Biophysical Chemistry 122 (2006) 221 – 231

Biophysical Chemistry

http://www.elsevier.com/locate/biophyschem

Sequestration of biogenic amines by alginic and fulvic acids

Concetta De Stefano [a], Antonio Gianguzza [b,*], Daniela Piazzese [b],
Nunziatina Porcino [a], Silvio Sammartano [a,*]

[a] *Dipartimento di Chimica Inorganica, Chimica Analitica e Chimica Fisica, Università di Messina, Salita Sperone 31, I-98166, Messina (Vill. S. Agata), Italy*
[b] *Dipartimento di Chimica Inorganica e Analitica "Stanislao Cannizzaro", Università di Palermo, Viale delle Scienze, I-90128 Palermo, Italy*

Received 22 February 2006; received in revised form 24 March 2006; accepted 24 March 2006

Abstract

The interaction of natural (alginic and fulvic acids) and synthetic (polyacrylic acid 2.0 kDa) polyelectrolytes with some protonated polyamines [diamines: ethylendiamine, 1,4-diaminobutane (or putrescine), 1,5-diaminopentane (or cadaverine); triamines: *N*-(3-aminopropyl)-1,4-diaminobutane (or spermidine), diethylenetriamine; tetramine: *N*,*N′*-bis(3-aminopropyl)-1,4-diaminobutane (or spermine); pentamine: tetraethylene-pentamine; hexamine: pentaethylenehexamine] was studied at $T=25\,°C$ by potentiometry and calorimetry. Measurements were performed without supporting electrolyte, in order to avoid interference, and results were reported at $I=0$ mol L^{-1}. For all the systems, the formation of $(am)L_2H_i$ species was found (am = amine; L = polyelectrolyte; $i=1...4$, depending on the amine considered). The stability of polyanion–polyammonium cation complexes is always significant, and for high-charged polycations, we observe a stability comparable to that of strong metal complexes. For example, by considering the formation reaction $(am)H_i+2L=(am)L_2H_i$ we found $\log K_i=6.0$, 6.5 and 10.8 for $i=1$, 2 and 3, respectively, in the system alginate–spermidine. Low and positive formation $\Delta H°$ values indicate that the main contribution to the stability is entropic in nature. The sequestering ability of polyelectrolytes toward amines was modelled by a sigmoid Boltzman type equation. Some empirical relationships between stability, charges and $\Delta G°$ and $T\Delta S°$ are reported. Mean values per salt bridge of formation thermodynamic parameters ($\Delta X°_n$) are $\Delta G°_n=-5.8\pm0.4$, $\Delta H°_n=0.7\pm0.5$ and $T\Delta S°_n=6.5\pm0.5\,kJmol^{-1}$ for all the systems studied in this work.

Keywords: Biogenic amines; Fulvic acid; Alginic acid; Polyacrylic acid; Sequestration; Polyammonium–polycarboxylate interactions

1. Introduction

Biogenic amines are low-molecular-weight naturally occurring amines containing amino groups separated by three to five methylene groups. They are formed by the amino acid decarboxylation or through transamination of aliphatic monoamines. Examples of biogenic amines are histamine, tyramine, serotonin, tryptamine, cadaverine and putrescine (diamines), derived from histidine, tyrosine, hydroxytryptophan, tryptophan, lysine and arginine, respectively, and spermidine (triamine) and spermine (tetramine) deriving from putrescine. Biogenic amines play a key role in many biological processes such as cell proliferation and differentiation, protein synthesis, aggregation, structural integrity, function of ribosomal subunits, DNA replication, membrane stabilization and in the activity of several enzymes [1,2]. They can be found in all animal and vegetal living organisms at different concentrations: total intracellular concentration range in animal cells is 2–3 mmol L^{-1} [2]. Investigations have also been carried out to evaluate the toxicity of biogenic amines in foods and beverages [3–5]. Due to their high basicity, biogenic amines are present in the protonated forms at physiological pH conditions in aqueous solution. Therefore, they behave as polyammonium cations differently charged, i.e., they can interact with organic and inorganic negatively charged ligands. Literature data on the interaction of polyammonium cations, particularly from biogenic amines, are relatively few and mainly concern the interaction with phosphate groups both free and present in molecules of biological interest [6–13]. A review on the coordination chemistry of polyamines, including biogenic amines, with nucleosides and nucleotides was reported recently by Lomozik et al. [14].

---

[*] Corresponding authors.
E-mail addresses: giang@unipa.it (A. Gianguzza), ssammartano@unime.it (S. Sammartano).

0301-4622/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.bpc.2006.03.015

The acid–base behaviour of the cadaverine, putrescine, spermidine and spermine biogenic amines, in different ionic media including aqueous media simulating the composition of natural waters and at different ionic strengths was previously investigated by our research group [15–17]. Results on the amines protonation were reported also considering the possible interaction of protonated amines with chloride and sulfate anions of ionic media used. Particular attention was paid in studying the interactions of polyammonium cations of biogenic amines and other polyamines with carboxylate and polycarboxylate low-molecular-weight ligands [18–21]. Results obtained from these investigations gave evidence for the formation of quite stable species $(am)L_2H_i$ [am = amine; L = negatively charged ligand; $i = 1, 2 \ldots (n_{am} + n_L - 1)$, with n_{am} and n_L = maximum number of protons in am and L, respectively], with significant formation percentages also for low reagent concentrations.

As an extension of previous investigations we report the results of a study carried out on the formation and stability of species formed by the interaction of biogenic amines with synthetic and naturally occurring high-molecular-weight polycarboxylate ligands. The study was performed with the aim to establish stability parameters of formed complex species and to define the chemical speciation of these classes of compounds in natural systems where carboxylic groups are by far the most common binding sites. Investigations were carried out on the interaction of the biogenic amines putrescine (1,4-diaminobutane), cadaverine (1,5-diaminopentane), spermidine [*N*-(3-aminopropyl)-1,4-diaminobutane] and spermine [*N*,*N*'-bis(3-aminopropyl)-1,4-diaminobutane] with synthetic polyacrylic acid [$(CH_2{-}CH{-}COOH)_n$, 2000 Da molecular weight] and naturally occurring fulvic and alginic acids. The structures of the biogenic amines under investigation in this work are reported in Fig. 1.

In order to have a more complete picture on the coordination chemistry of the polyamine class compounds and to define their speciation in natural waters, the interactions of the following other amines–ethylendiamine, diethylenetriamine, tetraethylenepentamine and pentaethylenehexamine with the same polycarboxylate ligands were also investigated. As it is well known, fulvic acid is the most soluble fraction of humic substances containing a high percentage of carboxylate (–COOH, 2–10 mmol/g) and phenolic (–OH, 1–5 mmol/g) groups [22,23], and a low, often negligible percentage, of –NH groups. In the fulvic fraction formed in oxic conditions, –SH groups are generally absent. A typical structure of fulvic acid containing the main binding groups is reported in Fig. 2.

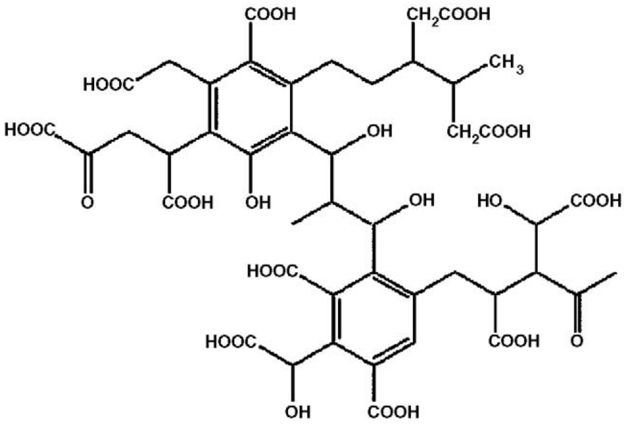


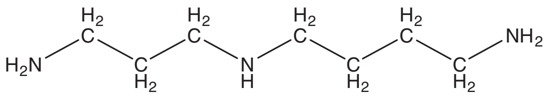
Fig. 2. Typical structure of fulvic acid containing the main binding groups.

