The Ca^{2+}-EDTA chelation as standard reaction to validate Isothermal Titration Calorimeter measurements (ITC)

C. Ràfols[a*], E. Bosch[a], R. Barbas[b], R. Prohens[b]

[a] Departament de Química Analítica i Institut de Biomedicina (IBUB), Universitat de Barcelona, Martí i Franquès 1-11, 08028 Barcelona, Barcelona (Spain)
[b] Unitat de Polimorfisme i Calorimetria, Centres Científics i Tecnològics, Universitat de Barcelona, Baldiri Reixac 10, 08028 Barcelona, Barcelona (Spain)

Abstract

A study about the suitability of the chelation reaction of Ca^{2+} with ethylendiamintetraacetic acid (EDTA) as a validation standard for Isothermal Titration Calorimeter measurements has been performed exploring the common experimental variables (buffer, pH, ionic strength and temperature). Results obtained in a variety of experimental conditions have been amended according to the side reactions involved in the main process and to the experimental ionic strength and, finally, validated by contrast with the potentiometric reference values. It is demonstrated that the chelation reaction performed in acetate buffer 0.1 M and 25ºC shows accurate and precise results and it is robust enough to be adopted as a standard calibration process.

Keywords

Isothermal Titration Calorimeter; ITC; ITC measurements validation; ITC chemical calibration; Energetics of Ca^{2+}-EDTA chelation

*Corresponding author: Clara Ràfols, e-mail: crafols@ub.edu

Introduction

Isothermal titration calorimetry is a very powerful technique to measure the energetics of chemical processes and it is widely used in studies of biochemical significance. It is able to measure directly thermodynamic quantities associated to any interaction event and this feature makes the technique very appreciated for research about interactions between non-simple chemical entities such as proteins, drug-protein, drug-RNA and others in fields as drug discovery or supramolecular chemistry (1).

However, accurate measurements of thermodynamic quantities associated to intermolecular interactions require a careful standardization of the calorimeter (2-4). A common way to evaluate the instrumental response is the measurement of a well-known physico-chemical process, which is taken as the standard. Several approaches, such as the dilution of NaCl (5) or propan-1-ol (6) with pure water, the protonation of 2-amino-2(hydroxymethyl)-1,3-propanediol (TRIS) (7, 8) or bicarbonate (9), the precipitation of silver halides (8), or the complexation of Ba^{2+} with 18-crown-6 (7) among others, were proposed with calibration purposes after careful selection of titration conditions. The calibration by means of a chemical reaction instead of a dilution process shows the advantage of the measurement not only of the process enthalpy variation but also the interaction stoichiometry and binding constant. Thus, several parameters can be used in the evaluation of the calorimeter response providing in this way a strongly robust procedure. However, several side reactions are involved in most pattern reactions and, consequently, the obtained data are a global measurement of the reaction energy including main and side processes (10, 11). Therefore, for a strict evaluation of the instrumental response, the experimental titration conditions (nature of the buffer, pH, ionic strength, temperature and others) must be rigorously controlled.

A promising and relevant reaction, the well-known chelation of Ca^{2+} with ethylendiamin tetraacetic acid (EDTA), was studied by Griko concluding that there is a strong dependence of binding thermodynamics on the buffer in which the reaction occurs (12). Nevertheless, the very convenient energetics of the reaction led MicroCal to test it as a calibration approach for their isothermal titration calorimeters and, in fact, the reaction was introduced as a test kit in the GE Healthcare (now Malvern Instruments). Later, Demarse *et al.* advised against the calibration application of this reaction arguing irreproducibility as a result of its high sensitivity to ionic strength and pH (9). In any case, the well-known complexing event and associated side reactions, the favorable binding energetics and the knowledge about the involved ionic equilibria lead us to explore the experimental titration conditions in order to establish a robust control of them. Thus, suitable validation methodology based in Ca^{2+}-EDTA chelation has been explored and finally proposed in this work. The achieved binding parameters are validated against the values accepted by the Critical Stability Constants compilation (13) confirming in this way the robustness of the suggested calibration procedure.

Experimental

Instruments

Two identical instruments VP-ITC (MicroCal, LLC, Northampton, Ma, USA) equipped with cells of 1.4047 mL and located in two different laboratories were used. ITC instruments were supplied with the ThermoVac accessory, a device for thermostating and degassing. The generated ITC data were collected automatically by the Windows-based Origin Software also supplied by MicroCal.

The pH measurements were performed by a GLP 22 potentiometer and a combined electrode Crison 5014 with a precision of ±0.002 pH units (Crison Instruments, Alella, Spain). The potentiometer was calibrated by means of ordinary commercial buffers of pH 4.01 and pH 7.00, from Crison Instruments.

Chemicals

HCl 1M and NaOH 0.5M Titrisols and sodium acetate anhydrous ≥ 99% were from Merck (Darmstat, Germany); 2-[N-morpholino]ethanesulphonic acid monohydrate (MES) >99% was purchased at Sigma (St. Louis, MO, USA); $CaCl_2 \cdot 2H_2O$ p.a. and $EDTA \cdot 2H_2O$ (disodium salt) p.a.-ACS 99% were from Panreac (Barcelona, Spain); water purified by a Milli-Q^R plus System from Millipore (Bedford, MA, USA) with a resistance higher than 18 MΩ is used.

