Speciation of chitosan with low and high molecular weight carboxylates in aqueous solution

Salvatore Cataldo[a], Concetta De Stefano[b], Antonio Gianguzza[ac], Daniela Piazzese[a] & Silvio Sammartano[b]

[a] Dipartimento di Chimica Inorganics e Analitica "Stanislao Cannizzaro", Universitàdi Palermo, Viale delle Scienze, I-90128 Palermo, Italy

[b] Dipartimento di Chimica Inorganica, Chimica Analitica e Chimica Fisica, Universitàdi Palermo, Salita Sperone 31, I-98166, Messina (Vill. S. Agata), Italy

[c] E-mail:
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Speciation of chitosan with low and high molecular weight carboxylates in aqueous solution

Salvatore Cataldo[a], Concetta De Stefano[b], Antonio Gianguzza[a*], Daniela Piazzese[a] and Silvio Sammartano[b]

[a]*Dipartimento di Chimica Inorganica e Analitica "Stanislao Cannizzaro". Università di Palermo, Viale delle Scienze, I-90128 Palermo, Italy*   **E-mail: giang@unipa.it*

[b]*Dipartimento di Chimica Inorganica, Chimica Analitica e Chimica Fisica. Università di Messina, Salita Sperone 31, I-98166, Messina (Vill. S. Agata), Italy*

ABSTRACT

Quantitative data on the speciation of chitosan (310 kDa) with low and high molecular weight carboxylates in aqueous solution are reported. The following carboxylic ligands were considered: monocarboxylate (butyrate); dicarboxylates (malonate, succinate, azelate); tricarboxylate (1,2,3-propanetricarboxylate); tetracarboxylate (1,2,3,4-butanetetracarboxylate); polyacrylates (2.0 and 20 kDa); polymethacrylate (5.4 kDa). The investigation was performed by potentiometry at $t = 25°C$, at low ionic strength (without addition of supporting electrolyte) and at $I = 0.15$ mol L^{-1} (NaCl). For all the systems the formation of (chitosan)LH$_i$ species was found (L = carboxylic ligand; i = 1 to 4 depending on the carboxylic ligand considered). The stability of proton–chitosan–carboxylate species depends on the number of carboxylic groups involved in the complexation, and it was possible to calculate a rough free energy value per bond $\Delta G_n = -15 \pm 2$ kJ mol^{-1}. By using the stability data, the quantitative sequestering capacity of chitosan towards the carboxylates here considered [expressed as the –log(total chitosan concentration) necessary to bind 50% of carboxylate, *i.e.*, pL$_{50}$] was calculated for different pH values, at low ionic strength and at $I = 0.15$ mol L^{-1}. The pL$_{50}$ values, ranging from 3 to 7, show that chitosan is quite a strong sequestering agent towards carboxylates. Evidences were also obtained for the different behaviour between low and high molecular weight carboxylates.

Keywords: chitosan, polyammonium-polycarboxylate interactions; chemical speciation, sequestration, effect of ionic strength on sequestration

1. INTROUCTION

Chitosan is a widely used biomaterial in industrial, environmental and pharmaceutical applications (Ravi Kumar, 2000; Ravi Kumar *et al.*, 2004). Literature data on chitosan, as well as the precursor chitin, also refer to its possible use as biosorbent material for metal ions in contaminated water and in wastewaters treatment (Wu *et al.*, 1999; Babel and Kumiawan, 2003; Crini, 2005; Navarro *et al.*, 2003; Varma *et al.*, 2004; Lee *et al.*, 2005). Other studies are focused on pharmaceutical properties of chitosan and its derivatives (Dodane and Vilivalam, 1998; Howling *et al.*, 2001; van der Lubben *et al.*, 2001). Chitosan is a linear copolymer of 2-amino-2-deoxy-D-glucopyranose and 2-acetamido-2-deoxy-glucopyranose (structure in Figure 1).
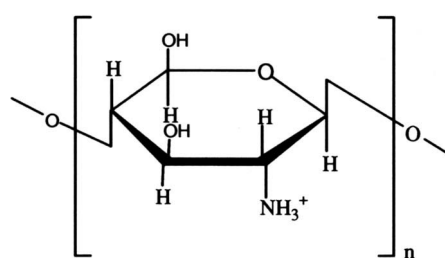

Figure 1 Structure of chitosan.

Due to the large number of amino groups in the chitosan structure, which can be protonated in the acidic pH range, it behaves in aqueous solution as a cation polyelectrolyte able to interact with organic and inorganic negatively charged ligands. Interactions between chitosan and carboxylic acids have been evidenced in the studies of carboxylates sorption by solid state or gel phase chitosan (Muzzarelli *et al.*, 2000; Torrado *et al.*, 2004; Riberlo *et al.*, 2005; de Yao *et al.*, 1996), as shown, for example, in the work of Shamov *et al.* (Shamov *et al.*, 2006) where complex species formation of chitosan with several carboxylic ligands was studied by fitting experimental sorption isotherms by Langmuir, Freundlich and Redlich-Peterson equations. Other papers, recently reported on the formation of different solid chitosan-carboxylate complexes, refer to the complex formation of chitosan with taurocholate and its derivatives (Muzzarelli *et al.*, 2006), lipoic acid (Kofuji *et al.*, 2008), lipopolysaccharides (Davydova *et al.*, 2008), carboxymethylcellulose (Gomez-Burgaz *et al.*, 2008; Peniche and Argüelles-Monal, 2001), polygalacturonic acid (Peniche and Argüelles-Monal, 2001), alginate (Peniche and Argüelles-Monal, 2001; Simsek-ege *et al.*, 2003; Saether *et al.*, 2008). In spite of such a number of investigations on chitosan–carboxylate systems, very few papers (Wang *et al.*, 1996; Wu *et al.*, 2007) are reported in literature on the complex species formation between water soluble chitosan and carboxylic ligands in aqueous solution, probably owing to the low solubility of chitosan (Ravindra *et al.*, 1998; Qin *et al.*, 2006) in water and no quantitative data on the stability of these systems are reported. Recent experimental data [unpublished data from this laboratory] show a solubility of chitosan (310 kDa MW) of about 0.1 mmol L^{-1} (referred to the monomer unit, see later) in 0.1 mol L^{-1} NaCl aqueous solution. On the other hand, chitosan is soluble in aqueous acidic solution where amino groups are protonated and this allows to study its interactions with negatively charged ligands in the acidic pH range and, in some cases at pH > 7, depending on the strength of interactions and the concentration of reagents involved.