Due to its chemical structure, fulvic fraction of humic substances strongly contributes to the mobilization (adsorption/release) process of metal ions and cations in soils and aquatic environments [24–28].

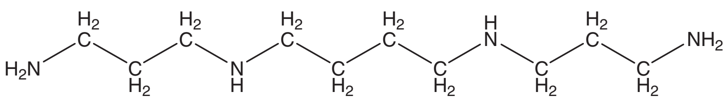
The alginic acid is one of the main components of brown algae and it is a copolymer of 1,4 linked β-D-mannuronic (M) and α-L-guluronic (G) acid residues, each containing one carboxylate group per monomeric unit [29] (Fig. 3). Sequestering capacity of alginate toward cations is well known and often used in metal ion biosorption technologies [30–33].

Proton and metal exchange in the polyelectrolyte polycarboxylate systems was studied using a model according to which protonation constants are considered as a function

1,4 - diamino-butane (putrescine, ***ptr***)   1,5-diamino-pentane (cadaverine, ***cdv***)



N-(3-aminopropyl)-1,4 - diamino-butane (spermidine, ***spd***)



N, N'-bis(3-aminopropyl)-1,4-diamino-butane (spermine, ***sper***)

Fig. 1. Structures of the biogenic amines under investigation.

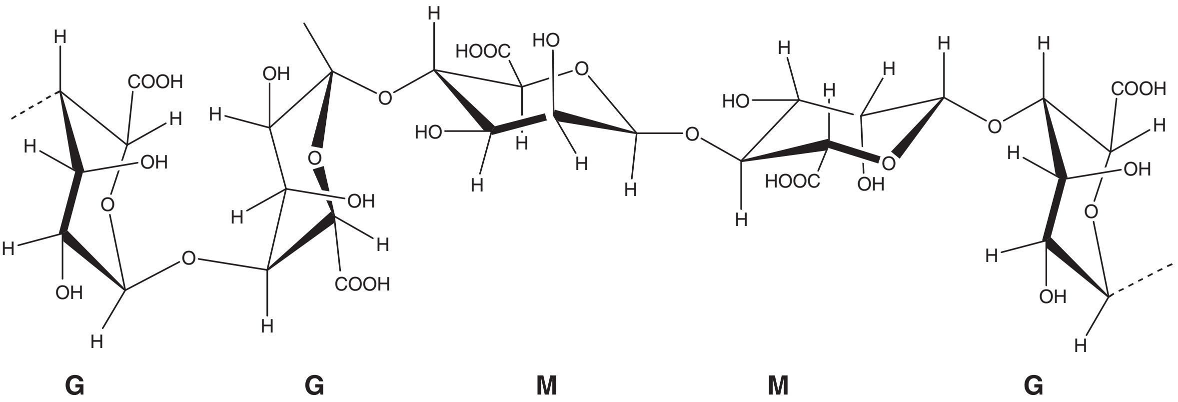


Fig. 3. Structure of alginic acid (G = guluronic acid, M = mannuronic acid units) [29].

depending on the dissociation degree of the polyelectrolyte (more details in Results). Results are reported in some papers where thermodynamic protonation parameters of synthetic polyacrylates and polymethacrylates have been examined as functions of ionic strength, ionic medium and molecular weight [34–39]. The above model was also applied more recently to naturally occurring fulvate and alginate systems in natural waters [40,41].

Stability data on the species formed in the systems investigated and their $\Delta H°$ formation were obtained by potentiometric ([H^+]-glass electrode) and calorimetric measurements, respectively, at $T = 25\,°C$. Since the dependence on the ionic medium composition of both polyammonium cations and polycarboxylate ligands is known [15–17,34–41,respectively], in order to avoid any interference, no ionic medium was used in the present study. Obtained results allowed us to find a relationship to predict the stability of any polyammonium–polycarboxylate system as a function of charge involved and number of salt bridge. Calculations were also performed to evaluate the sequestration ability of naturally occurring polycarboxylate systems towards polyammonium cations never before considered in the study of the role of organic matter in cation adsorption and removal processes.

2. Experimental section

2.1. Materials

All polyamines used in this work, ethylendiamine (en), 1,4 (putrescine, ptr), 1,5 (cadaverine, cdv), diethylenetriamine (dien), *N*-(3-aminopropyl)-1,4-diaminobutane (spermidine, spd) and *N*,*N*'-bis(3-aminopropyl)-1,4-diaminobutane (spermine, sper), tetraethylenepentamine (tetren) and pentaethylenehexamine (penten) were purchased by Fluka. Ethylenediamine, diethylenetriamine, tetraethylenepentamine and pentaethylenehexamine were purified by transformation in the corresponding hydrochloride species. Standard fulvic acid (FA) was supplied by the International Humic Standard Society IHSS (lot number 2S102F) with a water and ash content of 11.2% and 1.0%, respectively, and the following elemental composition: 50.12 (C %), 4.28 (H%), 42.61 (O%), 3.75 (N%), 0.89 (S%) and 0.12 (P %). The content of carboxylic groups in fulvic acid (meq mg^{-1}) was checked in our laboratories by potentiometric technique and by the use of the classic calcium acetate method to determine functional groups in humic substances [22,23]. Commercial alginic acid (AA) from *Macrocystis pyrifera* was supplied by Aldrich with an average content of mannuronic (M) and guluronic (G) acids of 61% and 39%, respectively, with an *M*/*G* ratio of 1.56. It was used as sodium soluble salt. Polyacrylic acid (PAA) (2 kDa) was supplied from Fluka with an average water content of 26%. Its purity, checked by potentiometric titrations, was always >99.5%. Fulvic, alginic and polyacrylic acids were used without further purification and their purity was always taken into account in the calculations. Sodium hydroxide and hydrochloric acid solutions were standardised against potassium hydrogen phthalate and sodium carbonate, respectively. All solutions were prepared using analytical grade water ($R = 18\,M\Omega$) and grade A glassware.

2.2. Procedure

Potentiometric measurements were carried out using a potentiometric apparatus consisting of Metrohm model 665 automatic titrant dispenser coupled with a Metrohm model 654 potentiometer and a combination Orion-Ross 8172 glass electrode. The estimated accuracy of the potentiometric system was ±0.15 mV and ±0.003 mL for emf and titrant volume readings, respectively. A volume of 25–50 mL of solution in turn containing alginic acid or fulvic acid (0.5–5 mmol L^{-1}) or polyacrylic acid (3–8 mmol L^{-1}) and amines (0.5–30 mmol L^{-1}) was titrated with NaOH standard solution. Results of investigations previously performed on the PAA–polyammonium cations system [20] were also taken into account and checked in this work by additional measurements (and calculations). All measurements were carried out in the pH range 5–9 where polycarboxylate ligands are present in their dissociated form and biogenic amines are present as ammonium cations. The experimental pH range was also chosen to avoid the formation of scarcely soluble species in the acidic pH range, being the alginic acid little soluble in its protonated form.

