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Polynuclear EDTA Complexes in Aqueous Solution: A Proof of Concept

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Abstract
Ethylenediaminetetraacetic acid (EDTA) is, so far, the most known and widely used chelating agent. It is well known to form very stable mononuclear complexes with several metal ions in aqueous solution. However, its multidentate nature opens the possibility to simultaneously bind more than one cation forming polynuclear species, rarely observed for EDTA, but common for its analogues like, *e.g.*, DTPA and TTHA. If formed, these species could significantly affect the speciation of both EDTA and metal ions in solution. As such, in this work we report the result of a potentiometric investigation on the chemical speciation of three model systems, namely: Sn^{2+}/Zn^{2+}/EDTA, Sn^{2+}/Fe^{2+}/EDTA and Zn^{2+}/Fe^{3+}/EDTA, at $T = 298.15$ K and $I = 0.15$ mol·dm^{-3} (in $NaNO_{3(aq)}$ or $NaClO_{4(aq)}$), as a proof of concept that simple and mixed polynuclear species can be also formed by EDTA. In fact, experimental data analysis evidenced the formation of not only the simple Sn_2(EDTA), Zn_2(EDTA) and $[Fe_2(EDTA)]^{2+}$ species, but also the mixed SnZn(EDTA), SnFe(EDTA) and $[ZnFe(EDTA)]^{+}$. The formation of latter species was then analyzed in terms of thermodynamic extra stability with respect to simple species, and their influence on the speciation of EDTA and the investigated cations was evaluated. Furthermore, quantum mechanical calculations were also performed for a better insight on the binding mode of EDTA when forming dinuclear species, and to support the evidence concerning the extra stability of mixed ones. We could conclude that, when forming dinuclear species, EDTA behaves as a tridentate ligand, binding cations through two carboxylates and one amino group (MIDA-like mode).

Keywords Ethylenediaminetetraacetic acid · Polynuclear complexes · Mixed complexes · Chemical speciation · Stability constants · Solution equilibria

1 Introduction

The chemical speciation (as defined by IUPAC [1]) of an element or compound in aqueous environments (*e.g.*, natural waters, process waters, wastewaters, biological fluids) depends on different chemical and physical parameters, which influence the stability and reactivity

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of the species that can be formed, due to the network of interactions with other components present in those systems [1, 2].

Thus, performing a speciation study consists in identifying and quantifying the different species occurring in a system, which is of fundamental importance in many strategic sectors and has several applications, since the properties, the activity and reactivity of various chemicals depend on the species they form in the system(s) of interest [1–3].

Among the possible approaches to speciation studies, the direct determination of individual species by different analytical techniques is generally difficult, often due to their very low concentration levels and the presence of possible interferences in the studied matrices. In addition, the variability of the different systems of interest for speciation studies would require the direct measurement of each individual species in each individual sample. In fact, real systems differ greatly from each other in terms of chemical composition and physico-chemical conditions, even within the same type of system. Natural waters and biological fluids, for example, can be considered as multi-component aqueous solutions in which several metal and organometal cations and inorganic and organic low-, medium- and high-molecular-weight ligands are present in different concentrations and ratios. In addition, such systems show a considerable variability of temperature, pH, pe (*i.e.*, redox conditions), ionic strength (I) and pressure (*e.g.*, deep waters), which significantly affect the speciation [1–5].

Thus, for obvious reasons of feasibility, cost-effectiveness and ability to obtain more general information and representative models of several systems, the most common approach to speciation studies is through modeling by thermodynamic equilibrium analysis. In this approach, thermodynamic parameters, such as equilibrium constants, enthalpy changes, activity coefficients, partial molar volumes, are used to determine and model the formation and percentage of different species as a function of the physico-chemical conditions (pH, temperature, pressure, ionic strength, composition) of certain systems. For example, any species formed by a given component may be expressed by means of its stoichiometric (*i.e.*, referred to free concentrations and not to activities) stability constant that, together with the analytical concentrations of all the other components, can be used to build a system of equations, whose solution gives the free concentrations of all the formed species as a function of an independent variable, such as pH [1–5].

Of course, even for seemingly simple systems, the formed species can be many. For example, depending on its acid–base properties (*i.e.*, hydrolysis), a generic metal cation (M^{m+}) may be present in water at different pH as different mono- and polynuclear hydrolytic species. The presence of the cation obviously implies that of one anion (at least), with whom it can interact to form further species (not necessarily just one). Then, the anion itself may have acid–base properties and form other differently protonated species which can, in turn, interact. As well, even neutral species (*e.g.*, some organic ligands) may interact. Thus, extending these considerations to multi-component systems such as natural waters and biological fluids, it is evident that the network of possible interactions is very wide [4–6].

Furthermore, the above example did not yet consider the fact that several natural and synthetic ligands contain in their structures more than one binding group, thus opening the possibility to form more complex species deriving from multiple interactions. Indeed, the formation of both simple and mixed cation and/or ligand polynuclear species, in addition to, or in substitution of, mononuclear ones, is very frequent, especially in multicomponent solutions like many real systems [5–11].

For example, the so-called complexones may form, other than simple ML chelates, further polynuclear species. This is known, *e.g.*, for diethylenetriaminepentaacetic (DTPA)

and triethylenetetraminehexaacetic (TTHA) acids, whose stability constants of several dinuclear complexes are reported for DTPA [12–17] and TTHA [12, 17–21] (for the latter, a mixed Ca^{2+}/Ga^{3+} species is also reported [20]), most of them reviewed by Anderegg et al. [22]. Concerning ethylenediaminetetraacetic acid (EDTA), probably the most important and famous complexone, the formation of its dinuclear species in aqueous solution has only been reported, to our knowledge, for Cd^{2+} [23] and UO_2^{2+} [12, 24–26]. In fact, its particular structure makes it suitable for coordinating a large number of cations as mononuclear complexes, due to the occurrence of two amino—and four carboxylic groups, which make it a hexadentate ligand, resulting in a high chelating capacity in a wide range of conditions [27–29]. Nevertheless, literature findings on dinuclear Cd^{2+}/EDTA and UO_2^{2+}/EDTA species in aqueous solution suggest that similar species with other cations may exist.

This fact should be of great concern because, due to its ability to form water-soluble, stable complexes with metal ions, EDTA is the most widely used synthetic chelating agent, causing huge quantities from many sources to be discharged into the aquatic environment [27–29], where it can simultaneously occur with several cations even present at high concentrations, significantly enhancing the probability to form simple and/or mixed polynuclear species that, if so, could affect both EDTA and cations speciation in those systems. In fact, in addition to its similarities with the behavior of many natural organic ligands, EDTA is very important because it is used in a wide range of industrial applications and common products where strong metal cation binding is required. Since EDTA is not removed during wastewater treatment and is poorly biodegradable, it has become ubiquitous in the aquatic environment, being, *e.g.*, the most highly concentrated anthropogenic organic compound in surface waters in Europe [27]. Although EDTA is not inherently toxic, it can remove toxic metals from sediments and therefore has a strong environmental impact [27–29].

