

Investigation of metal–buffer interactions using isothermal titration calorimetry

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Abstract Isothermal titration calorimetry (ITC) and potentiometric titration (PT) methods were used to study the interactions of cobalt(II) and nickel(II) ions with buffer substances 2-(*N*-morpholino)ethanesulfonic acid (Mes), dimethylarsenic acid (Caco), and piperazine-*N,N'*-bis(2-ethanesulfonic acid) (Pipes). Based on the results of PT data, the stability constants were calculated for the metal–buffer complexes ($T = 298.15$ K, ionic strength $I = 100$ mM NaClO₄). Furthermore, calorimetric measurements (ITC) were run in 100 mM Mes, Caco, and Pipes solutions with pH 6, at 298.15 K. The enthalpies (ΔH) of the metal–buffer complexation reactions were calculated indirectly by displacement titration using nitrilotriacetic acid (H₃N₃T₃A) as a strong-binding, competitive ligand. Finally, to verify obtained results, the number of protons released by H₃N₃T₃A due to complexation of the cobalt(II) and nickel(II) ions was determined from calorimetric data and compared with results of calculations.

Keywords Isothermal titration calorimetry · Metal–buffer interactions · Potentiometric titration · Proton exchange · Thermodynamic parameters

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Introduction

Most of the biological and biochemical processes are proton-donor or proton-acceptor type of reaction. For this reason, biochemical experiments are usually carried out in a buffer solution [1, 2]. The same mechanism takes place during calorimetric measurements when the binding enthalpy (under isobaric conditions) is directly measured. Calorimetric methods such as the isothermal titration calorimetry (ITC) are now widely used to quantify stoichiometry, binding constants, and thermodynamics on the field of life science investigations (<http://www.microcal.com/reference-center/reference-list.asp>).

When a reaction involves the release (or uptake) of protons, additional heat is usually generated. This heat (thermal effect) is not connected with intermolecular interactions and should be taken into account while interpreting calorimetric data. The situation is slightly more complicated when metal ions are involved in biological processes [3–7]. In such cases, to determine condition-independent thermodynamic values (K , ΔH), the effect of buffer competition with the ligand for the metal as well as proton competition with the metal for the ligand must be taken into consideration.

2-(*N*-Morpholino)ethanesulfonic acid (Mes), dimethylarsenic acid (Caco), and piperazine-*N,N'*-bis(2-ethanesulfonic acid) (Pipes) (Fig. 1) are the buffer substances widely used in biological experiments as well as in calorimetric studies [8]. However, to the best of our knowledge, there are few reports on the interactions of such buffers with metal ions. This was, among others, the main reason that prompted us to embark on these studies.

In this article, thermodynamic parameters for complexation reactions of the Co²⁺ and Ni²⁺ ions with Mes, Pipes, and Caco buffer are presented. Metal–buffer (Mes, Pipes,

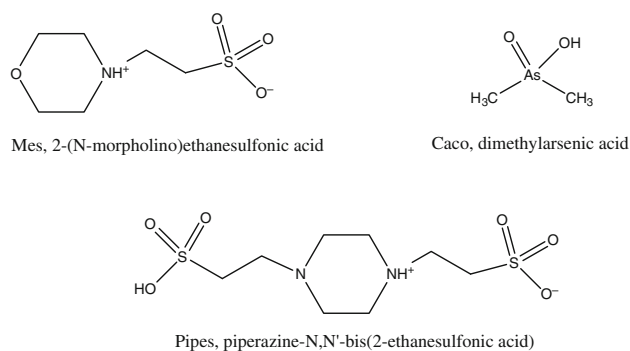


Fig. 1 Buffer substances used in this study

Caco) formation constants were determined by potentiometric titration (PT). Then, the enthalpies of the metal–buffer interaction were determined by displacement titration using the ITC technique [9]. Nitrilotriacetic acid (H_3NTA) was used in the displacement experiments as a strong-binding, competitive ligand. Finally, the effect of pH and the type of buffer on the enthalpy reaction and the stability constants of the resulting complexes were discussed.

Experimental

Materials

All reagents, namely 2-(*N*-morpholino)ethanesulfonic acid hydrate ($\geq 99\%$) (Mes), dimethylarsenic acid ($\geq 99\%$) (Caco), piperazine-*N,N'*-bis(2-ethanesulfonic acid) ($\geq 99\%$) (Pipes), $Co(NO_3)_2 \cdot 6H_2O$ ($\geq 99\%$), $Ni(NO_3)_2 \cdot 6H_2O$ ($\geq 99.99\%$), H_3NTA ($\geq 99\%$), $HClO_4$, and $NaClO_4$ were purchased from Sigma-Aldrich Chemical Corp. and used as received. Double distilled water with conductivity not exceeding $0.18 \mu S cm^{-1}$ was used for preparation of aqueous solutions of titrant and titrand.

