



Matteo Marafante, Silvia Berto
Università degli Studi di Torino, Italy
Gabriele Lando, Clemente Bretti, Concetta De Stefano, Demetrio Milea
Università degli Studi di Messina, Italy
Antonio Bianchi
Università degli Studi di Firenze, Italy
Katalin Varnágy
University of Debrecen, Hungary
Enrique Garcia-España
University of Valencia, Spain
Jana Havlíčková, Vojtěch Kubíček, Petr Hermann
Univerita Karlova (Charles Univesity Prague), Czech Republic

Recommended procedures for potentiometric determination of equilibrium constants of polydentate ligands and their metal ion complexes

<u>Content:</u>	<u>Page</u>
Introduction	3
1. General conditions	
1.1 Materials and stock solutions	
1.1.1 Strong hydroxide solution	4
1.1.2 Strong acid solution	5
1.1.3 Water	6
1.1.4 Background salt	6
1.1.5 Ligands	7
1.1.6 Metal ion stock solutions	7
1.2 Electrode system calibration & proton concentrations at high/low pH	8
1.3 Titrations	9
1.4 Data checking and fitting procedure	10
1.5 Publishing practice	12
1.6 General remarks	13
2. Titrations of H ₄ edta	
2.1 Introduction	13
2.2 Experimental	14
2.3 Results and discussion	15
2.3.2 Experimental conditions	16
2.3.3 Calibration of the electrode system	16

2.3.4 Evaluation of the H ₄ edta titrations	17
2.3.5 Evaluation of the Zn(II)-H ₄ edta titrations	18
2.3.5 Protonation constants of H ₄ edta	19
2.3.7 Stability constants in the Zn(II)-H ₄ edta system	20
2.4 Recommended values of the equilibrium constants	22
3. Conclusions	23
4. References	24

Introduction

In many research fields, not only in chemistry, numerous scientists are involved in the determination of stability constants for metal ion complexes in aqueous solution. The extent of these studies is represented by numerous compilations published under the auspices of the International Union of Pure and Applied Chemistry (IUPAC) [1–5] as well as several software containing critical [6] and non-critical [7,8] data. Interest in the formation of metal ion complexes is currently involved in numerous applied fields, ranging from the formation of complexes in biological fluids to the treatment of sewage. The example reported by Nancollas and Tomson [9] more than 40 years ago is still valid: “...the toxicity of cadmium to the grass shrimp (*Palaemonetes pugio*) was studied in the presence of chloride ion and the chelating ligand, nitrile-triacetic acid (NTA). Taking into account both chloride and NTA complexes of the metal ion, it was shown that the degree of toxicity was directly related to the activity of the free cadmium ion. In these cases, stability data are used simply as tools to control systems or processes or to understand the mechanism of biological and other reactions.”. Authors predicted correctly that “In view of the increasing need to understand the interactions between ions in diverse mixed electrolytes, investigators in almost all fields of science require reliable stability constants for their systems and conditions. Most workers in areas outside physical and inorganic chemistry are not trained in methods of stability constant determination.”.

Few years later, Braibanti *et al.*[10] published some recommended procedure for testing the potentiometric apparatus and technique for the pH-metric measurement of equilibrium constants of metal ion complexes by means of the well-known “**Nickel-Glycine Project**” in which seven laboratories active in the field of stability constant measurements were involved. The project investigated well-known systems, glycine (Hgly) and Ni(II)–Hgly. The general experimental conditions, agreed by all participants to be $t = 25\text{ }^{\circ}\text{C}$ and $I = 1.00\text{ mol dm}^{-3}$ (NaCl), aimed to assess those variables that influence the stability constants determination. The other experimental conditions (such as electrode type, methods for electrode system calibration, purity of reagents etc) were left to the arbitrary choice of individual researchers. All authors used doubly distilled deionised water and considered different reliable procedures for the preparation of solutions of strong acid, strong base, background electrolyte, NiCl₂ and glycine. Calibration of the glass electrodes in terms of hydrogen ion concentration was performed by means of a titration of standard hydrochloric acid solution with standard sodium hydroxide solution in the pH range 1.6–11.2. Parameters checked were E^0 , slope S and $\log K_w$, moreover corrections for the effects of junction potential changes, E_j , were included in the calibration procedure in the acidic pH region. The results of that intercalibration network were range of variation of each relevant equilibrium constant under the selected experimental conditions as follows in Table 1. The general discussion in the “Nickel Glycine Project” has been only recommendation of titration procedure till now. However over the years, a range of titrated systems of various properties has highly spread and simple amino acid – metal ion systems seems to be not sufficient for the current needs.

Table 1. Recommended ranges of values of equilibrium constants in the “Nickel Glycine Project”.[10]

Protonation constants	Stability constants
$\log K_1^H = 9.63 - 9.67$	$\log K_{NiL} = 5.54 - 5.68$
$\log \beta_2^H = 12.02 - 12.12$	$\log \beta_{NiL_2} = 10.24 - 10.48$

The present article is intended not to substitute the pioneering work of Braibanti *et al.* but it is more properly a refinement of their work as well as a reminder for all the scientific community. In fact, the reasons that pushed some of the masters in this discipline to publish such work are still valid and their purpose has not been accomplished yet (as also demonstrated by the only 131 citations of this paper in 36 years; September 2023). Thus, our aim is to refresh the necessity of such recommended procedures in which the system under analysis will cover problems connected with nowadays frequently studied systems with polydentate ligands those protonation/dissociation constants are spread through whole titration pH range and stability constants of their complexes are high (i.e. complexes start to form in acidic solutions) and chemical model can involve several complex species.

We choose well-known chelating H₄edta (EDTA) as a ligand and Zn(II)-H₄edta system as a test system. The reagents are generally widely available in a sufficient quality. Thus, the protonation of H₄edta and its zinc(II) complex stability constants were determined. Comparing to the “Nickel-Glycine Project”, improvement introduced in this exercise is a more relevant role of electrode system calibration, a robust statistical approach in evaluation of repeatability and significant differences of the results. The recommendation points are more widely discussed to make them easier to understand for a non-specialist researcher. Moreover, each phase of the process (i.e., solution preparation, experimental plan, analysis of the calibrations, analysis of the measurements, data elaboration) is standardized to avoid systematic variation due to different setups, experimental conditions etc. As for the “Nickel-Glycine Project”, this work provides intervals of variation within which the equilibrium constants of the H⁺/Zn²⁺/H₄edta system, determined in any laboratory should fall into, provided that the procedures used for the experiments strictly follow the recommendations in this report.

Although the recommendations below are especially necessary for examination of systems where whole pH range has to be used to obtain correct data for determination of equilibrium constants (and for the H⁺/Zn²⁺/H₄edta system as well), most of them are generally valid for determination of equilibrium constants by potentiometry (specifically, pH-metry) in aqueous solutions, as potentiometry is the most common method for determination of equilibrium constants.

1. General conditions

1.1 Materials and stock solutions

1.1.1 Strong hydroxide solution

- The main problem of stock strong hydroxide solutions is absorption of CO₂ from air.

- Hydroxide should have max. carbonate content below 0.5% w/w. Quality of the hydroxide solution should be quantitatively checked by Gran's method [11,12] regularly (weekly, or any time before a new set of titrations if the hydroxide solution was stored in a container directly on ambient air for a time longer than about one week). On daily basis, it is recommended to check the carbonate content in the stock hydroxide solution by calibration titrations (the general electrode system behaviour is tested by the same calibration titration, see below Chapter 2 “Electrode system calibration”). The unacceptable carbonate content influences behaviour of the electrode system in alkaline region (change of calibration parameters, change in logK_w value, systematic deviation of fit from the experimental points at pH >11). Solution with the high carbonate content must be discharged.

- Commercial “dilution-ready” NaOH or KOH solutions from well-established vendors can be generally recommended as “no-carbonate” hydroxide sources. The (Me₄N)OH solutions are not widely available and the commercial solutions

have to be carefully checked for carbonate and trimethylamine content. Alternatively, it can be prepared in laboratory from pure (with no Me₃N) salts (see below, Chapter 1.4).

- To prepare any hydroxide stock solution, only decarbonated water with a high resistivity ($> 18 \text{ mOhm cm}^{-1}$) must be used. The simplest procedure is: (i) boil water, (ii) bubble Ar/nitrogen (purity at least 99.99 %) through the hot water with cooling until water reaches room temperature and (iii) use it immediately for the hydroxide solution preparation. This decarbonated water can be stored directly on air in a well-closed flask for only very short period of time, max. hours, or under nitrogen or argon for months. It would be useful to add a soda lime trap mounted onto the stopper to avoid the presence of CO₂ after re-equilibration.

- Solutions used during titrations or for a short-term storage (days to about a week) must be stored in airtight alkali-resistant glass or PE/PP flask. **Caution!** Carbon dioxide slowly diffuses through polyethylene (PE) and polypropylene (PP) flask wall [13] and the PE/PP flasks with the stock hydroxide solution should not be in direct contact with air for a long time (a week or more). **Caution!** Some kinds of chemical glass are slowly dissolving in the stock hydroxide solution during a long contact time.

- It is not recommended to store the hydroxide stock solutions for weeks or months. If the long-storage is required, the stock solution has to be stored in airtight PE/PP flask under conditions which ensure no or minimal contact with CO₂ from ambient air (CO₂ content has to be checked in such long-time stored stock solution before its use). It can be ensured by storage of the well-closed plastic flasks inside a common glass desiccator over pellets of solid KOH or using soda lime traps mounted onto the stopper.