No background salt was added in order to avoid interactions of alkali metal cations with alginic acid or anions (for instance, Cl^-) with polyammonium cations. Measurements were performed at low and variable ionic strength values ($0.002<I$/mol $L^{-1}<0.025$). For each experiment, independent titrations of HCl solutions were performed in the same experimental conditions of ionic strength and temperature as the systems under study, in order to determine the formal electrode potential $E°_{ext}$. The free hydrogen ion concentration scale was used ($pH=-\log[H^+]$). All titrations were carried out by magnetically stirring and bubbling pure nitrogen through the solution, in order to avoid O_2 and CO_2 inside. Calorimetric measurements were carried out using a model 450 Tronac Isoperbol Titration calorimeter, coupled with a Keithley 196 system Dmm digital multimeter. The calorimetric titrations were carried out by adding the protonated amine to the solution vessel containing polycarboxylate salts. Also in this case no supporting electrolyte was used in the measurements. The titrant was delivered by a 2.5 mL capacity Hamilton syringe, model 1002TLL. Accuracy was checked by titrating a THAM (tris-(hydroxymethyl)amino-methane) buffer solution with HCl. The heat of dilution was measured before each experiment. The estimated accuracy of the calorimetric system was ±0.008 J and ±0.001 mL. For the acquisition of both potentiometric and calorimetric experimental data, suitable home-made computer programs were used.

2.3. Calculations

Calculations relative to the standardisation of potentiometric equipment were performed by the computer program ESAB2M [42]. The stability constant values for polyammonium cation complexes were calculated by using both computer programs BSTAC and STACO [43]; ES4ECI [44] was used to calculate formation percentages of the species and to plot speciation diagrams. The modified computer program ES5CM [45] was used to analyze calorimetric data for polyelectrolytes. Protonation constants of polyamines [15–19] and polycarboxylic anions under investigation [34,37,40,41], previously reported, were used in the calculations for determining the formation constants of polyacrylate, alginate and fulvate-polyamines complexes; moreover, the interactions of Cl^- with polyamines and of Na^+ with polycarboxylic anions were also taken into account. Formation constants and thermodynamic parameters were reported as extrapolated values at infinite dilution ($I=0$ mol L^{-1}) using a Debye–Hückel type Eq. (1) [46,47]

$$\log K = \log {}^{T}K\ z^* I^{1/2}(2+3I^{1/2})^{-1} + CI + DI^{3/2} \qquad (1)$$

where K is the formation constant; ${}^{T}K$ is the formation constant at infinite dilution; C and D are empirical parameters that can be expressed as

$$C = c_0 p^* + c_1 z^* \text{ and } D = d_0 p^* + d_1 z^*$$

with

$$p^* = \sum(\text{moles})_{\text{reactants}} - \sum(\text{moles})_{\text{products}}$$

$$z^* = \sum(\text{charges})^2_{\text{reactants}} - \sum(\text{charges})^2_{\text{products}}$$

where the empirical parameters c_0, c_1, d_0, d_1 are dependent only on the stoichiometry of the reaction in the range $0 \leq I$ (mol L^{-1}) ≤ 1: $C=0.1\ p^*+0.20z^*$ and $D=-0.075z^*$. These parameters are valid when almost all the weak interactions (polyammonium-chloride and polycarboxylate-sodium interactions) were taken into account in the calculations. The error due to extrapolation of formation constants and formation enthalpies is, in our experimental conditions, fairly low and generally negligible with respect to experimental uncertainties.

3. Results

3.1. Protonation of polycarboxylates

The acid–base behaviour of synthetic and natural polyelectrolytes can be expressed as function of the dissociation degree, α, [41], using the following three-parameter Eq. (2) proposed by Högfeldt et al. [48]

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

where K_1^H and K_0^H are the protonation constants at $\alpha \to 1$ and $\alpha \to 0$, respectively, and K_m^H represents a further protonation parameter that accounts for the non-linearity of the function log K^H vs α. When this function is linear, $\log K_m^H = (\log K_1^H + \log K_0^H)/2$, and therefore, we can write (Eq. (3))

$$\begin{aligned}\log K^{H} &= \alpha \log K_1^{H} + (1-\alpha)\log K_0^{H} \\ &= 2(1-\alpha)\log K_m^{H} + (2\alpha-1)\log K_1^{H}\end{aligned} \qquad (3)$$

The protonation constants of polyacrylate, alginate and fulvate ligands were determined in different ionic media at different ionic strengths using Eq. (2) for the calculation of protonation constants of polyacrylate (PAA) [34,37] and Eq. (3) for alginate (AA) [40] and fulvate (FA) [41] ligands, respectively. In particular, at $I=0$ mol L^{-1} and $T=25$ °C, protonation constant values are

for PAA: $\log K_1^{H°}=6.91$, $\log K_0^{H°}=4.85$, $\log K_m^{H°}=6.00$;
for AA: $\log K_1^{H°}=4.77$, $\log K_m^{H°}=3.686$;
for FA: $\log K_1^{H°}=3.36$, $\log K_m^{H°}=4.47$.

In the pH range of measurements for all the systems investigated (pH=5–9), only a small fraction of polycarboxylates is protonated, and therefore, one can consider, as for a monoprotic low-molecular-weight acid, a single protonation constant ($\log K_1^H$, $\alpha \to 1$).

3.2. Polycarboxylate–polyammonium complexes

Polyamines and synthetic and natural polycarboxylates form several protonated species $(am)(L)_2H_i$ in the experimental pH range investigated. In general, the maximum number of species formed in each system is related to the protonation extent of amines at pH>5. For example, penten forms only four species since the last two protonation steps occur at pH < 5. The results, at 25 °C and $I = 0$ mol L^{-1}, are reported in Table 1 for overall (β_i) and partial (K_i) reactions:

$$\mathrm{am}^0 + 2\mathrm{L}^{z-} + i\mathrm{H}^+ = [(\mathrm{am})(\mathrm{L})_2\mathrm{H}_i]^{(i-2z)} \qquad (\beta_i)$$

$$\mathrm{amH}_i^{i+} + 2\mathrm{L}^{z-} = [(\mathrm{am})(\mathrm{L})_2\mathrm{H}_i]^{(i-2z)} \qquad (K_i)$$

In the calculations we used, the charge values z, at $\alpha = 1$, equal to -4, -3.5 and -3.3 for alginate, fulvate and polyacrylate, respectively, as previously reported [41].

Calorimetric experiments were also performed in order to obtain the enthalpic changes for the formation of these species. The measurements were carried out (see Experimental section) in a way to obtain only the formation of the species having the maximum number of protons. The relative results are reported in Table 2.

By analyzing formation constants of Table 1, the following considerations can be made. The stability of polyanion–polyammonium cation complexes is always significant and, for high-charged polycations, we have a stability comparable to that of strong metal complexes. Moreover, the formation constants (log K_i) are linearly dependent on the charge of polyammonium cation. In fact, by considering the product charge parameter ζ

$$\zeta = |2\, z_{\mathrm{cation}}\, z_{\mathrm{anion}}| \tag{4}$$

there is quite a constant value of $\log K_i/\zeta$ (see Table 1), i.e., formation constants can be expressed by the simple linear relationship

$$\log K_i = a\zeta \tag{5}$$

for all the complexes of a single polycarboxylate system. Small differences can be observed for the three carboxylate systems. The trend is

$$\mathrm{PAA} > \mathrm{FA} > \mathrm{AA}.$$

By considering the linear Eq. (5) we have

$$a = 0.58 \pm 0.05 \quad \mathrm{PAA}$$

$$a = 0.50 \pm 0.06 \quad \mathrm{FA}$$

$$a = 0.44 \pm 0.06 \quad \mathrm{AA}$$

and for all the systems considered,

$$a = 0.51 \pm 0.04 \quad \mathrm{PAA} + \mathrm{FA} + \mathrm{AA}.$$

Enthalpy changes (Table 2), always small and positive, show the same trend as the formation constants. For the linear equation

$$\Delta H^\circ = b\zeta \tag{6}$$

we calculated

$$b = 0.40 \pm 0.10 \quad \mathrm{PAA}$$

$$b = 0.25 \pm 0.15 \quad \mathrm{AA}$$

$$b = 0.7 \pm 0.2 \quad \mathrm{AA} + \mathrm{PAA}.$$

Table 1

Overall (β) and partial (K) formation constants of polyacrylate (2.0 kDa), alginate and fulvate-polyammonium cation complexes at $I = 0$ mol L^{-1} and $T = 25$ °C