Isothermal titration calorimetry

All ITC experiments were performed at 298.15 K using an AutoITC isothermal titration calorimeter (MicroCal Inc. GE Healthcare, Northampton, USA) with a 1.4491 mL sample and the reference cells. The reference cell contained distilled water. The data, specifically the heat normalized per mole of injectant, were processed with Origin 7 from MicroCal. An initial 2 μL injection was discarded from each data set to remove the effect of titrant diffusion across the syringe tip during the equilibration process. All reagents were dissolved directly into 100 mM buffer solution of Mes, Caco, or Pipes. The pH of the buffer solution was adjusted to 6 with 0.1 M $HClO_4$. The

experiment consisted of injecting 10.02 μL (29 injections, 2 μL for the first injection only) of ca 5 mM buffered solution of H_3NTA into the reaction cell which initially contained ca 0.25–0.5 mM buffered solution of suitable salt. A background titration, consisting of an identical titrant solution but with the buffer solution in the reaction cell only, was removed from each experimental titration to account for the heat of dilution. All the solutions were degassed prior to titration. The titrant was injected at 5-min interval to ensure that the titration peak returned to the baseline before the next injection. Each injection lasted 20 s. For homogeneous mixing in the cell, the stirrer speed was kept constant at 300 rpm. Calibration of the AutoITC calorimeter was carried out electrically by using electrically generated heat pulses. The $CaCl_2$ –EDTA titration was performed to check the apparatus and the results (n —stoichiometry, K , ΔH) were compared with those obtained for the same samples (test kit) at MicroCal.

Potentiometric measurements

PTs were performed in 30 mL thermostated (298.15 ± 0.10 K) cell using Cerko Lab System microtitration unit fitted with 0.5-mL Hamilton's syringe, pH combined electrode (Schott – BlueLine 16 pH type) and a self-made measuring cell equipped with magnetic stirrer. The temperature was controlled using the Lauda E100 circulation thermostat. The electrode was calibrated according to IUPAC recommendations [10]. Syringe was calibrated by weight method. All the solutions were prepared immediately before measurements (ionic strength $I = 100$ mM $NaClO_4$). The compositions of the titrand solutions used in the experiments were as follows: (1) Mes (18.16 mM) and $HClO_4$ (3.11 mM), (2) Caco (10.01 mM) and $HClO_4$ (3.10 mM), (3) Pipes (2 mM), (4) Co^{2+} (2.49 mM), Mes (18.16 mM), and $HClO_4$ (3.11 mM), (5) Ni^{2+} (2.46 mM), Mes (18.16 mM), and $HClO_4$ (3.11 mM), (6) Co^{2+} (1.97 mM), Caco (10.01 mM), and $HClO_4$ (3.10 mM), (7) Ni^{2+} (1.95 mM), Caco (10.01 mM), and $HClO_4$ (3.10 mM), (8) Co^{2+} (1.68 mM), Pipes (2 mM), (9) Ni^{2+} (1.69 mM), Pipes (2.00 mM), and (10) H_3NTA (2.01 mM). The solutions were potentiometrically titrated with a standardized KOH solution (100.43 mM) with pH ranging from 2.5 to 12.0. The stability constants of the complexes were determined using CVEQUID program [11] by minimization of the differences between the theoretical model and the experimental data, according to Gauss–Newton–Marquart for nonlinear equations (see Ref. [12] for more details). The original CVEQUID algorithm was combined with CerkoLab software. After titration the acquired data was processed and the equilibria model was symbolically described by set of equations. The formalism used to define a “pK model” was based on a simplified way customary

used to describe equilibrium state in the mixture. To further simplify the preparation of data required for numerical procedures the “p*K* model” included statements describing the composition of titrant, titrand, electrode parameters, and solvent. The stoichiometric matrix required for the procedure was automatically generated from the model.

Results and discussion

The conditions under which ITC experiments are carried out influence both the binding constants (*K*) and the enthalpies (ΔH) of the reaction. To compare ITC thermodynamic values with other methods one should take into account the pH, temperature as well as the kind of buffer solution in which the measurements are done. In systems where metal ions (*M*) are involved, metal–buffer complex formation must also be considered during ITC data analysis.

To determine thermodynamic values (*K*, ΔH) of metal–buffer interactions, displacement ITC experiments were carried out. Thus, a weak ligand (buffer Mes, Caco, or Pipes) was replaced by a strong one in the coordination sphere of the central ion. H₃NTA was used in our study as the strong-binding, competitive ligand. The nitrilotriacetate ions (NTA³⁻) act as tetradentate ligands and form 1:1 metal–ligand complexes (ML) with most ions [13, 14]. Oxygen atoms of three carboxylic groups and a central nitrogen atom participate in the metal binding (Fig. 2). Moreover, the acid–base dissociation constants of H₃NTA, the enthalpies of proton–ligand dissociation as well as the enthalpies of complexation of cobalt(II) and nickel(II) ions with H₃NTA are known [15]. These values are required to determine the thermodynamic values of metal–buffer interactions by a displacement ITC experiment [9].