As mentioned above, our literature findings show that the formation of polynuclear species of EDTA in aqueous solution has never been reported except for Cd^{2+} [23] and UO_2^{2+} [12, 24–26], and, therefore, not even that of mixed species. However, under the typical conditions of many complex real matrices, this is likely to occur, altering the speciation of both EDTA and the metal cations interacting with it. For this reason, the possible formation equilibria of simple and mixed polynuclear species was studied in this work in three model systems, namely: Zn^{2+}/Sn^{2+}/EDTA, Zn^{2+}/Fe^{3+}/EDTA and Sn^{2+}/Fe^{2+}/EDTA. Potentiometric titrations at $T = 298.15$ K and $I = 0.15$ mol·dm^{-3} (in $NaNO_{3(aq)}$ or $NaClO_{4(aq)}$) were thus designed considering different concentration ratios of the two cations and EDTA, to attempt the maximization of both simple and mixed polynuclear species, as a proof of concept that EDTA can not only form mononuclear species (acting as the well-known hexadentate chelant). Zn^{2+}, Sn^{2+}, Fe^{3+} and Fe^{2+} were selected as investigated cations for their known solution properties and importance from a biological, environmental and industrial/technological point of view [30–34]. Results were thus discussed in terms of extrastability of mixed species and of their influence on the speciation of both the cations and EDTA.

2 Experimental

2.1 Chemicals

HCl and NaOH solutions were prepared by dilution from the concentrated ampoules and standardized with Na_2CO_3 and KH(phthalate), respectively, both previously dried at

$T = 383.15$ K for at least 2 h. The aqueous solutions of $NaNO_3$ and $NaClO_4$ (used as supporting electrolytes) were prepared by weight, after drying them in oven at $T = 383.15$ K for at least 2 h. EDTA solutions were prepared by weighing the disodium salt dihydrate ($Na_2H_2EDTA{\cdot}2H_2O$) and standardized by metallic Zn [35]. Sn^{2+}, Zn^{2+} and Fe^{3+} solutions were prepared daily from $SnCl_2{\cdot}2H_2O$, $ZnCl_2$, and $FeCl_3{\cdot}6H_2O$ salts, respectively, and standardized with EDTA [35]. Known amounts of HCl were added to Sn^{2+} and Fe^{3+} solutions to avoid cation hydrolysis. Furthermore, Sn^{2+} solutions were prevented from oxidation to Sn(IV) as reported elsewhere [33]. Concerning Fe^{2+} solutions, they were daily prepared from the Mohr's salt ($(NH_4)_2Fe(SO_4)_2{\cdot}6H_2O$), since the latter is known as one of the purest and most stable Fe(II) salts [36]. Ultra-pure (resistance = 18 m$\Omega{\cdot}$cm^{-1}) water was always used. All chemicals were obtained from Merck KGaA (Darmstadt, Germany) and/or its branches, at the highest possible purity.

2.2 Apparatus and Procedure for Potentiometric Titrations

Three totally independent systems and three different operators were employed to perform potentiometric titrations, at $T = 298.15 \pm 0.1$ K in double-jacketed cells thermostatted by water circulation, under inert atmosphere of high-purity $N_{2(g)}$, bubbled into solutions to avoid $O_{2(g)}$ and $CO_{2(g)}$, and under magnetic stirring. The first apparatus was a Thermo Scientific Orion Star T940 potentiometric titration system, equipped with a 10 cm^3 burette and a Thermo Scientific Orion 8102BNUWP ROSS Ultra Combination glass electrode. The second and third were two Metrohm 809 Titrando systems equipped with 10 cm^3 burettes and a Metrohm 6.0262.100 Electrode Plus combination glass electrode or a single junction Thermo Scientific Orion 8101BNWP ROSS Half-Cell glass electrode coupled with a Thermo Scientific Orion 900200 Sure-Flow Reference Half-Cell double junction reference electrode. According to the manufacturers, burette resolution was 0.001 cm^3 with a titration precision of $\pm$0.2–0.5% RSD, with an e.m.f. readings resolution of 0.1 mV and a relative accuracy of $\pm$0.2 mV.

Titrand solutions were prepared with EDTA ($1.0 \le c_{EDTA}$ / mmol$\cdot$dm^{-3} ≤ 4.0), mixtures of two cations M and M' (Sn^{2+} and Zn^{2+}; Sn^{2+} and Fe^{2+}; Zn^{2+} and Fe^{3+}, $0.5 \le c_M$ / mmol$\cdot$dm^{-3} ≤ 2.0, $0.5 \le c_{M'}$ / mmol$\cdot$dm^{-3} ≤ 2.0) in different c_M: $c_{M'}$: c_{EDTA} ratios, HCl ($5.0 \le c_H$ / mmol$\cdot$dm^{-3} ≤ 10.0) and ionic medium ($NaNO_{3(aq)}$ for Sn^{2+}/Zn^{2+}/EDTA and Zn^{2+}/Fe^{3+}/EDTA systems, $NaClO_{4(aq)}$ for Sn^{2+}/Fe^{2+}/EDTA, to avoid fast Fe^{2+} oxidation by NO_3^-) to reach a final ionic strength value of $I = 0.15$ mol$\cdot$dm^{-3}.

Cations and ligand concentrations used during experiments are reported in Table 1, and were selected by means of software for experimental design [37], to attempt to maximize the possible formation of both simple and mixed polynuclear species. 25 cm^3 of titrand solutions prepared as described were titrated by standard NaOH up to pH ~ 10.5, each in one of the three apparatus, randomly selected to minimize sources of systematic errors. In cases when precipitation was observed at pH < 10.5, titrations were stopped at that point. The equilibrium conditions were checked as usual, performing back titrations and testing different waiting times between successive titrant additions. Every titration consisted of 100–120 experimental points.

Before each experiment, electrodes were calibrated in [H^+] (free concentration instead of activity, *i.e.*, pH $\equiv -\log$ [H^+]) by independent HCl/NaOH titrations in the same temperature and ionic strength conditions of experiments, to determine formal electrodes potential (E^0), eventual acidic junction coefficients (j_a) and to check electrodes performance in the alkaline range by calculating pK_w, which always resulted p$K_w = 13.78 \pm 0.02$ ($\pm$95%

Table 1 Analytical concentrations of metal cations M and M' (Sn^{2+} and Zn^{2+}; Sn^{2+} and Fe^{2+}; Zn^{2+} and Fe^{3+}) and EDTA used to prepare titrand solutions

c_M [a]	$c_{M'}$ [a]	c_{EDTA} [a]
0.5	0.5	1.0
0.5	0.5	2.0
1.0	0.5	2.0
0.5	1.0	2.0
1.0	1.0	2.0
0.5	2.0	2.0
1.0	0.5	4.0
2.0	0.5	2.0
2.0	0.5	4.0
0.5	1.0	4.0
1.0	1.0	4.0
2.0	1.0	4.0
0.5	2.0	4.0
1.0	2.0	4.0
2.0	2.0	4.0

[a] c_X in mmol·dm^{-3}

confidence interval), in excellent agreement with literature values for the same conditions in Na^+ ionic media [38].

2.3 Data Analysis and Expression of Results

The experimental data analysis and the refinement of parameters from potentiometric titrations (*e.g.*, E^0, j_a, pK_w and other stability constants, analytical concentrations of components) was performed by BSTAC software [39]. Speciation diagrams and species free concentrations were calculated by PyES software [40].

The protonation constants ($p = p' = 0$) of EDTA, hydrolysis constants of cations ($p' = q = 0$, $r < 0$), stability constants of homocationic (simple, $p' = 0$) and heterocationic (mixed) species are given according to the general equilibrium (charges omitted):

$$pM + p'M' + qL + rH \rightleftharpoons M_pM'_{p'}L_qH_r \quad \beta_{pp'qr} \tag{1}$$

with $L = EDTA^{4-}$ and M or M' = Sn^{2+}, Zn^{2+}, Fe^{2+} or Fe^{3+}.

