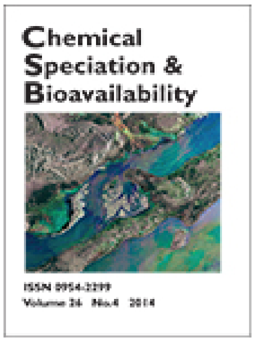

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Speciation of poly-amino carboxylic compounds in seawater

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ABSTRACT

In this work quantitative data on the interaction of *EDTA* and *EGTA* with the major inorganic components of seawater are reported. Protonation constants and alkali and alkaline earth metal complex formation constants are reported at different ionic strengths ($0< I/\text{mol L}^{-1} \leq 1$). These formation data were obtained from potentiometric measurements in several single and mixed electrolyte media (such as artificial seawater). Potentiometric measurements in artificial seawater (containing Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^- and SO_4^{2-}) were analysed by using the single salt approximation (the artificial seawater being considered as a single salt BA whose ions have fractional charge $-z_{\text{anion}} = z_{\text{cation}} = 1.117$). Several species B_pH_rL (L = *EDTA* or *EGTA*) are formed and their formation constants are reported at different salinities. Present results, together with similar data on the complexing capability of *NTA*, *DTPA* and *TTHA* towards inorganic components of natural fluids, allowed to find general conclusions on the important class of complexons.

Keywords: complexons, complexes with the major inorganic components of seawater, single salt approximation for artificial seawater, speciation

INTRODUCTION

Some poly-amino carboxylic compounds, such as ethylenediaminetetraacetic (*EDTA*), ethylenebis (dioxydiethylenediamine)tetraacetic (*EGTA*), diethylenetriaminepentaacetic (*DTPA*), triethylene tetraamine-hexaacetic (*TTHA*) and nitrilotriacetic (*NTA*) acids, generally called "complexons" (structures reported in Scheme 1) are employed in many fields of application because of their strong sequestering ability towards almost all metal ions.

Some examples of their application are listed as follows. In analytical chemistry, complexons (in particular *EDTA*) are used for quantitative determination of many metal cations (Flaschka, 1959). The interaction of metals with different chelating agents is used in agriculture to avoid the effects of trace element deficiency: it is known that (Bould, 1957) when essential metals for plants are not available from the soil, owing to their low solubility, the addition of sequestering agents, such as poly-amino carboxylic ligands, is an useful means of extracting metal ions from the soil and transporting them as chelates, *via* water, into the plants. The influence of heavy metal complexation on toxicity was investigated using *DTPA* as a sequestering agent (Oikari and Sillanpaa, 1996), and poly-amino polycarboxylic ligands are also used to enhance the extraction of metals from natural waters for analytical purposes. *EDTA* exchange was used to investigate the stability of humic substance – metal complexes (Burba *et al.*, 2001). Complexons are involved in many other areas, for example, in clinical practice where they are used as contrast agents in computed tomography (Carvalho *et al.*, 1997; Adline *et al.*, 1997; Bauer *et al.*, 1996; Grunder *et al.*, 1997); moreover solutions of boron-*DTPA* have been used for studies on micronutrient metals supplied for plants growth (Asad *et al.*, 1997). Recent applications of poly-aminocarboxylic

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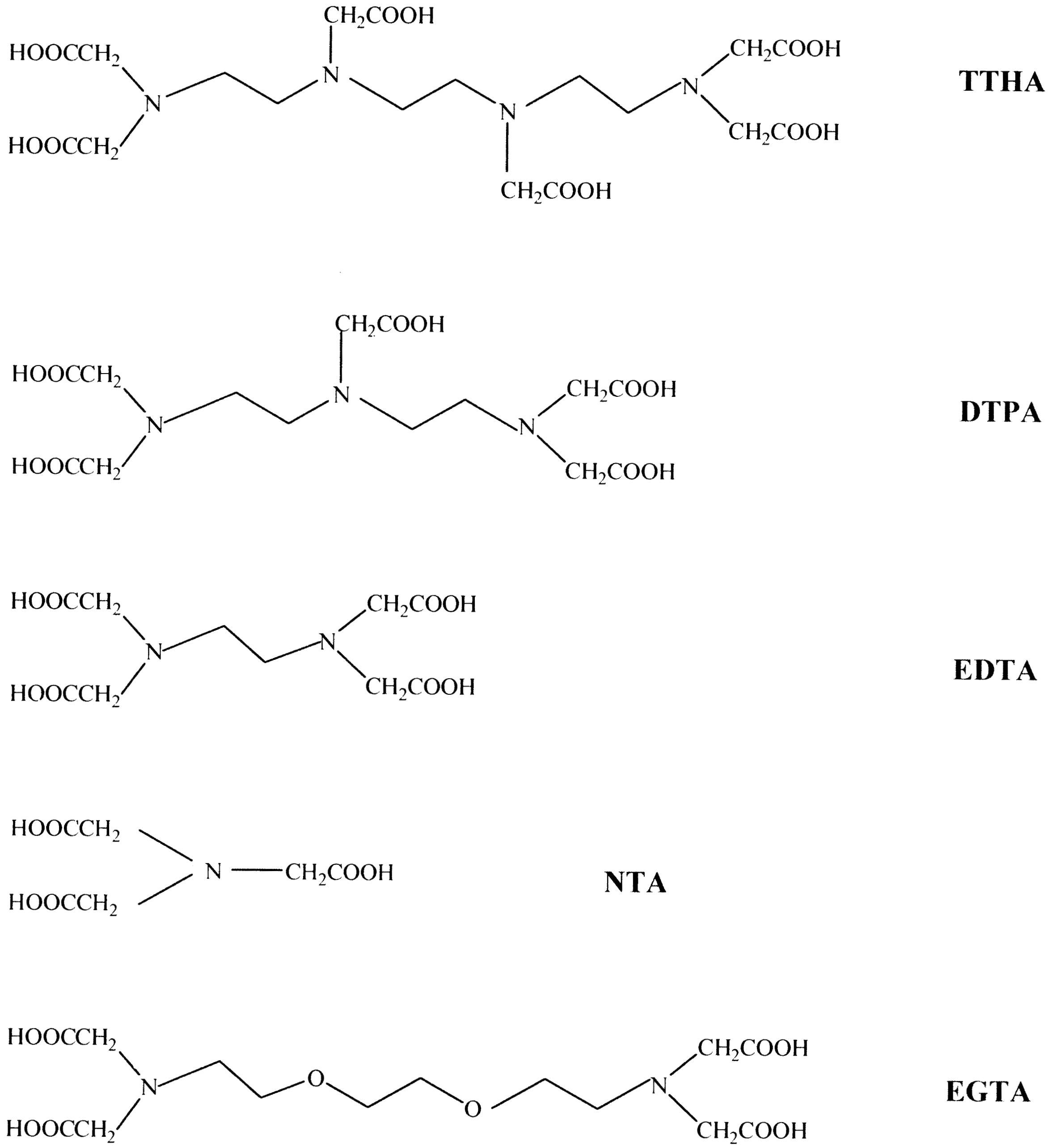