- To be used with background electrolyte ionic strength $I = 0.100 \text{ M}$, concentration of hydroxide stock solution should be 0.1–0.2 M. In the cases if more diluted strong hydroxide solutions have to be used, to keep desired ionic strength of the titrated solutions the background electrolyte salt can be added into the hydroxide stock solution (e.g., for $I = 0.1 \text{ M}$ it is KOH = 0.05 M + KCl = 0.05 M).

- Exact concentration of the stock hydroxide solution has to be always determined by an independent method and its precision should be below 0.5 relative % to the final hydroxide concentration – i.e. for instance $0.1012 \pm 0.0005 \text{ M}$. The recommended standard for determination of the hydroxide concentration is potassium hydrogen phthalate (with the highest available quality, dried at 110 °C for at least 2 h or to a constant weight before use).

- For preparation of the stock (Me₄N)OH solution, see another part (Chapter 1.4).

1.1.2 Strong acid solutions

- Commercial HCl (HNO₃ or HClO₄) solutions of the highest available quality.

- Concentration of the acid stock solutions should be adjusted according to the system to be used for, mostly 0.03 – 0.2 M (to be used with background electrolyte ionic strength $I = 0.100 \text{ M}$). However, solutions with higher acid concentration in the range are recommended since, with the higher the acid concentration, the lower the percentage error is.

- Exact concentration of the acid stock solution should be determined by an independent method with precision below 0.5 relative % to the final acid concentration – i.e., for instance $0.0998 \pm 0.0005 \text{ M}$. It should be determined against independent titrimetric standard, e.g., TRIS (TRIS = [tris(hydroxomethyl)methylamine] or anhydrous Na₂CO₃). These compounds should be used in the highest available quality, dried at 110 °C for at least 2 h or to the constant weight.

- The acid stock solution should be stored at tightly closed glass flask/container.

- If HNO₃ is used, attention should be paid to a possible occurrence of NO_x in the acid stock solution and presence of the oxides must be avoided.

1.1.3 Water

- Low-conductance water (>18 mOhm cm⁻¹) must be used for any stock solutions (hydroxide, acid, background electrolyte, ligands, metal ions) and for any titrations.

1.1.4 Background salt

- Commonly used salts are NaCl, NaNO₃, NaClO₄, KCl, KNO₃, or salts of organic quarternary cations (R₄N)X (the tetramethylammonium chloride - (Me₄N)Cl or tetraethylammonium perchlorate - (Et₄N)ClO₄ are the most commonly used organic salts).

- The background electrolyte salts should be of the highest analytical quality (residual content of any other metal ions in the bulk salt is important). Problematic salts are those with the (R₄N)⁺ cation; see also below for more details.

- Generally, quality of (Me₄N)Cl or other (R₄N)⁺ salts requires special attention. Main problem arises from residuals of methyl-amines (or other tertiary amines). Commercial samples of the salts, even with a high declared purity (99%), have to be purified to be used in titrations. For the most commonly used (Me₄N)Cl, recrystallization from an organic solvent (methanol or 2-propanol) is suitable (see below).

- Recrystallization of (Me₄N)Cl from boiling 2-propanol is based on a significant difference in solubility at about 90 °C vs. about 5 °C (fridge). Solution saturated at the high temperature is cooled and the crystallized salt is quickly filtered with minimal direct air access to the solid. Still “wet” solid is washed with minimal amount of cold 2-propanol (0–5 °C) and such “wet” salt is transferred into desiccator with P₂O₅ to completely remove any 2-propanol (1–2 days, vacuum can be applied). During the filtration, air should not go through the bed of the solid salt for a long time as this recrystallized salt is extremely hygroscopic. This recrystallized (NMe₄)Cl can be stored in desiccator with a good drying agent (e.g. P₂O₅).

- Recrystallization of (R₄N)X from boiling methanol is based on Perrin’s book.[14] Solution saturated at the high temperature is cooled and the crystallized salt is quickly filtered with minimal direct air access to the solid. Still “wet” solid is washed with minimal amount of cold MeOH (0–5 °C) and such “wet” salt is transferred into desiccator under vacuum. During the filtration, air should not go through the bed of the solid salt for a long time as this recrystallized salt is extremely hygroscopic. This recrystallized (Me₄N)Cl can be stored in desiccator kept under vacuum and with a good drying agent (e.g., P₂O₅).

- There is no universal background electrolyte. The background salt should be chosen according to the titrated system and/or expected area of application of the constants. Mostly, interference of the cations and/or anions of the background salt with the titrated systems should be preferentially taken into account.

- Examples of the interferences: Alkali metal ions strongly interact with polydentate ligands containing hard donor atoms (oxygen atoms); utilization of organic cations is then recommended. Protonated polyamines can form complexes with the anions (e.g. with nitrates); perchlorates should be chosen for such systems. Chlorides form stable complexes with soft metal ions as e.g. Pd²⁺ (perchlorates or nitrates should be chosen for such systems) or nitrates interacts strongly with hard metal ions as e.g. trivalent metal ions (perchlorates or chlorides should be chosen for such systems).

1.1.5 Ligands

- The ligand samples should be as pure as possible.
- Generally, the ligands must be chemically pure (> 99 %; checked by multinuclear NMR, HPLC, TLC etc.). Attention should be paid to minor impurities chemically similar to the titrated ligand.
- Special attention should be paid to “acid-base impurities” as non-stoichiometric amounts of acids (e.g. HCl, CF₃CO₂H or CH₃COOH), or bases as NH₃ (commonly as (NH₄)⁺ cation) and/or alkali metal ions (due to partially neutralized ligand in the sample) can be present. These “impurities” cannot be mostly detected by common spectral/chromatographic methods. There is no general method how to determine content of these impurities and methodology should be carefully chosen according to the studied system. Used form of ligands should minimize presence of such impurities. Examples can be well-characterized salts as M₂H₂edta (M = Na, K) or zwitter-ions (it is generally preferred form of amino acids and similar ligands for equilibrium studies).
- Ligands can be used in titrations as precisely weighted solids or as a stock solution with exactly known concentration (solution is recommended mainly if the solid is not fully stable, e.g. if it is hygroscopic). The ligand stock solutions should not be stored for a long time (for weeks or even months).
- Polydentate zwitter-ionic/anionic ligands (e.g. H₄edta) may cause leaching of glass components (Na⁺, Ca²⁺ and/or Mg²⁺) into the ligand stock solution if the solution is stored in a glass container over extended period of time. For such reason, after the preparation into a glass volumetric flask, LDPE or PP container should be preferred for storage.
- Contents/concentrations of the ligand and strong/weak acid/base in the ligand stock solution must be known. Generally, precision of the ligand concentration has to be below 0.5 relative % to the final ligand concentration. However, the precision of the ligand concentration has to be even higher for more-valent amines or (amino)acids (*i.e.*, if more dissociable protons are present on the ligand molecule itself, *e.g.*, more than four).
- Exact concentration of the all “strong” protons of polydentate ligands as polycarboxylic acids or polyamino-polycarboxylates (e.g. H₄edta or H₄dota), or polyamines (often obtained as hydrochlorides/hydrobromides) has to be determined independently wherever possible. It can be determined e.g. from elemental analysis (HCl/HBr content) and/or by acid-base titration to determine strong acid-strong base equivalent point.
- Exact ligand concentration should be determined by an independent method, according to nature of the ligand.
- Cations or anions present in the ligand stock solution should be the same as cations/anions used in the background electrolyte to ensure uniformity of ionic strength in the titrated solution.
- Stock solutions of “basic” ligands (e.g. organic amines or some amino acids) have to be prepared in decarbonized water (see above) and the stock solution has to be protected from CO₂ (see above). Alternatively, the amines can be neutralized with known amount of standard strong acid solution down to pH < ~4 to prevent the CO₂ absorption.
- For the H₄edta stock solution, commercial salts as Na₂H₂edta or K₂H₂edta with the highest available purity (e.g. “for analysis”) are pure enough and well defined for a direct preparation of the stock solution. The exact ligand concentration has to be determined with common standard chelatometric titration.

1.1.6 Metal ion stock solutions.

- Used metal salts should be as pure as possible.
- Contents of the metal ion and strong acid (e.g. added to stabilize the solution) in the stock metal ion solution must be known.
- The metal ion concentration in their stock solutions must be determined by a precise independent method(s) as gravimetry or chelatometry. Precision should be below 0.5 relative % to the final metal ion concentration. Similarly, the strong acid content should be known with precision below 0.5 relative % to the final proton concentration.
- Anions of the metal ion salts should be the same as those in the background electrolyte. If salt with such anion is not available, all interaction constants of salt anion in the titrated system should be known and considered in the final calculations.