2.4 Quantum Mechanical Calculations

Quantum mechanical computations in this study were carried out at the density functional theory (DFT) level using the ORCA program [41]. Geometry optimizations of the complexes were performed using the B3LYP hybrid functional [42, 43], with Grimmes's D4 correction [43] using the effective core potential LanL2DZ [44] and 6–31 + G(d) basis sets for the metal and nonmetal atoms, respectively. Solvation (water) effects were accounted through the Conductor-Like Polarizable Continuum Model (CPCM) [45, 46].

3 Results

3.1 Acid–Base Properties of EDTA and Investigated Cations and Stability Constants of Their Simple Mononuclear M/EDTA Species

Experimental data analysis for the determination of the stability constants of eventual polynuclear species needs, as input, the inclusion in the speciation model of log β of other species that may be formed in the investigated systems. These include the protonation constants of EDTA, the hydrolysis constants of the investigated cations, and the stability constants of the known M/EDTA species, *i.e.*, the mononuclear MLH_r. For the determination of more accurate log β, these constants must be known in the exact conditions as the systems to be investigated, *i.e.*, $T = 298.15$ K and $I = 0.15$ mol·dm^{-3}, in $NaNO_{3(aq)}$ or $NaClO_{4(aq)}$ (for the Sn^{2+}/Fe^{2+}/EDTA system). Many data are available in literature in single papers and in the most known databases [22, 33, 36, 38, 47–50], though they are not always reported in the above-mentioned conditions. However, there is sufficient amount of data (*e.g.*, constants in other conditions, other thermodynamic parameters like formation enthalpy changes, activity coefficients, interaction coefficients) to estimate these values by usual models for the dependence on temperature, medium and ionic strength (*e.g.*, van't Hoff, Specific ion Interaction Theory, Extended Debye–Hückel, Pitzer) of the desired constants [5]. The so-calculated stability constants used in this work are reported in Tables 2, 3 and 4, respectively for the protonation of EDTA (Table 2), the hydrolysis of investigated cations (Table 3) with those of their chloride complexes (which is present, though in relatively low concentration, as counter-ion of the salts used during experiments), and the stability constants of MLH_r species of Sn^{2+}, Zn^{2+}, Fe^{2+} or Fe^{3+} with EDTA (Table 4).

As can be noted, all these tables generically refer to Na^+ ionic media. This is because reported values can be considered as reasonable and valid in both $NaNO_{3(aq)}$ and $NaClO_{4(aq)}$, used in this work, for simple reasons. ClO_4^- is not (or very weakly) interacting with cationic species and, to a lower extent, neither NO_3^-, though the formation of very weak ion pairs with cations are sometimes reported for the latter [5]. However, the existing slightly different interacting ability of these two anions becomes negligible at ionic strengths (*i.e.*, low anions concentrations) as low as that used in this work (*i.e.*, $I = 0.15$ mol·dm^{-3}). This usually turns into differences of the stability constants and other thermodynamic parameters in the two media that are of the same order of magnitude of the uncertainties obtained during their determination and, thus, they are not-statistically significant. For example, JESS database [38] calculates, at $T = 298.15$ K and $I = 0.15$ mol·dm^{-3}, $pK_w = 13.78$ in both $NaNO_{3(aq)}$ and $NaClO_{4(aq)}$, while the proton activity coefficients calculated in the same conditions by the Pitzer model [51] are $\gamma = 0.773$ and 0.776, in $NaNO_{3(aq)}$ and $NaClO_{4(aq)}$, respectively. These considerations hold for all stability constants reported in this work.

Table 2 Protonation constants of EDTA used in this work, calculated from literature data at $T = 298.15$ K and $I = 0.15$ mol·dm^{-3} in Na^+ ionic media [22, 38, 47–49]

Equilibrium	log β
$EDTA^{4-} + H^+ \rightleftharpoons [(EDTA)H]^{3-}$	9.22
$EDTA^{4-} + 2\,H^+ \rightleftharpoons [(EDTA)H_2]^{2-}$	15.26
$EDTA^{4-} + 3H^+ \rightleftharpoons [(EDTA)H_3]^{-}$	17.92
$EDTA^{4-} + 4\,H^+ \rightleftharpoons (EDTA)H_4$	20.03

Table 3 Hydrolysis constants and stability constants of Cl^- complexes of Sn^{2+}, Zn^{2+}, Fe^{2+} and Fe^{3+} used in this work, calculated from literature data at $T = 298.15$ K and $I = 0.15$ mol·dm^{-3} in Na^+ ionic media [33, 36, 38, 48–50]

Equilibrium	log β
$Sn^{2+} + H_2O \rightleftharpoons [Sn(OH)]^+ + H^+$	− 3.78
$Sn^{2+} + 2\,H_2O \rightleftharpoons Sn(OH)_{2(aq)} + 2\,H^+$	− 6.53
$Sn^{2+} + 3\,H_2O \rightleftharpoons [Sn(OH)_3]^- + 3\,H^+$	− 16.97
$2\,Sn^{2+} + 2\,H_2O \rightleftharpoons [Sn_2(OH)_2]^{2+} + 2\,H^+$	− 5.06
$3\,Sn^{2+} + 4\,H_2O \rightleftharpoons [Sn_3(OH)_4]^{2+} + 4\,H^+$	− 6.39
$Sn^{2+} + Cl^- \rightleftharpoons [Sn(Cl)]^+$	0.78
$Sn^{2+} + 2\,Cl^- \rightleftharpoons SnCl_{2(aq)}$	1.54
$Sn^{2+} + 3\,Cl^- \rightleftharpoons [SnCl_3]^-$	1.53
$Sn^{2+} + Cl^- + H_2O \rightleftharpoons SnCl(OH) + H^+$	− 2.05
$Zn^{2+} + H_2O \rightleftharpoons [Zn(OH)]^+ + H^+$	− 9.16
$Zn^{2+} + 2\,H_2O \rightleftharpoons Zn(OH)_{2(aq)} + 2\,H^+$	− 17.11
$Zn^{2+} + 3\,H_2O \rightleftharpoons [Zn(OH)_3]^- + 3\,H^+$	− 28.37
$Zn^{2+} + 4\,H_2O \rightleftharpoons [Zn(OH)_4]^{2-} + 4\,H^+$	− 40.63
$2\,Zn^{2+} + H_2O \rightleftharpoons [Zn_2(OH)]^{3+} + H^+$	− 8.92
$2\,Zn^{2+} + 6\,H_2O \rightleftharpoons [Zn_2(OH)_6]^{2-} + 6\,H^+$	− 57.48
$Zn^{2+} + Cl^- \rightleftharpoons [Zn(Cl)]^+$	0.04
$Fe^{2+} + H_2O \rightleftharpoons [Fe(OH)]^+ + H^+$	− 9.71
$Fe^{2+} + 2\,H_2O \rightleftharpoons Fe(OH)_{2(aq)} + 2\,H^+$	− 20.82
$Fe^{2+} + 3\,H_2O \rightleftharpoons [Fe(OH)_3]^- + 3\,H^+$	− 31.01
$Fe^{2+} + 4\,H_2O \rightleftharpoons [Fe(OH)_4]^{2-} + 4\,H^+$	− 45.54
$Fe^{2+} + Cl^- \rightleftharpoons [Fe(Cl)]^+$	0.04
$Fe^{3+} + H_2O \rightleftharpoons [Fe(OH)]^{2+} + H^+$	− 2.27
$Fe^{3+} + 2\,H_2O \rightleftharpoons \left[Fe(OH)_2\right]^+ + 2\,H^+$	− 6.4
$Fe^{3+} + 3\,H_2O \rightleftharpoons Fe(OH)_{3(aq)} + 3\,H^+$	− 14.36
$Fe^{3+} + 4\,H_2O \rightleftharpoons [Fe(OH)_4]^- + 4\,H^+$	− 22.71
$2\,Fe^{3+} + 2\,H_2O \rightleftharpoons [Fe_2(OH)_2]^{2+} + 2\,H^+$	− 2.86
$3\,Fe^{3+} + 4\,H_2O \rightleftharpoons [Fe_3(OH)_4]^{2+} + 4\,H^+$	− 5.98

