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Determination of conditional stability constants for some divalent transition metal ion-EDTA complexes by electrospray ionization mass spectrometry

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Conditional stability constants of coordination complexes comprising divalent transition metals, Cu^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+}, and ethylenediaminetetraacetic acid (EDTA) were determined utilizing electrospray ionization mass spectrometry. The deviation of signal response of a reference complex was monitored at addition of a second metal ion. The conditional stability constant for the competing metal was then determined through solution equilibria equations. The method showed to be applicable to a system where Co^{2+} and Zn^{2+} competed for EDTA at pH 5. When Cu^{2+} and Ni^{2+} competed for EDTA, the equilibrium changed over time. This change was shown to be affected in rate and size by the type of organic solvent added. In this work, 30% of either methanol or acetonitrile was used. It was found that if calibration curves are prepared for both metal complexes in solution and the measurements are repeated with sufficient time space, any change in equilibrium of sample solutions will be discovered. Copyright © 2014 John Wiley & Sons, Ltd.

Keywords: chelating agent; EDTA; electrospray ionization mass spectrometry; conditional stability constant; mixed solvent

Introduction

Conditional stability constants (K_{ML}) describe the equilibria of coordination complex formation at ambient solution conditions.[1,2] A coordination complex consists of a central atom (M) surrounded by ligand/ligands (L), for example, water and ethylenediaminetetraacetic acid (EDTA). Some ligands, for example, EDTA, are able to have multiple connections with the central atom, this is defined as chelation.[3] In general, more connections lead to a stronger complex. EDTA is an extensively used and investigated polyaminopolycarboxylic acid that forms strong complexes with, for instance, divalent transition metals. [1,4–8] When the solution conditions, for example, pH, ionic strength, and temperature are changed, the complex formation capacity of the ligand is altered, which leads to a change in the magnitude of the stability constant.[1]

In determinations of the conditional stability constants between different metal ions and ligands, analytical methods such as potentiometry,[9–14] calorimetry,[15–18] polarography,[12,19–21] spectrophotometry,[13,14,22,23] and 1H NMR[14] have been applied. It is not unusual that combinations of analytical methods are used in the determination of conditional stability constants for a complex.[12–14,23] In potentiometric titrations, the ligands and the metal ions are dissolved in an acidic solution, and the pH is monitored during titration with hydroxide ions.[14,24] Alternatively, pH is kept constant and the ionic strength is changed.[11,25] Several parameters have to be taken into account before titration of the metal complex. For example, the protonation constants (pK_a values) of the ligand must be known[26,27] and the concentrations of the free ligands and metal ions need to be

determined. In addition, temperature control, inert atmosphere, and inert background electrolyte are also needed during determination procedures.[24,28,29] Different assumptions are often made to simplify the calculations although such operations might affect the results.[28,30]

In calorimetry, for example, isothermal titration calorimetry, the conditional stability constant and enthalpy is determined through several preceding steps. For example, the exact analyte and titrant concentrations must be known. In addition, several titrations without the analyte are performed in order to subtract the influence of heat of mixing, heat of dilution, and heat of protonation. After the measurements, the formed binding isotherm is fitted with an appropriate binding model, and the conditional stability constant is then determined.[31]

Thus, potentiometric and calorimetric determinations of conditional stability constants are time-consuming,[32] because subsequent determinations of many different parameters are needed before the actual determination of the conditional stability constant is performed.[11] In recent years, electrospray ionization mass spectrometry (ESI-MS) has been applied in determination of stability constants.[32,33] In ESI-MS, the time of

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analysis is relatively short per run; in the order of seconds to minutes and the sample volumes and concentrations needed are very low (μl–ml and μM). ESI-MS is often used as a structure determining technique of complexes, but a good option is to quantify a coordination complex, because the complex, to a high extent, can be kept intact during the ionization process and mass analysis.[34–36] A drawback of quantitative determinations is that different ions often have different responses.[34,35,37–39] To overcome the latter, different strategies have been applied. Assumptions such as the coordination complex formed have the same response factor either as the unbound ligand or the central atom that is one approach, which requires that the solvation energies and detections efficiencies are similar in order to obtain accurate results.[40,41] Other approaches address the differences by assuming response factors[42] or determining the response factors by the use of internal standards.[32,39] The internal standard must resemble the analyte ion to be measured with ESI-MS but not bind to the ligand or central atom.[35] Also, the ratio between the analyte and the internal standard responses needs to be stable in order to accurately determine the analyte concentration when the internal standard signal changes.[32,39] Unfortunately, the determination of response factors leads to multiple steps and is time-consuming.