1.2 Electrode system calibration & proton concentrations at high/low pH

- A pair of the indicating Ion Selective Electrode- H^+ (ISE- H^+) and the standard electrode, or a combined electrode can be used. The electrode system has to be appropriate for the pH range used and for the titrated system itself.
- Two-electrode systems with an appropriate salt bridge (the salt bridge should use the same salt as that in the background electrolyte in the titrated solution) are generally more precise and more stable than the combined pH electrode and thus recommended for measurements.
- Reference electrodes use concentrated solutions of salts (mostly KCl) which can diffuse into titrated solutions and thus the salts can alter conditions during potentiometric titrations. It should be taken into account mainly if combined electrodes are used. Alternatively, double junction combined electrodes are suitable to this purpose, since they allow for two junctions: the first between the reference electrode and the outer chamber of the probe and the second between the outer chamber and the solution. In this configuration it is recommended to change the solution into the outer chamber with a solution of the same electrolyte and at the same ionic strength of the measurement (*i.e.* 0.1 M KCl). In any case, the junction must be completely immersed into the solution to allow for the ion exchange and any hole present in the upper part of a combined electrode should be maintained open.
- Electrode system must have a fast electrical response to the proton concentration changes.
- Electrode calibration based on only two/three pH points with buffers should be avoided as it is, generally, not precise enough.
- Concentration pH scale should be used (as definition of reactant activity is generally problematic).
- Electrode calibration should be done by a titration of strong acid solution with a strong hydroxide solution of known concentrations under conditions of the equilibrium studies (mainly, the same ionic strength) and within the same pH range as for the measurements. The pH/titration points around the equivalence point should be skipped due to their large errors in the potential reading. A number of titration points in acidic and alkaline regions should be ideally close each other; at least 15 points in each region is recommended.
- The calibration should be done in EMF (electromotive force) readings, not pH. The electrode system has to be stable, *i.e.* calibration parameters should not significantly change in two consecutive calibrations (*e.g.*, $\Delta E_0 < 1$ mV, $\Delta S < 0.15$ mV / pH unit at $t = 25$ °C).
- For titration pH range approx. 2.3–11.0, a simple Nernst equation (1)

$$E = E_0 + S \times \log[H^+] \quad (1)$$

is sufficient for data fitting to get calibration parameters (*i.e.*, E_0 and S) of the electrode system. Generally, response of most of electrode systems to the proton concentration is approximately linear in the pH range.

- For lower (pH < 2.3) and higher (pH > 11.0) pH ranges than that above, corrections for acid and alkaline electrode errors have to be introduced and fitting should be carried out with the extended Nernst equation (2)

$$E = E_0 + S \times \log[\text{H}^+] + j_A \times [\text{H}^+] + j_B \times K_w / [\text{H}^+] \quad (2)$$

where E_0 and S are as above, and j_A and j_B are corrections for the acid and alkaline electrode errors, respectively, and K_w is the water ionic product. Despite commonly large errors of the j_A and j_B values, it is recommended to use these parameters for titrations extended into the more acidic/alkaline pH.

- In both cases, the $\log K_w$ value should be calculated from the calibration titrations and compared with credible value of K_w from literature (or independently determined) under the same titration conditions (ionic strength, temperature). If the values are well comparable, it is an independent check of validity of the calibration procedure of the electrode system. However, the $\log K_w$ value should be fixed during final fitting of the calibration parameters.

- As a summary, if the calibration pH range is 2.3–11.0, the fit of E^0 and S is sufficient. If more acidic solutions are required (but no more acidic than pH ~1.7), j_a should be also determined. If more alkaline pH values are required (but no more than pH ~12), the determination of j_b or $\log K_w$ is relevant. Moreover, the refinement of the acid analytical concentration in the vessel (c_H^0) could be useful to check if acid or alkaline impurities are present.

- The alkaline error is the most pronounced in the presence of Na^+ in the background electrolyte and/or with titration with NaOH.

1.3 Titrations

- Titration solution temperature should be kept with precision ± 0.1 °C. Usually, double-wall thermostated equipment is used.

- Carbon dioxide must be avoided during the titrations. It is ensured by a constant pass of N_2 or Ar (purity at least 99.99%) through the titration vessel which is otherwise closed system. The gas has to be saturated with water vapours by prior passing through a solution of the same ionic strength as the titrated solution (thus, there are no concentration changes of the titrated solution due to water addition/removal).

- The titrated solution has to be well stirred.

- All stock solutions should be added to the titration vessel with the highest available precision and reproducibility; generally, in a microliter range. Uncertainty of the solution addition should be maximally 0.1 relative% of the pipetted volume.

- The electrode system should have a fast electric response (= stable EMF reading is reached quickly) to a pH change (usually in less than ~10 sec.) to distinguish between response of the electrodes and a possibly slow chemical reaction in the titrated systems. Slow continuous change of EMF reading (over several minutes or even longer) may indicate a slow chemical reaction involving release/consumption of protons (it should be checked by an independent method) and/or non-stability of the electrode system (a drift of the electrode system with time, see below). Chemical equilibrium must be reached before the EMF reading to get correct results.

- In general, EMF of electrode systems tends to spontaneously drift with time. Therefore, very long titrations (>12 h) could produce unreliable results and are not recommended.

- If slow chemical reactions are present in the titrated system (generally, it means that the reaction is not in equilibrium during several minutes in a particular titration point, pH is often continuously changing), “common” titration cannot be used, and general procedures described in this text are not reliable. In such cases, a special titration procedure (e.g. “batch” / “out-of-cell” titrations) is more suitable.
- Repeatability of the titration procedure itself should be checked first, e.g. running several titrations under identical conditions. Such titrations should be “identical”, ideally influenced only by “non-significant” random errors. In this sense, repeatability of the calibrations can be evaluated by running three calibrations before performing a measurement and variations of the refined E^0 should be within 1 mV. Moreover, linear fitting of calibration parameters (E^0 , S , j_a , j_b , $\log K_w$) with time should produce not significant slopes. This can be evaluated by a F-tests (at 95 % C.I.) comparing the Model Sum of Squares (MSS) and the Residual Sum of Squares (RSS) taking into account the degrees of freedom.
- The titrations have to meet requirements for the final statistical data treatment. Therefore, titrations carried out under one general conditions should be run with slightly changed starting conditions (e.g., slightly different overall concentration of the ligand or of the mutual ratio of the analytes). Only such set of titrations should be used for equilibrium constant calculations (see below, the Data Treatment chapter).
- At least two repeatable titrations obtained under identical general conditions (using mutually slightly different conditions) in (at least) two different sets of titrations (with significantly different conditions) have to be used for the final equilibrium constant calculations.
- Electrode system should be calibrated by an appropriate method and frequently (see also above). Three calibration titrations should be run at first after any long storage of electrodes (e.g. overnight) to check for E^0 stability before starting measurements (see above). Then, calibration should be run before each titration to get calibration-titration pairs which are then used for the titration data treatment. As a whole, a one day recommended workflow could be e.g. as follows: Calibration01 – Calibration02 – Calibration1 – analysis of the calibrations – Measurement1 – Calibration2 - Measurement2 – Calibration3 and so on until the end of the day.
- A number of titration points should meet a number of expected equilibrium constant (a gold rule is at least 10 titration points per constant in the pH range where the constant influences the solution composition).
- The titration pH range should cover pH’s where changes in solution composition (= speciation) are expected.
- Titrated ligand-to-metal ion ratios should cover stoichiometry of species expected in the chemical model for whole system. In each metal-to-ligand ratio, several titrations have to be carried out to meet requirements of statistical treatment of the experimental data (see below).
- Any precipitation in the titrated solution must be avoided. Titration points with suspected precipitation may not be used for data treatment. The precipitate might be hardly detectable. Continuous EMF reading drift in pH region where precipitate (e.g., metal hydroxide, insoluble ligand or complex) is expected can indicate it before visual detection. The possible precipitation can be also checked by other method (e.g. spectrophotometry).
- Contact of electrodes (mainly the glass electrode) with any precipitate should be minimalized as it can lead to non-reproducible EMF reading if the same electrode system is used in the following titrations.

1.4 Data checking and fitting procedure

- Well established program should be used for the equilibrium constant calculations. Nowadays, several programs able to calculate the equilibrium constants from a titration dataset are available (both free or licensed): MICROQL [15], SUPERQUAD [16], CHESS [17], BSTAC [18], HYPERQUAD [19], PHREEQC [20], OPIUM [21], ReactlabTM suite [22] and KEV [23].
- The approach used by the software to calculate the equilibrium constants is common to all of them: with given certain fixed parameters related to the instrumental apparatus and the nature of the samples, stoichiometry of the species formed, and the initial guess of values of the related formation constants, the software traces calculated titration curve. The calculated curve is compared with the experimental one and another curve is traced changing the parameters that the user sets as free (to allow their change during the fitting). The optimization of parameters is carried out minimizing the differences between the calculated and experimental curves and it will continue iteratively until the convergence criteria are satisfied. The user can choose the parameters to be refined and let their value free to change during the calculation. For example, if the user is interested in the study of the protonation constants of an acid, the value of the equilibrium constants of the protonated species will be set free to be optimized.
- The input data given to the program are the titration points obtained experimentally, as volume of the titrant added vs. EMF registered. Each analysed solution needs to be described in term of concentration of its components, initial volume of the solution, ionic strength, temperature and calibration parameters (see above, calibration parameters E_0 , S , j_A , j_B ; Equation 2). The calibration parameters should be determined from calibration titration by the appropriate software. Unfortunately, not all mentioned softwares are able to optimize the calibration parameters, and only few are able to estimate also j_A and j_B .
- Among the programs mentioned above, not all are able to consider all calibration parameters during the calculation. Therefore, the user needs to critically evaluate which parameters have to be considered in relation with the type of the titration performed (pH range, ionic strength changes etc.) and, consequently, use the most suitable software. Lastly, every time when a titration is computed (including the calibration one), an indication of the uncertainty on the fitting procedure and on the refined parameters must be given by the software and reported in the outcome report.
- A minimal number of titration (data) points per titration and a minimal number of various ligand-to-metal ratios depend on the system. At least 10–15 titration points should cover 20–80% of the maximal abundance of the each particular species in solution. Conditions in which any species has a maximum formation percentage below 10 % should be avoided. Similarly, conditions in which a species is formed at 100 % should be avoided as well, since no proton concentration change is guaranteed in such conditions with the consequence of fully unreliable results.
- Only repeatable titrations should be used for further data treatment and equilibrium constants calculations. Repeatability of the measurements should be evaluated by analysing each titration separately, and the results (*i.e.*, the refined constants) should be critically compared to avoid the presence of outliers. In particular, outliers Q-tests performed on each $\log K$ (at 95 % C.I.) are suitable to this purpose. If all tests pass, measurements can be further analysed; contrarily, measurements with outliers should be eliminated. Moreover, the statistical parameters of the fit, in terms of mean deviation (md) of the whole fit should be of the same order of magnitude of the uncertainty in the EMF reading (commonly 0.2–1.0 mV).
- All titrations should be analysed simultaneously for the final data treatment and the refinement of the final set of equilibrium constants ($\log K_w$ used in the final fitting should be computed taking into account values obtained from all calibrations and its value has to be explicitly declared). Uncertainties associated with the final values of equilibrium