Amines	Ligands	I	log β_i [a]	log K_i [b]	log K_i/ζ
en	PAA 2.0 kDa	1	15.16±0.04 [c]	5.2	0.8
		2	24.49±0.04	7.7	0.6
ptr		2	27.00±0.04	7.4	0.6
cdv		2	27.07±0.06	6.7	0.5
spd		1	16.13±0.10	5.2	0.8
		2	26.72±0.05	6.3	0.5
		3	39.4±0.2	9.9	0.5
sper		1	15.30±0.26	4.6	0.7
		2	27.78±0.05	6.3	0.6
		3	38.31±0.07	9.6	0.5
		4	47.10±0.10	11.2	0.4
dien		1	14.53±0.04	4.7	0.7
		2	25.04±0.03	6.5	0.5
tetren		1	14.80±0.20	4.9	0.7
		2	24.89±0.10	6.1	0.5
		3	35.40±0.13	8.8	0.4
penten		1	14.86±0.10	5.0	0.8
		2	25.70±0.07	6.8	0.5
		3	35.53±0.12	8.5	0.4
		4	45.75±0.10	12.9	0.5
en	AA	1	15.11±0.04	5.2	0.7
		2	25.36±0.04	8.7	0.5
cdv		2	26.13±0.04	5.8	0.4
ptr		1	14.99±0.08	4.5	0.6
		2	25.32±0.04	5.7	0.4
spd		1	16.83±0.14	6.0	0.8
		2	26.99±0.08	6.5	0.4
		3	39.3±02	10.8	0.45
sper		3	36.62±0.04	7.9	0.3
		4	44.63±0.08	8.7	0.3
dien		1	12.79±0.12	4.2	0.5
		2	24.78±0.02	6.2	0.4
tetren		1	15.36±0.16	5.5	0.7
		2	26.08±0.10	7.2	0.4
		3	35.55±0.12	8.9	0.4
penten		2	25.81±0.02	6.9	0.4
		3	35.82±0.02	8.8	0.4
		4	43.81±0.12	10.9	0.3
		5	52.11±0.08	16.2	0.4
en	FA	1	13.73±0.12	3.8	0.5
		2	24.09±0.06	7.3	0.5
cdv		1	15.44±0.08	4.7	0.7
		2	27.4±0.2	7.0	0.5
ptr		1	13.96±0.12	3.5	0.5
		2	26.7±0.1	7.1	0.5
spd		1	17.01±0.20	6.2	0.9
		2	26.87±0.18	6.4	0.5
		3	39.0±0.2	10.5	0.5
sper		2	26.93±0.20	6.5	0.5
		3	37.07±0.12	8.4	0.4
		4	45.42±0.10	9.5	0.3
dien		1	14.57±0.14	4.8	0.7
		2	23.84±0.16	5.3	0.4
tetren		2	25.66±0.04	6.8	0.5
		3	34.96±0.06	8.4	0.4
		4	42.01±0.12	11.5	0.4
penten		1	16.10±0.20	6.2	0.9
		2	26.09±0.14	7.2	0.5
		3	36.12±0.16	9.1	0.4
		4	44.75±0.18	11.9	0.4

[a] log β_i refer to equilibrium $(\mathrm{am})^0 + 2\mathrm{L}^{z-} + i\mathrm{H}^+ = [(\mathrm{am})(\mathrm{L})_2\mathrm{H}_i]^{(i-2z)}$.

[b] log K_i refer to equilibrium $(\mathrm{am})\mathrm{H}_i^{i+} + 2\mathrm{L}^{z-} = [(\mathrm{am})(\mathrm{L})_2\mathrm{H}_i]^{(i-2z)}$.

[c] ±95% confidence intervals.

Table 2
Formation $\Delta G°$, $\Delta H°$ and $T\Delta S°$ values for PAA and AA complexes with different amines, at $I=0$ mol L^{-1} and $T=25$ °C

	Amines	$\Delta G°$	$\Delta H° \pm 0.5$ kJ mol^{-1}	$T\Delta S° \pm 1$	i[a]
PAA	en	−44.0	6.4	50.4	2
	dien	−37.1	3.9	41.0	2
	spd	−72.5	7.2	79.7	3
	sper	−63.9	9.6	73.5	4
AA	en	−49.7	3.2	52.9	2
	sper	−49.7	10.2	59.9	4

[a] According to the reaction: $(am)H_i^{i+} + 2L^{z-} = (am)L_2H_i^{(i-2z)}$.

Analogous calculations can be made for $T\Delta S°$:

$$T\Delta S° = b\zeta \tag{6a}$$

$$b = 3.25 \pm 0.25 \quad \text{AA} + \text{PAA}.$$

Low formation enthalpies together with high $T\Delta S°$ values indicate that the main forces for the interaction of polyammonium cations with polyanions are electrostatic in nature.

4. Discussion

4.1. Sequestration of polyamines by polycarboxylate ligands

The strong binding ability of polycarboxylates towards polyammonium cations can be discussed in terms of sequestration of amines by natural or synthetic polyelectrolytes. Some examples of speciation profiles are shown in Figs. 4–6. In the pH range of interest for biological fluids and natural waters (blood, pH = 7.4; seawater, pH = 8.1), yields of mixed amino-polycarboxylate species are always quite high and testify the importance of these complexes in the speciation studies of natural systems. For example, in the alginate–spermine system (Fig. 5), we observe that at pH = 7.4 and $C_{sper} = C_{AA} = 2$ mmol L^{-1}, the sum of sper(AA)$_2$H$_3$ and sper(AA)$_2$H$_4$ percentages is >50%. Nevertheless, from these diagrams, plotted in the experimental conditions used which are very different from the naturally occurring ones, it is difficult to establish the sequestering capability of polycarboxylate ligands in these systems. Another possible way is to consider the function $(\%)_{\text{complexation}}$ (P) vs. the sequestering agent (L) concentration. To this end, we may use the Boltzman type equation for sigmoid functions in the form:

$$P = P_\infty + \frac{P_0 - P_\infty}{1 + \exp[(\text{pL} - \text{pL}_{50})/S]} \tag{7}$$

where P is the total percentage of AL$_2$H$_i$ species calculated with respect to amine concentration; $\text{pL} = -\log[\text{L}]_\text{T}$; P_0 and P_∞ are the percentages for pL → 0 and pL → ∞, respectively; $\text{pL}_{50} = \text{pL}$ (for $P = 50\%$); S is an adjustable parameter which accounts for the slope in the flex of sigmoid curve. Being in our case, $P_\infty = 0$ and $P_0 = 100$, Eq. (7) becomes

$$P = \frac{100}{1 + \exp[(\text{pL} - \text{pL}_{50})/S]} \tag{7a}$$

In Figs. 7 and 8, we report the function P (%) of putrescine (Fig. 7) and spermine (Fig. 8) vs. the concentration of all the sequestering polycarboxylate agents used at different pH values; in particular, the Boltzman curves are drawn at pH = 7 for AA, FA and PAA and at pH = 8, for AA and FA, respectively,

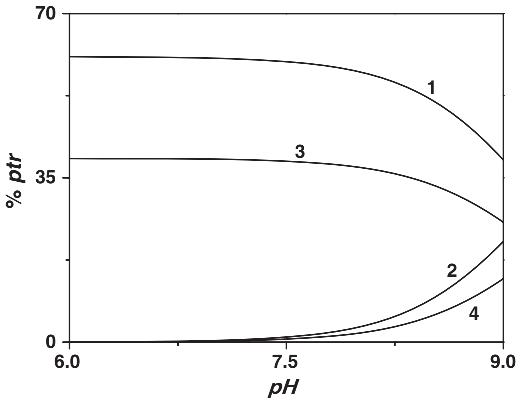


Fig. 4. Speciation diagram for the ptr–AA–H$^+$ system at $T=25$ °C and $I=0$ mol L^{-1}. $C_{ptr}=0.005$ mol L^{-1}; $C_{AA}=0.005$ mol L^{-1} Curves: (1) ptr(H)$_2$; (2) ptr(H); (3) ptr(AA)$_2$H$_2$; (4) ptr(AA)$_2$H.