In this study, an ESI-MS-based method with low analyte consumption is investigated for the determination of conditional stability constants of complexes formed by Cu^{2+}, Ni^{2+}, Zn^{2+}, or Co^{2+}, and EDTA. The study is based on measurements of competing complexation between two different transition metal ions, where one of the complexes' conditional stability constant is known and used in the calculations of the other complex. By only monitoring the response of the ionized coordination complex, the difference in responses from ESI-MS determinations is overcome and the analysis becomes fast and simple.

Experimental section

Material and reagents

Dehydrated $CuCl_2$ and $NiOAc_2 \cdot 4\,H_2O$, both with a purity of 99%, were purchased from Reidel de Haën (Germany), $NiCl_2 \cdot 6\,H_2O$ was bought from J.T. Baker (The Netherlands) with a purity of 98.7%, $ZnCl_2$ was purchased from Fisher Scientific (Sweden), and $CoCl_2 \cdot 6\,H_2O$ from Kebo, both with a declared purity of 98%. EDTA was purchased from Sigma-Aldrich (Sweden) in 99.4–100.06% purity. Methanol (MeOH) and acetonitrile (ACN) were both of HPLC grade. The hydrochloric acid (37%) (analytical reagent) was from VWR Prolab (Sweden); acetic acid (>99.8%), ammonium hydroxide (~25% NH_3), and triethylamine (GC grade) were purchased from Fluka (Sweden). All chemicals were used without further purification. Water of MilliQ grade (Merck Chemicals and Life Science AB, Sweden) was used in the preparation of all solutions. All glassware used was acid-washed for at least $2 \times 6\,h$; first in 0.1 M HCl then in 0.1 M HNO_3 and thereafter soaked in water of MilliQ grade, three times for more than 2 h in each bath.

Electrospray ionization mass spectrometry

Electrospray ionization mass spectrometry was performed on Micromass Quattro II Triple Quadrupole instrument (Waters Corp., Sweden) coupled to a Harvard Syringe Pump (Harvard Apparatus, MA, USA). The mass spectrometer was operated in ESI negative ionization mode. The ESI solution was delivered to the coaxial probe with a relatively high flow rate of 20 μl/min. In all the

measurements, the setting of the nebulization gas, N_2, was 10 l/h, the capillary potential was 2.50 kV, the drying gas was 350 l/h, the skimmer was set to $-1.5\,V$, the RF lens was set to $-0.2\,V$, and the ion energy was $-2.0\,V$. To optimize the stability as well as the signal of the complex at individual measurements series, the counter electrode (HV lens) parameter was set from -0.49 to $-0.65\,kV$, sample cone voltage was set from -20 to $-30\,V$, and source temperature was either 80 °C or 100 °C.

Calculation of conditional stability constants

Divalent metal ions and EDTA complexes are very strong within the pH range used. Therefore, it was assumed that each ligand formed a complex with one divalent metal ion, as the metal ion/EDTA relationship was 1 : 1 and 2 : 1 in standard and sample solutions, respectively. Two equilibria between the ligand and added metal ions are presented in the sample solution and can be described by the following expressions:

$$K_{M_a} = \frac{[LM_a]}{[L][M_a]} \tag{1}$$

$$K_{M_b} = \frac{[LM_b]}{[L][M_b]} \tag{2}$$

In Eqns. (1), (2), K_{Ma} and K_{Mb} are the equilibrium constants of the complex formation between the ligand L and the two competing metal ions M_a and M_b, respectively. $[LM_a]$ and $[LM_b]$ are the concentration of the complexes. $[L]$, $[M_a]$, and $[M_b]$ denote the free concentrations of ligands and metal ions, respectively. In a competition between two different metal ions for the ligand, an expression for the stability constant of an unknown complex can be described by first combining Eqns. (1) and (2) into Eqn. (3) and thereafter developing the expression further into Eqn. (4).

$$\frac{K_{M_b}}{K_{M_a}} = \frac{[LM_b][M_a]}{[LM_a][M_b]} \tag{3}$$

$$K_{M_b} = K_{M_a} \frac{[LM_b]([M_a]_{tot} - [LM_a])}{[LM_a]([M_b]_{tot} - [LM_b])} \tag{4}$$