Procedure

Solutions 0.1 M and 0.2 M of acetate buffer were prepared dissolving the anhydrous salt in water, adjusting the pH with HCl, and diluting to the final volume. Working in this way the ionic strength keeps constant and equals the buffer concentration. Solutions at pH 5.5 and: a) I=0.1 M, b) I=0.2 M were prepared. Solutions of MES buffer at pH=5.5 were obtained by partial neutralization of the basic form of MES with HCl (since the acidic form of MES is the commercial product, previous neutralization with NaOH is required). Buffer solution is diluted to get I=0.1 M.

The concentrations of $CaCl_2$ and EDTA were about 10^{-2} M and 10^{-3} M in acetic and MES buffer solutions, respectively, to keep the optimal titration conditions, that is the value of Wiseman parameter, C, between 5 and 500 (3), ($C = n K_{b(ITC)} c_s$, being n and $K_{b(ITC)}$ the expected reaction stoichiometry and binding constant, respectively, and c_s the concentration of the solution in the titration cell) (1, 3). All solutions were degassed before use.

To carry out the main titrations, the syringe is filled with $CaCl_2$ solution and the working cell with EDTA solution. Background titrations, performed with identical $CaCl_2$ solution but with the sample cell filled just by the buffer, allowed the determination of the dilution heat to be subtracted from the main experiment. The dilution heat of EDTA solution has been also investigated resulting in a negligible heat contribution. Titrations were performed with the two mentioned instruments randomly. The solution in the cell was stirred at 290 rpm by the syringe to ensure rapid mixing. Typically, 7.5 – 10 µL of titrant were injected during 20 seconds under control into a known volume of sample placed in the cell. The number of additions was from 30 to 40 with an adequate interval of 240 seconds between injections to allow complete

equilibrations. In addition, some ITC titrations were carried out at various temperatures (18.0, 25.0 and 29.5ºC, measured with a precision of ±0.2 ºC).

Calculations

Data were collected automatically and analyzed with the Origin program (one set of sites binding model) which uses a nonlinear least-squares algorithm (minimization of χ^2). To fit the heat flow per injection into an equilibrium binding equation, the software uses titrant and sample concentrations. It provides best fit values of the stoichiometry (n), involved enthalpy ($\Delta H_{b(ITC)}$), and binding constant ($K_{b(ITC)}$) at working conditions. Calculated parameters are conditional values since they are referred to the particular conditions of measurement and, in this work, are labelled with the subscript "ITC".

Results and discussion

It is well known that ITC allows the measurement of the global energy involved in any chemical interaction, resulting in an energetic evaluation of main and side reactions as a whole. For instance, most reactions of interest require buffered media, additional complexing agents or take place with any other concomitant process, all of them contributing to the finally estimated values. Obviously, this is not a minor detail when the energetics of an isolated process is required. In order to evaluate the quality of Ca^{2+}-EDTA chelate formation as a calibration standard, it is convenient the determination of the isolated reaction energetic parameters from a variety of experimental conditions. Thus, conditions such as buffer agent, working pH, ionic strength and temperature should be strictly controlled to subtract their effective contribution from the measured energetics. Finally, the confluence in thermodynamic final values should confirm the methodology and calculation approaches and allow the establishment of a robust working procedure.

a) Evaluation of Ca^{2+}-buffer interactions from literature data

The ITC binding parameters referred to the interactions of Ca^{2+} with common buffers at several pH values, from 6 to 9, were determined and are gathered in Table 1, which shows that only tricine and citric acid display significant binding constants with Ca^{2+} at working pH ($K_{b(ITC)(Ca^{2+}\text{-Buffer})}$) (14). Since the experimental ionic strength was not reported, concentration binding constants, that is, values corrected by the pH effect but not by the ionic strength ($K^{c}_{b(Ca^{2+}\text{-Buffer})}$) have been derived and included in Table 1, which also shows $K^{c}_{b(Ca^{2+}\text{-Buffer})}$ values calculated in this work for acetate buffer (13) and for Ca^{2+}-OH^- complex formation (16). To bear in mind the buffer capacity and energetics of used buffers, thermodynamic acidity constants and deprotonation enthalpies are also shown.

b) Evaluation of Ca^{2+}-EDTA chelate formation from literature data

Binding parameters (n, $\Delta H_{b(ITC)}$, $K_{b(ITC)}$) derived in Griko's work (12) together with working pH and ionic strength are given in Table 2 (note the simplified notation used for parameters

referred to the main reaction). Since selected buffers (PIPES, Imidazole, MOPS and TRIS) show low interactions with Ca^{2+} (Table1), values for experimental binding constants depend, almost exclusively, on the working pH, and they are about $2x10^6$ at pH 6.25 and $2x10^8$ at pH 7.5. This is because $K_{b(ITC)}$ is a conditional constant mainly affected by EDTA protonation degree.