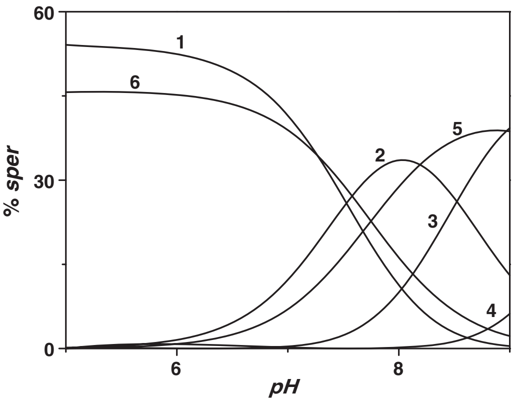


Fig. 5. Speciation diagram for the sper–AA–H$^+$ system at $T=25$ °C and $I=0$ mol L^{-1}. $C_{sper}=0.002$ mol L^{-1}; $C_{AA}=0.002$ mol L^{-1}. Curves: (1) sper(H)$_4$; (2) sper (H)$_3$; (3) sper(H)$_2$; (4) sper(H); (5) sper(AA)$_2$H$_3$; (6) sper(AA)$_2$H$_4$.

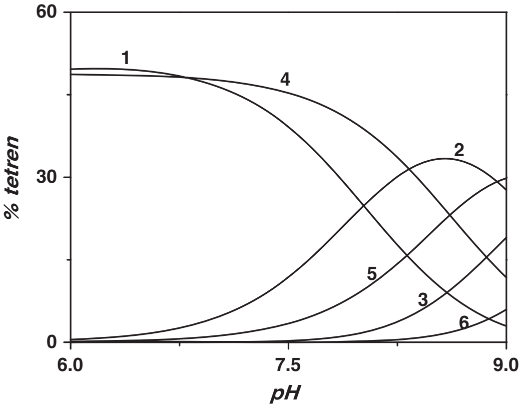


Fig. 6. Speciation diagram for the tetren–AA–H$^+$ system at $T=25$ °C and $I=0$ mol L^{-1}. $C_{penten}=0.005$ mol L^{-1}; $C_{AA}=0.005$ mol L^{-1}. Curves: (1) tetren (H)$_3$; (2) tetren(H)$_2$; (3) tetren(H); (4) tetren(AA)$_2$(H)$_3$; (5) tetren(AA)$_2$(H)$_2$; (6) tetren (AA)$_2$H.

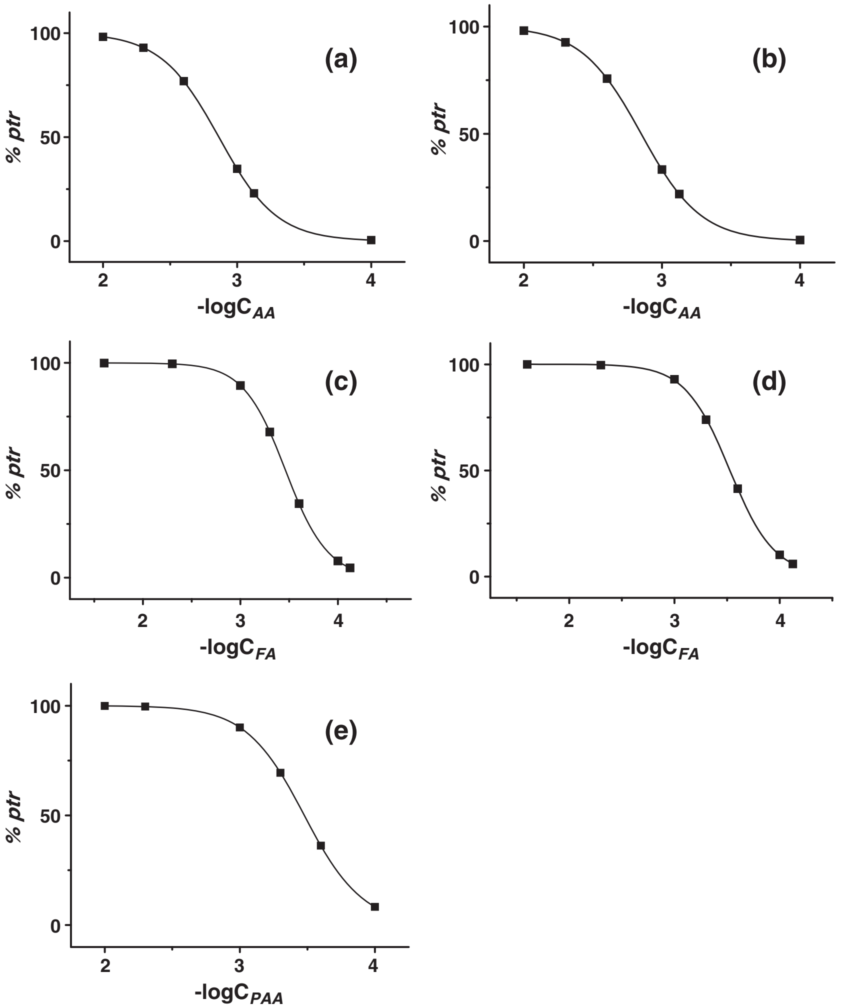


Fig. 7. Percentage of ptr as polycarboxylate species vs. sequestering agent concentration at $I=0\,\mathrm{mol\,L^{-1}}$ and $T=25\,°\mathrm{C}$. (a) AA at pH 7; (b) AA at pH 8; (c) FA at pH 7; (d) FA at pH 8; (e) PAA at pH 7.

for the putrescine and spermine complex species. In Table 3, pL_{50} values are reported for all the systems considered together with the ratio $\mathrm{pL}_{50}/i_{\max}$ ($i_{\max}$ = maximum number of protons in the complex species) and the slope (S) of the sigmoid curve. By analyzing the data of Table 3, the following considerations can be made:

(a) The slope in the flex in the sigmoid curves is quite similar for all the carboxylate and it results quite independent of the pH values; by considering the Eq. (7a) we calculated

For FA $S = 0.216 \pm 0.002$ (pH = 7)

$S = 0.215 \pm 0.003$ (pH = 8)

For AA $S = 0.220 \pm 0.001$ (pH = 7)

$S = 0.218 \pm 0.001$ (pH = 8)

For PAA $S = 0.217 \pm 0.001$ (pH = 7)

Calculations were made considering for each carboxylate all the amines at the same time.