Table 4 Stability constants of MLH_r.species of EDTA with Sn^{2+}, Zn^{2+}, Fe^{2+} and Fe^{3+} used in this work, calculated from literature data at $T = 298.15$ K and $I = 0.15$ mol·dm^{-3} in Na^+ ionic media [22, 36, 38, 47–50]

Equilibrium	log β
$Sn^{2+} + EDTA^{4-} + H^+ \rightleftharpoons [Sn(EDTA)H]^-$	21.26
$Sn^{2+} + EDTA^{4-} \rightleftharpoons [Sn(EDTA)]^{2-}$	18.69
$Zn^{2+} + EDTA^{4-} + H^+ \rightleftharpoons [Zn(EDTA)H]^-$	19.50
$Zn^{2+} + EDTA^{4-} \rightleftharpoons [Zn(EDTA)]^{2-}$	16.50
$Zn^{2+} + EDTA^{4-} + H_2O \rightleftharpoons [Zn(EDTA)(OH)]^{3-} + H^+$	4.90
$Fe^{2+} + EDTA^{4-} + H^+ \rightleftharpoons [Fe(EDTA)H]^-$	17.89
$Fe^{2+} + EDTA^{4-} \rightleftharpoons [Fe(EDTA)]^{2-}$	10.61
$Fe^{2+} + EDTA^{4-} + H_2O \rightleftharpoons [Fe(EDTA)(OH)]^{3-} + H^+$	1.34
$Fe^{3+} + EDTA^{4-} \rightleftharpoons [Fe(EDTA)]^-$	23.88
$Fe^{3+} + EDTA^{4-} + H_2O \rightleftharpoons [Fe(EDTA)(OH)]^{2-} + H^+$	16.43

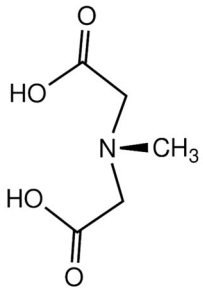
Table 5 Stability constants of $M_pM'_{p'}LH_r$ species of EDTA with Sn^{2+}, Zn^{2+}, Fe^{2+} and Fe^{3+}, at $T = 298.15$ K and $I = 0.15$ mol·dm^{-3} in Na^+ ionic media

Equilibrium	log β [a]
$2\,Sn^{2+} + EDTA^{4-} \rightleftharpoons Sn_2(EDTA)$	24.77 ± 0.05
$2\,Zn^{2+} + EDTA^{4-} \rightleftharpoons Zn_2(EDTA)$	21.94 ± 0.06
$2\,Fe^{3+} + EDTA^{4-} \rightleftharpoons [Fe_2(EDTA)]^{2+}$	37.26 ± 0.03
$Sn^{2+} + Zn^{2+} + EDTA^{4-} \rightleftharpoons SnZn(EDTA)$	24.99 ± 0.02
$Sn^{2+} + Fe^{2+} + EDTA^{4-} \rightleftharpoons SnFe(EDTA)$	24.52 ± 0.08
$Zn^{2+} + Fe^{3+} + EDTA^{4-} \rightleftharpoons [ZnFe(EDTA)]^{+}$	32.28 ± 0.02

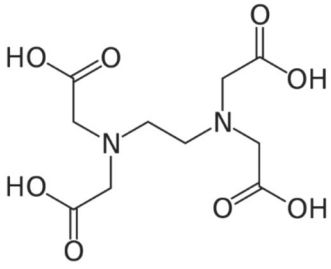
[a] ± standard deviation

Scheme 1. Structures of methyliminodiacetic acid (MIDA, (**a**) and EDTA (**b**)

a)



b)



3.2 Stability Constants of Simple Dinuclear M_2LH_r Species

The simultaneous analysis of all experimental data by BSTAC software evidenced the formation of simple (M_2LH_r) and mixed $(M_pM'_{p'}LH_r)$ dinuclear species of the kind (M_2L) or $(MM'L)$ (no protonated or hydroxo-species), reported in Table 5 together with corresponding stability constants. Mixed species will be discussed in next section.

As can be noted, in the experimental conditions of this work, the formation of simple polynuclear species of Fe^{2+} was not observed. For other investigated cations, the stability of the M_2L species follows the trend:

$$Fe^{3+} > Sn^{2+} > Zn^{2+}$$

in line with that of their mononuclear analogues. As expected, the binding of a second cation to the simple ML species has a stability that is more than half of the binding of M by free $EDTA^{4-}$. In fact, for the equilibrium

$$M + ML \rightleftharpoons M_2L \tag{2}$$

it is log $K = 5.44$, 6.08 e 13.38, for $M = Zn^{2+}$, Sn^{2+}, and Fe^{3+}, respectively, *vs.* log $\beta_{1010} = 16.50$, 18.69 and 23.88 for the same cations in the same order. Indeed, the binding of a second cation by EDTA implies that part of the binding groups forming the ML chelate are involved in the further binding of another cation. This turns EDTA, de facto, similar to a dicarboxylate with the eventual support to binding of one of the two nitrogen atoms of the ethylenediamine moiety. In fact, the constants relative to equilibrium (2) for Zn^{2+} and Fe^{3+} are comparable, for example, to those of methyliminodiacetic acid (MIDA, Scheme 1a), which is structurally similar to "half" EDTA (Scheme 1b), with a log

$K = 7.69$ (at $T = 298.15$ K and $I = 0.1$ mol·dm^{-3}) and log $K = 10.99$ (at $T = 298.15$ K and $I = 0.5$ mol·dm^{-3}) for Zn(MIDA) and [Fe(MIDA)]$^{+}$, respectively [38, 48, 49]. This binding mode of EDTA is known, for example, for dicationic complexes of Cu^{2+} in the solid state [52].

3.3 Stability Constants of Mixed $M_pM'_{p'}LH_r$ Species

As reported in Table 5, experimental data analysis showed the formation of the mixed SnZn(EDTA), SnFe(EDTA) and [ZnFe(EDTA)]$^{+}$ species for the three investigated systems. As well known for many different mixed cation (or mixed ligand) systems [6–11, 53, 54], the formation of mixed species is often thermodynamically favored with respect to that of simple analogues. Given a ligand L and two metal cations M and M' (as for the systems investigated in this work) forming both mononuclear (ML and M′L) and simple dinuclear (M_2L and M'_2L) species according to equilibria

$$M + L \rightleftharpoons ML \quad K_{ML} \tag{3}$$

$$M' + L \rightleftharpoons M'L \quad K_{M'L} \tag{4}$$

and (like Eq. 2)

$$M + ML \rightleftharpoons M_2L \quad K_{M_2L} \tag{5}$$

$$M' + M'L \rightleftharpoons M'_2L \quad K_{M'_2L} \tag{6}$$

the statistical probability X_{stat} that a mixed MM′L species is formed is given by [5, 6]:

$$X_{stat} = 2 + \frac{K_{ML}}{K_{M'L}}\sqrt{\frac{K_{M'_2L}}{K_{M_2L}}} + \frac{K_{M'L}}{K_{ML}}\sqrt{\frac{K_{M_2L}}{K_{M'_2L}}} \tag{7}$$

This value can be used to estimate the overall stability constant of the mixed species, as follows (please note that logβ are given according to Eq. 1, while equilibria in Eqs. 3–6 are "stepwise")

$$2\log\beta_{1110} = \log X_{stat} + \log\beta_{2010} + \log\beta_{0210} \tag{8}$$

Reversely, if logβ_{1110} is known (*e.g.*, experimentally determined like in this work) together with logβ_{2010} and logβ_{0210}, Eq. 8 can be rearranged to calculate the "experimental" extrastability (logX_{exp}):

$$\log X_{exp} = 2\log\beta_{1110} - \log\beta_{2010} - \log\beta_{0210} \tag{9}$$

The positive difference between logX_{exp} and logX_{stat} is an index that the formation of the mixed species is thermodynamically favored respect to what predicted statistically.