Table 2 includes also the values published by Christensen et al. (14), that is, the binding parameters for Ca^{2+}-EDTA chelation corrected for metal-buffer interactions (n, $\Delta H'_{b(ITC)}$, $K'_{b(ITC)}$), as well as the experimental conditions of each titration. Even in this instance, the higher the pH the higher the $K'_{b(ITC)}$ value. As carefully demonstrated by Tellinghuisen (3), ITC titrations yield accurate parameter values (less than 5% of uncertainty) when right experimental conditions are chosen and these conditions imply measured binding constants in the $10\text{-}10^8$ range. Thus, as shown in Table 2, several results obtained at pH 8 were derived from displacement reactions whereas those resulting from direct titrations are only tentative values ($>2x10^6$). It should be noticed that the whole set of measurements (12, 14) were performed at low buffer concentration ($c \leq 0.02M$) and, then, no correction for ionic strength was considered by the authors. However, this assumption is not right for citric acid buffer since a significant ionic strength can be achieved at pH 6 (c = 0.02 M, I= 80 mM).

In this work, the Ca^{2+}-EDTA binding constant for the neat chelation reaction has been calculated from the whole set of values given in Table 2, according to:

$$K_{b(ITC)} = K_b^c \frac{\alpha_{CaY^{2-}}^{H^+}}{\alpha_{Ca^{2+}}^{buffer}\alpha_{Y^{4-}}^{H^+}} \quad (1)$$

$$K'_{b(ITC)} = K_{b(ITC)} \cdot \alpha_{Ca^{2+}}^{buffer} = K_b^c \frac{\alpha_{CaY^{2-}}^{H^+}}{\alpha_{Y^{4-}}^{H^+}} \quad (2)$$

and

$$K_b = K_b^c \frac{\gamma_{CaY^{2-}}}{\gamma_{Ca^{2+}}\gamma_{Y^{4-}}} \quad (3)$$

where the fully deprotonated EDTA is symbolized by Y^{4-}, K_b^c is the concentration constant of the isolated chelation reaction, α stands for the side reaction coefficient of the subscript species with the one indicated in the superscript (16), γ accounts for the activity coefficients of the indicated species which have been computed according to the Debye-Hückel expression and, finally, K_b is the thermodynamic binding constant, that is, calculated at zero ionic strength.

To get the thermodynamic formation constant of Ca-EDTA chelate, the whole set of K_b^c values given in Table 2 have been corrected according to Eq. (3). It should be noticed, however, that buffer preparation procedures are not reported in the original works (12, 14) and only their concentrations are given. Therefore, working ionic strength cannot be accurately estimated

and calculations were performed under the assumption that ionic strength equals the reported buffer concentration. Consequently, values obtained from citric buffer (pH 5.9) were omitted to compute the mean K_b value. Table 2 also includes the reported binding values resulting from displacement titrations buffered by NaOH at pH 13 and ionic strength 0.1 M, not corrected by metal-buffer binding (14). No EDTA protonation is expected at this very basic pH and, then, only corrections for Ca^{2+}-OH^- interaction (16) and ionic strength are involved in the derived thermodynamic constant, 10.84. However, this value is significantly lower than expected and, then, it is not included in final computation. This discrepancy could be explained by the high and steep increase of $\alpha_{Ca^{2+}}^{OH^-}$ parameter with the increase of pH in the very basic pH range (16) and the unavoidable poor precision in the pH measurement at pH around or higher than 13. Final K_b results show strong consistency.

Regarding to the enthalpy values, the following expressions have been used

$$\Delta H'_{b(ITC)} = \Delta H_{b(ITC)} - (1-\alpha_{Ca^{2+}})\Delta H^c_{b(Ca^{2+}-Buffer)} \quad (4)$$

and

$$\Delta H^c_b = \Delta H'_{b(ITC)} - \alpha_{H_2Y^{2-}}\Delta H_{H_2Y^{2-}} - \Delta H_{HY^{3-}} + (1+\alpha_{H_2Y^{2-}})\Delta H_{b(H^+-Buffer)} \quad (5)$$

where $\Delta H^c_{b(Ca^{2+}-Buffer)}$ is the binding enthalpy of metal-buffer interaction and ΔH^c_b stands for the enthalpy of the isolated main process. This last one involves the $\Delta H'_{b(ITC)}$ quantity and the contributions of EDTA deprotonation ($\Delta H_{H_2Y^{2-}}$ and $\Delta H_{HY^{3-}}$) and buffer protonation ($\Delta H_{b(H^+-Buffer)}$) (17). The symbols $\alpha_{Ca^{2+}}$ and $\alpha_{H_2Y^{2-}}$ stand for the mole fraction of the species pointed out in the subscripts. Thus, calculated ΔH^c_b depends only of the ionic strength of the solution.

Nevertheless, in this work it is assumed that $\Delta H_b = \Delta H^c_b$ since the reaction enthalpy can be considered independent of the medium ionic strength in the present working range. Thus, Samartano et al. (18-20) reported only slight enthalpy variations for several protonation processes along wide and higher ionic strength ranges, phytate (0.1-1M), several polycarboxylate anions (1-5M) and also constant values for the protonation of several amines (0-0.5M). Note that these studies refer exclusively to protonation reactions because of reliable studies about the effect of ionic strength on the enthalpy in the 0-0.1 M range for complexing reactions are not available in literature. In fact, our own results show only a small dispersion in the enthalpy values derived from various experimental conditions including the one computed from strongly basic solutions, pH=13, and ionic strength about 0.1 M (Table2). Then, the only exclusion in the enthalpy mean value computation has been the one derived from solutions buffered by imidazole because of the lack of information about the enthalpy associated to Ca^{2+}-imidazole interaction.