H₃NTA is a weak triprotonated acid which dissociates in three steps presented by Eqs. 1–3:

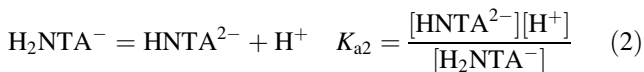
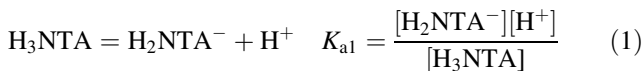
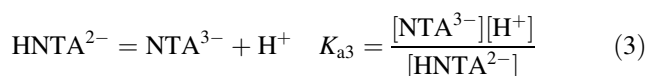
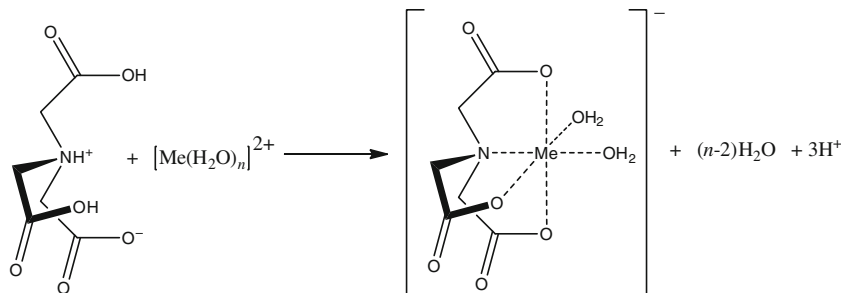
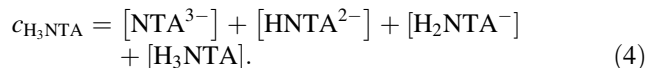


Fig. 2 The coordination mode of metal(II) ion to nitrilotriacetate anion (NTA³⁻)



At 298.15 K equilibrium constants pK_{a1} , pK_{a2} , and pK_{a3} are 1.68–2.08, 2.67–2.95, and 9.49–9.95, respectively [15]. For calculations, we used the pK_{a1} , pK_{a2} , and pK_{a3} values obtained in our laboratory. They are as follows: $pK_{a1} = 2.28$ (± 0.17), $pK_{a2} = 2.88$ (± 0.19), and $pK_{a3} = 9.55$ (± 0.07).

The concentration of H₃NTA is equal to the sum of the concentrations of all the particular components involved in equilibrium (Eq. 4). The concentration of these chemical species depends on the pH of a solution.



Knowing the pK_a values of H₃NTA and using appropriate formulas (Eqs. 5a–5c) one can find an expression that describes the relationship between the pH of a solution and the stability constant (Eq. 6).

$$c_{\text{H}_3\text{NTA}} = [\text{NTA}^{3-}] + [\text{HNTA}^{2-}] + [\text{H}_2\text{NTA}^-] + [\text{H}_3\text{NTA}] = [\text{NTA}^{3-}] \left(1 + \frac{[\text{H}^+]}{K_{a3}} + \frac{[\text{H}^+]^2}{K_{a3} \cdot K_{a2}} + \frac{[\text{H}^+]^3}{K_{a3} \cdot K_{a2} \cdot K_{a1}} \right) \quad (5a)$$

$$\alpha_{\text{proton}} = 1 + \frac{[\text{H}^+]}{K_{a3}} + \frac{[\text{H}^+]^2}{K_{a3} \cdot K_{a2}} + \frac{[\text{H}^+]^3}{K_{a3} \cdot K_{a2} \cdot K_{a1}} \quad (5b)$$

$$c_{\text{H}_3\text{NTA}} = [\text{NTA}^{3-}] \cdot \alpha_{\text{proton}} \quad (5c)$$

$$K_{\text{ITC}} = \frac{[\text{MNTA}]}{[\text{M}] \cdot c_{\text{H}_3\text{NTA}}} = \frac{[\text{MNTA}]}{[\text{M}][\text{NTA}^{3-}]} \cdot \frac{1}{\alpha_{\text{proton}}} = K_{\text{MNTA}} \cdot \frac{1}{\alpha_{\text{proton}}} \quad (6)$$

where *M* denotes metal ion, K_{ITC} is the conditional (observed) binding constant obtained directly from the ITC experiment. K_{ITC} depends on the pH of a solution and the pK_a values of a ligand (as well as ionic strength and temperature), α_{proton} is the function of pH and pK_a 's, K_{MNTA} is the pH-independent metal (M^{2+})–ligand (NTA^{3-}) binding constant ($\text{M}^{2+} + \text{NTA}^{3-} = \text{MNTA}^-$) and can be compared to *K* values obtained by other methods. It can also be extrapolated to different conditions.

The situation is slightly more complicated when metal ions are involved in the system under study. If one assumes that the metal ions (M) with buffer type of H_nB form complexes having a molar metal to ligand ratio of 1:1 (MB) the following equations should be used during calorimetric data analysis (Eqs. 7a–7c):

$$M + B = MB \quad K_{MB} = \frac{[MB]}{[M][B]} \quad (7a)$$

$$[M]_{ITC} = [M] + [MB] = [M] + K_{MB}[M][B] \\ = [M](1 + K_{MB}[B]) \quad (7b)$$

$$[M]_{ITC} = [M] \cdot \alpha_{buffer} \quad (7c)$$

where $[M]_{ITC}$ indicates the concentration of the metal species not involved in the MB complex, K_{MB} indicates the stability constant for the metal–buffer complex (MB), $[B]$ indicates the concentration of a buffer solution, $\alpha_{buffer} = 1 + K_{MB}[B]$ is a function of the K_{MB} and $[B]$.