Investigations on the formation and stability of complex species between polyammonium cations and polycarboxylic ligands are of great importance in the light of defining the speciation of both these classes of substances which are involved in several biological processes. As an extension of our previous studies on different polyammonium–polyanion (both

low (De Robertis *et al.*, 1996; De Robertis *et al.*, 1998; De Stefano *et al.*, 1998a; Cascio *et al.*, 2000b; De Robertis *et al.*, 2001a; De Robertis *et al.*, 2001b; De Stefano *et al.*, 2002) and high (Crea *et al.*, 2001; De Stefano *et al.*, 2006; Crea *et al.*, 2008a) molecular weight) systems of biological and environmental interest, in this paper we report an investigation on the interaction of chitosan with mono-, di-, tri- and tetra-carboxylates and with high molecular weight polycarboxylates such as polyacrylates and polymethacrylates. Results are given in terms of formation constants of complex species in aqueous solution. In particular, the following low molecular weight carboxylates were investigated: butyric acid [(**but**) $CH_3(CH_2)_2COOH$], as monocarboxylate; malonic acid [(**mal**) $(HOOC)–(CH_2)–(COOH)$], succinic acid [(**suc**) $(HOOC)–(CH_2)_2(COOH)$], azelaic acid [(**az**) $(HOOC)–(CH_2)_7–(COOH)$], as dicarboxylates; 1,2,3-propanetricarboxylic acid [(**tca**) $(HOOC–CH_2)–(CH–COOH)–(CH_2–COOH)$] as tricarboxylate; 1,2,3,4-butanetetracarboxylic acid [(**btc**) $(HOOC–CH_2)–(CH–COOH)_2–(CH_2–COOH)$] as tetracarboxylate. High molecular weight polycarboxylates investigated were: polyacrylates with average molecular weight 2 and 20 kDa [$(–CH_2–CH(COOH)–)_n$, **paa-2** and **paa-20**, respectively] and polymethacrylate 5.4 kDa [(**pma-5.4**) $(–CH_2–C(CH_3)(COOH)–)_n$]. Average molecular weight of chitosan used in this study was 310 kDa. Measurements were performed by potentiometry ([H^+]-glass electrode) in aqueous solution at 25°C. To define the complexation model for the systems investigated, the protonation constants of low (De Robertis *et al.*, 1993; De Stefano *et al.*, 1994; Crea *et al.*, 2004) and high (Crea *et al.*, 2009 and refs. therein) molecular weight carboxylic ligands previously reported, as well as those of chitosan [unpublished data from this laboratory] were used. Results are reported at infinite dilution and at I = 0.15 mol L^{-1}. Speciation profiles were drawn for all the systems considered in this study and were interpreted in terms of sequestering ability of chitosan towards polycarboxylates, using a sigmoid Boltzman type equation.

2. EXPERIMENTAL

2.1 Chemicals

Chitosan was supplied by Aldrich (lot. n. 448877, average molecular weight 310 kDa). Its water content, determined by thermogravimetric analysis,

Table 1 Experimental conditions for measurements on chitosan$-$low and high molecular weight carboxylate systems at $T = 25°C$

L	C_{chit} (mmol L^{-1})	C_L (mmol L^{-1})	C_H (mmol L^{-1})	Total number of points	independent titrations	pH range
but	0.7$-$1.4	0.5$-$1.3	7.0$-$12.0	434	7	2.5$-$7
az	0.6$-$1.0	0.5$-$1.7	7.0$-$10.0	651	7	2.5$-$8
mal	0.6$-$1.3	1.0$-$1.5	9.0$-$11.0	582	6	2.5$-$8
suc	0.7$-$1.4	1.30$-$2.5	12.0$-$15.0	638	7	2.5$-$8
tca	0.7$-$2.0	0.9$-$2.0	12.0$-$22.0	543	7	2.5$-$8
btc	0.6$-$1.9	0.6$-$1.70	12.0$-$22.0	498	8	2.5$-$7
paa-2	0.4$-$1.5	0.5$-$2.5	10.0$-$13.0	551	8	2.5$-$6
paa-20	0.6$-$1.9	0.7$-$1.1	10.0$-$12.0	527	7	2.5$-$6
pma-5.4	0.8$-$1.2	0.3$-$1.2	8.0$-$10.0	520	6	2.5$-$6

TGA, was about 27%. The purity of all the carboxylic acids used (butyric acid by Aldrich; azelaic acid by Fluka, malonic acid by Carlo Erba, succinic acid by U.S. Biochemical Corp., propane tricarboxylic and butanetetracarboxylic acids by Fluka), checked by potentiometric titrations, was always $>99\%$. The values of effective concentration of chitosan and carboxylic acids were considered in the calculations. Hydrochloric acid (Merck), and sodium hydroxide (Merck) solutions were prepared by diluting concentrated standard solutions. Acid and hydroxide solutions were standardised against sodium carbonate and potassium biphthalate, respectively. All solutions were prepared using analytical grade water (R = 18 MΩ) and grade A glassware was employed.