Here, $[M_a]_{tot}$ and $[M_b]_{tot}$ denote the total concentrations of the different metal ions. Eqn. (3) can thus be used to calculate the conditional stability constant for metal b when the stability constant, K_{Ma}, together with the concentrations of added metals and both competing complexes in solution are known. If the concentration of one M-EDTA complex is unknown, Eqn. (5) can determine the K_{Mb}.

$$K_{M_b} = \frac{[L]_{tot} - \frac{[LM_a]}{K_{M_a} \cdot ([Ma]_{tot} - [LM_a])} - [LM_a]}{\frac{[LM_a]}{K_{M_a}([M_a]_{tot} - [LM_a])} \cdot \left([M_b]_{tot} - [L]_{tot} + \frac{[LM_a]}{K_{M_a} \cdot ([M_a]_{tot} - [LM_a])} + [LM_a]\right)} \tag{5}$$

where $[L]_{tot}$ denotes the total concentration of the ligand.

The use of Eqn. (4) instead of Eqn. (5) in determination of K_{Mb} contains less error because the calculation has fewer unknown parameters and is thus preferred when the concentrations of both complexes in the solution is known. The advantage of Eqn. (5) is that the time of analysis is shorter because only one complex concentration, the reference complex, must be determined.

Sample preparation and ESI-MS measurements

For all experiments, 10 mM stock solutions of the divalent metals and EDTA were prepared: EDTA was dissolved in low amounts of NH_4OH and further diluted with water; $ZnCl_2$ was dissolved and diluted with the buffer used in the experiment, adjusted to pH 5; and all other metal salts were dissolved and diluted with water. From the stock solutions, sample solutions with specific concentrations in different solvents were prepared. The amount of organic solvent needed for a stable electrospray was 30 vol%. In this work, three different combinations of organic solvent and buffer were used: 30 vol% MeOH and 70 vol% 5.0 mM ammonium acetate, 30 vol% ACN and 70 vol% 2.5 mM triethylamine acetic acid (TEAA), or 30 vol% MeOH and 70 vol% 2.5 mM TEAA. The pHs of the solvent systems were adjusted to 5, 6, or 7 with combined glass electrode containing 3 M KCl electrolyte. The same acid or base present in the buffer was used, except for in the experiments presented in Table 1, with Cu-EDTA as the reference complex and in $MeOH/NH_4OH$, which was adjusted with HCl (aq) and NH_4OH (aq). The analysis with ESI-MS was initiated by conditioning the sample solution for about 10 min, followed by 5 or 10 min of acquisition. The instrument was washed between the measurements with either MeOH or ACN. A maximum of 60–100% fluctuation, in counts per second, of the reference complex chromatogram recordings were permitted. Examples of recorded chromatograms are presented in Fig. 1. The integrated spectra of the chromatograms in Fig. 1 are shown in Fig. 2. The resulting signal peaks in integrated spectra of the reference complex, M_a-EDTA, were used to create a standard curve (Fig. 3). Three concentrations from 2 to 50 μM were chosen for each standard curve, the concentrations were determined by the detection level and linear range in each experiment. The sample solution was prepared in 1 : 1 : 1 relationship, M_a/M_b/EDTA. In addition, for some experimental series, a second standard curve with M_b-EDTA was created. The concentration of reference complex could then be determined, and thereafter, the conditional stability constant was calculated with either Eqn. (4) or Eqn. (5). According to Kempen *et al.*,[33] the limitations in accuracy of this method is reached when the absolute value of the difference in log K is larger than 2 for the competing metal ion-ligand complexes.