In such a case, to determine the pH and buffer-independent stability constant K_{MNTA} the following equation must be applied (Eq. 8):

$$K_{ITC} = \frac{[ML]}{[M]_{ITC} c_{H_3NTA}} = \frac{[ML]}{[M][L]} \cdot \frac{1}{\alpha_{proton} \cdot \alpha_{buffer}} \\ = \frac{K_{MNTA}}{\alpha_{proton} \cdot \alpha_{buffer}} \quad (8)$$

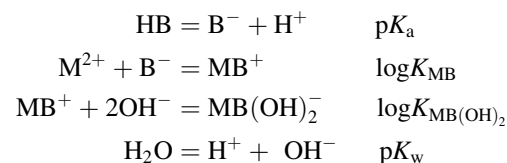
which finally leads to an expression (Eq. 9) for the condition-independent K_{MNTA} :

$$K_{MNTA} = K_{ITC} \cdot \alpha_{proton} \cdot \alpha_{buffer} \quad (9)$$

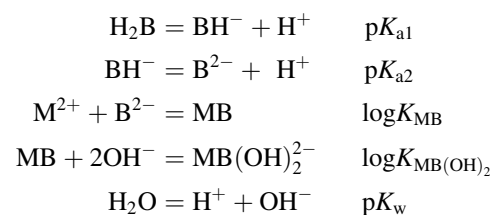
The assumption that in the systems under study, 1:1 metal–buffer complexes (MB) are formed, was verified by

PT. The following equilibria were assumed and used to calculate the metal–buffer stability constants.

Equilibrium models for interactions of the metal ions (M) with the Mes or Caco buffers (HB):



Equilibrium models for interactions of the metal ions (M) with the Pipes buffer (H_2B):



The pK and $\log K$ values of the individual equilibria that contribute to the experimental equilibrium involving a metal (M) binding to a buffer (B), together with their standard deviations, are summarized in Table 1. The K_{MB} values obtained from PTs are the pH-independent stability constants and can be extrapolated to the conditions, under which the calorimetric measurements were carried out. The metal–buffer conditional stability constants K'_{MB} at pH 6 ($I = 0.100$ M $NaClO_4$) were calculated using Eq. 10.

Table 1 pK and $\log K$ values (standard deviation in parentheses) for the metal–buffer interactions in water at $T = 298.15$ K (ionic strength $I = 0.100$ M $NaClO_4$) obtained by adapting the equilibrium model to PT data

pK or $\log K$	Equilibrium model	$M^{2+} = Co^{2+}$	$M^{2+} = Ni^{2+}$
<i>Mes buffer</i>			
pK_{a1}	$MesH = Mes^- + H^+$	6.10 (± 0.02)	6.11 (± 0.03)
$\log K_{MB}$	$M^{2+} + Mes^- = MMes^+$	2.04 (± 0.17)	2.06 (± 0.19)
$\log K_{MB(OH)_2}$	$MMes^+ + 2OH^- = MMes(OH)_2^-$	10.38 (± 0.09)	10.79 (± 0.09)
pK_w	$H_2O = OH^- + H^+$	13.74 (± 0.02)	13.76 (± 0.03)
<i>Caco buffer</i>			
pK_{a1}	$CacoH = Caco^- + H^+$	6.09 (± 0.04)	6.13 (± 0.03)
$\log K_{MB}$	$M^{2+} + Caco^- = MCaco^+$	2.30 (± 0.22)	2.33 (± 0.15)
$\log K_{MB(OH)_2}$	$MCaco^+ + 2OH^- = MCaco(OH)_2^-$	10.16 (± 0.10)	10.60 (± 0.05)
pK_w	$H_2O = OH^- + H^+$	13.70 (± 0.02)	13.71 (± 0.01)
<i>Pipes buffer</i>			
pK_{a1}	$PipesH_2 = PipesH^- + H^+$	2.61 (± 0.13)	2.86 (± 0.15)
pK_{a2}	$PipesH^- = Pipes^{2-} + H^+$	7.06 (± 0.09)	7.15 (± 0.10)
$\log K_{MB}$	$M^{2+} + Pipes^{2-} = MPipes$	3.12 (± 0.15)	3.20 (± 0.14)
$\log K_{MB(OH)_2}$	$MPipes + 2OH^- = MPipes(OH)_2^{2-}$	10.18 (± 0.07)	10.57 (± 0.07)
pK_w	$H_2O = OH^- + H^+$	13.77 (± 0.06)	13.76 (± 0.07)

Table 2 Individual equilibria that contribute to the overall equilibrium for the formation of metal–H₃NTA complex in the Mes, Caco, or Pipes buffer solution (B) (the charges of ions are omitted for the sake of clarity)

No.	Reaction ^a	Coefficient	ΔH^b
1	MB = M + B	α_{MB}	$-\Delta H_{MB}^0$
2	HNTA = NTA + H	α_{HNTA}	$-\Delta H_{HNTA}^0$
3	H ₂ NTA = NTA + 2H	α_{H_2NTA}	$-\Delta H_{H_2NTA}^0$
4	H ₃ NTA = NTA + 3H	α_{H_3NTA}	$-\Delta H_{H_3NTA}^0$
5	M + NTA = MNTA	1	ΔH_{MNTA}^0
6	B + H = HB	$\alpha_{HNTA} + 2\alpha_{H_2NTA} + 3\alpha_{H_3NTA}$	ΔH_{BH}^0

^a Equilibria are written in the direction that the reaction occurs (1–4 are dissociations, 5 and 6 are associations)

^b $\Delta H^0/\text{kcal mol}^{-1}$ values are for the association reactions

$$K'_{MB} = \frac{[MB]}{[M] \cdot c_B} = \frac{[MB]}{[M][B]} \cdot \frac{1}{\alpha_{proton}} = K_{MB} \cdot \frac{1}{\alpha_{proton}}, \quad (10)$$

where c_B is the concentration of buffer (B) and equals $c_B = [HB] + [B]$ for the Mes and Caco buffer or $c_B = [H_2B] + [HB] + [B]$ for the Pipes buffer.