2.2 Equipments and experimental procedure

Potentiometric titrations were carried out (at $25.0 \pm 0.1°C$) using an apparatus consisting of a model 809 Metrohm *Titrando*, equipped with a combined glass electrode (Ross type 8102, from Orion). The estimated accuracy was ± 0.20 mV and ± 0.02 mL for e.m.f. and titrant volume readings, respectively. The apparatus was connected to a PC, and automatic titrations were performed using a Metrohm TiAMO 1.0 software to control titrant delivery, data acquisition and to check for e.m.f. stability. All solutions were prepared by dissolving different concentrations of chitosan, in the presence of small amounts of hydrochloric acid, and carboxylic acids. Concentration ratios chit/L (L = carboxylic acids) were always about 1 : 1 when L was a low molecular weight polycarboxylic acid. For these systems measurements were carried out in the pH range 2.5 to 8. For systems of chitosan with high molecular weight polycarboxylic acids, different concentration ratios were used ranging from

chit/L = 1 : 1 to 1 : 4 and to 2 : 1. These last systems were investigated in the pH range 2.5$-$6. Table 1 shows the experimental conditions used in all systems investigated.

Most of measurements were performed without using background salt, in order to avoid possible interactions between cation (such as Na$^+$) and anion (for instance Cl$^-$) components of ionic medium with carboxylic groups of carboxylic ligands and amino groups of chitosan, respectively. Some measurements were carried out by adding NaCl as supporting electrolyte in order to obtain 0.15 mol L^{-1} ionic strength, and to evaluate medium effects on the stability. All titrations were carried out under magnetic stirring and bubbling N$_2$ through the solutions in order to exclude O$_2$ and CO$_2$ inside. A volume of 20$-$25 mL of aqueous solution containing chitosan in the presence of different carboxylic ligands was titrated with standard sodium hydroxide solution. For each experiment, independent titrations of HCl solutions were performed with standard hydroxide solutions in the same experimental conditions of ionic strength and temperature as the systems under investigation to determine the formal electrode potential $E°_{ext}$. The free hydrogen ion concentration scale was used (pH $= -\log [H^+]$).

2.3 Calculations

The computer program ESAB2M (De Stefano *et al.*, 1987) was used to calculate all the parameters (E°, K$_w$, analytical concentration of reagents) for the standardisation of potentiometric equipment. BSTAC and STACO (De Stefano *et al.*, 1996) computer programs were used to determine protonation and formation constants for all systems investigated. The ES4ECI (De Stefano *et al.*, 1993) program was used to calculate formation percentages of the species and to plot speciation diagrams.

Table 2 Protonation constants of different polyelectrolytes at $I \to 0 \, \text{mol L}^{-1}$ and $T = 25°C$

Ligand	$\log \beta_1^H$	$\log \beta_2^H$	z	Refs
paa-2	6.46 ± 0.06	11.30 ± 0.1	-3.3	Crea *et al.*, 2008a
paa-20	7.21 ± 0.04	12.56 ± 0.05	-4.6	Crea *et al.*, 2008a
pma-5.4	7.44 ± 0.05	13.28 ± 0.09	-4.4	Crea *et al.*, 2008a
chit	6.82 ± 0.01	12.54 ± 0.02	0	This work

Formation constants at infinite dilution ($I \to 0 \, \text{mol L}^{-1}$) were calculated using the following simple Debye–Hückel type equation

$$\log K = \log^T K - z*0.51 I^{1/2}/(1 + 1.5 I^{1/2}) + CI \quad (1)$$

where K is the formation constant; $^T K$ is the formation constant at infinite dilution; C is an empirical parameter whose numerical value, at low ionic strength, depends mainly on the ligand class considered (De Stefano *et al.*, 1993 and refs therein); and $z* = \Sigma(\text{charges})^2_{\text{reactants}} - \Sigma(\text{charges})^2_{\text{products}}$.

3. RESULTS AND DISCUSSION
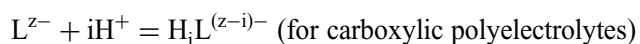
3.1 Protonation of polyelectrolytes

In the calculations of formation constants values for chitosan-carboxylate complex species we used protonation constants previously determined of low (De Robertis *et al.*, 1993; De Stefano *et al.*, 1994; Crea *et al.*, 2004) and high (Crea *et al.*, 2009 and refs. therein) molecular weight polycarboxylic ligands here investigated, and protonation constants of chitosan [unpublished data from this laboratory]. Here we report only a brief enlightenment on the model we considered to explain the acid–base behaviour of polyelectrolytes. In a previous paper (Crea *et al.*, 2009), we interpreted the protonation equilibrium of several polyelectrolyte ligands using some equations proposed by different authors. In particular, we used the modified Henderson–Hasselbalch equation (Katchalsky and Spitnik, 1947; Katchalsky, 1954)

$$\log K^{H*} = \log K_n^{H*} - (n-1)\log[(1-\alpha)/\alpha] \quad (2)$$

and the three parameter equation proposed by Högfeldt *et al.* (1989)

$$\log K^{H*} = \alpha^2 \log K_1^{H*} + (1-\alpha)^2 \log K_0^{H*}$$
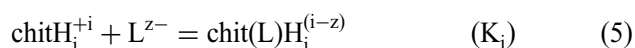$$+ 2\alpha(1-\alpha) \log K_m^{H*} \quad (3)$$