constants should be assigned considering the repeatability of the measurements. As an example, the simultaneous fitting of four titrations lead to the value of $\log K^H = 10.202 \pm 0.002$, whereas data analysis of each single titration in the set gave the following values: 10.20 ± 0.01 , 10.21 ± 0.01 , 10.19 ± 0.02 , 10.19 ± 0.02 . Then, the actual value of the equilibrium constant should be: 10.20 ± 0.02 ; the “10.20” represents the value obtained considering all titrations, “0.02” is the confidence interval obtained multiplying the standard error of the mean by the critical t-value (for $\alpha = 0.025$ and $\nu = 3$).

- If several sets of titrations (*i.e.*, groups of titrations differing for a factor among each other in terms of instrumental apparatus, operator, reagents solutions etc.) of the same system were obtained, each set should be first treated separately as above reported and the results critically compared to check for statically relevant differences (*e.g.*, using t-test for paired samples).

- Metal ion hydrolysis constants should be used in calculation of systems involving metal ions (mainly, if the metal ion was in excess over the ligand and/or metal ion-ligand interaction is weak). Their values should be obtained under the same or, at least, very similar conditions. The values are generally taken from literature [8,24–26] and should be explicitly reported. Sometimes, it might be necessary to revise the metal ion-hydroxide stability constants found in databases/literature to be in accord with the ionic strength or temperature used for the measurements (*e.g.* if the recommended values collected in the database are thermodynamic constants at the infinite dilution). In this case, the calculations applied must be declared.

- For calculations of ligand-metal ion systems, the ligand protonation constants and the metal ion hydrolysis constants are usually fixed.

- For a correct titration set and relatively simple chemical model, standard deviation (in logarithmic scale) of the equilibrium constant values obtained directly from the data fitting should be in the second decimal digit. It indicates good repeatability of the titrations but it is not real uncertainty/errors of values of the constants; these uncertainties originate from mathematical treatment of the data and/or repeatability of the titration procedure. These errors of equilibrium constants have to be published/presented together with values of the constants (see also below).

- Real errors of values of the equilibrium constants depend on the system studied, experimental conditions, data treatment etc. and cannot be easily defined.

- Fitting the experimental data with various chemical models (a number of the species involved, various stoichiometries of the species, mutually interchanged species etc.) should be done. The final chemical model must have the statistics as good as possible and, mainly, it must be “chemically sound” (*i.e.* it must be chemically “allowed” in the studied system).

- Correctness of the chemical model should be checked (wherever it is possible) by independent methods, mostly by the spectroscopic ones.

- The data for the final chemical model should be used for further discussions as *e.g.* calculation of distribution diagrams.

1.5 Publishing practice

- Values of the published constants have to be presented together with their fitting errors (= the standard deviations).

- Potentiometric “Experimental” should contain the following information:

- Source and purity of chemicals used for the stock solution preparation.
- Source and purity of the investigated ligands.
- If specifically important, information about preparation, composition and storage of the stock solutions.
- Information about equipment used in the titrations (electrodes, pipetting, titration system itself).
- Temperature and ionic strength (the salt and its concentration).
- Information about the electrode system calibration. Water ionic product value has to be listed explicitly.
- Information about the titrations themselves: ligand and metal ion concentrations in the titration vessel, metal-ion-ligand ratio(s), pH ranges of the titrations, a number of data points per titration, a number of parallel titrations run under identical conditions, gas used to keep inert atmosphere, nature and concentration of the titrand (the most commonly, it is a strong hydroxide solution), starting volume of the titrated solution.
- Time necessary to reach equilibrium (= stable pH reading) in particular pH range(s) after the titrand addition.
- Source and values of equilibrium constants used as fixed constants during data treatment. Typically, metal ion hydrolytic constants.
- Information about strategy used for the data treatment.
- Information about programs/software used for the data treatment.
- Information how the published chemical model was chosen and confirmed.

1.6 General remarks

The recommendations and comments above are considered as the best practise for determination of equilibrium constants by pH-metry as it is the most common method in studies dealing with metal ion-ligand equilibria. Most of the recommendations are applicable for other potentiometric methods used in studies of equilibrium constants. If the rules above would be followed, the equilibrium studies should produce reproducible data and the obtained constants should be reliable.

The rules above are especially important for systems where chemical composition of titrated solutions is changed over a very wide pH range and titration points from acidic and/or alkaline regions are necessary. It is a common case for polydentate ligands containing multiple protonation/metal ion-binding sites. Typically, polyamino-polyacids, polyacids or polyamines can be classified as such ligands. Following the rules is also very important for systems where the speciation chemical model is complicated and really “good” experimental titration data are required.

2. Titrations of H₄edta

2.1 Introduction

Polydentate ligands contain a number of donor atoms which can be bound to the central metal ion. Due to a possible formation of a number of chelate rings, they are considered as strong chelating agents and they found a number of

applications in various scientific and industrial fields. Therefore, solution chemistry of the ligands and their complexes has been widely studied and the systems belong among the most frequently investigated. Equilibrium studies involving these ligands bring several own problems, not so important for other families of ligands, and the text below is trying to address them.

Most of these ligands can be considered as poly-acids or poly-bases and, the most commonly, they contain several acid as well as basic sites in their structures. As the ligands contain a lot of donor atoms binding metal ion(s) and the same donor atoms can be protonated in aqueous solutions, a number of the ligand protonations can be observed. In addition, the protonations are often spread over entire water pH range and they also take place in very acidic and/or very alkaline solutions. Knowledge of the corresponding protonation constants is the first challenge which has to be solved. Thus, there are a lot of protonation constants, frequently with similar values, and knowledge of the very low and/or very high protonation constants is often a key parameter for a correct evaluation of ligand-metal ion systems involving the ligands. The complexes are often very thermodynamically stable (i.e. their stability constants are high) and, therefore, the complexes are formed even in very acidic solutions. The ligand-metal ion interactions can lead to a complicated chemical models involving a number of species: “normal” $[M(L)]$ complexes, protonated complexes, polynuclear complexes, hydroxidospecies, isomeric complexes, coordination polymers etc. These properties call for very good titration data to determine stability constants and to choose the right chemical model. There is necessary to obtain data even from low and high pH regions, close to limits for utilization of glass electrode for determination of concentration of free protons in the solutions. In such cases, the above rules have to be followed as much as possible to obtain reliable and reproducible titration data.

The $H_4\text{edta}$ (EDTA) is well known polydentate ligand and its salts are available anywhere with a very good purity. It contains six donor atoms which are binding to a single central metal ion. Six protonations are possible and $H_4\text{edta}$ protonation constants are spread through a wide pH range. Its Zn^{2+} complexes are formed in an accessible pH range, and the chemical model also involves protonated and hydroxidospecies present in the $Zn(II)-H_4\text{edta}$ system at a various solution pH. Its complexes are formed quickly and no problems with a slow kinetics of complex formation are expected in the system. Zinc(II) hydrolysis is relatively well defined. Thus, it fulfils requirements for a ligand suitable for testing of whole procedure for determination of equilibrium constants of polydentate ligands, with the recommendations listed above in mind.

The following procedure is intended to be used mostly by newcomers to the field or those who wants to test whole aspects of determination of equilibrium constants in a lab. Therefore, the ligand ($H_4\text{edta}$) and metal ion (Zn^{2+}) were chosen due to their availability and relatively accessible solution behaviour.

2.2 Experimental

Chemicals: Deionized (high-resistive) decarbonated water (as defined above) was used throughout the experiments. “Dilute-in” aq. KOH and/or aq. HCl solutions purchased from established suppliers of laboratory analytical chemicals. The KOH solution may not contain any carbonates (checked by Gran method). Other chemicals: $K_2H_2\text{edta}\cdot 2H_2O$ (at least 99%, extra pure), $ZnCl_2$ (analytical grade, better than 99%), KCl (for analysis 99+%), potassium hydrogen phthalate (99.99%, acidimetric standard, must dried to constant weight), anhydrous Na_2CO_3 or TRIS (99.99%, acidimetric standard, must dried to constant weight). The pH is defined as $pH = -\log[H^+]$ for all systems.