To find α_{proton} (Eq. 5b), the values of the acid-based dissociation constants of the buffers were taken from Table 1. The logarithms of the K'_{MB} value equal to 1.68, 1.95, and 2.03 for the Co–Mes, Co–Caco, and Co–Pipes complex, respectively, and 1.69, 1.96, and 2.03 for the Ni–Mes, Ni–Caco, and Ni–Pipes complex, respectively. Then, the K'_{MB} constants were used to determine the enthalpies of the metal–buffer interaction. Assuming, based on potentiometric data, that the Co²⁺ and Ni²⁺ ions form 1:1 complexes with buffer (B) as well as taking into account four protonation states of H₃NTA ligand (Eq. 4), the individual equilibria that contribute to the overall equilibrium, as well as the coefficients that indicate the percentage of the

particular chemical species in solution under experimental conditions are presented in Table 2. The overall reaction for the formation of metal–H₃NTA complex is given by general equation 11.

$$(1 - \alpha_{MB})M + \alpha_{MB}MB + (1 - \alpha_{HNTA} - \alpha_{H_2NTA} - \alpha_{H_3NTA}) \times NTA + \alpha_{HNTA}HNTA + \alpha_{H_2NTA}H_2NTA + \alpha_{H_3NTA}H_3NTA + (\alpha_{HNTA} + 2\alpha_{H_2NTA} + 3\alpha_{H_3NTA})B = MNTA + (\alpha_{HNTA} + 2\alpha_{H_2NTA} + 3\alpha_{H_3NTA})BH \quad (11)$$

In Eq. 11, the sum $(\alpha_{HNTA} + 2\alpha_{H_2NTA} + 3\alpha_{H_3NTA})$ corresponds to the number of protons transferred. The coefficients in Table 2 indicate the percentage of the metal and ligands in solution under experimental conditions and are defined as follows (Eqs. 12–15):

$$\alpha_{MB} = \frac{K'_{MB}[B]}{1 + K'_{MB}[B]} \quad (12)$$

$$\alpha_{HNTA} = \frac{[HNTA^{2-}]}{c_{H_3NTA}} = \frac{[H_3O^+]}{K_{a3} \cdot \alpha_{proton}} \quad (13)$$

$$\alpha_{H_2NTA} = \frac{[H_2NTA^-]}{c_{H_3NTA}} = \frac{[H_3O^+]^2}{K_{a3} \cdot K_{a2} \cdot \alpha_{proton}} \quad (14)$$

$$\alpha_{H_3NTA} = \frac{[H_3NTA]}{c_{H_3NTA}} = \frac{[H_3O^+]^3}{K_{a3} \cdot K_{a2} \cdot K_{a1} \cdot \alpha_{proton}} \quad (15)$$

In pH 6, the $(\alpha_{HNTA} + 2\alpha_{H_2NTA} + 3\alpha_{H_3NTA})$ calculated according to Eqs. 13–15, equals 1. This means that during the experiment the number of moles of the protons released by 1 mol of H₃NTA during complexation of the Co²⁺ or Ni²⁺ ions equals to 1.

When a proton is released from a ligand during complexation a new, additional energy is generated. It is connected with the transfer of the proton from the ligand to the

Table 3 Thermodynamic parameters ($\log K_{ITC}$, ΔH_{ITC}) of the Co²⁺ and Ni²⁺ ions binding to H₃NTA and the ΔH_{MB} values of the metal–buffer (Mes, Caco, and Pipes) interactions in solutions of pH 6, at 298.15 K

Buffer	Metal ion	$\log K_{ITC}$ (MNTA)/M ^{-1a}	ΔH_{ITC} (MNTA)/kcal mol ⁻¹	$\log K$ (MNTA)/M ^{-1b}	$\Delta H_{MB}^c/\text{kcal mol}^{-1}$
Mes	Co ²⁺	6.45 (±0.07)	+1.04 (±0.01)	10.76 (±0.01)	0.29
Caco	Co ²⁺	6.29 (±0.02)	+4.41 (±0.01)	10.84 (±0.02)	1.25
Pipes	Co ²⁺	6.22 (±0.03)	+1.62 (±0.01)	10.84 (±0.02)	0.58
Mes	Ni ²⁺	7.12 (±0.12)	-1.50 (±0.01)	11.45 (±0.12)	0.40
Caco	Ni ²⁺	7.31 (±0.07)	+2.01 (±0.01)	11.86 (±0.07)	1.18
Pipes	Ni ²⁺	6.49 (±0.12)	-0.89 (±0.01)	11.11 (±0.07)	0.63

^a The equilibrium binding constant K_{ITC} and binding enthalpy ΔH_{ITC} for the metal–H₃NTA interaction were obtained from ITC experiments by fitting binding isotherms, using nonlinear least-squares procedures, to a model that assumes a single set of identical binding sites

^b The logarithms of metal–H₃NTA formation constants corrected for both buffer competition with the strong ligand (H₃NTA) for the metal ion and proton competition with the metal ion for the ligand