More recently, we studied the acid-base properties of carboxylic (Crea *et al.*, 2009), and amino (Crea *et al.*, 2008a) polyelectrolytes using a model according to which the protonation constants are independent of dissociation degree α. Protonation parameters have been calculated considering the repetitive unit of polyelectrolytes as a diprotic monomeric carboxylic or amino unit and two protonation constants, corresponding to the two different acidic binding sites (β_1^H and β_2^H), have been determined. The equilibrium constants β_i^H were defined as follows:

$$L^{z-} + iH^+ = H_iL^{(z-i)-} \quad \text{(for carboxylic polyelectrolytes)}$$

$$L + iH^+ = H_iL^{i+} \quad \text{(for amino polyelectrolyte)}$$

The effective charge z on the monomeric unit can be determined using Eqn (1), as reported by Crea *et al.* (2008a). Protonation constants for all the polyelectrolytes used in this study, together with the corresponding z values, are reported in Table 2, at $I = 0 \, \text{mol L}^{-1}$.

3.2 Formation constants of chitosan–carboxylate complex species

In the experimental pH range investigated calculations gave evidence for the formation of quite stable species (**chit**)LH$_i$ with low and high molecular weight polycarboxylate ligands, (i = 1 to 4 depending on the carboxylate ligand considered) according to the reactions below reported

$$\text{chit}^0 + L^{z-} + iH^+ = \text{chit(L)H}_i^{(i-z)} \quad (\beta_i) \quad (4)$$

$$\text{chitH}_i^{+i} + L^{z-} = \text{chit(L)H}_i^{(i-z)} \quad (K_i) \quad (5)$$

$$\text{chitH}_i^{+i} + H_jL^{z-j} = \text{chit(L)H}_{(i+j)}^{(i-+j-z)} \quad (K_{i+j}) \quad (5a)$$
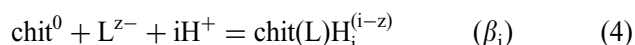
According to the general complexation model, the following complex species were found in the different systems investigated: **chit**(L)(H) (for L = butyrate); **chit**(L)(H) and **chit**(L)(H)$_2$ (for L = malonate, succinate and azelate); **chit**(L)(H);

Table 3 Overall and partial formation constants of chitosan (310 kDa)−carboxylates complexes at $I \rightarrow 0\,\mathrm{mol\,L^{-1}}$ and $T = 25°C$

L	$\log \beta_1^a$	$\log K_1^b$	$\log \beta_2^a$	$\log K_2^b$	$\log \beta_3^a$	$\log K_3^c$	$\log \beta_4^a$	$\log K_4^c$
but	9.77 ± 0.03^d	3.0	–	–	–	–	–	–
suc	10.29 ± 0.01	3.5	16.29 ± 0.01	3.9	–	–	–	–
mal	10.58 ± 0.01	3.8	16.51 ± 0.01	4.1	–	–	–	–
az	10.48 ± 0.02	3.7	16.42 ± 0.01	4.0	–	–	–	–
tca	10.83 ± 0.02	4.0	17.21 ± 0.02	4.8	22.67 ± 0.02	3.7	–	–
btc	11.30 ± 0.03	4.5	18.59 ± 0.01	6.2	24.71 ± 0.01	5.1	29.06 ± 0.02	3.6
paa-2	–	–	20.26 ± 0.01	7.8	23.17 ± 0.05	4.3	–	–
paa-20	–	–	22.08 ± 0.02	9.6	25.61 ± 0.02	6.0	–	–
pma-5.4	–	–	21.15 ± 0.01	8.7	25.68 ± 0.01	5.8	–	–

[a]Refers to equilibrium (4); [b]refers to equilibrium (5); [c]refers to equilibrium (5a); [d] $\pm$ SD.

chit(L)(H)$_2$ and **chit**(L)(H)$_3$ (for L = tricarballylate); **chit**(L)(H) **chit**(L)(H)$_2$, **chit**(L)(H)$_3$, and **chit**(L)(H)$_4$ (for L = butanetetracarboxylate); **chit**(L)(H)$_2$, and **chit**(L)(H)$_3$; (for L = polyacrylate and polymethacrylate). The formation constant values of all these species are reported at $I \rightarrow 0\,\mathrm{mol\,L^{-1}}$ and $T = 25°C$ in Table 3 for both overall (4) and partial (5, 5a) reactions.

For all the complex species formed by chitosan with mono-, di-, tri-, tetra-carboxylates the maximum number of protons i in the complexes is equal to the number of carboxylic groups; for polyelectrolyte ligands i = 2, 3 and this can be attributed to the effective charge, higher than the formal one for a dicarboxylic unit. Data reported in Table 2 show a general quite high stability of chitosan−carboxylate complex species which increases with increasing the number of carboxylic binding groups. The stability follows the trend

$$\mathbf{but < suc \sim mal \sim az < tca < btc < paa-2}$$
$$\mathbf{< pma-5.4 < paa-20.}$$
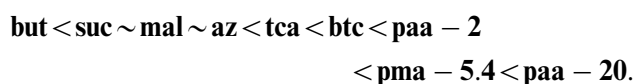
The higher stability of complexes with polyelectrolyte ligands can be explained considering that the stability of complex species could be dependent not only on the ionic interactions between amino groups of chitosan and carboxylic groups of ligands, but even on interactions between hydrophobic skeleton of chitosan and polyelectrolytes (Shamov *et al.*, 2002). Formation constants are increasing regularly for each type of complex species as a function of the charge z. For example, the stability of monoprotonated species can be expressed by the simple linear equation

$$\log K_1 = 3.1 + 0.5(|\,z-\,|\,1)$$

By analyzing altogether the stability data for the different systems studied, it is possible to calculate a rough estimate of the free energy per bond (ΔG_n). In fact the ratio $\log K/\zeta$ (where ζ is the product of the charge of reactants) is fairly constant with a mean value $\log K/\zeta = 1.3 \pm 0.3$, and, by assuming $\zeta = 2n$ (n =number of salt bridges) (De Stefano *et al.*, 2006), one obtain $\Delta G_n = -15 \pm 2\,\mathrm{kJ\,mol^{-1}}$.