Results and discussion

Determinations of stability constants in different solvent system

Ethylenediaminetetraacetic acid has the capacity to coordinate a metal ion with two nitrogen atoms and four carboxylic groups. The strength of the coordination complex has an optimum value at a specific pH. As the pH decreases, EDTA is gradually protonated,[7] and the value of the conditional stability constant, log K_{M-EDTA}, decreases because of increased competition between metal ions and protons for the coordination atoms in the ligand. The conditional stability constants of *M*-EDTA complexes also decrease above a certain pH, because metal hydroxide complexes form in alkaline solutions.[1,7] Thus, to keep a stable pH in the solutions during preparation and analysis with ESI-MS, a buffer and a high flow rate during measurements were used, both precautions contribute to minimizing pH changes due to redox reactions in the stainless steel capillary.[42] In Table 1, initial results from two of the buffered solvent systems are shown. The reference complex in these measurements was either Cu-EDTA or Ni-EDTA. All determined conditional stability constants increased with pH for both reference complexes as expected. With Cu-EDTA as the reference complex, the determined conditional stability constants in both solvent systems were similar when compared. At all measured pHs, determined conditional stability constants for competing Zn^{2+} in ACN/TEAA were higher than in $MeOH/NH_4OH$. This was also the case with competing Ni^{2+} at pH 5 but with competing Ni^{2+} at pH 6 and 7, instead, the determined values were higher in $MeOH/NH_4OH$. The deviations in determined conditional stability constant between the solvent systems were found random and not dependent on the pH. For example, the largest difference between the solvent systems in determined log K_{Mb} for Zn^{2+} was at pH 6, and at the same pH, the differences in determined log K_{Mb} for competing Ni^{2+} were the smallest. Actually, all determined values using Cu-EDTA were consistently larger than literature values. Literature values are determined in 100% water, and the effect of this is discussed further. The conditional stability constants determined for Zn^{2+} in competition with both Cu^{2+} and Ni^{2+} were similar but all deviated from the literature values.

Table 1. Determined conditional stability constants, log K_M, for some metal ion-EDTA complexes.

Competing metal ion, M_b	Reference complex	pH	Determined logK_{Mb} MeOH/ NH_4OAc solvent	Determined log$_{Mb}$ ACN/ TEAA solvent	Literature values logK_{Mb}* aqueous solvent
Zn^{2+}	Cu-EDTA	5	11.1 ± 0.3	11.4 ± 0.2	9.9
Zn^{2+}		6	13.1 ± 0.3	13.8 ± 0.3	11.7
Zn^{2+}		7	15.7 ± 0.2	15.9 ± 0.3	13.1
Ni^{2+}		5	12.6 ± 0.5	13.8 ± 0.0	12.0
Ni^{2+}		6	15.2 ± 0.1	14.9 ± 0.1	13.8
Ni^{2+}		7	17.1 ± 0.5	16.5 ± 0.1	15.2
Cu^{2+}	Ni-EDTA	5	nd	12.1 ± 0.3	12.2
Cu^{2+}		6	nd	14.1 ± 0.2	14.0
Cu^{2+}		7	nd	15.0 ± 0.1	15.4
Zn^{2+}		5	nd	11.5 ± 0.1	9.9
Zn^{2+}		6	nd	14.0 ± 0.2	11.7
Zn^{2+}		7	nd	14.6 ± 0.3	13.1