Stock solutions: The KOH stock solutions (~ 0.1 M; required precision is defined above) were prepared by dilution of the commercial solution with decarbonized water and were standardized against potassium hydrogen phthalate.[27] The HCl stock solutions (~ 0.02 – 0.04 M, required precision is defined above) were prepared from commercial aq. HCl and were standardized against Na_2CO_3 or TRIS.[27] The $\text{K}_2\text{H}_2\text{EDTA}$ stock solutions were prepared by dissolving of exactly weighed commercial salt. The ligand concentration was determined from the weight of $\text{K}_2\text{H}_2\text{edta}$ and was mostly precise enough for the determination of the equilibrium constants. Alternatively, the concentration of the stock H_4edta solution can be determined by a standard chelatometric titration (the H_4edta concentration determined by chelatometry is generally more precise).

Electrode system and its calibration. Two-electrode set-up with a salt bridge (0.100 M KCl) or combination electrode were used (depending on experimental set-up in the labs). Calibration of the electrode systems was done by strong acid-strong base titration (here HCl-KOH, pH range from ~ 1.7 to ~ 11.8 – 12.0) with fitting of E_0 , S , j_A and j_B . Several calibration titrations were run in the beginning of titration experiments until the calibration parameters were stable. Calibration titrations were then run before and after each ligand or ligand-metal ion titration. This procedure gives calibration-titration pairs which were used for the data fitting. Through the text, “pH” means $-\log[\text{H}^+]$ and, thus, all constants are mentioned in the text are concentration constants.

Potentiometric titrations: For EMF reading, pH meter with a resolution of 0.1 mV has to be used. Ionic strength $I = 0.100$ M KCl and $25.0(1)$ °C (double-walled titration flask) was used for all titrations. Titration solutions were prepared from the appropriate amounts of other stock solutions and/or water to get the recommended starting volume ~ 10 – 25 ml. Electrode system should be the best available, response of the electrode system should be quick. Automatic burette to deliver the stock KOH solutions should be used (accuracy at least 0.3 relative %). The potential reading was considered to be stable within 0.2–0.3 mV per one minute, waiting time to read the potential was minimally about 25 seconds (depending on experimental titration set up in each lab); the waiting time should not exceed about two minutes. The N_2 or Ar (99.99%) saturated with water vapours by bubbling through KCl of the same ionic strength was used to protect titration solution against air CO_2 . The H_4edta concentrations in the titration vessel were ~ 0.002 and ~ 0.003 M. For the H_4edta -metal ion titrations, the L: Zn^{2+} ratio 1: ~ 0.95 – 0.98 was used. For each system, two sets of at least three titrations for each H_4edta concentration were obtained (i.e. at least 3+3 titrations were used for calculations of the constants for each system). The pH ranges were from ~ 1.7 – 1.8 to ~ 11.8 – 12 . for determination of H_4edta protonation constants and for the Zn(II)- H_4edta systems, respectively. At least about 60 points per each titration was obtained (recommendation is to get at least 10 points per constant; for the H_4edta systems, it means ~ 60 titration points per titration). Calibrations were carried out in the same pH ranges.

Data fitting: To estimate the desired constants, a proper software for titration processing has to be used. Some software were listed before. The program can be used for two main achievements. (i) To compute the electrode calibration curve refining the calibration parameters used to process the H_4edta titrations (E_0 , S , j_A , j_B and $\log K_w$). (ii) To determine the desired equilibrium constants from the H_4edta and Zn(II)- H_4edta titrations. Furthermore, the software should be able to assess the uncertainty of estimated constants related to the calculation process. Here, several programs, BSTAC,[18] HYPERQUAD [19] and OPIUM,[21] were used for the data treatment. The used water ionic product was $\log K_w = -13.78$:[28] the constant was fixed during determination of equilibrium constants. The calibration HCl-KOH titrations (see above) were used to obtain E_0 and the other calibration parameters defined by Equation 1. These parameters were then applied to the corresponding H_4edta /Zn(II)- H_4edta titrations (calibration-titration pairs) used to estimate the desired

equilibrium constants. The Zn(II) aqua-ion hydrolytic constants were taken from literature.[8,26] The final equilibrium constants were obtained from a global fit involving all six titration-calibration pairs for both H₄edta and Zn(II)-H₄edta systems. The ligand protonation constants were fixed in the calculation of stability constants of the complexes.

2.3 Results and discussion

As already described above, the aim of this text is to establish, describe and test experimental procedures and important aspect connected with equilibrium constant determination by means of potentiometry. The H₄edta was chosen as a good representative of polydentate ligands where the principles important for potentiometric titrations can be shown and tested. The H₄edta is one of the most frequently investigated ligands and values of its protonation constants and values of a lot of metal ion stability constants are known with a generally accepted correctness. [8,24] The constants have been determined under various conditions according to diverse applications of H₄edta. Solution properties of the ligand are a good illustration of behaviour of polyamino-polycarboxylates and other polydentate ligands. The H₄edta forms very stable complexes and, for most of metal ions requiring octahedral arrangement, anions of H₄edta can fill-up all donor positions and, thus, can form mostly only M:L 1:1 complexes. Work with H₄edta and metal ion-H₄edta systems is thus representative for this ligand family and can be considered as good systems to show various aspects and problems with determination of equilibrium data for polydentate ligands. The H₄edta presents constants in a wide pH range what is a common situation for such polyvalent acid/base. As its complexes are very thermodynamically stable, they are formed in acidic solutions. In the Zn(II)-H₄edta system, [Zn(L)]²⁻ as well as protonated, [Zn(HL)]⁻, and hydroxide-species, [Zn(L)(OH)]³⁻, are present and their stability constant can be determined. Such chemical models are commonly possible for systems containing polydentate ligands as H₄edta. The Zn²⁺ cation has a rather simple hydrolysis model and, thus, the Zn(II)-OH⁻ system has been reasonably well described.[8]

2.3.2 Experimental conditions

The conditions for the titration were chosen having in mind several aspects. Ionic strength 0.100 M KCl was chosen as K⁺ is less complexing cation than Na⁺. The HCl/chlorides are generally better defined reagents than HNO₃/nitrates and perchlorates cannot be used with K⁺ ion. The 0.1–0.2 M KOH were used as higher pH is easily accessible with this hydroxide concentration; there is a smaller dilution of the titrant at the end of titration and ionic strength still can be considered as almost constant (~10 rel.% change). Chlorides do not interact with Zn²⁺ cations and H₄edta only very weakly interact with K⁺. All stock solutions were standardized according to recommendation mentioned above. The 0.002 and 0.003 M concentrations of H₄edta were used as such concentration (H₄edta is a four-valent acid) is compatible with *I* = 0.100 M ionic strength up to end of titrations. Thus, the constants will be obtained at two different ligand concentrations, these ligand concentrations will not significantly contribute to ionic strength and the solutions are still enough concentrated to get detectable changes of solution composition during titrations with glass electrode. Starting volume of the titrated solution should more be than 10 ml, as lower starting volume requires high precision of all volumes of solutions added into the titration vessel (for the volumes, ionic strength change during titrations will not exceed approx. 10 rel.%). However, with very precise (±1 µl) and reproducible pipetting, the starting volume could be decreased (down to ~5 ml). A small excess of the ligand over the metal ion was used to avoid any precipitation of the metal hydroxide during titrations. The number of titrations and a number of titration points is a reasonably robust set of

experimental data, suitable for fitting. All the constants considered here are “concentration” equilibrium constants as only concentrations of all reagents are used here.

2.3.3 Calibration of the electrode system

Calibration of the electrode system has to be done by titration of a strong acid by a strong base (or much less frequently, *vice versa*). In most of cases, this method of calibration is more suitable than 2- or 3-buffer calibration; pH of buffer solutions is expressed in activity but most of determinations of equilibrium constants use concentration scale where pH is defined as $-\log[H^+]$. The electrode calibration by acid-base titration also allows for mathematical description of behaviour of the electrode system. Even more important, it also allows for a fast and easy check if electrode system suddenly starts to behave in an improper way. It is the main reason why the raw of calibration-titration-calibration-titrations etc. should be used. Calibration titrations must be run under the same conditions and in the same (or at least very close) pH range as the titrations of the studied systems.

The only equilibrium considered in the calibration model is the dissociation of water. Trying to fit the computed curve with the experimental one permits to refine the desired calibration parameters. In the pH range $\sim 2.0 - \sim 11.0$, considering only E_0 , S and the $\log K_w$ is sufficient to achieve good results. However in the case of $H_4\text{edta}$ titrations, the pH window has to be enlarged at least from 1.7 to 12.0. In this case, the contribution of liquid-junction potential described by j_A and j_B should be checked and taken into account. In this pH range, the extended Nernst Equation 2 is able reasonably describe acid and alkaline glass electrode errors. Unfortunately, as reported in Section 5.0, not all available software allow the user to refine all parameters. The ionic strength can be considered constant during the titration but some available software can consider also variations of the ionic strength along the titration. In this case, the initial ionic strength of the solutions has to be defined in the input data. In all the programs, the input data that have to be inserted into the calculation are the calibration curve as “added volume [ml]” vs “measured EMF [mV]” and the initial H^+ concentration in the solution. The titration points close to the titration equivalence point should be excluded as, in fact, they are affected by high uncertainty and, therefore, bearing less information about the parameters considered.