In order to verify the influence of ionic strength on the interactions between chitosan and polycarboxylates, stability constant values were also determined at the typical ionic strength of biological fluids, *i.e.* $I = 0.15\,\mathrm{mol\,L^{-1}}$ (NaCl) and are reported in Table 4.

Table 4 Overall formation constants of chitosan−carboxylate complexes at $I = 0.15\,\mathrm{mol\,L^{-1}}$ and $T = 25°C$

L	$\log \beta_1^a$	$\log \beta_2^a$	$\log \beta_3^a$	$\log \beta_4^a$
but	9.49 ± 0.05^b	–	–	–
suc	9.89 ± 0.01	15.69 ± 0.01	–	–
mal	9.73 ± 0.03	16.00 ± 0.02	–	–
az	10.08 ± 0.01	15.82 ± 0.01	–	–
tca	10.22 ± 0.02	16.18 ± 0.02	21.44 ± 0.01	–
btc	10.41 ± 0.03	17.10 ± 0.01	22.82 ± 0.01	26.98 ± 0.02
paa-2	–	19.54 ± 0.02	23.01 ± 0.02	–
paa-20	–	20.13 ± 0.04	24.17 ± 0.03	–
pma-5.4	–	19.18 ± 0.01	23.87 ± 0.01	–

[a]Refers to equilibrium (4); [b] $\pm$ SD.

A comparison between data reported in Tables 3 and 4 shows a substantial lowering of stability constant values calculated at $I = 0.15$ mol L^{-1}. This effect can be explained by considering both the interference of Na$^+$ and Cl$^-$, leading to the formation of weak alkali metal complexes with low [Daniele *et al.*, 2008 and refs therein] and high molecular weight polycarboxylic ligands (De Stefano *et al.*, 2002; De Stefano *et al.*, 2004) and of polyammonium cations with chloride ion (De Robertis *et al.*, 1998; Cascio *et al.*, 2000a), in addition to the lowering of activity coefficients.

3.3 Speciation and sequestration profiles

In Figure 2 we report some speciation profiles in very dilute solutions, $I < 0.01$ mol L^{-1}. Being in all the systems investigated the stability of chitosan–carboxylate complexes quite relevant, high formation percentage are observed, even at low concentrations of chitosan and carboxylates.

In Table 5 the formation percentages of the species **chit**-L–H$^+$ complexes at $I \to 0$ mol L^{-1} and $T = 25°C$ (C$_{chit}$ = C$_L$ = 1 mmol L^{-1}) are reported for different pH values.

To show the effect of ionic strength on the sequestering capacity of chitosan, we report in Figure 3, as an example, the speciation diagrams at $I = 0.15$ mol L^{-1} (dotted lines) and in pure water (full lines) for the H-**chit-btc** and H-**chit-pma-5.4** systems.

As can be seen it is evident the lowering effect mainly due to ion pair formation Na$^+$–L^{z-} and, in particular, to the formation of different chloride (**chit**)H$_r$Cl$^{(r-1)+}$ species, especially in the H-**chit-pma-5.4** system.

The quantitative sequestering capacity of chitosan towards low and high molecular weight carboxylic acids, at different pH values, was derived from stability data by defining the function (%) complexation (P) *vs.* − log [total concentration] of sequestering agent (chitosan, in our case). To this end the following sigmoid Boltzman type equation, already successfully tested for different systems (De Stefano *et al.*, 2006; Crea *et al.*, 2008a; De Stefano *et al.*, 2007; Crea *et al.*, 2008b), was used:

$$P = P_\infty - \frac{P_0 - P_\infty}{1 - \exp[(pL - pL_{50})/S]} \qquad (6)$$

where P_0 and P_∞ are the percentages for pL $\to 0$ and pL $\to \infty$, respectively; pL$_{50}$ = pL for P = 50%; and

S is an adjustable parameter which accounts for the slope in the flex of sigmoid curve. Being in our case $P_\infty = 0$ and $P_0 = 100$, Eqn (6) becomes

$$P = \frac{100}{1 - \exp[(pL - pL_{50})/S]} \qquad (6a)$$

In Table 6 we report pL$_{50}$ values for the sequestration of low and high molecular weight carboxylates by chitosan at different pH values and at different ionic strengths. At $I < 0.01$ mol L^{-1} results are reported at pH = 5 and 6 for all the carboxylates investigated; since high molecular weight polyelectrolyte complex species are scarcely soluble at pH > 6, we report at pH = 7 only sequestration parameters relative to chitosan–low molecular weight carboxylate systems.

Results obtained show that the sequestration ability of chitosan is higher at pH ∼ 6, in correspondence to the formation of the more stable **chit**(L)H complexes due to the favourable presence of both deprotonated carboxylic groups and protonated amino groups in the chitosan molecule. In Figure 4 the sequestration percentages of **but**, **mal**, **tca**, **btc** and **pma-5.4** carboxylates by chitosan are reported at pH = 6 and $I \to 0$ mol L^{-1}.