^c The enthalpies of metal–buffer interactions based on Eq. 17

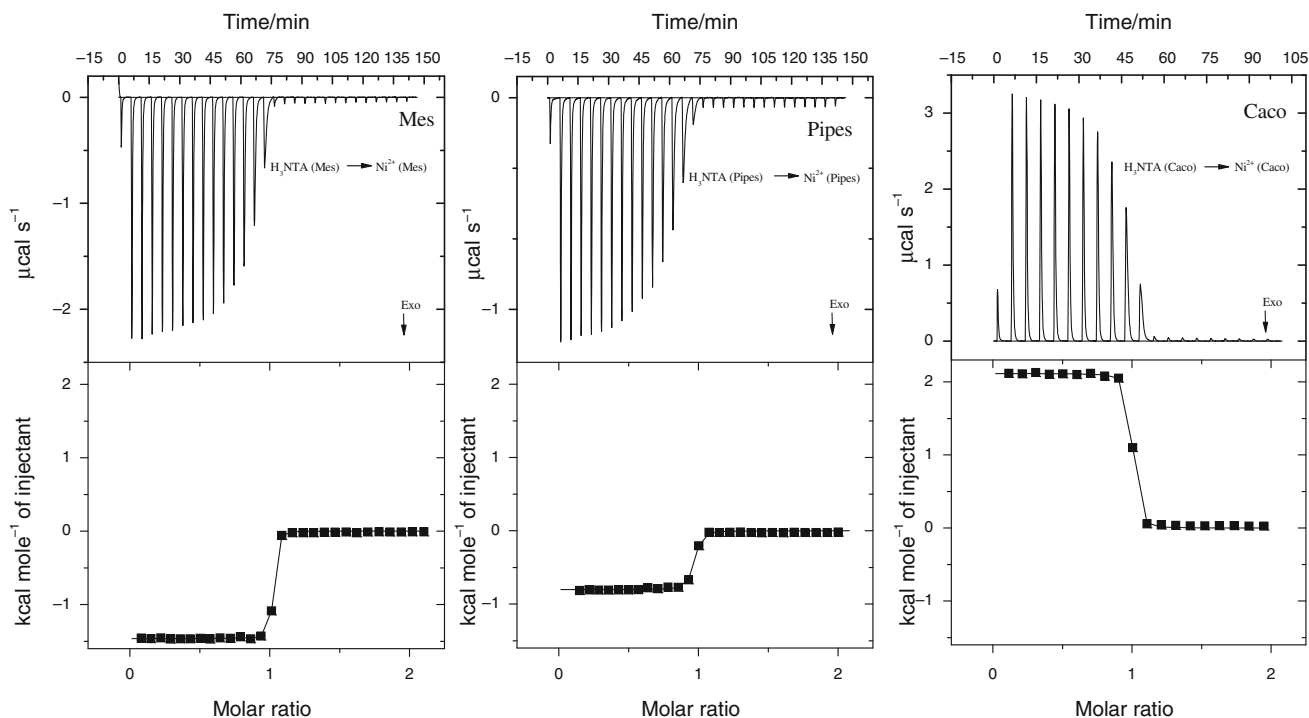


Fig. 3 Calorimetric titration isotherms of the binding interaction between Ni^{2+} and H_3NTA in buffers with different ionization enthalpies (Mes, Pipes, Caco, 100 mM each) of pH 6, at 298.15 K

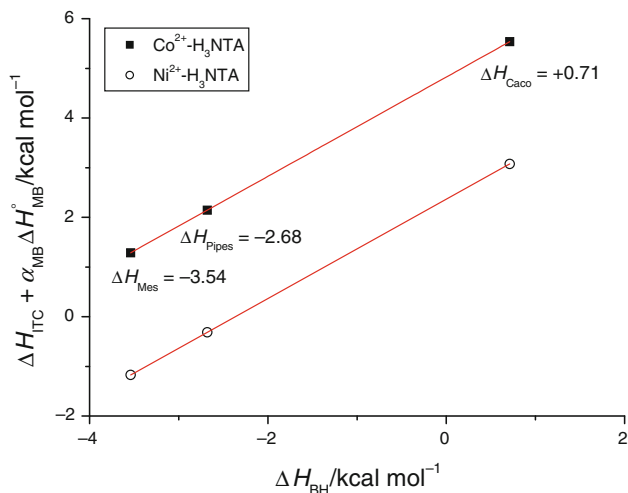


Fig. 4 Plot of $(\Delta H_{\text{ITC}} + \alpha_{\text{MB}} \Delta H_{\text{MB}}^{\circ})$ versus $\Delta H_{\text{BH}}^{\circ}$ for the Co^{2+} and Ni^{2+} - H_3NTA interactions in 100 mM Mes, Caco, and Pipes at pH 6, 298.15 K

buffer. Carrying out the ITC measurements in buffer solutions of equal pH but different enthalpies of proton association of its components (Mes, Caco, Pipes), ΔH_{BH} , made it possible to determine the enthalpy of complex formation, independent of the nature of the buffer used [16–18]. Moreover, when the enthalpy of the metal–ligand (strong ligand) reaction is known, $\Delta H_{\text{MNTA}}^{\circ}$, the metal–buffer (weak ligand) enthalpy can be calculated, $\Delta H_{\text{MB}}^{\circ}$. The observed enthalpy of binding, ΔH_{ITC} , obtained from