Once that the calibration parameters are obtained, the water ionic constant, $\log K_w$, should be checked in order to verify the quality of the output data of calibration titration fitting. The slope S should be no far than ~ 1 mV with respect the Nernstian theoretical value (for $t = 25$ °C, $S = 59.16$ mV) and the $\log K_w$ value should be comparable with the tabulated one for the same experimental conditions. Here, $\log K_w -13.78$ was used in the calculations (the best value for $I = 0.100$ KCl).[26,28] Moreover, the calibration parameters should be quite stable during the same working days if no maintenance operations were applied (e.g. salt bridge solution refilling). It also means that robust electrode system has to be used and it is mostly fulfilled only with high-end electrodes. It should be a good practice to evaluate the agreement between the calibration parameters collected on the same electrodes during the time of titrations experiments filling a control chart. This allows to verify if there are some outliers and to discard the corresponding calibrations and possibly also the corresponding titration of the measured system as pairs of calibration-titration are used for equilibrium constant determination (see below).

2.3.4 Evaluation of the $H_4\text{edta}$ titrations

Every titration considered should be coupled with a calibration (see above), that must be done right before or after the considered titration. The calibration has to be treated as mentioned before and the refined parameters obtained are used

for the analysis of the corresponding H₄edta titration (mostly) as a fixed parameters. The K⁺ ion forms a very weak complex with H₄edta and, thus, the protonation constants (manly the first one) determined according to the suggested procedure are slightly influenced. Therefore, the equilibrium constants determined here have to be considered as conditional constants (see also below). The interaction (variably strong) between cations (K⁺, Na⁺) of the background salt is a general phenomenon for polydentate ligands and it must be taken into account. The formation of these weak complexes leads to decreased values of the “highest” protonation constants and, consequently, to decrease of values of ML stability constants (values of metal ion stability constants directly correlate with values of ligand protonation constants). The get thermodynamic constants, non-coordination cations have to be used, e.g. quarternary ammonium cations.

While in the calibration, the only important concentration was the concentration of H⁺ in the titrated solution and in the titrant (expressed as OH⁻ concentration), the H₄edta concentration in the titration vessel must be known for the titrations of ligand containing solutions. The considered H₄edta-related equilibria during the calculation process include the water autoprotolysis and the formation of the variously protonated forms of the ligand. The most of the software optimize the overall protonation constants β_n defined as Equation 3 (charges of the species are omitted for clarity).

$$\beta_n = \frac{[H_nL]}{[H^+]^n \cdot [L]} \quad (3)$$

In the commercially available forms of H₄edta, it binds only a certain number of H⁺ equivalents, only these protons are considered in the total amount of available H⁺ in the titrated solution at start of titration. Here dipotassium salt of H₄edta was used and, thus two protons have to be counted for. Moreover, the total amount of H⁺ available to be titrated is not only influenced by the ligand amount ($c_{\text{ligand}}^{\text{H}^+}$) but also by the amount of the strong acid added before the titration. In fact, a certain amount of strong acid ($c_{\text{acid}}^{\text{H}^+}$) need to be added to the titration vessel in order to reach really acidic pH where the H₄edta is almost totally protonated. The quantity of H⁺ then will be given by Equation 4 and this amount is one of input parameters for the programs.

$$c_{\text{tot}}^{\text{H}^+} = c_{\text{acid}}^{\text{H}^+} + c_{\text{ligand}}^{\text{H}^+} \quad (4)$$

Therefore, the parameters to consider in the calculation routine are: (i) the calibration parameters (E_0, S, j_A, j_B) and $\log K_w$; (ii) the concentration/molar amount of ligand; (iii) the concentration/molar amount of available protons $c_{\text{tot}}^{\text{H}^+} / n_{\text{tot}}^{\text{H}^+}$; (iv) the concentration of the titrant; and (v) the starting volume of the titrated solution. The temperature and the ionic strength have to be inserted as information but they do not enter in the calculation if not explicitly requested. Once all the parameters are inserted into the input file together with the titration points, the refinement of the constants can be launched. The obtained values for the constants should be the same whereas the calculations consider different starting estimates of values of the constants; in fact, the obtained values should be the result of an iterative routine that reached convergence if the difference between the experimental and fitted curves is in minimum. The program should also be able to calculate and express an uncertainty (usually the standard deviation) associated to each equilibrium constant. That uncertainty is a useful indicator to evaluate the goodness of the refinement and the reliability of the estimated equilibrium constant.

2.3.5 Evaluation of the Zn(II)-H₄edta titrations

As above, every titration considered here should be coupled with an appropriate calibration. While studying the interaction between Zn^{2+} and $H_4\text{edta}$, more aspects need to be considered comparing to $H_4\text{edta}$ titrations. The appropriate calibration parameters are fixed. In this fitting, the ligand protonation constants have to be entered, mostly as fixed parameters. For $H_4\text{edta}$, they were determined as discussed in Section 8.3.4. Beside the acid-base equilibria of $H_4\text{edta}$ itself and the desired formation of $Zn(\text{II})\text{-}H_4\text{edta}$ complexes, the hydrolytic behaviour of Zn^{2+} cation needs to be taken into account. The most common hydrolytic species of Zn^{2+} are $[Zn(\text{OH})]^+$, $[Zn(\text{OH})_2]$, $[Zn(\text{OH})_3]^-$ and $[Zn(\text{OH})_4]^{2-}$. When computing the titration data, all these equilibria must be taken into account to obtain a correct and reliable estimation of the desired stability constants. Usually, the constants are taken from reliable sources, as e.g. those mentioned above.[8] The hydrolysis constants are entered into the calculations usually as fixed parameters. In the present calculations, the following values were used ($\log\beta_{Zn(\text{OH})} -9.15$, $\log\beta_{Zn(\text{OH})_2} -17.1$, $\log\beta_{Zn(\text{OH})_3} -28.39$ and $\log\beta_{Zn(\text{OH})_4} -40.7$).

Besides initial H^+ concentration/molar amount defined in Equation 3 (here, any additional protons present in the metal ion stock solution, e.g. to prevent metal ion hydrolysis, has to be added to the overall H^+ count) and the initial ligand concentration/molar amount, the initial concentration/molar amount of the metal ion must be known. All the data are the input parameters for the calculations. In general, approach to calculation of the stability constants is the same as it is used for the determination of the $H_4\text{edta}$ protonation constants.

Here in the $Zn(\text{II})\text{-}H_4\text{edta}$ system, there are more species to be considered in the chemical speciation model; the components are the Zn^{2+} , Cl^- (as counterion of Zn^{2+} and component of the inert salt defining ionic strength), $H_4\text{edta}$ (considered as totally non-protonated, $(\text{edta})^{4-}$), K^+ (as counterion of $H_4\text{edta}$ and the inert salt) and H^+ . The ions in the inert salt, K^+ and Cl^- , can be considered in the calculation as they are able to form weak ionic pairs with $H_4\text{edta}$ and Zn^{2+} , respectively. If those species are not considered in the speciation model, the constants obtained according this procedure are conditional constants valid for that particular system (where the medium is rich in K^+ and Cl^-). On the contrary, if these weak interactions would be considered, the obtained stability constants for the $Zn(\text{II})\text{-}H_4\text{edta}$ system would be valid for systems with different media or different counterions. As these weak interactions could be only very hardly defined, they are generally not considered for systems where very “strong” complexes (complexes with a high stability constant) are formed, as in the $Zn(\text{II})\text{-}H_4\text{edta}$ system. The ligand protonation constants has to be determined under identical (or at least very close) conditions as they mostly are, as in the present case, conditional protonation constants. Suitable chemical speciation model for the $Zn(\text{II})\text{-}H_4\text{edta}$ system involve three species.[8,24] Monoprotonated complex $[Zn(\text{Hedta})]^-$ is present in the acidic media. Complex $[Zn(\text{edta})]^{2-}$ is expected in the slightly acidic and neutral pH. Lastly, $[Zn(\text{OH})(\text{edta})]^{3-}$ hydroxide species was suggested to be present in alkaline solutions.

Taking these considerations into account, titration data for the $Zn(\text{II})\text{-}H_4\text{edta}$ system were calculated with the calibration parameters, protonation constants of $H_4\text{edta}$ and $Zn(\text{II})$ hydrolysis constants as fixed parameters, and with the above three $Zn(\text{II})$ complexes constants as fitted parameters.

2.3.6 Protonation constants of $H_4\text{edta}$

With data from the investigated pH range, with used equipment, with the above described electrode system calibration and data treatment, only four protonation constants ($\log K_{1-4} \sim 2-10$) could be reliably obtained (Table 1). The next protonation constant ($\log K_5 \sim 1.3-1.4$) [8,24] lays outside of the used pH range and, with the obtained set of titrations, its value cannot be determined fully correctly. It is caused by a relatively low abundance of the species in the investigated

pH range (< ~15–20%; see distribution diagrams, Figure 1). Its value strongly depends on correctness of calibration of the electrode system and, thus, on correct determination of acid error of glass electrode described by j_A parameter. As different software were used here, dispersion of its values is thus rather high. To determine the constant correctly, the titrations have to start at slightly lower pH with a special electrode calibration or another (spectral) method(s) have to be used. However, involving or omitting of the five-protonated species in the speciation model (and thus $\log K_5$ in the data treatment), also influences value of the closest constants, here value of $\log K_4$. In the most acidic solutions, involving/omitting the $(H_5L)^+$ species somewhat changes proton mass balance and values of the other constants (mainly $\log K_4$ here) are adjusted accordingly during the calculations (omitting $\log K_5$ increases value of $\log K_4$). Thus, the fit involving $\log K_5$ (despite to its scattered range) leads to a more reliable value of $\log K_4$. Anyway, equilibrium constants obtained in this work are values accessible under the used experimental conditions and data treatment, and value of $\log K_4$ as well as $\log K_5$ should not be considered as fully correct one for the fourth protonation constant of $H_4\text{edta}$. However, the values are primarily intended to be used for testing of whole titration/calculation procedure as they are determinable under the experimental conditions defined above; they are suitable for this purpose.