Scheme 1 *Simplified structure of polyaminocarboxylic acids.*

ligands are: (i) suppression of unfavourable catalytic effects of some metal ions in production of chemicals, or as cleaning agents, used to maintain the efficiency of filters and to avoid their occlusion by metallic salts, (ii) in the cosmetic and pharmaceutical industries, as well as in the production of foodstuffs and beverages, to prevent the formation of insoluble metal precipitates, or the catalytic degradation enhanced by some metal ions such as copper and iron; (iii) as softening agents in the treatment of water supplies and in heat transfer equipment; (iv) in the leather industry, for the prevention and removal of stains caused by iron compounds; (v) in all chemical processes where the presence of free calcium and other metal ions must be avoided; (vi) in analytical chemistry procedures, for the sequestration or removal of interfering metal ions, for masking metals by chelation in quantitative complexometric titrations.

The large number of applications justifies the intense interest of chemists in the binding ability of complexons, as demonstrated by the amount of papers published in the last decades (for example: Alderighi *et al.*, 1996; Arena *et al.*, 1978; Baumann, 1974; Bohigian and Martell, 1965; Brucher *et al.*, 1996; Carson *et al.*, 1968a, 1968b; Chaberek *et al.* 1959; Choppin *et al.*, 1977; Delgado *et al.*, 1997; Grimes *et al.*, 1963; Gritmon *et al.*, 1977; Harju, 1970; Harju and Ringbom 1970; Harris and Martell, 1976; Letkeman and Martell, 1979; Liu *et al.*, 1997; Lund, 1971; Lund and Overoll, 1982; Moeller and Thompson, 1962; Martell and Motekaitis, 1980; Rizkalla *et al.*, 1989), and chemical stability data reported in several compilations (Anderegg, 1977 and refs therein; Martell and Sillén, 1964, 1971; Martell and Smith, 1977; Martell *et al.*, 1997; May and Murray, 2000; Perrin, 1979; Pettit and Powell 1997). A further important aspect of the aqueous solution chemistry of poly-amino carboxylic acids concerns their structure, which makes them useful models in speciation studies of naturally occurring amino-carboxylic ligands, such as humic substances, in aquatic environments. For example, Turner and Withfield (1987) reported an equilibrium speciation model for natural waters based on *EDTA*–metal complexation.

As a further contribution to the speciation of organic matter in natural fluids, we report here a chemical speciation study of *EDTA*, *EGTA*, *DTPA*, *TTHA* and *NTA* in aqueous multi-component solutions containing the cation macro-components of natural waters. In particular, (i) the protonation and complexation ability of *EGTA* was investigated in different ionic media (NaCl, tetraethylammonium iodide and in mixtures of $MgCl_2$–NaCl or $CaCl_2$–NaCl) at different ionic strengths ($0< I/\mathrm{mol\ L^{-1}} \leq 1$) and $T = 25$°C; (ii) formation constants for the interactions of Na^+, Ca^{2+}, Mg^{2+} with *EGTA* are reported at different ionic strengths ($0< I/\mathrm{mol\ L^{-1}} \leq 1$); (iii) a multicomponent ionic medium simulating the major inorganic composition of natural seawater (Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, SO_4^{2-}, Synthetic Sea Water for Equilibrium studies, SSWE) has been used in order to define the speciation of *EGTA* and *EDTA* in seawater. To simplify calculations SSWE has been considered as a single salt BA with ion charge $z = \pm 1.117$ (De Stefano *et al.*, 1998a). All measurements were carried out by potentiometry ([H^+]-glass electrode) at $T = 25$°C. For purpose of comparison some literature data are reported too. In the discussion of results the analogous data already reported for *NTA*, *DTPA* and *TTHA* are taken into account to find general behaviour of complexons in natural fluids.

METHODOLOGY

Materials and reagents

All the reagents were from Fluka. Solutions of ethylenediaminetetraacetic acid (*EDTA*) and ethylenebis(dioxydiethylenediamine)tetraacetic acid (*EGTA*) were prepared from commercial products without further purification, and their purity, checked alkalimetrically, was found to be >99%. Tetraethylammonium iodide was re-crystallised from methanol–acetone and solvent was completely eliminated before use. Sodium, potassium, calcium and magnesium chlorides and sodium sulfate were used without further purification. Calcium and magnesium chloride concentrations were checked by complexometric titrations using *EDTA*. Sodium and potassium chlorides were used after drying at 140°C. The sulfate concentration in sodium sulfate solutions was checked by a ion chromatographic analyser (Dionex Sunnyvale, CA, USA; model DX500) coupled with a conductometric detector. Hydrochloric acid and sodium or tetramethylammonium hydroxide solutions were standardised against sodium carbonate and potassium biphthalate, respectively. Tetramethylammonium hydroxide solutions were used when tetraethylammonium iodide was used as background salt. All the solutions were prepared using analytical grade water ($R = 18\ \mathrm{M\Omega\ cm^{-1}}$) and grade A glassware.