In summary, derived thermodynamic quantities for isolated EDTA-Ca^{2+} chelation at 25ºC are: $\log K_b$ = 12.24±0.18 (N=10) and ΔH_b = -6.35±0.85 Kcal mol^{-1} (N=13), being N the number of measurements involved in the mean values computation (Table 2). Literature values for these quantities are: $\log K_b$ = 12.42 at 25ºC and I=0; ΔH_b = -6.5±0.1 Kcal mol^{-1} at 20ºC and I=0.1 M; and ΔH_b = -7.2 Kcal mol^{-1} at 25ºC and I=1 M. Note that no ΔH_b value at I=0 is published but the one estimated in this work is close to those reported despite these last ones were obtained at higher ionic strength and at 20 or 25ºC (13). Thus, the quality of the obtained values, derived from measurements taken in a variety of experimental conditions, confirms this reaction as a right validation standard.

c) Evaluation of Ca^{2+}-EDTA chelate formation as a chemical calibration process from "in-house" experimental data

In this work, most ITC titrations were performed in 0.1 M buffers to ensure well buffered solutions and, then, the concentration of each species present in working conditions. No significant differences were obtained from the two curve-fitting approaches assayed (to subtract from the titration curve the entire blank curve point by point or just a constant value such as the mean of the last titration points). Then, the simpler second procedure has been adopted for further calculations.

To get proper and robust experimental $K_{b(ITC)}$ values, that is, close to the central value of the recommended range (3), acetate and MES buffers at identical pH (5.5), ionic strength (0.1 M) and temperature (25ºC) were used. Both buffers show low but measurable binding ability with Ca^{2+} (Table 1). Then, the differences, if any, in the experimental binding parameters should be attributed to the effect of the buffers. Nevertheless, as shown in Table 3, binding constant values measured from both solutions are strongly consistent and lower than those calculated from titrations at higher pH (Table 2), whereas the difference in enthalpy values should be attributed to the differences in acidic dissociation of buffers themselves plus the Ca^{2+}-buffer interactions in working conditions. Table 3 shows also the agreement of derived values, $\log K_b^c$ and ΔH_b^c, with those from Critical Stability Constants compendium (13) ($\log K_b^c$ = 10.65±0.08, I=0.1M and ΔH_b = -7.2 Kcal mol^{-1}, I=1 M, both quantities measured at 25ºC) and confirms the suitability of selected reaction and experimental conditions as a calibration tool. In addition, the thermodynamic $\log K_b$ value determined in this work (12.65±0.09; N=30) is consistent with that derived from literature data, 12.24 (Table 2), and with the reference one, 12.42 (13). However, the ΔH_b^c value is slightly different for both buffered solutions (about 1 kcal mol^{-1}) due, probably, to the used enthalpy values for buffers deprotonation. For instance, literature shows a variety of values for this quantity for acetate buffer at I=0.1 M, from 0.09 to 0.28, and, consequently, derived ΔH_b^c ranges between -7.12 to -6.77. In any case, a small but non-negligible dispersion is shown by ΔH_b^c among the complete data pool reported in Tables 2 and 3.

In summary, both assayed buffers seem to be suitable to support the chelating standard reaction, Fig 1, but acetate buffer is selected because of the higher simplicity in buffer

preparation. Thus, in order to evaluate the robustness of the measurements in a wider interval of experimental conditions, titrations in 0.2 M acetate buffer and several temperatures were also performed and results included in Table 3. Thus, measurements at 25ºC show a small decrease in log K_b^c value with the increase of ionic strength, whereas, as expected, ΔH_b^c keeps constant. On the other hand, log K_b^c values are temperature independent, but a slight decrease in ΔH_b^c is noticed with the temperature increase from 18 to 30ºC. Thus, Tables 2 and 3 summarize the robustness of the parameters obtained for the selected chelating reaction with respect to the buffer, pH, ionic strength and temperature and allow recommend it for ITC validation purposes. In particular, the measurements made in this work allow the conclusion that acetate or MES buffers can be successfully used for ITC instruments calibration. However, acetate is preferred because of the simplicity in the buffer preparation.

d) Recommended standardization procedure

Prepare acetate buffer 0.1 M and pH 5.5 by partial neutralization of sodium acetate with HCl solution. Prepare $CaCl_2$ and EDTA solutions about 10^{-2} M and 10^{-3} M, respectively, in acetate buffer and proceed to titration at 25ºC. Subtract the energy contribution of last point of titration from the whole titration curve. Use the appropriate software to calculate the titration parameters which should be in the following ranges: n= 1.07± 0.05, $\Delta H_{b(ITC)}$ = 1.80 ± 0.07 Kcal mol^{-1} and log $K_{b(ITC)}$ = 5.08 ± 0.02 or $K_{b(ITC)} = (1.2 \pm 0.1)10^5$ M^{-1}.

Conclusions

The study about the main experimental chemical conditions involved in the ITC titration of EDTA with Ca^{2+} shows the robustness of the reaction and allow the proper evaluation of thermodynamic parameters of the isolated chelating reaction. Thus, three interaction parameters (stoichiometry, reaction enthalpy and binding constant) can be determined in a well-designed single titration. Obtained values allow direct comparison with the reference quantities and, then, the easy evaluation of the instrumental response. Therefore, it is demonstrated the rightness of the selected chelation reaction as a calibration standard and it is strongly recommended as a useful and easy tool to calibrate ITC titration instruments.