* The Dow Chemical Company, Versene product information, 1974, Form nr 298-717-80.
nd, not determined.
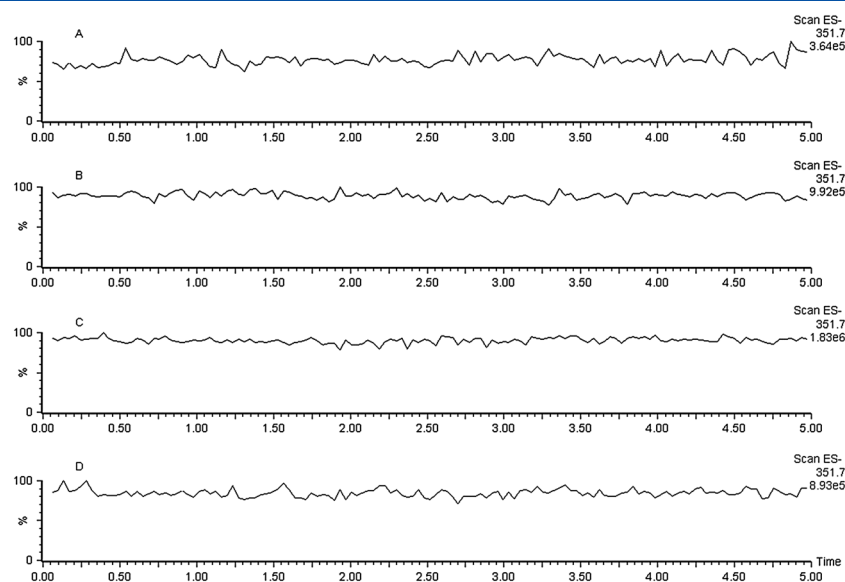
Figure 1. Signals [counts per second (cps)] recorded over time of Cu-EDTA ($m/z = 351.7$ in negative mode ESI) at concentrations 5, 10, and 20 µM (A, B, and C, respectively). The solvent system used in these measurements was 30 vol% ACN and 70 vol% 2.5 mM TEAA at pH 6.
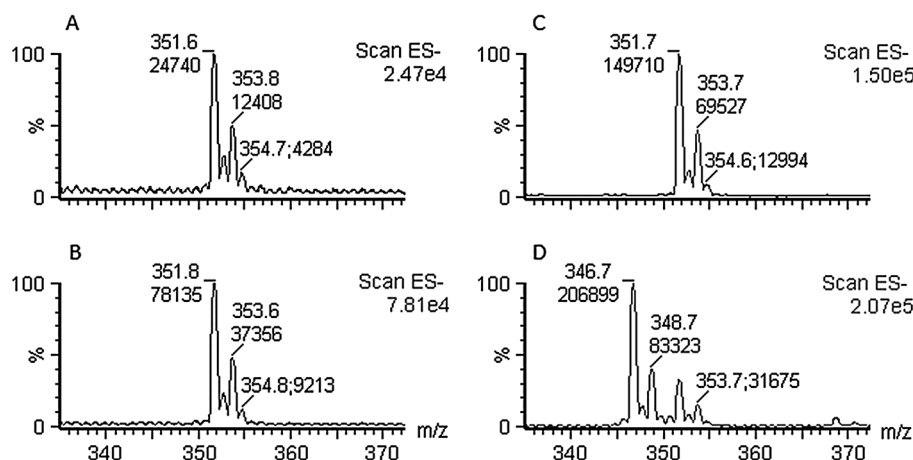
Figure 2. Integrated negative mode ESI-MS spectra for 5, 10, and 20 µM Cu-EDTA solutions and sample solution with 40 : 40 : 40 µM $Cu^{2+}/Ni^{2+}/EDTA$ (A, B, C, and D, respectively, c.f. Fig. 1). Cu-EDTA shows a typical isotopic pattern from m/z 352 to 355.

With competing Cu^{2+} against Ni-EDTA instead, the results were close to the literature values. When considering the selectivity from the results with Cu-EDTA as the reference complex at pH 6, Ni-EDTA was much stronger than both Zn-EDTA and Cu-EDTA; the latter two were determined to be equal in strength. This can be compared with the order of selectivity in the literature which is instead $Zn^{2+} < Ni^{2+} < Cu^{2+}$. Because the measurements were performed in organic solvent–water mixtures, deviations from literature values that are derived from 100% aqueous solutions with higher electrolyte concentration were expected. The literature values in water system were chosen for comparison as no conditional stability constants with this system of ligands, metals, organic solvent, and buffers have been determined before. The decrease in the dielectric constant (ε) of the solvent system, due to the added amount of organic solvent, has been shown earlier to only change the conditional stability constants slightly.[43,44] In general, log K has a linear dependency to $1/\varepsilon$, and when the dielectric constant is reduced through addition of MeOH or ACN, the conditional stability constant is slightly increased for all metal complexes in this work. This has thus only a minor effect on the ratio log $K_{Cu\text{-}EDTA}$/logs $K_{Ni\text{-}EDTA}$, as well as on the ratio log $K_{Zn\text{-}EDTA}$/log $K_{Co\text{-}EDTA}$. In addition, other deviations from reference solution that can have affected the results are the lower concentrations of the electrolytes used and a slightly different temperature. Still, the strength of the complexes should be in the same order as in the literature.