ITC titration, can be expressed by Eq. 16, which is based on Hess's law [19]:

$$\begin{aligned} \Delta H_{\text{ITC}} = & -\alpha_{\text{MB}} \Delta H_{\text{MB}}^{\circ} - \alpha_{\text{HNTA}} \Delta H_{\text{HNTA}}^{\circ} - \alpha_{\text{H}_2\text{NTA}} \Delta H_{\text{H}_2\text{NTA}}^{\circ} \\ & - \alpha_{\text{H}_3\text{NTA}} \Delta H_{\text{H}_3\text{NTA}}^{\circ} + (\alpha_{\text{HNTA}} + 2\alpha_{\text{H}_2\text{NTA}} \\ & + 3\alpha_{\text{H}_3\text{NTA}}) \Delta H_{\text{BH}} + \Delta H_{\text{MNTA}}^{\circ} \end{aligned} \quad (16)$$

Finally, the metal–buffer enthalpy, $\Delta H_{\text{MB}}^{\circ}$, can be found by transformation of Eq. 16:

$$\begin{aligned} \Delta H_{\text{MB}}^{\circ} = & [-\Delta H_{\text{ITC}} - \alpha_{\text{HNTA}} \Delta H_{\text{HNTA}}^{\circ} - \alpha_{\text{H}_2\text{NTA}} \Delta H_{\text{H}_2\text{NTA}}^{\circ} \\ & - \alpha_{\text{H}_3\text{NTA}} \Delta H_{\text{H}_3\text{NTA}}^{\circ} + (\alpha_{\text{HNTA}} + 2\alpha_{\text{H}_2\text{NTA}} \\ & + 3\alpha_{\text{H}_3\text{NTA}}) \Delta H_{\text{BH}} + \Delta H_{\text{MNTA}}^{\circ}] / \alpha_{\text{MB}} \end{aligned} \quad (17)$$

To do this, the ΔH_{ITC} of Co^{2+} - H_3NTA and Ni^{2+} - H_3NTA interactions were measured in Mes, Caco, and Pipes buffered solutions. The obtained results ($\log K_{\text{ITC}}$, ΔH_{ITC}) as well as the $\Delta H_{\text{MB}}^{\circ}$ values calculated from Eq. 17 are listed in Table 3. Representative binding isotherms for Ni- H_3NTA interactions are shown in Fig. 3. Calorimetric titration isotherms of the binding interaction between Co^{2+} and H_3NTA are presented in the Supplementary Material (Fig. S1). The proton-association enthalpies of buffers, ΔH_{BH} ($\text{H}^+ + \text{B}^- = \text{HB}^{\pm}$), used in this study are +0.71, -2.68, and -3.54 kcal mol⁻¹ for Caco, Pipes, and Mes, respectively [20]. The metal- H_3NTA formation constants, the enthalpies of metal- H_3NTA interactions, $\Delta H_{\text{MNTA}}^{\circ}$, as well as the enthalpies of the proton association to the ligand

used in the calculations were taken from literature [15, 21]. They are listed below:

$$\begin{aligned} \log K_{\text{CoNTA}}(\text{Co}^{2+} + \text{NTA}^{3-} = \text{CoNTA}^-) &= 10.5 \\ \log K_{\text{NiNTA}}(\text{Ni}^{2+} + \text{NTA}^{3-} = \text{NiNTA}^-) &= 11.4 \\ \Delta H_{\text{CoNTA}}^\circ(\text{Co}^{2+} + \text{NTA}^{3-} = \text{CoNTA}^-) \\ &= -0.07 \text{ kcal mol}^{-1} \Delta H_{\text{NiNTA}}^\circ(\text{Ni}^{2+} + \text{NTA}^{3-} = \text{NiNTA}^-) \\ &= -2.53 \text{ kcal mol}^{-1} \Delta H_{\text{HNTA}}^\circ(\text{NTA}^{3-} + \text{H}^+ = \text{HNTA}^{2-}) \\ &= -4.90 \text{ kcal mol}^{-1} \Delta H_{\text{H}_2\text{NTA}}^\circ(\text{HNTA}^{2-} + \text{H}^+ = \text{H}_2\text{NTA}^-) \\ &= +0.80 \text{ kcal mol}^{-1} \Delta H_{\text{H}_3\text{NTA}}^\circ(\text{H}_2\text{NTA}^- + \text{H}^+ = \text{H}_3\text{NTA}) \\ &= +0.75 \text{ kcal mol}^{-1} \end{aligned}$$

The ΔH_{ITC} determined from the ITC experiment depends on the nature of the buffer solution in which the measurement was carried out (Table 3). The drop of the ΔH_{ITC} value with decreasing buffer association enthalpy, ΔH_{BH} , shows that during complexation of the metal ions, the protons are transferred from the ligand to the buffer. For this reason, energetic effects due to metal–ligand interaction in buffer solutions of negative association enthalpies (Mes, Pipes) are reduced by the energy (heat) released during proton binding to a buffer component. With the Caco buffer of positive association enthalpy (+0.71 kcal mol⁻¹), the proton transfer during complexation of the metal ions results in an increase in ΔH_{ITC} . It is especially seen in the case of Ni²⁺–H₃NTA interactions, where ΔH_{ITC} is negative when titration is carried out in the Mes and Pipes buffer solutions, then changes to positive in the Caco buffer (Table 3; Fig. 3).