Symbols list

$K_{b(ITC)(Ca^{2+}\text{-Buffer})}$: Ca^{2+}-Buffer conditional binding constant (involving side reactions, pH and ionic strength, I)

$K^c_{b(Ca^{2+}\text{-Buffer})}$: Ca^{2+}-Buffer concentration binding constant (involving ionic strength, I)

$\Delta H_{b(ITC)(Ca^{2+}\text{-Buffer})}$: Ca^{2+}-Buffer conditional binding e (involving side reactions, pH and ionic strength, I)

$\Delta H_{b(H^{+}-\text{Buffer})}$: Buffer protonation enthalpy

$\Delta H^{c}_{b(Ca^{2+}\text{-Buffer})}$: Ca^{2+}-Buffer binding enthalpy (involving ionic strength, I)

n: stoichiometry of Ca^{2+}-EDTA chelation

$K_{b(ITC)}$: Ca^{2+}-EDTA conditional binding constant (involving side reactions, pH and ionic strength, I)

$K'_{b(ITC)}$: Ca^{2+}-EDTA conditional binding constant (involving pH and ionic strength, I)

K^{c}_{b} : Ca^{2+}-EDTA concentration binding constant (involving ionic strength, I)

K_{b} : Ca^{2+}-EDTA thermodynamic binding constant (I=0)

$\Delta H_{b(ITC)}$: Ca^{2+}-EDTA conditional enthalpy variation (involving side reactions, working pH and ionic strength, I)

$\Delta H'_{b(ITC)}$: Ca^{2+}-EDTA conditional enthalpy variation (involving working pH and ionic strength, I)

ΔH^{c}_{b} : Ca^{2+}-EDTA concentration binding enthalpy variation (involving ionic strength, I)

ΔH_{b} : Ca^{2+}-EDTA thermodynamic binding enthalpy variation (I=0)

Acknowledgments

This work was supported by funds from the Spanish Government (Grant CTQ2014-56253-P). The experimental measurements of Mr. Pedro Trechera are also acknowledged.

References

(1) J.E. Ladbury, M.L. Doyre, Biocalorimetry 2, Wiley, Chichester, 2004

(2) I. Wadsö, L. Wadsö, Systematic errors in isothermal micro- and nanocalorimetry, J. Therm. Anal. Cal., 82 (2005) 553-558

(3) J. Tellinghuisen, Designing isothermal titration calorimetry experiments for the study of 1:1 binding. Problems with the “standard protocol”, Anal. Biochem., 424 (2012) 211-220

(4) L.D. Hansen, G.W. Fellingham, D.J. Russell, Simultaneous determination of equilibrium constants and enthalpy changes by titration calorimetry: Methods, instruments, and uncertainties, Anal Biochem., 409 (2011) 220-229

(5) J. Tellinghuisen, Calibration in isothermal titration calorimetry; Heat and cell volume from heat dilution of NaCl (aq), Anal. Biochem., 360 (2007) 47-55

(6) R. Adão, G. Bai, W. Loh, M. Bastos, Chemical calibration of isothermal titration calorimeters: An evaluation of the dilution of propan-1-ol into water as a test reaction using different calorimeters, concentrations, and temperatures, J. Chem. Thermodynamics, 52 (2012) 57-63

(7) C. Sgarlata, V. Zito, G. Arena, Conditions for calibration of an isothermal titration calorimeter using chemical reactions, Anal. Bioanal. Chem., 405 (2013) 1085-1094

(8) L. Baranauskiene, V. Petrikaite, D. Matulis, Titration calorimetry standards and the precision of isothermal titration calorimetry data, Int. J. Mol. Sci. 10 (2009) 2752-2762

(9) N.A. Demarse, C.F. Quinn, D.L. Eggett, D.J. Russell, L.D. Hansen, Calibration of nanowatt isothermal titration calorimeters with overflow reaction vessels, Anal. Biochem., 417 (2011) 247-255

(10) Y. Zhang, S. Akilesh, D.E. Wilkox, Isothermal titration calorimetry measurements of Ni(II) and Cu(II) binding to His, GlyGlyHis, HisGlyHis and bovine serum albumin: A critical evaluation, Inorg. Chem., 39 (2000) 3057-3064

(11) G. Garrido, C. Ràfols, E. Bosch, Isothermal titration calorimetry of Ni(II) binding to histidine and to N-2-aminoethylglycine, Talanta, 84 (2011) 347-354

(12) Y.V. Griko, Biophys. Chem., Energetics of Ca^{2+}-EDTA interactions: calorimetric study, 79 (1999) 117-127

(13) M.R. Smith, A.E. Martell, Critical Stability Constants, Vol 6 (Second Supplement), Plenum press, New York, 1989

(14) J.J. Christensen, D.M. Gooden, J.E Kung, E.J. Toone, Additivity and the Physical Bases of Multivalence Effects: A Thermodynamic Investigation of the Calcium EDTA Interaction, J. Am. Chem. Soc. 125 (2003) 7357-736

(15) R. N. Goldberg, N. Kishore, R.M. Lennen, Thermodynamic Quantities for the Ionization Reactions of Buffers, J. Phys. Chem Ref. Data, 31, (2002), 231-370

(16) A. Ringbom, “Complexation in Analytical Chemistry”, J. Wiley, NY (1963)