Evaluation of ion suppression

The reduction in Cu-EDTA signals at addition of either Zn^{2+} or Ni^{2+} and also in Ni-EDTA signals at addition of Zn^{2+} leads to higher conditional stability constants compared with literature values. If M_a-EDTA's response factor in sample solution is suppressed, determined log K_{Mb} becomes higher than the actual log K_{Mb}. It is therefore necessary, for an accurate determination, that the response factor for M_a-EDTA is the same in the sample as in the standards, despite the higher ionic strength in the samples. These highly reduced M_a-EDTA signals could thus indicate ion
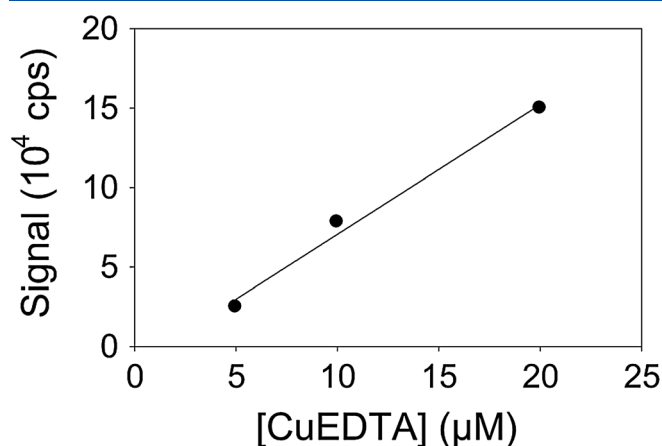
Figure 3. A typical standard curve from measurements in solvent system MeOH/TEAA. The concentrations of the reference complex are plotted *versus* signal intensity in counts per second (cps). Each data point is derived from the corresponding mass spectrum in Fig. 2. $R^2 > 0.96$ in all standard curves.

suppression at addition of $M_b Cl_2$, a reduction not because of the complexation between the competing metal ion and EDTA. Electrolytes are well known to cause decrease in analyte response.[45] For example, the signal intensity in ESI-MS measurements of pure Mn-EDTA and Th-EDTA has been observed by Reinso-Maset *et al.* to be suppressed at addition of the other metal salt, $Th(NO_3)_4$, and $MnCl_2$, respectively.[46] Because the concentration of Cl^- in sample solution is twice as high compared with standard solutions, experiments were performed to investigate if any suppression in M_a-EDTA signal response was caused by the extra addition of Cl^-. In Fig. 4, results of measurements at pH 5 with Cu-EDTA and at pH 6 with $NiOAc_2$ are shown. Small increases in signal for both M_a-EDTA complexes were observed when the concentration of each salt was doubled. This increase could possibly be due to an equilibrium change toward the complex because of excess M_a in the solution, and therefore, these results neither support nor exclude possible ion suppression in the ESI-MS measurements.
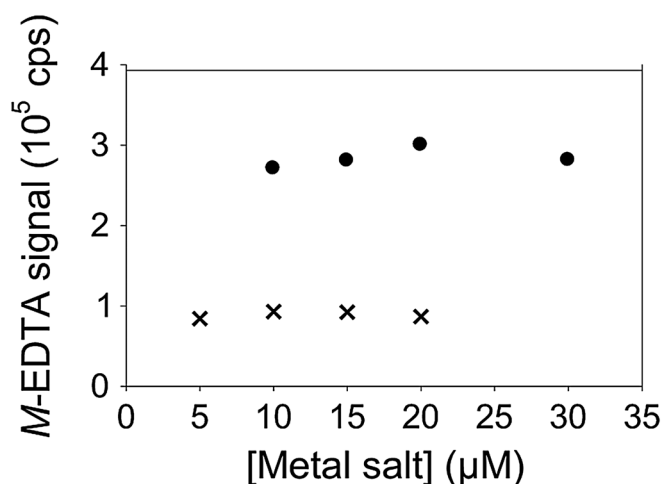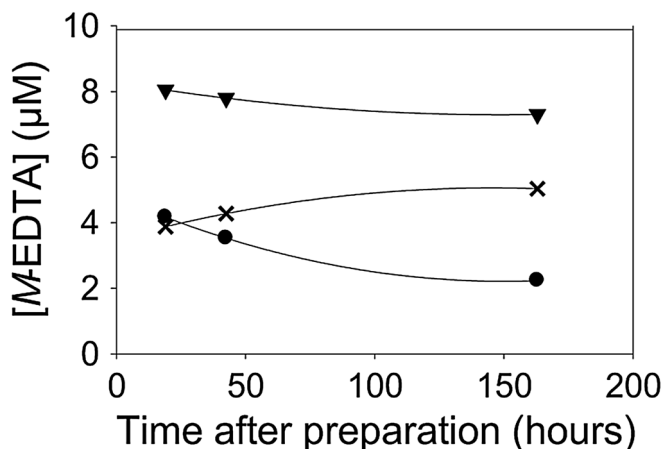
Observed changes in complexation equilibrium over time