Equipment and procedure

Potentiometric measurements were performed using an apparatus consisting of a Metrohm mod. 665 automatic titrant dispenser coupled with a Metrohm mod. 654 potentiometer equipped with a combination Orion-Ross 8102 glass-electrode. The estimated accuracy (95% confidence interval) of this system was ± 0.15 mV and ± 0.003 mL for E.M.F. and titrant volume readings, respectively. The burette and the potentiometer were connected through a personal computer, and suitable software allowed us to monitor all titrations. All measurements were performed in thermostatted cells at 25 ± 0.1 °C. In titration cells, pure nitrogen was bubbled through the solutions in order to avoid O_2 and CO_2 inside, and the solutions were magnetically stirred. In the measurements relative to the protonation of the ligands, a volume of 25–50 mL of solution, containing the poly-amino carboxylic acid (1–5 mmol L^{-1}) and the background salt (NaCl, Et_4NI, mixture of $MgCl_2$–NaCl or $CaCl_2$–NaCl or synthetic seawater), added to adjust the ionic strength or the salinity to the different desired values, was titrated with hydroxide solution (NaOH, Me_4NOH). In the measurements relative to alkaline earth metal complex formation, $CaCl_2$ or $MgCl_2$ concentration in the solution was $C_{MCl_2} = 1$–10 mmol L^{-1}. For each experiment, two or three titrations were carried out. For each experimental condition, independent titrations of hydrochloric acid solutions with NaOH or Me_4NOH solutions were performed in the same experimental ionic strength conditions as the systems under study, in order to determine formal electrode potential. All titrations were carried out up to pH ~11 (but for solutions containing Mg^{2+}, pH ≤ 8.5–10, depending on the concentration of this cation).

Calculations

All parameters of an acid–base titration (analytical concentration of the reagents, electrode formal potential E^0, the coefficient j_a for the junction potential E_j =

$j_a[H^+]$, ionic product of water K_w) were determined by using the non linear least squares computer program ESAB2M (De Stefano *et al.*, 1987). Computer programs BSTAC and STACO and ES4ECI (De Stefano *et al.*, 1993) were used to calculate protonation and formation constants in not constant ionic strength conditions and to draw distribution diagrams of the species in solution and to compute species formation percentages, respectively. Weak complex formation constants were calculated using the computer program ES2WC (De Robertis *et al.*, 1987). The errors associated with the parameters calculated in this work are given as ≥ 95% confidence intervals.

Equilibrium constants are defined as follows (charges are omitted for simplicity):

(1) overall protonation reaction:
$L + j\mathrm{H} = H_jL$ β_j^H
(2) stepwise protonation reaction:
$\mathrm{H}_{j-1}L + \mathrm{H} = \mathrm{H}_jL$ K_j^H
(3) overall metal complex reaction:
$pM + qL + r\mathrm{H} = M_pL_q\mathrm{H}_r$ β_{pqr}
(4) partial metal complex reactions:
$M_{p-1}L + M = M_pL$ K_{MpL}
$M + \mathrm{H}_rL = M\mathrm{H}_rL$ K_{MHrL}
(5) mixed metal complex formation
$M' + M\mathrm{H}_rL = M'MH_rL$ $K_{M'MHrL}$

All equilibrium constants are given in the molar (mol L^{-1}) concentration scale.

RESULTS

The protonation constants of *EGTA* at different ionic strengths (0.05≤ I/mol L^{-1} ≤1), in NaCl and Et_4NI, are reported in Table 1.

Table 1 Protonation constants of EGTA, at different ionic strengths, in NaCl and Et_4NI, at T = 25°C

	$\log\beta^H_1$	$\log\beta^H_2$	$\log\beta^H_3$	$\log\beta^H_4$
I^b		NaCl		
	(±0.03)$^{a)}$	(±0.03)	(±0.02)	(±0.04)
0.05	9.77	18.70	21.45	23.53
0.1	9.60	18.39	21.06	23.10
0.25	9.37	17.94	20.49	22.50
0.5	9.22	17.61	20.10	22.11
1	9.16	17.36	19.84	21.92
		Et_4NI		
	(±0.03)	(±0.02)	(±0.02)	(±0.05)
0.05	9.90	18.96	21.77	23.83
0.1	9.77	18.74	21.48	23.55
0.25	9.64	18.52	21.21	23.26
0.5	9.63	18.50	21.20	23.30
1	9.71	18.67	21.46	23.65

^{a}Uncertainties expressed as 95% confidence interval; ^{b}mol L^{-1}.

As expected, log β^H_j values obtained in the two media are very different, as shown in Figure 1 (where log β^H_3 values of *EGTA* are plotted *vs* $I^{½}$), owing to the different ability of Na^+ and Et_4N^+ to interact with complexons (Daniele *et al.*, 1983, 1985).

The dependence on ionic strength can be expresses by the Debye-Hückel type equation (Casale *et al.*, 1988; Daniele *et al.*, 1991, 1997).

$$\log \beta^H_j = \log {}^T\beta^H_j - z^* I^{½} (2 + 3\, I^{½})^{-1} + CI + DI^{3/2} \quad (1)$$

where $z^* = \Sigma\, z^2_{\mathrm{reactants}} - \Sigma\, z^2_{\mathrm{products}}$ (z = charges), log ${}^T\beta_j^H$ is the protonation constant at infinite diluition, C and D are empirical parameters that can be calculated by least squares calculations. These parameters are reported in Table 2.