(17) J.J. Christensen, L.D. Hansen, R.M.Izatt, “Handbook of Proton Ionization Heats and Related Thermodynamic Quantities", Wiley, New York, 1976

(18) C. de Stefano, D. Milea, S. Sammartano, Speciation of phytate ion in aqueous solution. Thermodynamic parameters for protonation in NaCl, Thermochim. Acta, 423, (2004), 63-69

(19) C. de Stefano, C. Foti, O. Guiffré, S. Sammartano, Dependence on ionic strength of protonation enthalpies, of polycarboxylate anions in NaCl aqueous solution, J. Chem. Eng. Data, 46, (2001), 1417-1424

(20) A. de Robertis, C. Foti, O. Guiffré, S. Sammartano, The dependence on ionic strength of enthalpies of protonation for polyamides in $NaCl_{(aq)}$, J. Chem. Eng. Data, 47, (2002), 1205-1212

FIGURE CAPTION

Figure 1. ITC titration of EDTA with Ca^{2+}at pH 5.5, I = 100 mM and 25^{o}C. A: 0.50 mM EDTA and 5.10 mM Ca^{2+} in Acetate buffer.B: 1.02 mM EDTA and 9.70 mM Ca^{2+} in MES buffer

Table

Table 1: Ca^{2+}-Buffer binding parameters measured by ITC at 25 °C

Buffer	pKa[a]	ΔH buffer deprot. (kcal mol^{-1})[a]	Working pH[b]	$\Delta H_{b(ITC)}$ (Ca^{2+}-Buffer) (kcal mol^{-1})	$K_{b(ITC)}$ (Ca^{2+}-Buffer) (M^{-1})[b]	K^{c}_{b}(Ca^{2+}-Buffer) (M^{-1})
Acetic Acid	4.75	-0.098	-	1±0[d]	-	1.18±0.06 [d]
MES	6.270	3.537	6	-0.095±0.016[b]	3.7±0.1	9.9[c]
Citric Acid	6.396	-0.808	6	-0.13±0.47[b]	170.8±11.6	383.9[c]
Imidazole	6.993	8.757	-	-	-	-
PIPES	7.141	2.677	-	-	-	-
MOPS	7.184	5.043	8	0.90±0.03[b]	3.9±0.1	4.4[c]
HEPES	7.564	4.876	8	0.67±0.03[b]	6.7±0.3	8.8[c]
TRIS	8.072	11.341	8	-1.17±0.01[b]	3.4±0.3	8.0[c]
TRICINE	8.135	7.498	8	-2.41±0.11[b]	99.6±8.5	218.0[c]
			9	-4.33±0.20[b]	215.8±80.3	241.5[c]
NaOH	-	-	13	-	-	1.55[e]

[a] Ref. 15
[b] Ref. 14
[c] This work
[d] Ref. 13
[e] Calculated from Refs. 14 and 16

Table 2. Ca^{2+}- EDTA binding parameters at 25ºC from literature sources

Buffer	pH	Buffer conc.(M)	n	$\Delta H_{b(ITC)}$ (kcal mol^{-1})	$K_{b(ITC)}$ (M^{-1})	$\Delta H'_{b(ITC)}$ (kcal mol^{-1})	$K'_{b(ITC)}$ (M^{-1})	log $K'_{b(ITC)}$	ΔH^c_b (kcal mol^{-1})	log K^c_b	log K_b
MES	6[b, B]	0.01	0.97±0.02	---	---	-4.08±0.23	(2.01±0.05)x10^6	6.30	-6.85	11.73	12.44
Citric acid	6[b, B,]	0.02	1.04±0.02	---	---	1.58±0.12	(1.66±0.06)x10^5	5.22	-7.80	10.50	11.46
PIPES	6[b, B]	0.02	1.02±0.02	---	---	-2.50±0.05	(7.96±0.34)x10^5	5.90	-6.90	11.18	12.21
	6.25[a]	0.01	0.95	-3.50±0.15	(3.12±0.4)x10^6	-3.50	3.12x10^6	6.49	-7.70	11.38	12.10
	7.5[a]	0.01	1.01	-3.32±0.15	(1.04±0.6)x10^8	-3.32	1.04x10^8	8.02	-6.47	11.36	12.07
Imidazole	6.25[a]	0.01	0.93	-11.15±0.15	(1.84±0.4)x10^6	---	---	6.26	-4.50	11.27	11.98
MOPS	6.25[a]	0.01	1.01	-6.38±0.15	(2.26±0.4)x10^6	-6.37	2.26x10^6	6.35	-6.58	11.36	12.07
	7.5[a]	0.01	0.99	-5.73±0.15	(2.35±0.6)x10^8	-5.22	2.40x10^8	8.38	-5.71	11.75	12.46
	8[b, B]	0.02	0.96±0.02	---	---	-5.64±0.08	>2x10^6	---	-6.15	---	---
TRIS	7.5[a]	0.01	1.0	-11.97±0.15	(1.98±0.6)x10^8	-12.09	1.99x10^8	8.30	-5.15	11.67	12.38
	8.0[b, B]	0.02	1.01±0.01	---	---	-11.67±0.09	>2x10^6	---	-5.17	---	---
HEPES	8.0[b, A]	0.02	0.95±0.02	---	---	-5.25±0.12	(5.91±0.26)x10^8	8.77	-5.42	11.51	12.47
TRICINE	8.0[b, A]	0.02	0.94±0.03	---	---	-8.58±0.05	(2.97±0.42)x10^8	8.47	-6.28	11.21	12.17
NaOH	13[b, A]	0.1	1.05±0.02	-6.15±0.05	(1.03±0.10)x10^9	-6.36±0.05[c]	1.19x10^9	9.07	-6.36	9.08	10.84
									-6.35[d]		12.24[e]
								Mean:			
								Standard deviation:	0.85		0.18