Exception done for the SnFe(EDTA) species (of Fe^{2+}), for which the simple dinuclear Fe_2(EDTA) was not detected, logX_{exp} and logX_{stat} can be calculated for both the SnZn(EDTA) and [ZnFe(EDTA)]$^{+}$ species. In the same order, X_{stat} is:

$$X_{\text{stat}} = 2 + \frac{K_{\text{SnL}}}{K_{\text{ZnL}}}\sqrt{\frac{K_{\text{Zn}_2\text{L}}}{K_{\text{Sn}_2\text{L}}}} + \frac{K_{\text{ZnL}}}{K_{\text{SnL}}}\sqrt{\frac{K_{\text{Sn}_2\text{L}}}{K_{\text{Zn}_2\text{L}}}} = 2 + \frac{10^{18.69}}{10^{16.50}}\sqrt{\frac{10^{5.44}}{10^{6.08}}} + \frac{10^{16.50}}{10^{18.69}}\sqrt{\frac{10^{6.08}}{10^{5.44}}} = 76.14 \tag{10}$$

and

$$X_{\text{stat}} = 2 + \frac{K_{\text{FeL}}}{K_{\text{ZnL}}}\sqrt{\frac{K_{\text{Zn}_2\text{L}}}{K_{\text{Fe}_2\text{L}}}} + \frac{K_{\text{ZnL}}}{K_{\text{FeL}}}\sqrt{\frac{K_{\text{Fe}_2\text{L}}}{K_{\text{Zn}_2\text{L}}}} = 2 + \frac{10^{23.88}}{10^{16.50}}\sqrt{\frac{10^{5.44}}{10^{13.38}}} + \frac{10^{16.50}}{10^{23.88}}\sqrt{\frac{10^{13.38}}{10^{5.44}}} = 2572.40 \tag{11}$$

resulting in $\log X_{\text{stat}} = 1.88$ and 3.41, while corresponding $\log X_{\text{exp}}$ are:

$$\log X_{\text{exp}} = 2\log\beta_{\text{SnZnL}} - \log\beta_{\text{Sn}_2\text{L}} - \log\beta_{Zn_2\text{L}} = 2 \cdot 24.99 - 24.77 - 21.94 = 3.27 \tag{12}$$

and

$$\log X_{\text{exp}} = 2\log\beta_{\text{ZnFeL}} - \log\beta_{\text{Zn}_2\text{L}} - \log\beta_{\text{Fe}_2\text{L}} = 2 \cdot 32.28 - 21.94 - 37.26 = 5.36 \tag{13}$$

As can be seen, the latter values for SnZn(EDTA) and $[\text{ZnFe(EDTA)}]^+$ species are much higher than corresponding $\log X_{\text{stat}}$, indicative of a thermodynamically favorable formation of mentioned species.

3.4 Influence of Mixed Species on Chemical Speciation

In terms of chemical speciation, the formation and the extrastability of the mixed species result in a higher cation complexation by EDTA, proving that the formation of these species cannot be neglected. In fact, considering or not the mixed species in the speciation model deeply affects the fraction of complexed cations, as shown in Figs. 1 and 2, for the Sn^{2+}/Zn^{2+}/EDTA (Fig. 1) and Zn^{2+}/Fe^{3+}/EDTA (Fig. 2) systems.

Including the mixed species in the model, both cations (Sn^{2+} and Zn^{2+} in Fig. 1 and Zn^{2+} and Fe^{3+} in Fig. 2) are fully complexed by EDTA already at pH = 2.0 (in the conditions of the figures), while this does not happen if their formation is neglected. This is better evidenced from the analysis if the speciation diagrams of Figs. 3, 4, 5, for Sn^{2+}/Zn^{2+}/EDTA, Sn^{2+}/Fe^{2+}/EDTA e Zn^{2+}/Fe^{3+}/EDTA systems, respectively.

In Sn^{2+}/Zn^{2+}/EDTA, in the conditions of the diagrams, the mixed SnZn(EDTA) species represents, at pH = 2.0, 60% of Sn^{2+} (Fig. 3b) and Zn^{2+} (Fig. 3c) species in solution (and 30% of EDTA, which has an analytical concentration that is double that of cations, Fig. 3a), until it reaches a negligible percentage at pH ~ 4.0, where mononuclear species dominate. Noteworthy, the simple dinuclear Sn_2(EDTA) and Zn_2(EDTA) are not formed in significant percentages in the conditions of the diagrams (but their formation was observed in other experimental conditions).

Similarly, in the conditions of the diagrams in Fig. 4 for the Sn^{2+}/Fe^{2+}/EDTA system, the mixed SnFe(EDTA) species reaches more than 85% of the two cations (and the half for EDTA) at pH = 2.0, regularly decreasing its percentage with increasing pH up to pH ~ 6.0, in favor of mononuclear species. Interestingly, in the case of Fe^{2+}

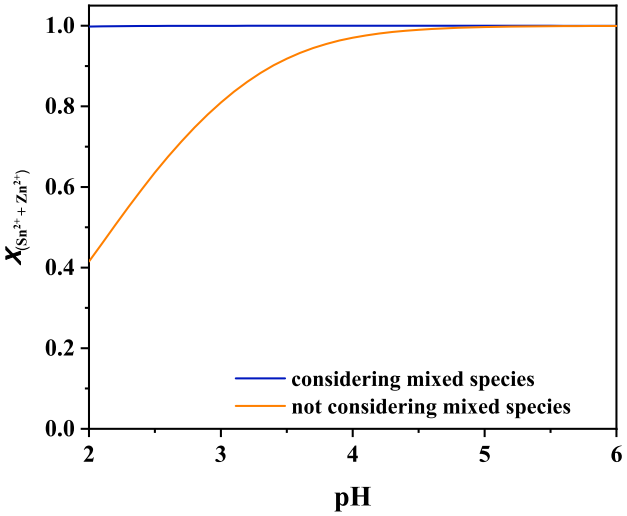


Fig. 1 Fraction ($Sn^{2+}+Zn^{2+}$) of cations complexed by EDTA *vs.* pH, in the Sn^{2+}/Zn^{2+}/EDTA system, considering (blue) or not (orange) the formation of mixed species. Conditions: $c_{Sn}=c_{Zn}=1$ mmol·dm^{-3}, $c_{EDTA}=2$ mmol·dm.$^{-3}$ (Color figure online)