As expected, chitosan shows a strong sequestering ability towards high molecular weight carboxylic ligands. The pL$_{50}$ values follow a fairly linear trend as a function of the number of carboxylic groups, n_{carb}, for low molecular weight ligands; in dilute solutions we have:

$$pL_{50} = 1.4 + 1.1 n_{carb}$$

For high molecular weight polycarboxylic ligands we observe an increase on pL$_{50}$ greater than 2 log units. This behaviour is due to the high number of carboxylic sites available to the interactions with protonated amino groups on chitosan. The values of slope S in the sigmoid curves are independent of pH and ionic strength values for low molecular weight carboxylate ligands.

Again, to evaluate the influence of ionic strength on the sequestering ability of chitosan towards low and high molecular weight carboxylates, pL$_{50}$ values are reported at the ionic strength of biological fluids ($I = 0.15$ mol L^{-1}). As an example, we show in Figure 5 the sequestration percentage of butane tetracarboxylate (at concentration 10^{-7} mol L^{-1}) by chitosan at $I \to 0$ and $I = 0.15$ mol L^{-1}.

We can observe an appreciable lowering of pL$_{50}$ at $I = 0.15$ mol L^{-1} in comparison with the corre-

(a)

Species: 1. **chit**(H)$_2$; 2. **chit**(H); 3. **chit(but)**(H)

(b)

Species: 1. **chit**(H)$_2$; 2. **chit(suc)**(H)$_2$; 3. **chit**(H); 4. **chit(suc)**(H)

(c)

Species: 1. **chit**(H)$_2$; 2. **chit(tca)**(H)$_3$; 3. **chit(tca)**(H)$_2$; 4. **chit**(H); 5. **chit(tca)**(H)

(d)

Species: 1. **chit**(H)$_2$; 2. **chit(btc)**(H)$_4$; 3. **chit(btc)**(H)$_3$; 4. **chit**(H); 5. **chit(btc)**(H)$_2$; 6. **chit(btc)**(H)

(e)

Species: 1. **chit**(H)$_2$; 2. **chit(paa-2)**(H)$_2$; 3. **chit(paa-2)**(H)$_3$

(f)

Species: 1. **chit**(H)$_2$; 2. **chit(pma-5.4)**H$_3$; 3. **chit(pma-5.4)**H$_2$
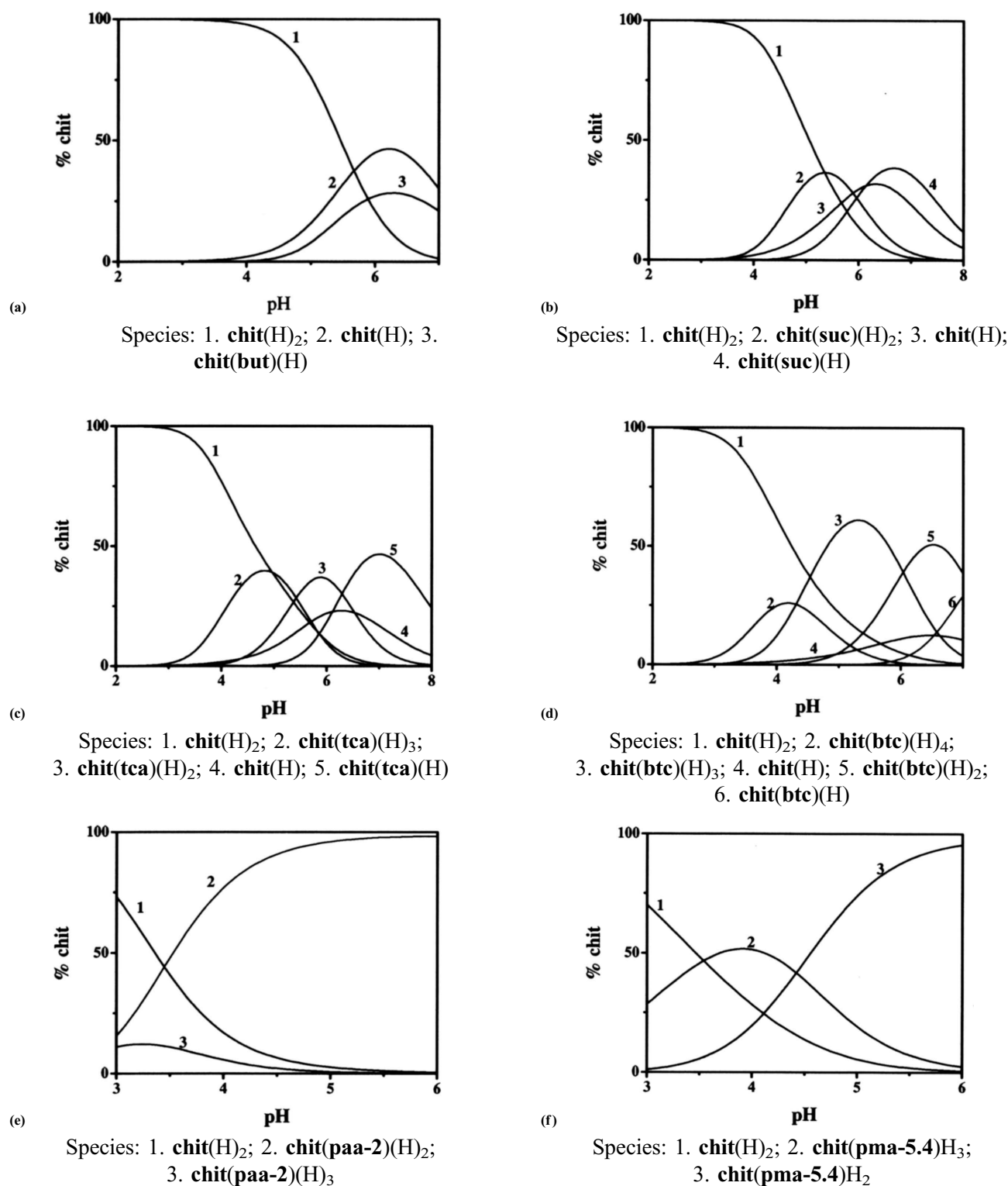
Figure 2 Speciation diagrams in the chitosan - L systems [(**a**) **but**; (**b**) **suc**; (**c**) **tca**; (**d**) **btc**; (**e**) **paa-2**; (**f**) **pma-5.4**] at $I \rightarrow 0$ mol L^{-1} and $T = 25°$C; C$_{chit}$ = 1 mmol L^{-1}, C$_L$ = 1 mmol L^{-1}.