Table 1 Range of values of stepwise protonation constants ($\log K_n$) of $H_4\text{edta}$ obtained in this work ($I = 0.1 \text{ M KCl}$, $25 \text{ }^\circ\text{C}$) and their comparison with the values found in the commonly used databases.

Constant	Values interval (this work)	Average (this work)	Confidence Interval (C.I.)	NIST database [24b] ^a	IUPAC database [8] ^b
$\log K_1$	10.15–10.18	10.19	± 0.08	10.19	10.12
$\log K_2$	6.12–6.23	6.17	± 0.07	6.13	6.13
$\log K_3$	2.66–2.87	2.75	± 0.14	2.69	2.77
$\log K_4$	2.02–2.25	2.11	± 0.17	2.00	2.01 ^c
$\log K_5$	(0.9–1.3)	1.15	± 0.57	(1.5)	1.4 ^c
$\log K_6$	–	–	–	–	0.1 ^c

^a $I = 0.1 \text{ M}$, $25 \text{ }^\circ\text{C}$. ^b $I = 0.1 \text{ M KCl}$, $25 \text{ }^\circ\text{C}$. ^c $20 \text{ }^\circ\text{C}$.

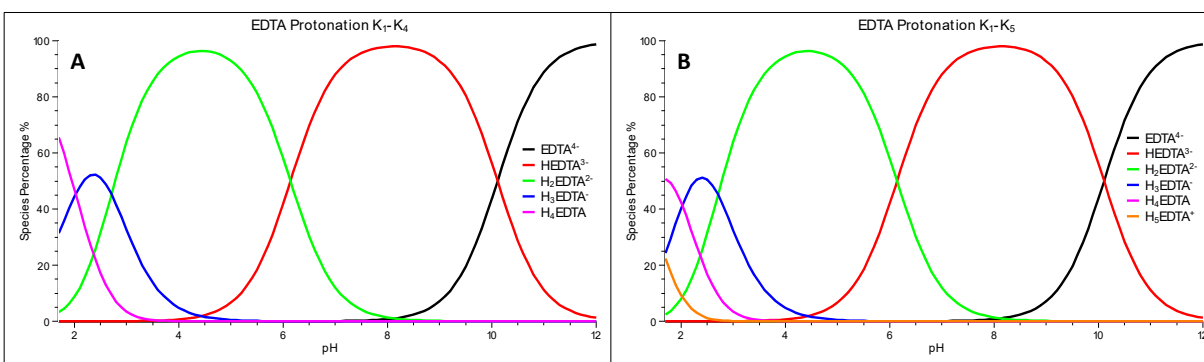


Figure 1: Distribution diagrams for speciation models involving four ($\log K_{1-4}$; **A**) of five ($\log K_{1-5}$; **B**) protonated ligand species ($0.003 \text{ M H}_4\text{edta}$, $I = 0.1 \text{ M KCl}$, $25 \text{ }^\circ\text{C}$) in the pH range used in this work. It was calculated with the average values of protonation constants given in Table 1.

2.3.7 Stability constants in the $\text{Zn(II)}\text{-H}_4\text{edta}$ system

The Zn(II)-H₄edta system was titrated only on one Zn:H₄L ratio as it is well-known that complexes with other stoichiometry than Zn:L 1:1 are not formed in the system. The system and entire experimental procedure shows several aspects which are important for systems of polydentate ligands with metal ions. It is necessary to titrate through a wide pH range to accumulate also suitable experimental data for determination of stability constants of the protonated complex and the hydroxido-species (see below).

Commonly, not all donor atoms of the polydentate ligands are bound to metal ions in acidic solutions and some of them can be protonated – it leads to a formation of protonated complexes. Mostly, various protonated complexes are present in solution together with differently protonated ligand species. To determine the stability of protonated complexes, there is necessary to know values of ligand protonation constants as all protonated species influence proton mass balance in the same pH range. For the Zn(II)-H₄edta system, the H₄L and (H₅L)⁺ species are present in the same range as protonated complex [Zn(HL)]⁻ and all species compete for protons. Therefore, to get the proper proton mass balance and correct $\log K_{\text{HZnL}} / \log \beta_{\text{HZnL}}$ constant, values of the acidic ligand protonation constants have to be known as correctly as possible. As the values of H₄edta protonation constants obtained in this work were used in the data treatment of the Zn(II)-H₄edta system, it is not fully true here. In the procedure used in this work, the $\log K_5$ was not determined correctly and $\log K_4$ is not known with a high correctness (see above). To get fully correct values of equilibrium constants in the Zn(II)-H₄edta system, the correct values of $\log K_5 / \log K_4$ have to be known and the titrations have to start from slightly lower pH (pH ~1.6). In the calculation here, five protonation constants of H₄edta were used as fixed parameters despite that correctness of the two acidic ones is not ideal. However as already stated above for the ligand protonation constants, the data presented here are intended for testing of procedures and it still fulfil the aim.

Values of stability constants of the hydroxido-species depend on correctness of metal hydrolysis constants used in the final calculations. The metal ion-hydroxido complexes influence proton mass balance in pH ranges where these species are present. It takes place mainly for weak complexes and in titrations with metal ion excess, mostly from slightly acidic pH up to high pH. The equilibrium constants describing the metal ion-hydroxide system should be known for identical conditions (or at least as close as possible conditions) as the performed titration experiment (see also above). For present data treatment, the values already mentioned above were used, as they we considered them as the most reliable.

In the current work, the chemical speciation model involving three complex species already defined for the system in literature was confirmed (Table 2). No other complex species could be detected. As it can be seen in the distribution diagram (Figure 2), there is about 30% of the “free” Zn²⁺ ion in the starting pH of the titrations. It is an ideal abundance for good determination of the stability constants of complexes. There is necessary to note that “free” metal ion abundance lower than ~15% in pH range of the titrations can lead to a systematic error of stability constant for the [M(L)] complex and, thus, to erroneous results. All complex species have abundance higher than the limiting once (~15–20%) and their stability constants were calculated with reasonable errors. The protonated complex is present in the same pH range as highly protonated ligand species (see also above). The hydroxide species start to be detected in pH higher than ~10 with maximal ~40% abundance at the end of titration. The distribution diagram confirmed that titration pH range is suitable for calculations of the stability constants. As stated above, these equilibrium constants have to be considered as conditional stability constants valid for the conditions used in the experiments. As with other amino acids, values of stability constant of the [Zn(L)] complex depends mainly on values of protonation constants which are assigned

to protonation of the amine groups; here, to the amino groups in the ethylenediamine backbone. Therefore, values of $\log K_1/\log K_2$ are decisive for values of $\log K_{ZnL}$.

There is a larger difference between the values for the complexes published in databases [8,24] and constants determined here comparing those for the ligand. Values of the stability constants depend not only on quality of the titration data but also on quality of the ligand protonation constants as also on quality of the metal ion hydrolysis constants.

Table 2. Range of values of stability constants of H₄edta obtained in this work ($I = 0.1$ M KCl, 25 °C) and their comparison with the values found in the commonly used databases.

Equilibrium ^a	Constant	This work	NIST database [24b] ^b	IUPAC database [8] ^c
$Zn + L = [Zn(L)]$	$\log K_{HZnL}$	16.40–16.50	16.5	16.25–16.5
$[Zn(L)] + H = [Zn(HL)]$	$\log K_{ZnL}$	3.10–3.20	3.0	3.0
$[Zn(L)(OH)] + H = [Zn(L)] + H_2O$	$\log K_{ZnLOH}$	11.8–12.2	11.6	—

^aCharges are omitted for clarity. ^b $I = 0.1$ M, 25 °C. ^c $I = 0.1$ M KNO₃, 25 °C.

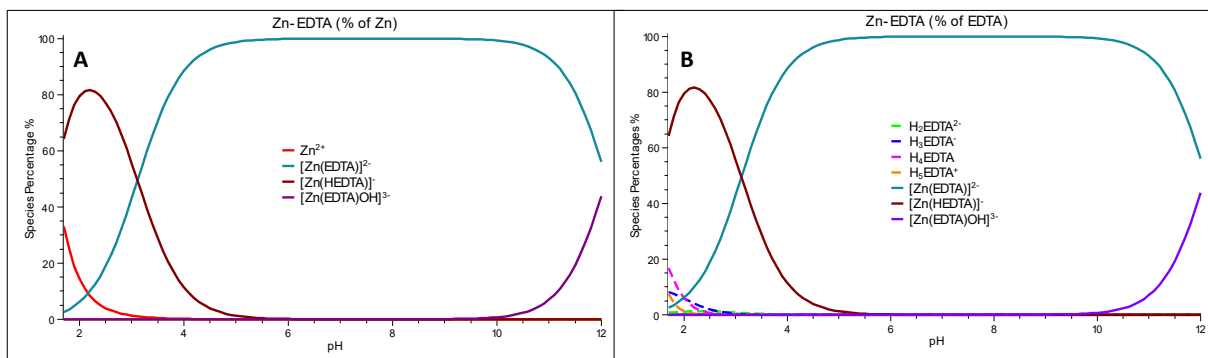


Figure 2: Distribution diagrams for the Zn(II)–H₄edta system ($c_{\text{edta}} = c_{\text{Zn}} = 0.003$ M, 0.1 M KCl, 25 °C); distribution diagram according to the metal ion (A) and distribution diagram according to the ligand (B). Calculated with average values of constants presented in Tables 1 and 2.