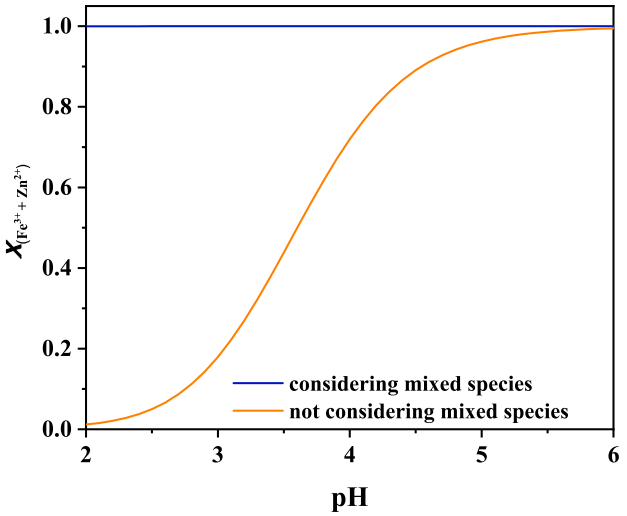


Fig. 2 Fraction ($Zn^{2+}+Fe^{3+}$) of cations complexed by EDTA *vs.* pH, in the Zn^{2+}/Fe^{3+}/EDTA system, considering (blue) or not (orange) the formation of mixed species. Conditions: $c_{Zn}=c_{Fe}=1$ mmol·dm^{-3}, $c_{EDTA}=2$ mmol·dm.$^{-3}$ (Color figure online)

(Fig. 4c), which is a weaker Lewis acid than Sn^{2+} (and, thus, the former hydrolyzes more weakly than the latter) the dominant mononuclear species in the pH range considered in diagrams (*i.e.*, $2.0 \le pH \le 6.0$) is the monoprotonated $[Fe(EDTA)H]^{-}$, while it is the fully deprotonate $[Sn(EDTA)]^{2-}$ for Sn^{2+} (analogously to all other investigated cations, in which it is always the ML species to dominate among the mononuclear).

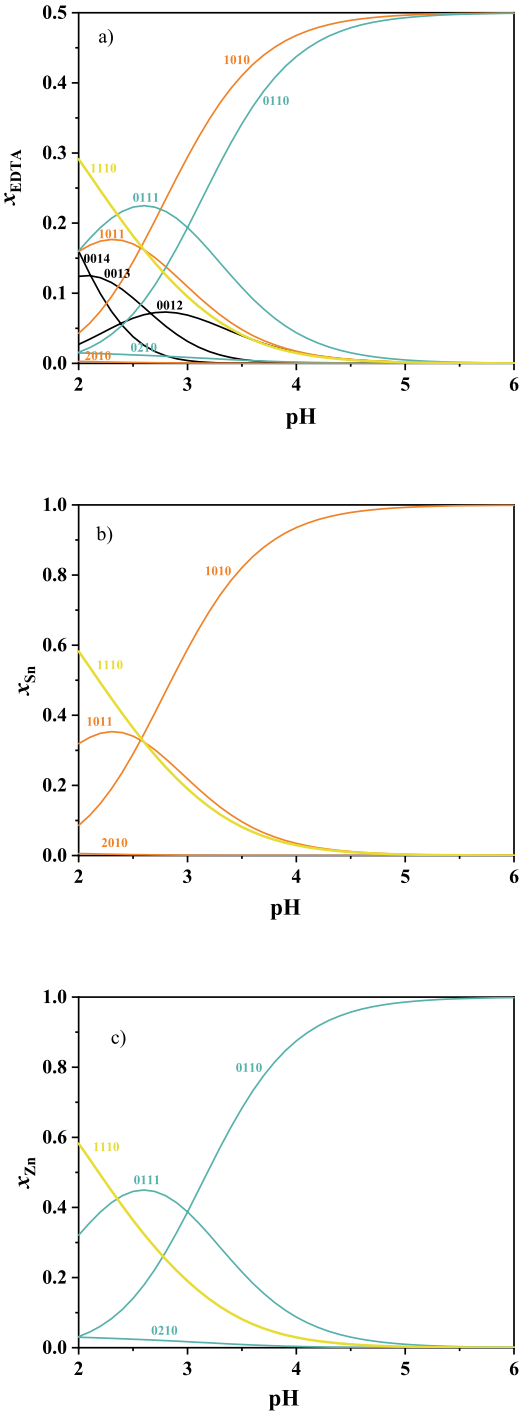


Fig. 3 Distribution diagrams of $Sn_pZn_{p'}(EDTA)_qH_r$ species *vs.* pH in the Sn^{2+}/Zn^{2+}/EDTA system. Conditions: $c_{Sn} = c_{Zn} = 1$ mmol·dm^{-3}, $c_{EDTA} = 2$ mmol·dm^{-3}, $T = 298.15$ K, $I = 0.15$ mol·dm^{-3} in $NaNO_{3(aq)}$. **a** fraction of EDTA; **b** fraction of Sn^{2+}; **c** fraction of $Zn.^{2+}$. *pp'qr* indexes refer to $Sn_pZn_{p'}(EDTA)_qH_r$ species: *e.g.*, 1110 is SnZn(EDTA)

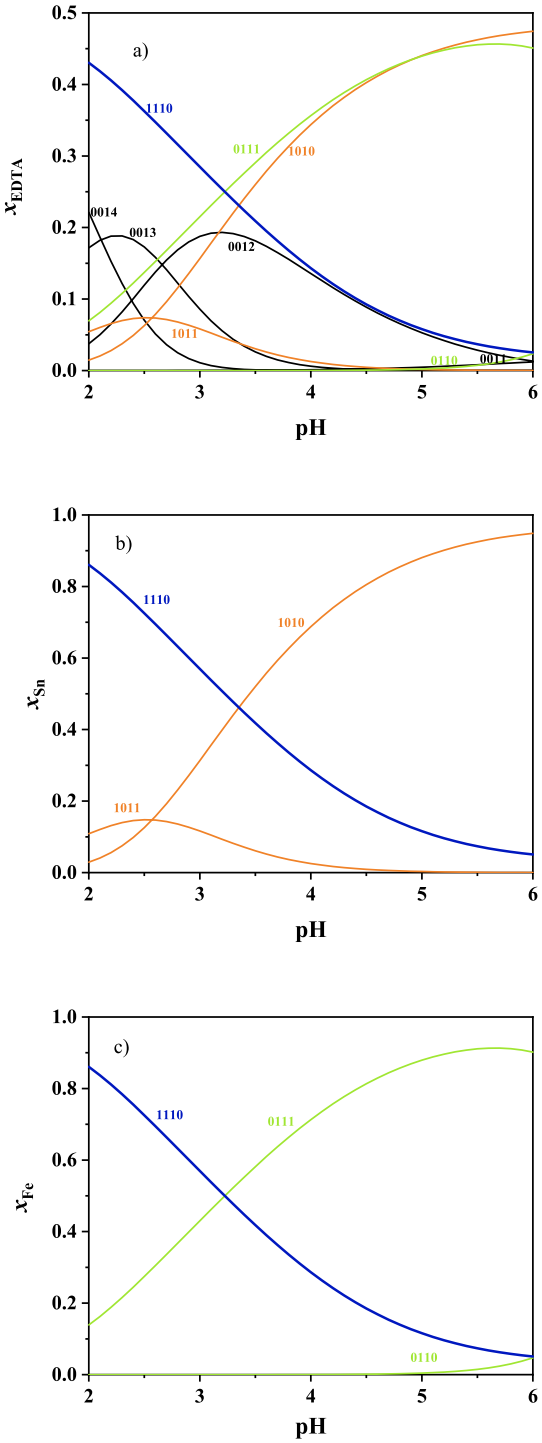
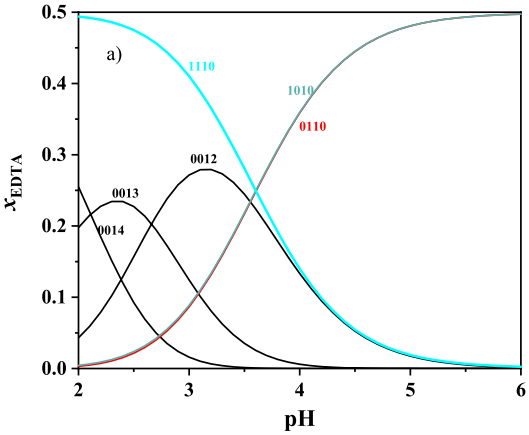
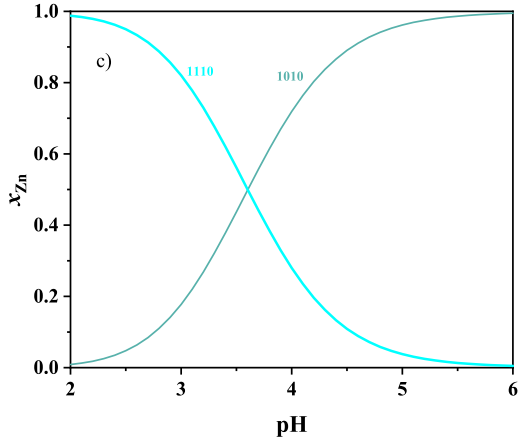