Table 5 Formation percentages of the species **chit**-L−H complexes at different pH values, $I \rightarrow 0 \, mol \, L^{-1}$, $C_{chit} = C_L = 1 \, mmol \, L^{-1}$, and $T = 25°C$

L	Σ%		
	pH = 5	pH = 6	pH = 7
but	7.9	27.0	20.9
suc	35.7	51.9	39.0
mal	44.8	60.9	50.2
az	44.0	59.3	46.6
tca	53.9	64.1	55.8
btc	73.3	82.0	71.9
paa-2	96.8	98.33	–
paa-20	98.7	99.5	–
pma-5.4	93.3	97.9	–

sponding one at $I \rightarrow 0 \, mol \, L^{-1}$. Results reported in Table 6 show that chitosan concentrations of $10^{-4.54}$ and of $10^{-3.57}$ are necessary to sequestrate 50% of butane tetracarboxylate at $I \rightarrow 0 \, mol \, L^{-1}$ and $I = 0.15 \, mol \, L^{-1}$, respectively.
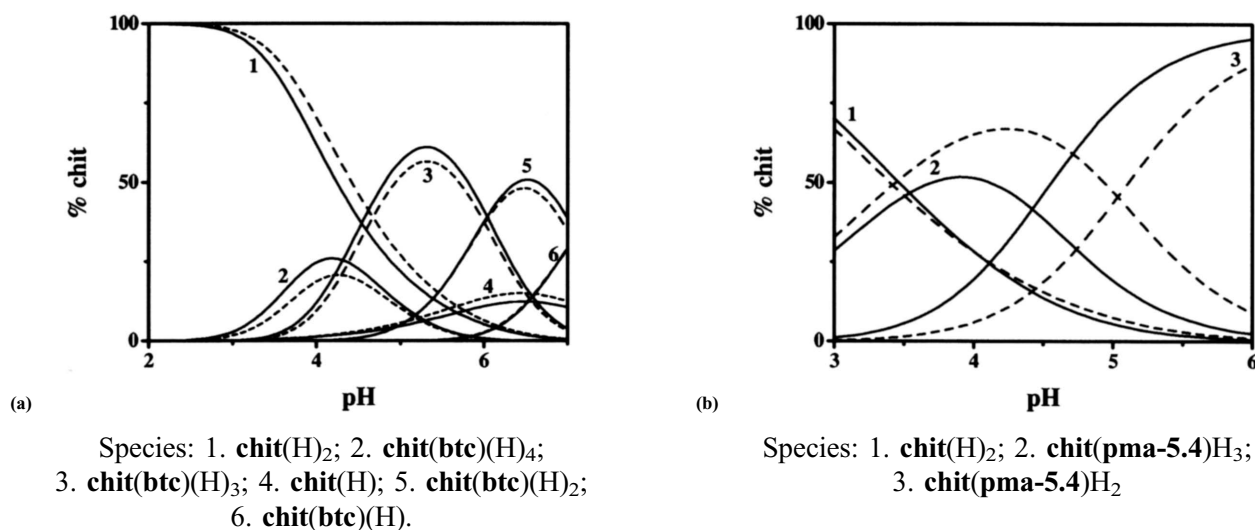
3.4 Comparison with analogous systems

The stability of polyammonium cation-polycarboxylic anion species strongly depend on charges involved in the formation reaction. We may compare the formation constants of chitosan−carboxylate complex species with those of analogous species of low molecular weight polycarboxylate ligands previously investigated in our laboratories (De Robertis *et al.*, 1996; De Stefano *et al.*, 1998a; Cascio *et al.*, 2000b; De Robertis *et al.*, 2001a; De

Robertis *et al.*, 2001b). For the interaction of carboxylates with low molecular weight polyammonium cations we found (De Robertis *et al.*, 1996) $\Delta G_n = -6.3 \, kJ \, mol^{-1}$ and, in particular, when biogenic polyammonium cations are involved (De Stefano *et al.*, 1998a; De Robertis *et al.*, 2001a) $\Delta G_n = -6.4 \, kJ \, mol^{-1}$. For the interaction of fulvate, alginate and polyacrylate with biogenic amines (De Stefano *et al.*, 2006) a value of $\Delta G_n = -5.8 \pm 0.4 \, kJ \, mol^{-1}$ was obtained. These values are quite different from $\Delta G_n = -15 \pm 2 \, kJ \, mol^{-1}$ here found. The significantly higher value of ΔG_n for chitosan complexes is, on the other hand, in accordance with the observed (De Stefano *et al.*, 1998b) higher effect of positive charge of ammonium cations with respect to the anions ones on the stability of these complexes.