(b) pL_{50} values for all the carboxylates is linearly dependent on the $i_{\max}$ of the complexes and can be expressed by a simple relationship:

$$\mathrm{pL}_{50} = c\,i_{\max} \tag{8}$$

Results reported in Table 3 show no significant differences in the $\mathrm{pL}_{50}/i_{\max}$ values; a mean value of this parameter c was calculated for all the polyammonium cations and polycarboxylates considered altogether, at different pH values considered in the calculations

$c = 1.49 \pm 0.05$ at pH = 7

$c = 1.48 \pm 0.05$ at pH = 7.4

$c = 1.45 \pm 0.08$at pH = 8

As can be seen, the mean values of sequestration parameters for all the systems investigated are practically the same in the pH range 7–8, including the physiological (7.4) pH value.

The result reported can be interpreted as a mean value, useful to predict the sequestering ability of natural and synthetic

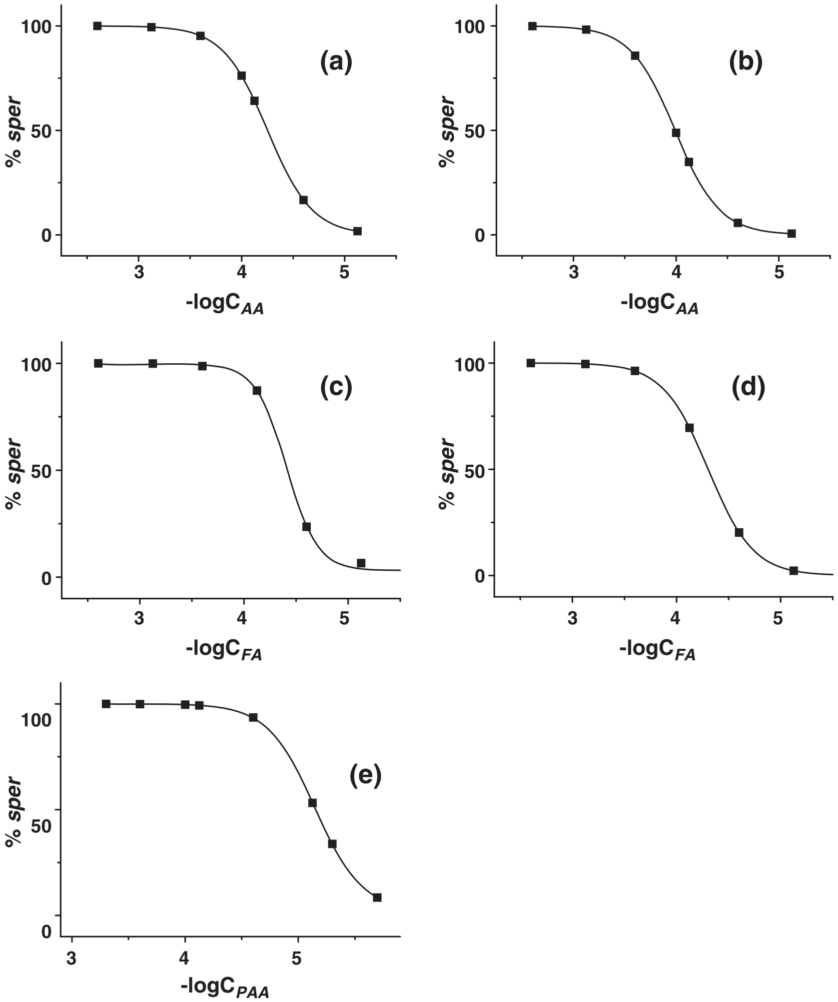


Fig. 8. Percentage of sper as polycarboxylate species vs. sequestering agent concentration at $I=0\,\mathrm{mol\,L^{-1}}$ and $T=25\,°\mathrm{C}$. (a) AA at pH 7; (b) AA at pH 8; (c) FA at pH 7; (d) FA at pH 8; (e) PAA at pH 7.

polycarboxylates towards polyammonium cations. For example, the sequestering ability of a tetramine (fully protonated) should be, on average, $\mathrm{pL_{50}}=6.0\pm0.2$ at $\mathrm{pH}=7$.

4.2. Predictive relationships

As reported in Results, both stability and enthalpy changes for the formation of high-molecular-weight polycarboxylate complexes with polyammonium cations are a simple (linear) function of the charges involved in the formation equilibrium reported in Eq. (2). This signifies that a mean value of $\Delta G°$ per $-\mathrm{NH}_x^+-\mathrm{OOC}^-$ salt bridge can be calculated, providing two assumptions are made: (a) each charged group participates to the binding of polyammonium cation by polyanion; and (b) the number of salt bridges (n) can be approximately calculated from the simple relationship

$$n = \zeta/2.$$

and therefore,

$$\Delta G°_n = \Delta G°/n \tag{9}$$

or

$$\Delta G°_n = 2\Delta G°/\zeta \tag{9a}$$

where $\Delta G°_n$ represents the contribution per bond to the stability of complexes. Analogous expressions can be written for $\Delta H°_n$ and $T\Delta S°_n$.

In Table 4, the mean values of $\Delta G°_n$, $\Delta H°_n$ and $T\Delta S°_n$ per salt bridge are reported for each amine–polycarboxylate system and for the three systems considered altogether. The differences between the various systems are quite low, also considering the fairly high errors associated to the thermodynamic parameters (about $\pm0.5\,\mathrm{kJ\,mol^{-1}}$). The mean $\Delta G°_n$ value, $-5.8\pm0.4\,\mathrm{kJ\,mol^{-1}}$ obtained for all the systems studied, can be compared with the one reported for several polyammonium–low-molecular-weight carboxylate systems [15,16,49]. For a series of di-pentammonium cations (with low-molecular-weight mono–tetra-carboxylates), we found [49] $\Delta G°_n=-6.3\,\mathrm{kJ\,mol^{-1}}$ and for biogenic polyammonium cations [16] with several organic anions we reported $\Delta G°_\mathrm{B}=-5.7\,\mathrm{kJ\,mol^{-1}}$. The mean value per salt bridge $T\Delta S°_n=6.8\,\mathrm{kJ}$

Table 3
pL_{50} values of different polyelectrolytes calculated at different pH values, according to Eq. (7a)

	pL_{50}	pL_{50}/i_{max}	pL_{50}	pL_{50}/i_{max}	pL_{50}	pL_{50}/i_{max}	i_{max}
	pH=7		pH=7.4		pH=8		
FA							
en	3.38	1.7	3.29	1.7	3.06	1.5	2
ptr	3.46	1.7	3.51	1.7	3.53	1.7	2
cdv	3.42	1.7	3.47	1.7	3.49	1.7	2
dien	2.56	1.3	2.61	1.3	2.61	1.3	2
spd	5.09	1.7	5.10	1.7	5.04	1.7	3
sper	4.42	1.1	4.49	1.1	4.30	1.1	4
tetren	4.22	1.1	4.14	1.0	3.97	1.0	4
penten	5.18	1.3	5.03	1.3	4.73	1.2	4
	$S^{a}=0.216\pm0.002^{b}$		$S^{a}=0.219\pm0.001^{b}$		$S^{a}=0.215\pm0.003^{b}$		
AA							
en	4.10	2.0	3.96	2.0	3.06	1.5	2
ptr	2.85	1.4	2.84	1.4	3.53	1.7	2
cdv	2.87	1.4	2.88	1.4	3.49	1.7	2
dien	3.11	1.6	3.11	1.6	2.61	1.3	2
spd	5.29	1.8	5.26	1.8	5.04	1.7	3
sper	4.25	1.1	4.17	1.1	4.30	1.1	4
tetren	4.44	1.5	4.39	1.5	3.97	1.3	3
penten	5.41	1.1	5.1	1.1	4.73	1.0	5
	$S^{a}=0.220\pm0.001^{b}$		$S^{a}=0.217\pm0.001^{b}$		$S^{a}=0.218\pm0.001^{b}$		
PAA							
en	3.41	1.7	3.41	1.7			2
ptr	3.44	1.7	3.57	1.8			2
cdv	3.09	1.6	3.22	1.6			2
dien	2.99	1.5	3.11	1.6			2
spd	5.11	1.7	5.19	1.7			3
sper	5.16	1.3	5.17	1.3			4
tetren	4.11	1.4	4.2	1.4			3
penten	5.75	1.4	5.37	1.3			4
	$S^{a}=0.217\pm0.001^{b}$		$S^{a}=0.218\pm0.001^{b}$				

[a] S=slope of the sigmoid curve.