Both C and D can be expressed as a function of the protonation step j and the difference in z^2 (z^*):

NaCl $C = 0.11\, j + 0.0033\, z^*$; $D = 0$

Et_4NI $C = 0.11\, j + 0.171\, z^*$; $D = -0.075\, z^*$

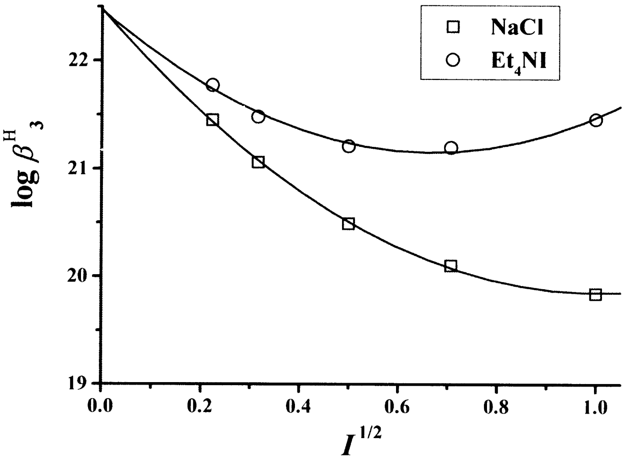

***Figure 1** Dependence on ionic strength of third overall protonation constant of* EGTA, *in (() Et_4NI and in (() NaCl, at* T = *25°C.*

Table 2 Parameters for the ionic strength dependence of EGTA protonation constants, at T = 25°C

j	z*	C	D	C	D
		NaCl		Et_4NI	
		(±0.06)^a		(±0.07)^a	(±0.10)^a
1	8	0.26	0	1.60	–0.77
2	14	0.16	0	2.97	–1.46
3	18	0.34	0	3.84	–1.83
4	20	0.58	0	4.26	–1.90

^{a}In parenthesis we report 95% confidence interval.

Values of log ${}^{T}\beta_j^{H}$ of *EGTA* calculated by using eqn (1) are reported in Table 3 together with those of other complexons, such as *NTA, EDTA* (Daniele *et al.*, 1985), *DTPA* and *TTHA* (De Stefano *et al.*, 2003), already studied in these laboratories.

All complexons (other poly-amino carboxylic ligands, whose protonation data are reported in literature, will be discussed later) show sharp differences between the first n protonation steps (n = number of substituted amino groups) and the other steps relating to the protonation of carboxylic groups. Another significant difference can be observed between simple poly-amino carboxylic acids, *NTA*, *EDTA*, *DTPA* and *TTHA*, having general formula $(CH_2)_{2n-2}N_n(CH_2COOH)_{n+2}$, and *EGTA*: the difference is due to both the presence of two -O- mojeties and to the length of the chain which separates protonable groups. As regards the first n protonation constants of simple poly-amino carboxylates we found a very simple relationship, as a function of n and j:

$$\log K^{H}_{j} = a + b_j^{2}/n$$

where a = 12.2 and b = 2.2, with a linear correlation coefficients r = 0.972.

Table 3 Protonation constants of *NTA*, *EDTA*, *EGTA*, *DTPA* and *TTHA* at I = 0 mol L^{-1} and T = 25°C

	NTA^a	*EDTA*^{a}	*EGTA*^{b}	*DTPA*^{c}	*TTHA*^{c}
$\log K^H_1$	10.39	11.09	10.48 ± 0.05$^{d)}$	11.52	11.71
$\log K^H_2$	2.94	6.72	9.48 ± 0.04	9.36	10.42
$\log K^H_3$	1.97	3.02	3.09 ± 0.02	4.83	6.83
$\log K^H_4$	–	2.12	2.24 ± 0.07	3.13	4.61
$\log K^H_5$	–	–	–	2.35	3.20
$\log K^H_6$	–	–	–	–	2.42
$\log \beta^H_2$	13.33	17.81	19.96	20.88	22.13
$\log \beta^H_3$	15.30	20.83	23.05	25.71	28.96
$\log \beta^H_4$		22.95	25.29	28.84	33.57
$\log \beta^H_5$	–	–		31.19	36.77
$\log \beta^H_6$		–	–	–	39.19

[a]Daniele *et al.*, 1985; [b]This work; [c]De Stefano *et al.*, 2003; [d]Uncertainties expressed as 95% confidence interval.

Sodium complexes

The difference between the protonation constant values in Et_4NI and in NaCl can be interpreted in terms of Na^+-complexes formation (De Robertis *et al.*, 1987), according to the equality: $\bar{P} = \bar{P}^*$ with

$$\bar{P} = \Sigma\, r\, P_{pqr}\,[H^+]^r / (1 + \Sigma\beta_{pqr}\,[Na^+]^p\,[H^+]^r)$$

and

$$\bar{P}^* = \Sigma_j\, \beta_j^{H*}\,[H^+]^j / (1 + \Sigma\beta_j^{H*}\,[H^+]^j)$$

where $\bar{P}^*$ are apparent protonation constants *i.e.* the ones calculated without allowing for the formation of weak complexes. The formation constants of alkaline metal complexes of *EGTA* and other different complexons are reported in Table 4. The stability of these species is intermediate between *EDTA*, *NTA* and *DTPA* and *TTHA*.

Considering or neglecting the formation of weak species implies the use of two different models:

(a) Model 1, which accounts for differences in $\log K^H_j$ values in terms of differences in activity coefficients in different background salts;
(b) Model 2, which accounts for the formation of weak complexes.