[a] Ref.12

[b] Ref.14, A: displacement titrations, B: direct titrations

[c] This value includes the Ca^{2+}-OH^- formation enthalpy (ref. 16)

[d] Value from imidazole solution was omitted in the mean calculation

[e] Values from citric acid and NaOH solutions were omitted in the mean calculation

Table 3. Ca^{2+}-EDTA binding parameters measured in this work

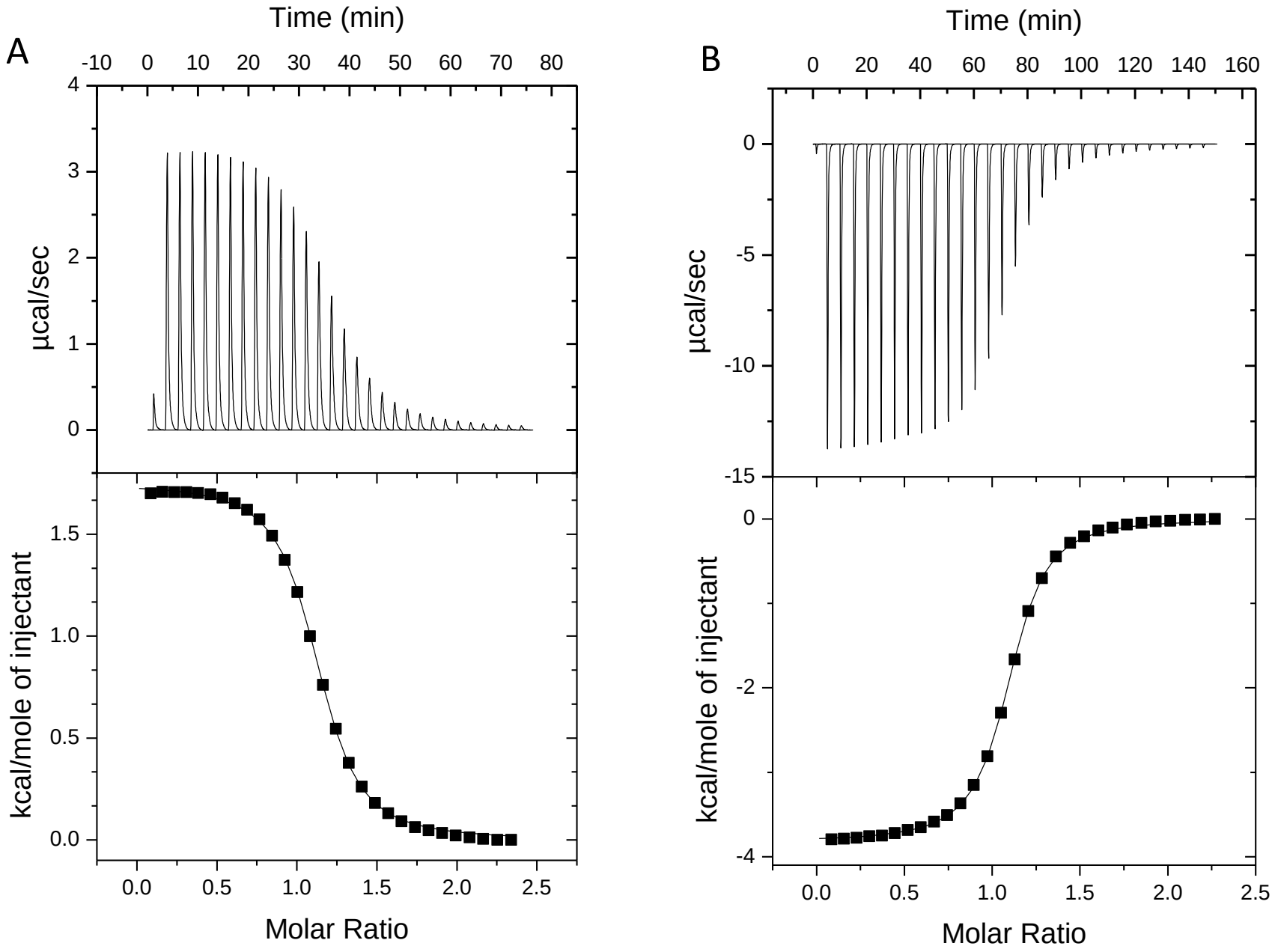
Buffer	pH	T (^{0}C)	I (M)	n	$\Delta H_{b(ITC)}$ (kcal mol^{-1})	$K_{b(ITC)}$ (M^{-1})	log $K_{b(ITC)}$	N	ΔH_b^c (kcal mol^{-1})[a]	log $K_b^{c\ b}$	log K_b
HAc/Ac	5.5	25.0	0.1	1.07 ± 0.05	1.80 ± 0.07	$(1.2 \pm 0.1)\cdot10^5$	5.08	24	-7.12	10.92	12.69
$MesH^+$/Mes	5.5	25.0	0.1	1.12 ± 0.01	-3.80 ± 0.03	$(9.5 \pm 0.2)\cdot10^4$	4.97	6	-6.23	10.73	12.49
HAc/Ac	5.5	18.0	0.2	1.10 ± 0.04	1.19 ± 0.02	$(1.05 \pm 0.1)\cdot10^5$	5.02	4	-6.91[c]	10.63	---
HAc/Ac	5.5	25.0	0.2	1.08 ± 0.01	1.48 ± 0.03	$(1.03 \pm 0.08)\cdot10^5$	5.01	14	-6.62[c]	10.62	---
HAc/Ac	5.5	29.5	0.2	1.04 ± 0.03	1.66 ± 0.07	$(1.2 \pm 0.2)\cdot10^5$	5.08	4	-6.64[c]	10.71	---