Measurements of M_a, M_b, and EDTA systems at pH 5 were performed to elucidate if the equilibrium changed over time. A standard curve for quantification of each *M*-EDTA complex was prepared with two different combinations of metals, one with competition between Cu^{2+} and Ni^{2+} and the other with competition between Co^{2+} and Zn^{2+}. These combinations of metal ions were chosen because of their closeness in complex binding strength. With Ni^{2+}, Cu^{2+}, and EDTA, two experiments were performed, one in MeOH/TEAA and the other in ACN/TEAA. In experiments performed with MeOH/TEAA, the determined conditional stability constants were the same for both Cu-EDTA and Ni-EDTA. Thus, both complexes seemed equal in strength, and they were also close to the literature values determined in water. This can be compared with the results in solutions with 30 vol% ACN that had a lower determined binding strength for Cu-EDTA than for Ni-EDTA. In competition measurements between Zn-EDTA and Co-EDTA complexes in ACN/TEAA, the logs K_{Mb} were 10.1 ± 0.2 and 9.5 ± 0.1 for Zn-EDTA and Co-EDTA, respectively. That is in the same selectivity order, and about the same magnitude, as in the literature, where Zn-EDTA has a log K of 9.9 and is thus stronger than Co-EDTA, which has a log K of 9.7.[47]

The amount *M*-EDTA *versus* equilibrium time for the Ni^{2+}, Cu^{2+}, EDTA systems in ACN/TEAA and in MeOH/TEAA are graphically presented in Figs 5 and 6, respectively. As all EDTA in the sample solution is assumed to form complex with added divalent metal ions, the total concentration of *M*-EDTA should be equal to the sum of both metal complexes. In Fig. 5, with ACN as the organic solvent, the amount of Cu-EDTA decreases over time, and simultaneously, the amount of Ni-EDTA increases. The graph shows that Cu-EDTA was more abundant than Ni-EDTA only in the first measurement, and it also shows decreasing total amounts of *M*-EDTA with time. In Fig. 6, where MeOH is the organic solvent used, the *M*-EDTA concentrations did not change at the same rate and to the same extent as in ACN. In these measurements, Cu-EDTA was more abundant than Ni-EDTA. Still, a slight decrease of the Cu-EDTA concentration over time can be noticed and the opposite trend is shown for the Ni-EDTA concentration. The total amount of *M*-EDTA is constant, but is summed up to 7 µM, instead of the expected 8 µM given by the concentrations

Figure 4. Change in signal response *versus* metal salt concentration. ● $CuCl_2$/Cu-EDTA, x $NiOAc_2$/Ni-EDTA. The lowest concentration in each series represent a metal ion/EDTA ratio of 1 : 1; thereafter, only the concentration of metal salt was increased.
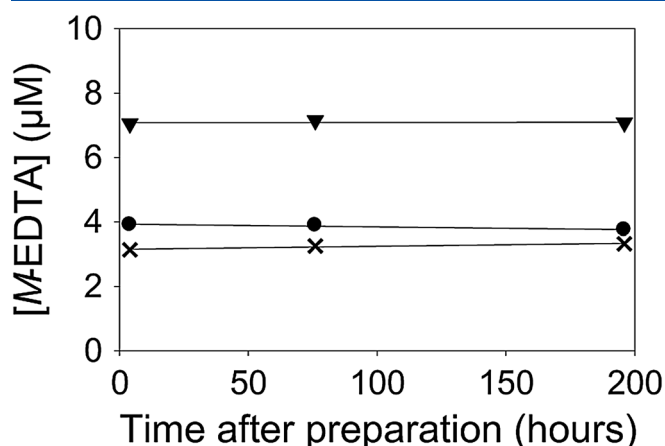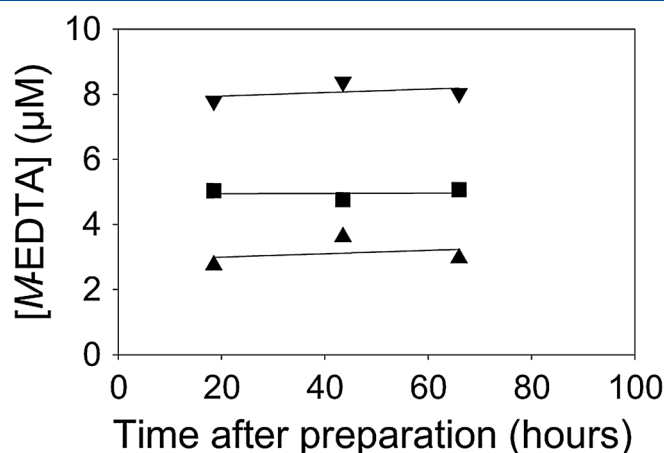

Figure 5. Metal complex concentration *versus* equilibrium time determined by ESI-MS in ACN/TEAA. ● [Cu-EDTA], x [Ni-EDTA], ▼ total amount [*M*-EDTA]. 8 µM EDTA was added in each sample solution.