Table 4 Formation constants of sodium complexes of *NTA*, *EDTA*, *EGTA*, *DTPA* and *TTHA*, at I = 0 mol L^{-1} and T = 25°C

	$\log \beta_{pr}$				
p r	*EDTA*^{a}	*EGTA*^{b}			
1 0	2.55 ± 0.15^d	2.09 ± 0.05^d	–	–	–
1 1	11.3 ± 0.2	11.95 ± 0.05	–	–	–
1 2	–	20.48 ± 0.10	–	–	–
1 3	–	22.0 ± 0.2	–	–	–
2 0	–	3.29 ± 0.07	–	–	–
2 1	–	12.14 ± 0.10	–	–	–
Equilibrium^f	*NTA*^{a}	*EDTA*^{a}	*EGTA*^{b}	*DTPA*^{c}	*TTHA*^{c}
L + Na = NaL^e	1.9	2.5	2.1	2.5	2.8
HL + Na = NaHL	-0.2	0.2	1.5	1.6	2.2
H_2L + Na = NaH_2L	–	–	0.5	1.0	1.7
H_3L + Na = NaH_3L	–	–	-1.0	0.4	1.4
NaL + Na = Na_2L	–	–	1.2	1.6	2.0
NaHL + Na = Na_2HL	–	–	0.2	0.5	1.6

[a]Values calculated from data in Daniele *et al.*, 1985; [b]This work; [c]De Stefano *et al.*, 2003; [d]uncertainties expressed as 95% confidence interval; [e]charges omitted for simplicity, [f)]according to partial metal complex reactions (4).

Both models are suitable, but equilibrium parameters obtained using Model 2 must be used with care. If we consider weak complex formation constants it is necessary to specify the reference background salt, *i.e.* the baseline salt, considered as not interacting with the ligand.

The tetralkylammonium cation seems to be the most convenient for these studies, since for a large series of different ligands we obtained very similar data for the dependence on ionic strength of protonation constants (Daniele *et al.*, 1994; Foti *et al.*, 1997).

Magnesium and calcium complexes

Potentiometric data obtained in Mg^{2+} and Ca^{2+} were analysed by least squares calculation and gave evidence for the formation of the following species ($L = EGTA^{4-}$, $M^{2+} = Mg^{2+}$ or Ca^{2+}): ML^{2-}, MHL^-, MH_2L^0, M_2L^0, M_2HL^+, $MNaL^-$, $MNaHL^0$. Formation constants obtained by using model 2, which allows for the formation of weak Na^+ complexes, are reported in Table 5. Formation constants for the interaction of Ca^{2+} or Mg^{2+} with *EDTA*, *DTPA*, *TTHA* and *NTA* are also reported in Table 5 for comparison.

Alkaline earth metal cations form quite strong complexes with all complexons (Arena *et al.*, 1983; De Stefano *et al.*, 1999; Foti *et al.*, 2002). For *EGTA*-Ca^{2+} system we found the formation of ML, MHL, MH_2L, and M_2L, while for *EGTA*-Mg^{2+} system we found the formation of ML, MHL, MH_2L, M_2L, and M_2HL. When model 2 is used the formation of weak species (Table 4) must be taken into account together with some mixed Na^+–metal complexes such as NaMgL or NaCaL, and NaCaHL, in the Na–Mg–*EGTA* and Na-Ca-*EGTA* system respectively. The stability of all these species is not negligible in a correct speciation model. In Tables 6 and 7 we report the formation constants for the interactions of Mg^{2+} and Ca^{2+} with *EGTA* obtained using model 1.

These stability data are obtained without taking into account the formation of weak Na^+ complexes but only considering the conditional protonation constants of *EGTA* in NaCl reported in Table 1.

In Figure 2 we plotted the speciation diagram of *EGTA*-Ca^{2+} system at different ionic strengths in *NaCl* and considering $C_{Ca^{2+}}$ = 10mmol L^{-1} and C_{EGTA} = 5 mmol L^{-1}. The formation percentages depend strongly on ionic strength. In fact, the percentages of the species CaHL decreases (from 60% to 20%) by increasing the ionic strength value and then the competition of Na^+ towards the ligand. Moreover, at pH > 6 the formation percentage of CaL is almost the same for both ionic strength values.

Complexation with seawater macro-components: speciation profiles

In principle, the speciation of complexons in seawater can be calculated by using formation data for single binary systems (Tables 1, 3 and 4) and some mixed systems (Tables 5–7). Nevertheless, when fairly concentrated mixed electrolyte solutions are taken into account, the speciation of big ligands, with several coordinating groups, cannot be calculated correctly using this simplified approach. In fact, in a recent study on the protonation of linear polyamines in synthetic seawater (De Stefano *et al.*, 2000) we showed that for $n > 3$ (n = number of protonated aminogroups) this simplified approach does not hold, probably due to the formation of several weak mixed species. In our laboratory carboxylates and aminoacids have been also studied in synthetic seawater to provide quantitative information on the interaction of these ligands with the major inorganic constituents of seawater (De Robertis *et al.*, 1997, 2000; De Stefano *et al.*, 1998, 1999, 2000, 2002a, 2002b; Foti *et al.*, 2002)

On the basis of previous experience, the speciation of poly-amino carboxylic acids has also been studied

Table 5 Formation constants for magnesium and calcium complexes of *EDTA*, *EGTA*, *DTPA*, *TTHA* and *NTA*, at $I = 0$ mol L^{-1} and $T = 25$°C

Equilibrium[f]	*EDTA*[c]	*EGTA*	*DTPA*[a]	*TTHA*[b]	*NTA*[c]
Ca + L = CaL[d]	12.57	12.81 ± 0.03[e]	12.76	12.31	7.6
Ca + HL = CaHL	5.0	6.52 ± 0.04	7.90	10.23	–
Ca +H_2L = CaH_2L	–	1.49 ± 0.06	–	5.21	–
Ca + CaL = Ca_2L	–	2.05 ± 0.04	2.87	6.21	–
Ca + CaHL = Ca_2HL	–	–	–	2.7	–
Na + CaL = NaCaL	–	0.65 ± 0.06	0.68	2.1	–
Na + CaHL = NaCaHL	–	0.3 ± 0.2	–	1.2	–
Mg + L = MgL[d]	10.70	7.08 ± 0.03	11.27	10.8	6.5
Mg + HL = MgHL	4.0	4.78 ± 0.02	7.39	9.37	–
Mg + H_2L = MgH_2L	–	1.83 ± 0.03	–	4.09	–
Mg + MgL = Mg_2L	–	2.38 ± 0.03	3.11	7.67	–
Mg + MgHL = Mg_2HL	–	0.82 ± 0.04	–	2.6	–
Na + MgL = NaMgL	–	0.2 ± 0.1	0.80	2.0	–
Na + MgHL = NaMgHL	–	–	–	1.2	–

[a,b]De Stefano *et al.*, 2003; [c]Daniele *et al.*, 1985; [d]charges omitted for simplicity; [e]uncertainties expressed as 95% confidence interval; [f]according to mixed metal complex formation (5).

