTG curves of chitosan hydrochloride, sodium carboxymethylcellulose, complex and 1:1 mixture.

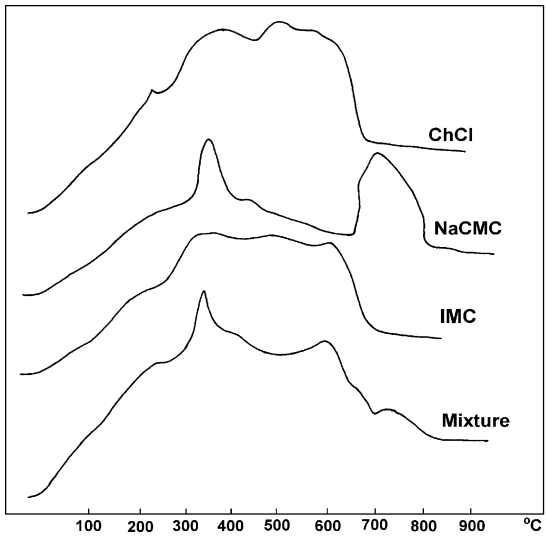


Fig. 8. DTA curves of chitosan hydrochloride, sodium carboxymethylcellulose, complex and 1:1 mixture.

in Table 3. Good agreement of the experimental data with the mathematical models is observed.

The Freeman–Caroll (Freeman & Caroll, 1958) differential method and the Coats–Redfern (Coats & Redfern, 1964) integral one has determined the kinetic data listed in Table 3, referring to the first stage of thermal degradation. In the case of chitosan and carboxymethylcellulose, the reaction order takes values between 0 and 0.6, while the activation energy in this first stage of thermal degradation lies between 125 and 133 kJ/mol. In the case of the Chi–CMC complex, higher differences are noticed between the values of the activation energies; thus, the chitosan–carboxymethylcellulose complex has a reaction order equal to two and activation energy of 144 kJ/mol, indicating a complex mechanism of degradation. The information obtained by thermal analysis is helpful for some applications (membranes, capsule wall) for which more experimental work is in progress.

4. Conclusions

The interaction of chitosan hydrochloride with sodium carboxymethylcellulose was studied in aqueous solution, by potentiometric, conductometric and turbidimetric titration, as well as in solid state, by FTIR spectra and thermogravimetric analysis. All the titration methods have evidenced the formation of complex in a stoichiometric ratio between partners, suggesting that the interaction proceeds through electrostatic forces. FTIR spectra of solid complex evidenced some bands characteristic to the electrostatic interaction, along with other bands, suggesting the partial recovery of the NH_2 and COOH groups. The thermal analysis of the partners and their complex or mixture confirmed the formation of the complex and offered useful information for further application.

Table 2
Thermogravimetric data regarding the decomposition of chitosan hydrochloride, sodium carboxymethylcellulose, complex and 1:1 mixture

Sample	Degradation stage	Characteristic of DTA curves	T_i (°C)	T_{max} (°C)	T_r (°C)	Weight loss
ChCl	I	exo	210	230	420	43.23
	II	exo	420	670	705	56.77
NaCMC	I	exo	300	360	400	56.25
	II	exo	400	710	830	43.75
IMC	I	exo	220	325	400	50.84
	II	exo	400	640	700	49.16
1:1 mixture	I	exo	210	230	300	12.86
	II	exo	300	360	400	24.02
	III	exo	400	640	880	63.12

Table 3
Kinetic data regarding the decomposition of chitosan hydrochloride, sodium carboxymethylcellulose, complex and 1:1 mixture

Sample	Freeman–Caroll method	Coats–Redfern method
ChCl	$n=1$; Ea = 133.98 kJ/mol; ln $A=30.13$; $r^2=0.97$	$n=1$; Ea = 129.80 kJ/mol; ln $A=29.24$; $r^2=0.95$
NaCMC	$n=1$; Ea = 125.66 kJ/mol; ln $A=30.06$; $r^2=0.95$	$n=1$; Ea = 130.45 kJ/mol; ln $A=31.25$; $r^2=0.98$
IMC	$n=2$; Ea = 147.00 kJ/mol; ln $A=26.79$; $r^2=0.97$	$n=2$; Ea = 142.68 kJ/mol; ln $A=26.04$; $r^2=0.96$
1:1 mixture	$n=1$; Ea = 131.45 kJ/mol; ln $A=32.88$; $r^2=0.98$	$n=1$; Ea = 132.82 kJ/mol; ln $A=33.97$; $r^2=0.97$
	$n=1$; Ea = 134.50 kJ/mol; ln $A=27.30$; $r^2=0.99$	$n=1$; Ea = 138.06 kJ/mol; ln $A=28.70$; $r^2=0.97$

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