2.4 Recommended values of the equilibrium constants

This work is intended to provide a guide for newcomers into the field of determination of equilibrium constants by means of potentiometry and/or less experienced researchers in the field. It should be also a text which should guide students utilizing this methodology.

If the experimental procedure and rules described above in this text will be strictly followed and a suitable equipment and program for the data treatment will be used, the obtained equilibrium constants should be in the ranges shown in the Table 3. If these values will be reproducibly obtained, the tested laboratory procedure could be considered

as a correct one and values of equilibrium constants for new systems originating from the laboratory could be considered as reliable ones.

Table 3. Recommended values of H₄edta stepwise protonation constants and stability constants in the Zn(II)-H₄edta system.

Constant	Recommended range of the values
$\log K_1$	10.1–10.2
$\log K_2$	6.1–6.2
$\log K_3$	2.6–2.8
$\log K_4$	2.0–2.2
$\log K_5$	(0.9–1.4)
$\log K_{\text{HZnL}}$	16.4–16.5
$\log K_{\text{ZnL}}$	13.1–13.2
$\log K_{\text{ZnLOH}}$	11.8–12.2

There is necessary to point out that more problematic systems, e.g. those with complicated chemical model and a lot of species with various stoichiometry in the model, those with very basic ($\log K_1 > \sim 12$) and/or very acidic ($\log K_n < \sim 1.5$) protonation constants of ligands, those with very stable complexes (no “free” metal ion is present in solution at pH ~ 2 or below), those containing easily hydrolysable metal ions (e.g. M^{4+} ions) or those where kinetics of complexation reactions has to be considered, requires a special treatment and procedures, and the testing procedure suggested here would not be suitable. Correctness of such experimental approaches has to be tested in a different and appropriate way.

3. Conclusions

As a part of COST CA18202 (NECTAR) project, our aim was to define and describe experimental aspects which are connected with potentiometric determination of equilibrium constants in systems involving polydentate ligands. The ligands generally present a number of protonation constants and chemical models for their metal ion-ligand system can involve more different metal ion-ligand species. The H₄edta, other chemicals and conditions were chosen to be accessible to most of the laboratories using, or intending to use, potentiometry for determination of equilibrium constants.

However, the described procedures and aspect are valid for utilization of potentiometry in general, for other ligand and metal ion families. The text is based on long-term experience of the participating laboratories with potentiometric determination of equilibrium constants. The recommendations are intended as a “the best laboratory practise” to get reproducible and correct results from potentiometric measurements. If the experimental procedures described in this text would be followed, the values of the constants should be in the recommended range. It should be a test of potentiometric practice in labs where potentiometry is more or less frequently used as well as a starting point for laboratories which want to start with potentiometric measurements.

The values in Table 3 are considered as values which should be obtained if the procedures recommended above will be strictly followed. The acceptable range for values of the constants is based on data from five laboratories participating in this project while the groups followed the experimental procedure described in the Experimental part.

The values should not be considered as reference values of equilibrium constants for H₄edta and the Zn(II)-H₄edta system as, mainly in acidic solutions, the used experimental conditions were not fully covering necessary requirements to get fully correct equilibrium constants, as discussed in the text.

We believe that this text will help to improve reproducibility and correctness of published equilibrium potentiometric data and general knowledge of potentiometry as the most common method for determination of acid-base and stability constants.

4. References

- [1] G. Anderegg, F. Arnaud-Neu, R. Delgado, J. Felcman and K. Popov, Critical evaluation of stability constants of metal complexes of complexones for biomedical and environmental applications (IUPAC Technical Report). *Pure Appl. Chem.* **2005**, *77*, 1445–1495.
- [2] I. Sóvágó, T. Kiss and A. Gergély, Critical survey of the stability constants of complexes of aliphatic amino acids (IUPAC Technical Report). *Pure Appl. Chem.* **1993**, *65*, 1029–1080.
- [3] T. Kiss, I. Sóvágó and A. Gergély, Critical survey of stability constants of complexes of glycine (IUPAC Technical Report). *Pure Appl. Chem.* **1991**, *63*, 597–638.
- [4] L. D. Pettit. Critical survey of formation constants of complexes of histidine, phenylalanine, tyrosine, L-DOPA and tryptophan. *Pure Appl. Chem.* **1984**, *56*, 247–292.
- [5] G. Anderegg. Critical survey of stability constants of NTA complexes. *Pure Appl. Chem.* **1982**, *54*, 2693–2758.
- [6] A. E. Martell and R. M. Smith, *Critical Stability Constants*, Plenum Press, New York, 1974–1989; Vols. 1–6. (b) *NIST Standard Reference Database 46 (Critically Selected Stability Constants of Metal Complexes)*, Version 7.0; NIST, Gaithersburg, MD, 2003.
- [7] P. M. May, D. Rowland, E. Königsberger and G. Hefter, JESS, a Joint Expert Speciation System - IV: A large database of aqueous solution physicochemical properties within automatic means of achieving thermodynamic consistency. *Talanta* **2010**, *81*, 142–148.
- [8] D. Pettit and K. Powell, *IUPAC Stability Constants Database*; Academic Software, Otley, UK, 2004.
- [9] G. H. Nancollas and M. B. Tomson, Guidelines for the determination of stability constants. *Pure Appl. Chem.* **1982**, *54*, 2675–2692.
- [10] A. Braibanti, G. Ostacoli, P. Paoletti, L. D. Pettit and S. Sammartano, Recommended procedure for testing the potentiometric apparatus and technique for the pH-metric measurement of metal-complex equilibrium constants. *Pure Appl. Chem.* **1987**, *59*, 1721–1728.
- [11] F. J. C. Rossotti and H. Rossotti, Potentiometric titrations using Gran plots. A textbook omission. *J. Chem. Educ.* **1965**, *42*, 375–378.
- [12] M. Inoue and Q. Fernando, Effect of dissolved CO₂ on Gran plots. *J. Chem. Educ.* **2001**, *78*, 1132–1135.
- [13] S. Pauly, Permeability and Diffusion Data, in *Polymer Handbook*, 4th Ed., Eds: J. Brandrup, E. H. Immergut and E. A. Grulke, Wiley-Interscience, Chichester 1999, VI / p. 435.
- [14] D. D. Perrin and W. L. F. Armarego, *Purification of Laboratory Chemicals*, 3rd Ed., Pergamon Press, Oxford, UK, 1988, p. 392.

- [15] J. C. Westall, *MICROQL - I. A Chemical Equilibrium Program in BASIC*, Version 2 for PC's. Report 86-02, 1986. Department of Chemistry, Oregon State University, Corvallis, Oregon.
- [16] P. Gans, A. Sabatini and A. Vacca, SUPERQUAD: an improved general program for computation of formation constants from potentiometric data. *J. Chem. Soc., Dalton Trans.* **1985**, 1195–1200.
- [17] <https://chess.geosciences.mines-paristech.fr/home>
- [18] C. De Stefano, S. Sammartano, P. Mineo, and C. Rigano, Marine Chemistry - An Environmental Analytical Chemistry Approach. In *Computer Tools for the Speciation of Natural Fluids*. Kluwer Academic Publishers, Amsterdam 1997, pp. 71–83.
- [19] P. Gans, A. Sabatini and A. Vacca, Investigation of equilibria in solution. Determination of equilibrium constants with the HYPERQUAD suite of programs. *Talanta*, **1996**, *43*, 1739–1753.
- [20] <https://www.usgs.gov/software/phreeqc-version-3>
- [21] (a) M. Kývala and I. Lukeš, *International Conference, Chemometrics '95*, Pardubice, Czech Republic, 1995, p. 63; (b) M. Kývala, P. Lubal and I. Lukeš, *IX. Spanish-Italian and Mediterranean Congress on Thermodynamics of Metal Complexes* (ISMEC 98), Girona, Spain, 1998. <http://www.natur.cuni.cz/~kyvala/opium.html>
- [22] <https://jplusconsulting.com/products/reactlab-equilibria/>
- [23] A. N. Meshkov and G. A. Gamov, KEV: A free software for calculating the equilibrium composition and determining the equilibrium constants using UV–Vis and potentiometric data. *Talanta* **2019**, *198*, 200–205. <https://doi.org/10.1016/j.talanta.2019.01.107>
- [24] (a) A. E. Martell and R. M. Smith, *Critical Stability Constants*; Plenum Press: New York, 1974–1989; Vols. 1–6. (b) *NIST Standard Reference Database 46 (Critically Selected Stability Constants of Metal Complexes)*, Version 7.0; NIST, Gaithersburg, MD, 2003.
- [26] C. F. Baes, Jr. and R. E. Mesmer, *The Hydrolysis of Cations*, Wiley, New York, 1976.
- [27] *First Transition Series Metals*. In *Hydrolysis of Metal Ions*. P. L. Brown and C. Ekberg, Eds., Wiley-VCH, Weinheim 2016, Chapter 11, pp. 499–716. <https://doi.org/https://doi.org/10.1002/9783527656189.ch11>
- [28] K. Pratt, *Appendix A: Standard Solutions and Reference Materials*, in G. W. Latimer, Jr. (Ed.), *Official Methods of Analysis of AOAC International* (22), New York, 2023; online Ed., Oxford Academic, Jan. 4th, 2023. <https://doi.org/10.1093/9780197610145.005.001>, accessed Oct. 21st, 2023.
- [29] R. Fisher and J. Bye, *Bull. Soc. Chim. Fr.* **1964**, 2920–2929.