Table 6 Formation constants for Mg^{2+}-*EGTA* complexes at T = 25°C (calculated without considering the formation of Na^+ complexes. Model 1)

Equilibrium[b]	I = 0.1 mol L^{-1}	I = 0.5 mol L^{-1}	I = 1.0 mol L^{-1}
$Mg^{2+} + L^{4-} = MgL^{2-}$	5.45 ± 0.03[a]	4.76 ± 0.03[a]	4.73 ± 0.05[a]
$Mg^{2+} + HL^{3-} = MgHL^{-}$	3.56 ± 0.02	3.06 ± 0.02	3.05 ± 0.03
$Mg^{2+} + H_2L^{2-} = MgH_2L$	1.02 ± 0.03	0.70 ± 0.03	0.71 ± 0.05
$Mg^{2+} + MgL^{2-} = Mg_2L$	1.57 ± 0.03	1.25 ± 0.03	1.26 ± 0.04

[a]Uncertainties expressed as 95% confidence interval; [b]according to partial metal complex reactions (4).

Table 7 Formation constants for Ca^{2+}-EGTA complexes at T = 25°C (calculated without considering the formation of Na^+ complexes. Model 1)

Equilibrium[b]	I = 0.1 mol L^{-1}	I = 0.5 mol L^{-1}	I = 1.0 mol L^{-1}
$Ca^{2+} + L^{4-} = CaL^{2-}$	11.14 ± 0.03[a]	10.31 ± 0.02[a]	10.09 ± 0.04[a]
$Ca^{2+} + HL^{3-} = CaHL^{-}$	5.27 ± 0.04	4.65 ± 0.03	4.51 ± 0.04
$Ca^{2+} + H_2L^{2-} = CaH_2L$	0.70 ± 0.05	0.30 ± 0.05	0.23 ± 0.08
$Ca^{2+} + CaL^{2-} = Ca_2L$	1.22 ± 0.03	0.82 ± 0.03	0.75 ± 0.05

[a]Uncertainties expressed as 95% confidence interval; [b]according to partial metal complex reactions (4).

in a multi-component ionic medium that simulates the basic composition of natural seawater (Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^- and SO_4^{2-}, Synthetic Sea Water for Equilibrium studies, SSWE), whose composition at S (salinity) = 35‰ is reported in Table 8. As can be seen, Na^+, Mg^{2+}, Cl^- are present in higher concentrations (C > 55 mmol L^{-1}) with respect to the other components.

In such a medium "internal ionic interactions", which lower the free concentration of calcium, magnesium and, to a lesser extent, sodium ions, cannot be neglected. Some investigations (De Robertis *et al.* 1994, 1998; De Stefano *et al.*, 1994,1998) carried out to evaluate internal interactions among the components of the ionic medium (SSWE) showed that at least the following 8 species: $NaCl^0$, KCl^0 (very weak, K<1 mol L^{-1}), $MgCl^+$, $CaCl^+$, $Na(SO_4)^-$, $Mg(OH)^+$ (weak), and $Mg(SO_4)^0$ and $Ca(SO_4)^0$ (fairly strong) must be taken into account. If we add to these species the other complex species formed in poly- aminocarboxylic systems, *i.e.*, protonated, Na^+, Ca^{2+}, Mg^{2+} and mixed species, a considerable number of species needs to be considered for each system. To simplify equilibrium calculations, we recently proposed a more simple approach (De Stefano *et al.*, 1998; 2000) designed to take into account all the interactions of the major components of seawater with low molecular weight ligands (valid in the range $5 \le S \le 45$) by considering the major inorganic components of seawater as a single seasalt. For this approach, therefore, the mixture SSWE has been considered as a single 1:1 salt BA whose concentration is $C_{BA} = 1/2\Sigma C_i$ (C_i = concentration of all the ions), with z charge for the anion and the cation calculated as $z = \pm (I/C_{BA})^{1/2} = \pm 1.117$. This salt shows slight self association (log K = -0.03, T = 25°C, I = 0 mol L^{-1}); anion $A^{1.117-}$ is weakly protonated (log K^H = 0.24, T =

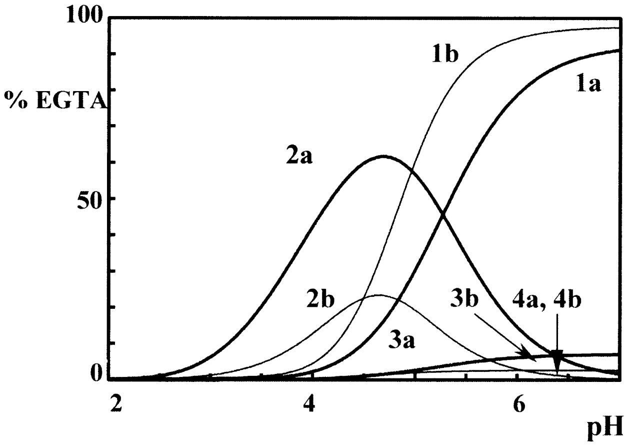

Figure 2 *Speciation diagram of the species* vs. *pH in the system* Ca^{2+}-EGTA *at different ionic strengths; (*L *= EGTA). 1a =* CaL*; 2a = CaLH; 3a =* $CaLH_2$*; 4a =* Ca_2L*. (I = 0.1 mol* L^{-1}*, NaCl); 1b =* CaL*; 2b = CaLH; 3b =* $CaLH_2$*; 4b =* Ca_2L*. (I = 1.0 mol* L^{-1}*, NaCl). Experimental conditions:* Ca^{2+} *= 0.01 mol* L^{-1}*;* L *= 0.005 mol* L^{-1}*.*