[b] ±std. dev.

mol^{-1} is also comparable with previous findings on low-molecular-weight systems [16,50,51]. For biogenic ammonium cation–polyanion system, we reported $T\Delta S°_n=8.7$ kJ mol^{-1}. The similarity, in terms of both strength of interactions and, in particular, in formation $T\Delta S°$, for high- and low-molecular-weight polycarboxylates complexes of biogenic polyammonium cations, is a further confirmation of the electrostatic nature of the involved bonds.

Table 4
Mean $\Delta G°_n$, $\Delta H°_n$ and $T\Delta S°_n$ values[a] per salt bridge, at $I=0$ mol L^{-1} and $T=25$ °C

	$\Delta G°_n$	$\Delta H°_n$	$T\Delta S°_n$
PAA[b]	-6.6 ± 0.5^{c}	0.8 ± 0.5^{c}	7.4 ± 0.6^{c}
AA[b]	-5.1 ± 0.5	0.5 ± 0.5	5.6 ± 0.6
FA[b]	-5.7 ± 0.5	–	–
Total[d]	-5.8 ± 0.4	0.7 ± 0.5	6.5 ± 0.5

[a] kJ mol^{-1}.

[b] All the amines considered simultaneously.

[c] ≥95% confidence intervals.

[d] All the complex species considered.

5. Final remarks

In this work we reported several formation thermodynamic data on the interaction between protonated polyamines and synthetic and natural polycarboxylates. The most important results are (a) the rather high stability of the formed species that leads to a strong sequestering ability of polycarboxylates towards polyammonium cations (see sequestration diagrams, Figs. 7 and 8) and (b) the similarity of the behavior of the systems containing these components and the relative low-molecular-weight ones. The predictive Eqs. (5), (6) and (8) allow to model other important amino-carboxylate systems. Calculations performed on the sequestration parameters using Eq. (8) showed that there is no significant difference in the sequestering capacity of all the polycarboxylates towards biogenic polyammonium cations in the pH range 7–8. These results can be also used for a better understanding of enzymatic reactions involving biogenic amines at physiological pH value (7.4).

A quite weak point in determining the stability of metal complexes of natural macromolecules, such as fulvates, is represented by the high concentration of reagents which gives an unrealistic picture of natural systems. In fact, when very low concentration are considered ($\ll 0.01\,\mathrm{mmol\ L^{-1}}$), the effect of residual binding sites may alter considerably the value of formation constants (in other words these must be considered as a function of reagents concentrations) [52]. This is not the case of present results, since alginate and polyacrylate polyelectrolytes contain only carboxylate binding groups, and fulvates contain very small percentages of –SH and –NH groups that do not significantly interfere in the binding with polyammonium cations. Also, the presence of –OH groups does not interfere since these are dissociated at quite high pH values (pH > 9).

Acknowledgements

This study was performed by the financial support of the Universities of Messina and Palermo (PRA) and MIUR (FIRB Project Number RBAU01HLFX_004 and _005).

References

[1] V. Zappia, in: A.E. Pegg (Ed.), Progress in Polyamine Research, Plenum Press, New York, 1988.

[2] S.S. Cohen, A Guide to the Polyamines, Oxford University Press, Oxford, UK, 1998.

[3] W.M. Dabrowski (Ed.), Toxins in Food. Chemical and Functional Properties of Food Components Series, C.H.I.P.S., New York, 2004.

[4] C. Ruiz-Capillas, F. Jimenez-Colmenero, Biogenic amines in meat and meat products, Crit. Rev. Food Sci. Nutr. 44 (2004) 489–599.

[5] C. den Brinker, M. Kerr, C. Rayner, Investigation of biogenic amines in fish and fish products., Public Health Division, Victoria Government Department of Human Services, 1995. Available on line, www.foodsafety.vic.gov.au.

[6] M.L. Antonelli, V. Carunchio, E. Cernia, R. Purrello, Noncovalent interactions in ternary complexes of spermine and copper(II) with adenosine 5′-triphosphate and tripolyphosphate, J. Inorg. Biochem. 37 (1989) 201–211.

[7] S. Bunce, E.S.W. Kong, The interactions between nucleic acids and polyamines. I. High resolution carbon-13 and hydrogen-1 nuclear magnetic resonance studies of spermidine and 5′-AMP, Biophys. Chem. 8 (1978) 357–368.

[8] D. Esposito, P. Del Vecchio, G. Barone, Interactions with natural polyamines and thermal stability of DNA. A DSC study and a theoretical reconsideration, J. Am. Chem. Soc. 119 (1997) 2606–2613.

[9] M.W. Hosseini, J.M. Lehn, Binding of AMP, ADP and ATP nucleotides by polyammomium macrocycles, Helv. Chim. Acta 70 (1987) 1312–1319.

[10] E. Kimura, M. Kodama, T. Yatsunami, Macromonocyclic polyamines as biological polyanion complexons: 2. Ion-pair association with phosphate and nucleotides, J. Am. Chem. Soc. 104 (1982) 3182–3187.

[11] E. Kimura, Y. Kuramoto, T. Koike, H. Fujioka, M. Kodama, A study of new bis(macrocyclic polyamine) ligands as inorganic and organic anion receptors, J. Org. Chem. 55 (1990) 42–46.

[12] I. Labadi, E. Jenei, R. Lahti, H. Lönnberg, Interaction of pyrophosphate ion with di-, tri-, and tetra-amines in aqueous solution: a potentiometric and calorimetric study, Acta Chem. Scand. 45 (1991) 1055–1059.

[13] K. Mernissi-Arifi, M. Zenkouar, G. Schlewer, B. Spiess, Quantitative investigation of the interactions between inositol-tris(phosphates) and polyamines, J. Chem. Soc., Faraday Trans. 92 (1996) 3101–3107.

[14] L. Lomozik, A. Gasowska, R. Bregier-Jarzebowska, R. Jastrzab, Coordination chemistry of polyamines and their interactions in ternary systems including metal ions, nucleosides and nucleotides, Coord. Chem. Rev. 249 (2005) 2335–2350.

[15] A. De Robertis, C. De Stefano, A. Gianguzza, S. Sammartano, Binding of polyanions by biogenic amines: I. Formation and stability of protonated putrescine and cadaverine complexes with inorganic anions, Talanta 46 (1998) 1085–1093.

[16] A. De Robertis, C. De Stefano, C. Foti, O. Giuffrè, S. Sammartano, Thermodynamic parameters for the binding of inorganic and organic anions by biogenic polyammonium cations, Talanta 54 (2001) 1135–1152.

[17] C. De Stefano, C. Foti, A. Gianguzza, S. Sammartano, Speciation of low molecular weight ligands in natural fluids: protonation constants and association of open chain polyamines with major components of seawater, Anal. Chim. Acta 418 (2000) 43–51.

[18] C. De Stefano, A. Gianguzza, R. Maniaci, D. Piazzese, S. Sammartano, Binding of polyanions by biogenic amines: II. Formation and stability of protonated putrescine and cadaverine complexes with carboxylic ligands, Talanta 46 (1998) 1079–1084.