4. FINAL REMARKS

The most relevant results obtained in this study can be summarized as follows:

- the quite high stability of chitosan−carboxylate species formed, with particular reference to high molecular weight carboxylic ligands, allows to consider chitosan as a good sequestering agent for this class of compounds;
- the stability of (**chit**)LH$_i$ species strongly depends on charges involved in the complexation reactions;
- the quantitative sequestering ability of chitosan

(a)   Species: 1. **chit**(H)$_2$; 2. **chit**(**btc**)(H)$_4$; 3. **chit**(**btc**)(H)$_3$; 4. **chit**(H); 5. **chit**(**btc**)(H)$_2$; 6. **chit**(**btc**)(H).

(b)   Species: 1. **chit**(H)$_2$; 2. **chit**(**pma-5.4**)H$_3$; 3. **chit**(**pma-5.4**)H$_2$

Figure 3 Speciation diagrams in the chitosan - L systems [(**a**) **btc**; (**b**) **pma-5.4**] at I (NaCl) = 0.15 mol L^{-1} and $T = 25°C$ (*dotted lines*); $I \rightarrow 0 \, mol \, L^{-1}$ and $T = 25°C$ (*full lines*); $C_{chit} = 1 \, mmol \, L^{-1}$, $C_L = 1 \, mmol \, L^{-1}$.

Table 6 pL_{50} values of different polycarboxylates calculated at different pH values and ionic strengths, according to Eqn (6a)

L	pL_{50}	S^a	pL_{50}	S^a	pL_{50}	S
			$I \sim 0 \, mol \, L^{-1}$			
	pH = 5		pH = 6		pH = 7	
but	2.02	0.434	2.73	0.434	2.54	0.434
suc	3.01	0.434	3.42	0.434	3.09	0.434
mal	3.23	0.434	3.66	0.434	3.37	0.434
az	3.06	0.434	3.62	0.434	3.28	0.434
tca	3.50	0.434	3.79	0.434	3.59	0.434
btc	4.11	0.434	4.54	0.434	4.17	0.434
paa-2	6.02	0.426	6.56	0.403	–	–
pma-5.4	5.40	0.432	6.42	0.412	–	–
paa-20	6.71	0.388	7.19	0.292	–	–
			$I = 0.15 \, mol \, L^{-1}$			
but	1.42	0.434	2.25	0.434	2.21	0.434
suc	2.36	0.434	2.81	0.434	2.63	0.434
mal	2.66	0.434	2.89	0.434	2.52	0.434
az	2.59	0.434	2.99	0.434	2.82	0.434
tca	2.69	0.434	3.07	0.434	2.95	0.434
btc	3.24	0.434	3.57	0.434	3.23	0.434
paa-2	5.70	0.430	6.08	0.424	–	–
pma-5.4	4.70	0.434	5.40	0.432	–	–
paa-20	5.88	0.428	6.42	0.412	–	–

[a]$S =$ slope of the sigmoid curve.
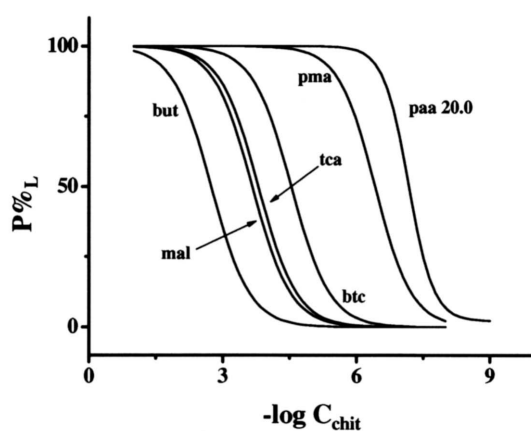
Figure 4 Carboxylates sequestration by chitosan at pH = 6, $I = 0 \, mol \, L^{-1}$, and $T = 25°C$. $C_L = 10^{-7} \, mol \, L^{-1}$.
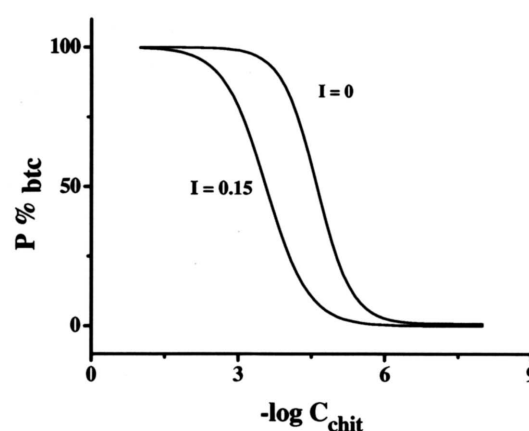
Figure 5. Sequestration of butane tetracarboxylate by chitosan at different ionic strength values at pH = 6 and $T = 25°C$. $C_{btc} = 10^{-7} \, mol \, L^{-1}$.

was determined taking into account the amount of chitosan necessary to sequestrate 50% of polycarboxylates (pL_{50}), according to a simple sigmoid Boltzman type equation;

- an empirical predictive relationship was found showing that pL_{50} values for low molecular weight carboxylate ligands depend linearly on the number of carboxylic groups;
- calculations on the stability of species at $I = 0.15 \, mol \, L^{-1}$ in NaCl medium show a lowering of the formation constants with respect to

those at $I = 0 \, mol \, L^{-1}$, owing to the ion pair formation between amino groups of chitosan with chloride ions and between carboxylic groups with sodium ions of the ionic medium.

Results obtained may be useful to make prevision on interacting properties of water soluble chitosan with different biological molecules containing carboxylic functions, as polypeptides, proteins, cholesterol and fatty acids.

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