Figure 6. Metal complex concentration *versus* equilibrium time determined by ESI-MS in MeOH/TEAA. ● [Cu-EDTA], x [Ni-EDTA], and ▼ total amount of [M-EDTA]. 8 µM EDTA was added in each sample solution.

Figure 7. Metal complex concentration *versus* equilibrium time determined by ESI-MS in ACN/TEAA. ▲ [Co-EDTA] µM, ■ [Zn-EDTA] µM, and ▼ total amount of [M-EDTA]. 8 µM EDTA was added in each sample solution.

prepared initially. This difference due to time at pH 5 could explain why the results between the systems for Ni-EDTA at pH 5 presented in Table 1 also differs, because the latter measurements were performed at least 1 day after sample preparation in ACN/TEAA. Thus, according to the results shown in Fig. 5, it is not surprising that the determined conditional stability constant for competing Ni^{2+} at pH 5 was higher compared with the Cu-EDTA literature value. The change in $MeOH/NH_4OH$, according to Fig. 6, probably would not be so drastic and therefore leads to lower determined conditional stability constants, which is also reflected in the results shown in Table 1. It has been observed by others that mixtures of Ni^{2+}, Cu^{2+}, and EDTA in aqueous solution show slow kinetics.[4,5] Usually, equilibrium was reached within a few days and Cu-EDTA was complex with higher stability. However, as shown in Figs 5 and 6, equilibrium was not reached after 8 and 9 days, respectively, and the order of stability was the opposite. According to the obtained results from determinations of conditional stability constants in this investigation together with the aforementioned observations in pure water solutions,[4,5] the magnitude of the Cu-EDTA stability constants in different solutions follow the series water > 70 vol% TEAA/30 vol% MeOH > 70 vol% TEAA/30 vol% ACN. The lower concentration of Cu-EDTA in solution over time could be due to reduction of free Cu^{2+} ions to Cu^+ ions because of the presence of triethylamine in the buffer. Triethylamine has been observed to reduce Cu^{2+} in water,[48] and Cu^+ could then be kept stable in ACN/TEAA solution because the nitrogen atom in ACN stabilizes Cu^+ with complex formation.[49] Ni^{2+} ions, due to their divalency, form stronger complexes with free EDTA than Cu^+ ions do.[1] This would lead to higher amounts of Ni-EDTA and lower amounts of Cu-EDTA. In standard solutions with only Cu-EDTA complex present in 1 : 1 M ratio, both monovalent and divalent copper ions form complexes with EDTA, not competing with other metals and are thus not affected with time. Moreover, there are also only low amounts of free Cu^{2+} that can be reduced to Cu^+. In MeOH/TEAA, reductions can still occur but formed Cu^+ is not stable in water[49] or MeOH[50] and this could, therefore, explain why the change in MeOH/TEAA is lower and slower, see Figs 5 and 6. It should, however, be mentioned that integrated mass spectra in either positive or negative ion mode did not indicate any type of Cu^+-ACN or Cu^+Cl complexes nor free copper ions stripped of its solvent. In the ESI-MS measurements

shown in Fig. 7, the Co^{2+}, Zn^{2+}, and EDTA system show determined concentrations close to the expected, and the mutual order of complex binding strength follows what is reported in the literature at this pH: Zn-EDTA > Co-EDTA.[47]

Conclusions

A fast metal ion competition method has been developed to determine the conditional stability constant for strong M-EDTA complexes with ESI-MS. The new analytical method works for systems with complexes of M-EDTA close in strength. To ensure that the system is stable over time, calibration curves for both M-EDTA complexes should be used and the series repeated with sufficient time space. The choice and preparation of the solvent system have been shown to have a great effect on solution equilibria. The type of organic solvent and concentration should be carefully chosen to secure the correct function of the ESI-MS system but kept as low as possible to reduce the impact of the solvent on the equilibria in the sample solution. The strength of M-EDTA complexes is highly dependent on pH, but with a buffer and a high flow rate, the pH changes are reduced. The counterion from the metal salts has been shown to be of minor importance to the analyte response in the micrometer levels used in the experiments presented.

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