Table 8 Composition of artificial seawater (SSWE) at 35 salinity[a] and T = 25°C

Component	c (mol L^{-1})	m (mol kg^{-1})
NaCl	0.4221	0.42740
Na_2SO_4	0.0288	0.02919
KCl	0.0110	0.01112
$CaCl_2$	0.0111	0.01121
$MgCl_2$	0.0548	0.05552
BA[b]	0.5751	0.58240
I_T	0.717	0.726

[a]Concentrations at different salinities are given by the relationship: $m_s = m_{35}$ 27.56572 S / (1000–1.005714 S); [b]Seawater salt 1:1.

25°C, I = 0 mol L^{-1}), and cation $B^{1.117+}$ undergoes hydrolysis [log K ($B + H_2O = B(OH) + H^+$) = –12.75, T = 25°C, I = 0 mol L^{-1}]. By using this approach we were able quantitatively to determine the interactions between poly-aminocarboxylic ligands and the B cation in "single salt" seawater. Formation constants for the species B_pH_rL (L = *EDTA*; *EGTA* with r = 0–2 and p = 1–2) are reported in Table 9 at I = 0 mol L^{-1} and in Table 10 are reported the formation constant at S = 35 and T = 25 °C for other poly-aminocarboxylc acids.

Figures 3–5 show the speciation diagrams of *EGTA*, *EDTA* and *TTHA* in synthetic seawater at S = 35. The speciation diagrams of *EDTA* and *EGTA* in SSWE are plotted in the range 2 ≤ pH ≤ 7. Above this pH the formation percentages of the species are constants.

Figure 3 shows the distribution diagram for the system *BA-EDTA* at S = 35 and C_{EDTA} = 0.1 mmol L^{-1}. All the species formed between *EDTA* (and its protonated forms) and $B^{1.117+}$ have significant yields and, at pH> 7, 100% of the ligand is complexed as *B*L. Instead, under the same conditions, for the system *BA-EGTA* (Figure 4) (80% of the ligand is complexed as B_2L and only 20% as *B*L. For comparison we report in Figure 5 the speciation profile in the same medium for *TTHA*. This is very similar to that of *EDTA*. When studying the formation of complexes of different metal cations with *EDTA* or *EGTA* in seawater, these data for the interaction with major inorganic components can be useful as the basis for successive calculations.

Table 9 Formation constants for *EDTA* and *EGTA* complexes with the cation of the single seawater salt $B^{1.117+}$, at I = 0 mol L^{-1} and T = 25°C

p r	log β_{pr}^{b}	
	EDTA	*EGTA*
1 0	9.79 ± 0.05 [a]	9.90 ± 0.10[a]
1 1	14.11 ± 0.05	15.56 ± 0.07
1 2	17.90 ± 0.10	20.94 ± 0.10
2 0	–	11.45 ± 0.12

[a]Uncertainties expressed as 95% confidence interval ;[b]according to overall metal complex reaction (3).

Table 10 Equilibrium constants for the formation of *EDTA*, *EGTA*, *DTPA* and *TTHA* complexes with the cation of the single seawater salt $B^{1.117+}$, at S = 35 and T = 25°C

Equilibrium[d]	*EDTA*[b]	*EGTA*[b]	*DTPA*[c]	*TTHA*[c]
$B + L = BL$[a]	9.1	9.2	9.6	12.3
$B + HL = BHL$	2.5	4.5	5.2	6.9
$B + H_2L = BH_2L$	–0.1	0.6	1.25	2.6
$B + H_3L = BH_3L$	–	–	0.75	1.2
$B + BL = B_2L$	–	1.0	0.5	0.6
$B + BHL = B_2HL$	–	–	0.1	0.9

[a]Charges omitted for simplicity; [b]this work; [c]De Stefano *et al.*, 2003; [d]according to partial metal complex reactions (4).

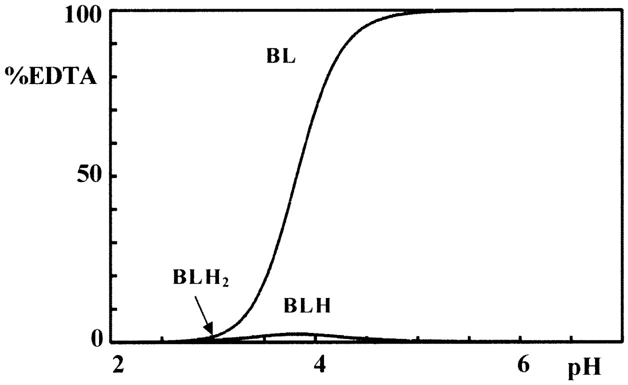

Figure 3 *Speciation diagram of the species* vs *pH for the system BA*-EDTA. *(L* = EDTA*)* *(*$C_A = C_B$ *= 0.57 mol L*$^{-1}$*;* C_{EDTA} *= 0.0001 mol L*$^{-1}$*); T = 25°C.*

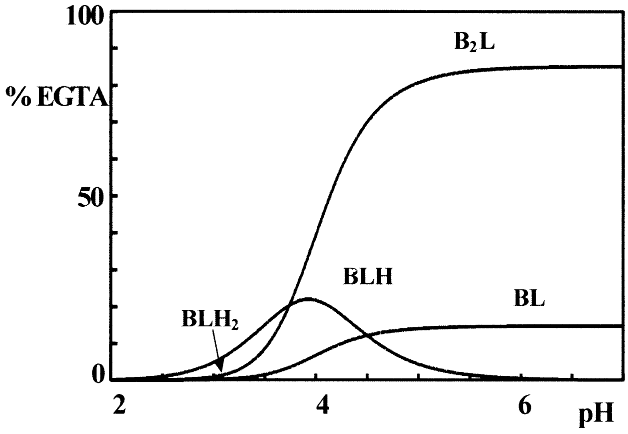

Figure 4 *Speciation diagram of the species* vs *pH in the system BA*–EGTA. *(L* = EGTA*)* *(*$C_A = C_B$ *= 0.57 mol L*$^{-1}$*;* C_{EGTA} *= 0.0001 mol L*$^{-1}$*); T = 25°C.*