Equation 16 can be transformed to the linear relationship $y = a + bx$ (Eq. 18) as seen below:

$$\begin{aligned} \Delta H_{\text{ITC}} + \alpha_{\text{MB}} \Delta H_{\text{MB}}^\circ &= (\alpha_{\text{HNTA}} + 2\alpha_{\text{H}_2\text{NTA}} + 3\alpha_{\text{H}_3\text{NTA}}) \\ &\times \Delta H_{\text{BH}}^\circ + \Delta H_{\text{MNTA}}^\circ - \alpha_{\text{HNTA}} \Delta H_{\text{HNTA}}^\circ \\ &- \alpha_{\text{H}_2\text{NTA}} \Delta H_{\text{H}_2\text{NTA}}^\circ - \alpha_{\text{H}_3\text{NTA}} \Delta H_{\text{H}_3\text{NTA}}^\circ \end{aligned} \quad (18)$$

where $y = \Delta H_{\text{ITC}} + \alpha_{\text{MB}} \Delta H_{\text{MB}}^\circ$, $a = \Delta H_{\text{MNTA}}^\circ - \alpha_{\text{HNTA}} \Delta H_{\text{HNTA}}^\circ - \alpha_{\text{H}_2\text{NTA}} \Delta H_{\text{H}_2\text{NTA}}^\circ - \alpha_{\text{H}_3\text{NTA}} \Delta H_{\text{H}_3\text{NTA}}^\circ$, $b = \alpha_{\text{HNTA}} + 2\alpha_{\text{H}_2\text{NTA}} + 3\alpha_{\text{H}_3\text{NTA}}$ —the number of proton transferred, and $x = \Delta H_{\text{BH}}^\circ$ —the proton-association enthalpy of buffer.

The plots of $(\Delta H_{\text{ITC}} + \alpha_{\text{MB}} \Delta H_{\text{MB}}^\circ)$ versus $\Delta H_{\text{BH}}^\circ$ for the Co²⁺ and the Ni²⁺ complexes are shown in Fig. 4. From a slope of the relationship described by Eq. 18, the number of protons transferred was determined and equaled 0.999 (± 0.001) for both the Co²⁺ and the Ni²⁺–H₃NTA complexes. These values agree with those calculated using Eqs. 13–15.

When the enthalpy of metal (M)–ligand (L) interaction is unknown, $\Delta H_{\text{ML}}^\circ$, it can be determined, for triprotonated ligand (H₃L), using the value at the point of interception of the plot presented in Fig. 4, $\Delta H_{\text{ML}}^\circ - \alpha_{\text{HL}} \Delta H_{\text{HL}}^\circ - \alpha_{\text{H}_2\text{L}} \Delta H_{\text{H}_2\text{L}}^\circ - \alpha_{\text{H}_3\text{L}} \Delta H_{\text{H}_3\text{L}}^\circ$.

Conclusions

ITC and PT methods have successfully been applied to determine thermodynamic parameters (K_{MB} , ΔH_{MB}) for complexation reactions of Co²⁺ and Ni²⁺ ions with buffer substances Mes, Caco, and Pipes. The metal–buffer formation constants K_{MB} were determined by PTs. The stability of the examined complexes increased in the following directions: CoMes ($\log K_{\text{CoMes}} = 2.04$) < CoCaco ($\log K_{\text{CoCaco}} = 2.30$) < CoPipes ($\log K_{\text{CoPipes}} = 3.12$) and NiMes ($\log K_{\text{NiMes}} = 2.06$) < NiCaco ($\log K_{\text{NiCaco}} = 2.33$) < NiPipes ($\log K_{\text{NiPipes}} = 3.20$). Furthermore, these values were extrapolated to the conditions under study (pH 6) and used for calculation of the enthalpies of metal–buffer interactions ΔH_{MB} . The enthalpies, ΔH_{MB} , were determined indirectly by ITC displacement titration using H₃NTA as a strong-binding, competitive ligand. Based on Hess's law the following values were obtained: $\Delta H_{\text{CoMes}} = 0.29$ kcal mol⁻¹, $\Delta H_{\text{CoPipes}} = 0.58$ kcal mol⁻¹, $\Delta H_{\text{CoCaco}} = 1.25$ kcal mol⁻¹, $\Delta H_{\text{NiMes}} = 0.40$ kcal mol⁻¹, $\Delta H_{\text{NiPipes}} = 0.63$ kcal mol⁻¹, and $\Delta H_{\text{NiCaco}} = 1.18$ kcal mol⁻¹.

The ITC method is a useful tool for investigation of the metal–ligand interactions in solution. It can be used as a supportive or alternative technique for other methods. However, the determination of thermodynamic parameters is not always easy, especially when metal ions are involved in the systems under study. During analysis of calorimetric data the experimental conditions must be taken into consideration. The most important energetic effects that are not connected with the metal–ligand interactions are the enthalpy of proton dissociation from the ligand, the enthalpy of buffer ionization as well as the enthalpy formation, and the stability constant of metal–buffer complex. In this article, it has been presented how to include these factors during calorimetric data analysis.

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