[a] Calculated from $\Delta H_{b(ITC)}$ and side reactions (EDTA protonation and Ca^{2+}-Buffer interaction)

[b] Calculated from log $K_{b(ITC)}$ and side reactions (EDTA protonation and Ca^{2+}-Buffer interaction)

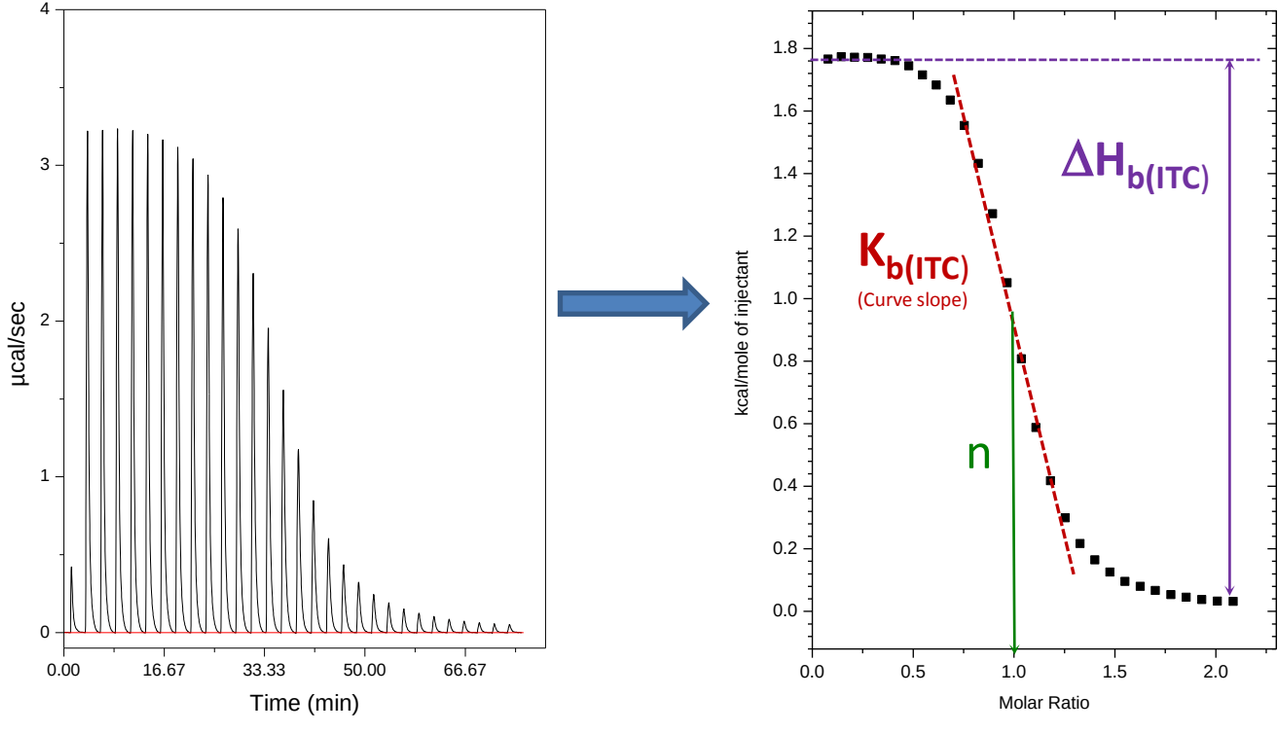
[c] Calculated using log $K_{Ca\text{-}Buffer}$ and $\Delta H_{Ca\text{-}Buffer}$ values at I=0.1M and T=25ºC

Figure


A
Time (min)
-10 0 10 20 30 40 50 60 70 80
µcal/sec
4 3 2 1 0
kcal/mole of injectant
1.5 1.0 0.5 0.0
0.0 0.5 1.0 1.5 2.0 2.5
Molar Ratio
B
Time (min)
0 20 40 60 80 100 120 140 160
µcal/sec
0 -5 -10 -15
kcal/mole of injectant
0 -2 -4
0.0 0.5 1.0 1.5 2.0 2.5
Molar Ratio

*Graphical Abstract (for review)

Isotermical Calorimetric Titrations



Chemical Standardization

$Ca^{2+} + EDTA \rightleftharpoons Ca\text{-}EDTA$

Buffer: Acetate, pH 5.5
Temperature: 25°C