Fig. 4 Distribution diagrams of $Sn_pFe_{p'}(EDTA)_qH_r$ species *vs.* pH in the Sn^{2+}/Fe^{2+}/EDTA system. Conditions: $c_{Sn}=c_{Fe}=1$ mmol·dm^{-3}, $c_{EDTA}=2$ mmol·dm^{-3}, $T=298.15$ K, $I=0.15$ mol·dm^{-3} in $NaClO_{4(aq)}$. **a** fraction of EDTA; **b** fraction of Sn^{2+}; **c** fraction of $Fe.^{2+}$. *pp'qr* indexes refer to $Sn_pFe_{p'}(EDTA)_qH_r$ species: *e.g.*, 1110 is SnFe(EDTA)





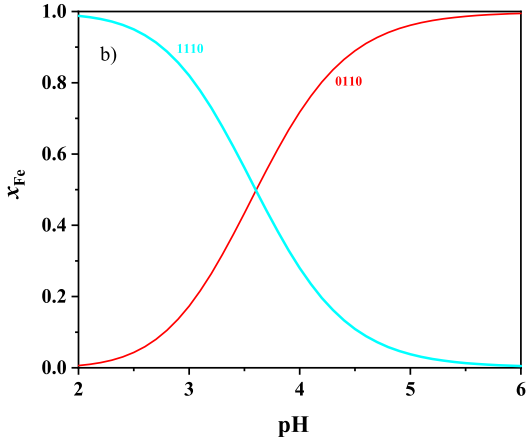


Fig. 5 Distribution diagrams of $Zn_pFe_{p'}(EDTA)_qH_r$ species *vs.* pH in the Zn^{2+}/Fe^{3+}/EDTA system. Conditions: $c_{Zn}=c_{Fe}=1$ mmol·dm^{-3}, $c_{EDTA}=2$ mmol·dm^{-3}, $T=298.15$ K, $I=0.15$ mol·dm^{-3} in $NaNO_{3(aq)}$. **a** fraction of EDTA; **b** fraction of Zn^{2+}; **c** fraction of Fe^{3+}. *pp'qr* indexes refer to $Zn_pFe_{p'}(EDTA)_qH_r$ species: *e.g.*, 1110 is $[ZnFe(EDTA)]^+$

The $[Sn(EDTA)H]^-$ species hardly reaches 10% of Sn^{2+}, with a maximum observable at pH ~ 2.5.

Finally, in Zn^{2+}/Fe^{3+}/EDTA system in the conditions of diagrams in Fig. 5, the mixed $[ZnFe(EDTA)]^+$ species represents almost 100% of both cations (and always the half for EDTA) at pH = 2.0, decreasing up to negligible percentages at pH ~ 6.0. Mononuclear $[Fe(EDTA)]^-$ and $[Zn(EDTA)]^{2-}$ species represent a bit more of 50% of the respective cations at pH slightly higher than pH ~ 3.5, until they reach 100% at pH = 6.0.

Also for the last two systems, simple dinuclear M_2(EDTA) species are not formed in the conditions of the diagrams (though they do in other experimentally investigated), further confirming that mixed species are much more favored than analogous simple ones.

3.5 Structural Models of Simple and Mixed Dinuclear Complexes

The above hypothesis concerning the possible analogy between MIDA and EDTA, when the latter forms dinuclear species, needed some further support. As such, in order to get deeper insights on the binding modes and the structure of the dinuclear EDTA complexes in aqueous solution, we also carried out quantum mechanical calculations at the DFT level on the dinuclear species of the Sn^{2+}/Zn^{2+}/EDTA system (*i.e.*, Sn_2(EDTA), Zn_2(EDTA) and SnZn(EDTA)). Solvent effects were implicitly included in the following calculations, hence explicit water molecules participating in the ion coordination shell were omitted from the structural models. First, we considered the Zn_2(EDTA) species, for which two hypothetical binding modes were investigated (Fig. 6a, b). In one case, each zinc ion was coordinated to the MIDA-like half of EDTA acting as a tridentate ligand, *i.e.*, the zinc ion was bound to two carboxylic oxygen atoms of the same nitrogen and nitrogen itself (Fig. 6a, structure "a"), while the remaining coordination shell was completed by water (not shown in Fig. 6). In the other case, only the carboxylic groups (two per zinc ion) of the two different nitrogens participated in ion coordination (Fig. 6b, structure "b") and each half of the EDTA was acting more like a bidentate group with a negligible contribution of nitrogens to coordination. Upon structural optimization, the corresponding two structural models depicted in Fig. 6a and b were obtained. While

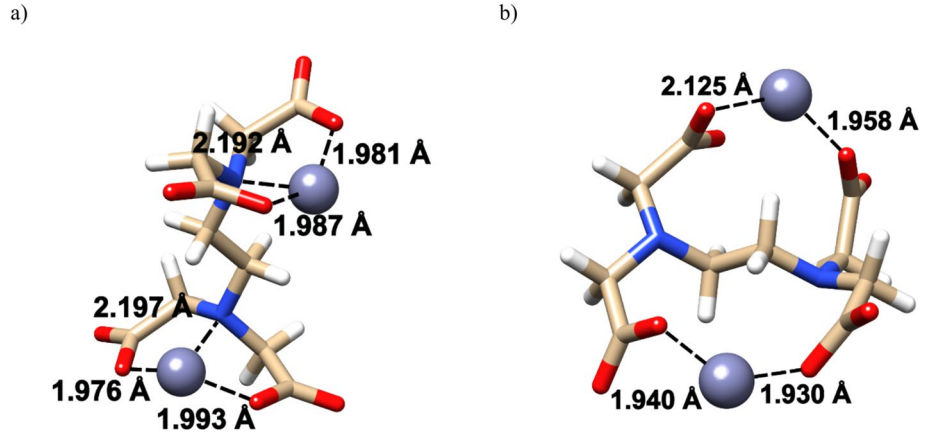


Fig. 6 Optimized structures of the Zn_2(EDTA) species obtained from quantum mechanical calculations. Structure "a": Each cation bound to two carboxylates of the same nitrogen of EDTA and nitrogen itself. Structure "b": Each cation bound to two carboxylates of a different nitrogen of EDTA