[19] A. De Robertis, C. De Stefano, A. Gianguzza, S. Sammartano, Binding of polyanions by biogenic amines: III. Formation and stability of protonated spermine and spermidine complexes with carboxylic ligands, Talanta 46 (1998) 1079–1084.

[20] F. Crea, A. De Robertis, C. De Stefano, A. Gianguzza, D. Piazzese, S. Sammartano, Binding of acrylic and sulphonic polyanions by open-chain polyammonium cations, Talanta 53 (2001) 1241–1248.

[21] S. Cascio, A. De Robertis, C. De Stefano, C. Foti, A. Gianguzza, S. Sammartano, Stability–charge and stability–structure relationships in the binding of dicarboxylic ligands by open-chain polyammonium cations, J. Chem. Eng. Data 45 (2000) 717–723.

[22] J.C. Masini, G. Abate, E.C. Lima, L.C. Hahn, J.L. Nakamura, H.R. Nagatomy, Comparison of methodologies for determination of carboxylic and phenolic groups in humic acids, Anal. Chim. Acta 364 (1998) 223–233.

[23] F.J. Stevenson, Humus Chemistry. Genesis, Composition, Reactions, John Wiley and Sons, New York, 1994.

[24] E. Tipping, Cation binding by humic substances, Cambridge Environmental Chemistry Series, vol. 12, Cambridge University Press, Cambridge, UK, 2002.

[25] H.M. Selim, W.L. Kingery (Eds.), Geochemical and Hydrological Reactivity of Heavy Metals in Soils, Lewis Publisher, Boca Raton, 2003.

[26] K. Kalbitz, R. Wennrich, Mobilization of heavy metals and arsenic in polluted wetland soils and its dependence on dissolved organic matter, Sci. Total Environ. 209 (1998) 27–31.

[27] S. Gaffney, N.M. Marley, S.B. Clark (Eds.), Humic and Fulvic Acids, Isolation, Structure and Environmental Role ACS Symposium Series, vol. 651, Am. Chem. Soc., Washington DC, 1996, p. 207.

[28] J. Buffle, Complexation Reactions in Aquatic Systems: An Analytical Approach, Ellis Horwood, Chichester, 1988.

[29] Kelco Inc., Alginate Products for Scientific Water Control. 3rd ed. Merck Division Inc., San Diego, CA, 1987.

[30] H. Eccles, S. Hunt (Eds.), Immobilisation of Ions by Biosorption, Hellis Horwood, Chichester, 1986.

[31] J. Wase, C. Forster (Eds.), Biosorbents for Metal Ions, Taylor and Francis, London, UK, 1997.

[32] B. Volesky (Ed.), Sorption and Biosorption, BV Sorbex Inc., Montreal – St. Lambert, Quebec, Canada, 2003.

[33] T.A. Davis, B. Volesky, A. Mucci, A review of the biochemistry of heavy metal biosorption by brown algae, Water Res. 37 (2003) 4311–4330.

[34] C. De Stefano, A. Gianguzza, D. Piazzese, S. Sammartano, Polyacrylate protonation in various aqueous ionic media at different temperatures and ionic strengths, J. Chem. Eng. Data 45 (2000) 876–888.

[35] A. De Robertis, C. De Stefano, C. Foti, A. Gianguzza, D. Piazzese, S. Sammartano, Protonation constants and association of polycarboxylic ligands with the major components of seawater, J. Chem. Eng. Data 45 (2000) 996–1000.

[36] C. De Stefano, A. Gianguzza, D. Piazzese, S. Sammartano, Speciation of polyelectrolytes in natural fluids. Protonation and interaction of polymethacrylates with major components of seawater, Talanta 58 (2002) 405–417.

[37] C. De Stefano, A. Gianguzza, D. Piazzese, S. Sammartano, Polyacrylates in aqueous solution. The dependence of protonation on molecular weight, ionic medium and ionic strength, React. Funct. Polym. 55 (2003) 9–20.

[38] C. De Stefano, A. Gianguzza, D. Piazzese, S. Sammartano, Quantitative parameters for the sequestering capacity of polyacrylates towards alcaline earth metal ions, Talanta 61 (2003) 181–194.

[39] C. De Stefano, A. Gianguzza, D. Piazzese, S. Sammartano, Speciation of organic matter in natural waters. Interaction of polyacrylates with the major cation components of seawater, Mar. Chem. 86 (2004) 33–44.

[40] C. De Stefano, A. Gianguzza, D. Piazzese, S. Sammartano, Modelling of proton and metal exchange in the alginate biopolymer, Anal. Bioanal. Chem. 383 (2005) 587–596.

[41] F. Crea, A. Giacalone, A. Gianguzza, D. Piazzese, S. Sammartano, Modelling of natural and synthetic polyelectrolyte interactions in natural waters, Mar. Chem. 99 (2006) 93–105.

[42] C. De Stefano, P. Princi, C. Rigano, S. Sammartano, Computer analysis of equilibrium data in solution. ESAB2M: an improved version of the ESAB program, Ann. Chim. 77 (1987) 643–675 (Rome).

[43] C. De Stefano, S. Sammartano, P. Mineo, C. Rigano, Computer tools for the speciation of natural fluids, in: A. Gianguzza, E. Pellizzetti, S. Sammartano (Eds.), Marine Chemistry—An Environmental Analytical Chemistry Approach, Kluwer Academic Publishers, Amsterdam, 1997, pp. 71–83.

[44] C. De Stefano, G. Mineo, C. Rigano, S. Sammartano, Ionic strength dependence of formation constants: XVII. The calculation of equilibrium concentrations and formation constants, Ann. Chim. 83 (1993) 243–277 (Rome).

[45] A. De Robertis, C. De Stefano, C. Rigano, S. Sammartano, Computer analysis of equilibrium data in solution. ES5CM Fortran and Basic programs for computing formation enthalpies from calorimetric measurements, Thermochim. Acta 138 (1986) 141–146.

[46] P.G. Daniele, A. De Robertis, C. De Stefano, S. Sammartano, Ionic strength dependence of formation constants: XIII. A critical examination of preceding results, in: S. Alegret, J.J. Arias, D. Barcelò, J. Casal, G. Router (Eds.), Miscellany of Scientific Papers Offered to Enric Casassas, Universidad Autonoma de Barcelona, Bellaterra (Barcelona), Spain, 1991, pp. 121–126.

[47] P.G. Daniele, C. De Stefano, C. Foti, S. Sammartano, The effect of ionic strength and ionic medium on thermodynamic parameters of protonation and complex formation, Curr. Topics Sol. Chem. 2 (1997) 253–274.

[48] E. Högfeldt, T. Miyajima, J.A. Marinsky, M. Muhammed, Application of a simple three parameter model to titration data for some polyelectrolytes, Acta Chem. Scand. 43 (1989) 496–499.

[49] A. De Robertis, C. De Stefano, O. Giuffrè, S. Sammartano, Binding of carboxylic ligands by protonated amines, J. Chem. Soc., Faraday Trans. 92 (1996) 4219–4226.

[50] C. De Stefano, O. Giuffrè, S. Sammartano, ΔG^0 and $T\Delta S^0$ charge relationships for the binding of carboxylic anions by open-chain polyammonium cations, J. Chem. Soc., Faraday Trans. 94 (1998) 2395–2398.

[51] A. De Robertis, C. De Stefano, O. Giuffrè, S. Sammartano, ΔG^0 and $T\Delta S^0$—charge relationships for the binding of inorganic and organic anions by open chain polyammonium cations: a short review, Ann. Chim. 89 (1999) 23–35 (Rome).

[52] R.M. Town, M. Filella, Implications of natural organic matter binding heterogeneity on understanding lead (II) complexation in aquatic systems, Sci. Total Environ. 300 (2002) 143–154.