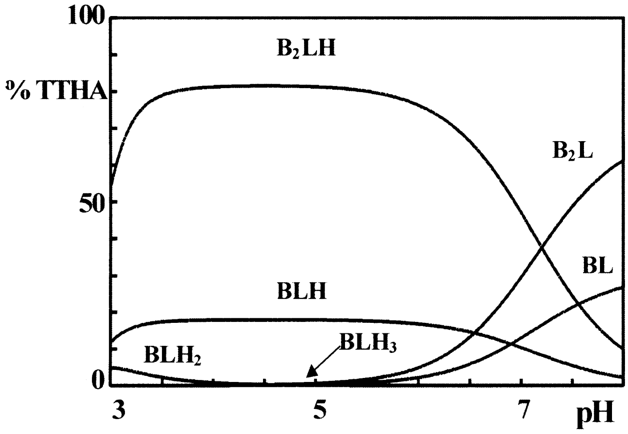

Figure 5 *Speciation diagram of the species* vs *pH for the system BA*-TTHA. *(L* = TTHA*)* *(*$C_A = C_B$ *= 0.57 mol L*$^{-1}$*;* C_{TTHA} *= 0.0001 mol L*$^{-1}$*); T = 25°C.*

LITERATURE COMPARISON

For purposes of comparison, Table 11 shows some literature data for the protonation of *EGTA* in different media and at different ionic strengths; the difference on protonation constants denotes a difference in stability of weak complexes.

It should be noted that protonation constants are considerably higher in Et_4NI than in Na^+ media, indicating a significant difference in the stability of weak Na^+ complexes. Table 12 reports some literature data for Mg^{2+} and Ca^{2+} complexes of *EGTA* compared with the present results.

Here again, data are fairly consistent for the main species whilst there is some uncertainty with regard to the minor species, particularly for poly-protonated complex species. Stability data reported in literature mostly refer to Model 1, and in particular, no data on the formation of mixed metal complexes can be found in literature. These data are reported here for the first time. It is not the aim of this work to make a detailed analysis of literature data, but we think that the body of results reported in many papers may be used to derive recommended or tentative values at low ionic strength. Systematic dependence on ionic strength has been reported only in this work.

CONCLUSIONS

The main results of this work can be summarised as follows:

- The protonation of *EGTA* was studied in different ionic media (NaCl, Et_4NI and in mixture of $CaCl_2$-NaCl and $MgCl_2$-NaCl) in the ionic strength range $0< I/\text{mol L}^{-1} \leq 1$. Significant differences (Table 1) in the protonation constants can be interpreted in terms of weak complex formation. The formal dependence on ionic strength and weak complex formation were quantified and the relative parameters are reported (Tables 2 and 4).
- Using two different models, Model 1, which considers the difference in protonation constants in terms of different activity coefficients, and Model 2, which accounts for the formation of weak Na^+ complexes, two sets of Mg^{2+} and Ca^{2+} complex formation constants were obtained at different ionic strengths in NaCl (Table 5 for Model 2; Tables 6 and 7 for Model 1). It is interesting to note that when Model 2 is used, it is possible to find mixed Na^+–M^{2+} complexes of significant stability.
- From measurements in synthetic seawater we obtained quantitative data at different salinities for the interaction of complexones (*EGTA*, *EDTA*) with the major inorganic components of seawater using the single salt approach (Tables 8, 9); the data are comparable with those reported for other complexones such as *DTPA* and *TTHA*.
- Many quantitative data (dependence on ionic strength, mixed metal complex formation, mean stability of inorganic species in seawater) have never been reported before.

Table 11 Literature data on the protonation of *EGTA*

I/mol L^{-1}	T/°C	$\log K^H_1$	$\log K^H_2$	$\log K^H_3$	$\log K^H_4$	Ref.
0.1 KNO_3	25	9.51	8.92	2.82	2.46	Felcman *et al.*, 1983
0.1 KNO_3	25	9.54	8.93	2.73	2.08	Ringbom *et al.*,1968
0.1 KCl	25	9.53	8.88	–	–	Boyd *et al.*, 1965
		(–26.5)[a]	(–20.4)			
0.1 KNO_3	20	9.46	8.85	2.65	2.0	Anderegg, 1964
		(–24.4)	(–24.1)			
0.1 NaCl	25	9.60	8.79	2.67	2.04	this work
0.5 NaCl	25	9.22	8.39	2.49	2.01	this work
0.5 $NaClO_4$	25	8.89	8.40	2.50	0.93	Napoli, 1975

[a] ΔH values in kJ mol^{-1} are given in parenthesis.

Table 12 Literature data on the formation of Mg^{2+}- and Ca^{2+}-*EGTA*

I/mol L^{-1}	T/°C	$\log K_{110}$	$\log K_{111}$	$\log K_{110}$	$\log K_{111}$	Ref.
		Mg^{2+}		Ca^{2+}		
0.1 KNO_3	25	5.2	–	11.0	–	Ringbom *et al.*, 1968
0.1 KNO_3	20	5.2	3.4	10.97	5.3	Anderegg, 1964
		(21.7)[a]	–	(-35.0)[a]		
0.1 KCl	20	5.21	3.37	11.0	5.33	Pettit and Powell, 1997
0.1 KCl	25	5.45	3.56	11.14	5.27	This work

[a] ΔH values in kJ mol^{-1} are given in parenthesis.

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