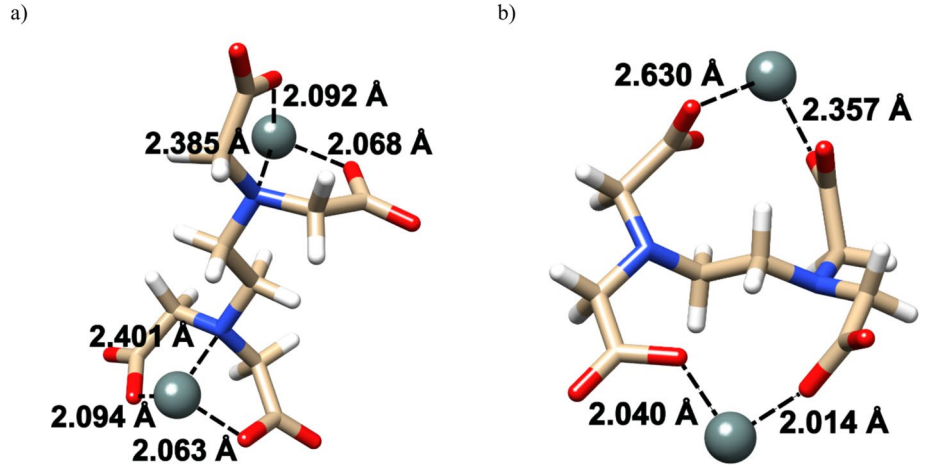


Fig. 7 Optimized structures of the Sn_2(EDTA) species obtained from quantum mechanical calculations. Structure "a": Each cation bound to two carboxylates of the same nitrogen of EDTA and nitrogen itself. Structure "b": Each cation bound to two carboxylates of a different nitrogen of EDTA

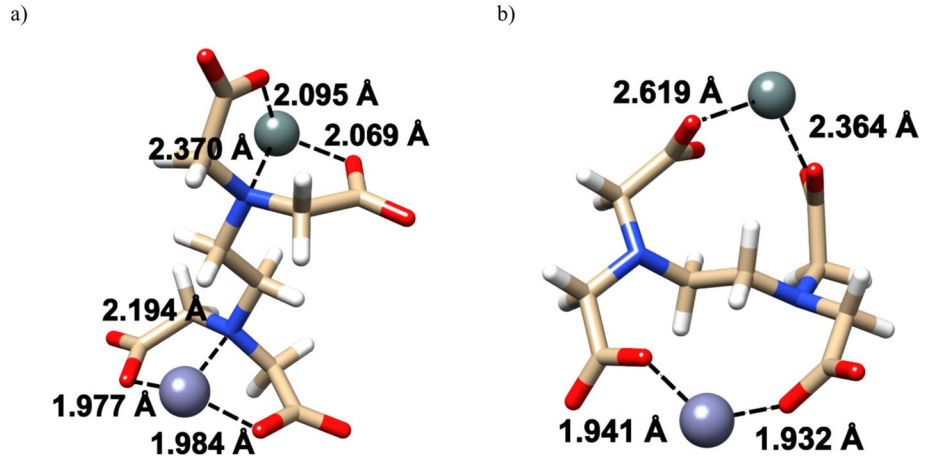


Fig. 8 Optimized structures of the SnZn(EDTA) species obtained from quantum mechanical calculations. Structure "a": Each cation bound to two carboxylates of the same nitrogen of EDTA and nitrogen itself. Structure "b": Each cation bound to two carboxylates of a different nitrogen of EDTA

both models resulted in relatively stable structures; the structure "a" was significantly more stable than the "b" by about 147 kJ·mol^{-1}. This finding is in agreement with the observed solid state structure of dicationic copper complexes [52] and is consistent with the stability constants obtained in this work, as previously discussed.

Similar calculations were performed on Sn_2(EDTA) (Fig. 7a, b) and SnZn(EDTA) (Fig. 8a, b), with analogous results, *i.e.*, structures "a" was found to always be

more stable than "b". The computed energy difference is 175 and 146 kJ·mol^{-1}, for Sn_2(EDTA) and SnZn(EDTA), respectively.

In a previous section, we also discussed the extra thermodynamic stability of the mixed complex species with respect to simple ones. In particular, $\log X_{exp} = 3.27$ Eq. 12 for Sn^{2+}/Zn^{2+}/EDTA corresponds to 18.7 kJ·mol^{-1}, which is 7.9 kJ·mol^{-1} more stable than $\log X_{stat} = 1.88$ Eq. 10, *i.e.*, what predicted on solely statistical considerations (see discussion above). Remarkably, quantum mechanical calculations on the complexes structural models provided an enhanced energetic stability of the mixed species by 7.8 kJ·mol^{-1} $\left(\Delta E = 2 \cdot \Delta E(\text{SnZn(EDTA)}) - \Delta E\left(\text{Sn}_2\text{(EDTA)}\right) - \Delta E\left(\text{Zn}_2\text{(EDTA)}\right)\right)$, in excellent agreement with that estimate, thus supporting the evidence of an extra stability arising from the combination of both entropic (statistical) and enthalpic contributions.

4 Conclusions

Despite literature findings on dinuclear complexes of some complexones in aqueous solution, as well as dinuclear Cd^{2+} and UO_2^{2+} EDTA complexes in aqueous solution, the work here presented is, to our knowledge the first attempt to identify and determine the stability of simple and mixed dinuclear EDTA species in aqueous solution for other cations. The chemical speciation of three model systems, namely Sn^{2+}/Zn^{2+}/EDTA, Sn^{2+}/Fe^{2+}/EDTA and Zn^{2+}/Fe^{3+}/EDTA, has been investigated at $T = 298.15$ K in $NaNO_{3(aq)}$ and in $NaClO_{4(aq)}$ (for the second) at $I = 0.15$ mol·dm^{-3}, designing potentiometric titrations in concentration ratios able to enhance the eventual formation of simple and mixed polynuclear complexes. The analysis of experimental data evidenced the formation of the simple Zn_2(EDTA), Sn_2(EDTA) and $[Fe_2(EDTA)]^{2+}$ species, as well as the mixed SnZn(EDTA), SnFe(EDTA) and $[ZnFe(EDTA)]^{+}$. In the experimental conditions adopted, the simple Fe_2(EDTA) (*i.e.*, of Fe^{2+}) species was not observed. The comparison between the equilibrium constants related to the binding of a further cation by the mononuclear cation EDTA complexes (to form the dinuclear species) and the stability constant of ML species of MIDA (which can be considered as "half" EDTA) suggests that EDTA behaves, in this case, as a tridentate ligand, binding cations through two carboxylates and one amino group (MIDA-like mode), as observed in the solid state for similar complexes [52] and confirmed by quantum mechanical calculations. Concerning the formation of mixed MM′EDTA species, they showed an extra thermodynamic stability when compared to that statistically predicted, as also confirmed by quantum mechanical calculations for SnZn(EDTA). This extrastability turns into a high formation percentage of these mixed species at pH < 6.0, significantly affecting the chemical speciation of both EDTA and cations up to this pH, as an indication that mixed species cannot be neglected in a correct speciation model of systems where different cations are simultaneously present, as it happens for many real ones.

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Data Availability Data will be available upon request.

Declarations

Competing Interests The authors declare no competing interests.

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