



Analyzing ITC Data for the Enthalpy of Binding Metal Ions to Ligands

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Abstract

Isothermal titration calorimetry (ITC) has become increasingly popular because it is the only method that directly measures the enthalpy change for a reaction. When analysis is done properly and accurate thermodynamic values are obtained, ITC results can be compared to thermodynamic values collected by other methods and can be extrapolated to different conditions. However, incorrect assumptions are sometimes made in metal binding data analysis. To illustrate the importance of data analysis for enthalpy changes, copper (II) was titrated into ethylenediaminetetraacetic acid (EDTA) in three different buffers, allowing for accurate quantification of a metal-buffer interaction (ΔH_{MB}).

Introduction

The enthalpy, ΔH_{ITC} , reported from the fit of titration curve represents the overall heat in the reaction cell. In order to account for all of the processes during a reaction it is recommended to analyze the data and account for each separate chemical event. Analysis may seem overwhelming at first, but it can be broken down into specific incremental events that may occur simultaneously in the reaction cell.

Several papers have been published that have reviewed how to analyze ITC data and quantify protons released during a binding event in the ITC (1,2). However, when metal binding reactions are studied, an additional component in ITC data analysis is required due to other heat generating events occurring in the reaction cell. Quantification of the additional buffer-metal chemistry and its added complexity has been previously incorporated into data analysis, yet how these values were calculated is not always easily understood or straightforward (3,4). It is the goal of this paper to show the experimentalist how ΔH_{MB} can be quantified and incorporated in data analysis.

One of the major heat producing events in a reaction can be related to the displacement of protons from a binding pocket or binding ligand. Proton coupled equilibria, can originate from protonation of buffer and needs to be understood and quantified as a large portion of ΔH_{ITC} . The buffer protonation event can work in favor to the experimentalist as large amounts of heat are

recorded for reactions that would otherwise produce minimal or non-detectable amount of heat. In order to be able to quantify the event, the reaction buffer needs to be varied. At least two different buffers should be used under identical reaction conditions. This addresses the proton coupled equilibria problem but requires that the proton-buffer and metal-buffer interactions, as quantified by ΔH_{HB} and ΔH_{MB} , be known. It is also important to note that ΔH_{MB} is not the heat of dilution – this is a different quantity that also must be measured and subtracted from the raw data.

Data analysis begins by listing the components that make up the overall binding enthalpy, ΔH_{ITC} , which comes from fitting the data (equation 1).



Scheme 1 is a simple model for a 1:1 interaction, but more complex model can be built into the simple model to account for the chemistry occurring in the reaction cell by adding additional equations. The first reaction in scheme 1 is typically the reaction of interest, metal (M) binding to ligand (L).

Reaction	Enthalpic Designation
$mM + L \rightleftharpoons mML$	$m \Delta H_{ML}, K_{ML}^m$
$mMB \rightleftharpoons mM + B$	$-m\Delta H_{MB}, K_{MB}^{-m}$
$n(H + B \rightleftharpoons HB)$	$n\Delta H_{HB}, K_{HB}^n$
$H_nL \rightleftharpoons L + nH$	$-n\Delta H_{HL}, K_{HL}^{-n}$

Scheme 1. Set of coupled reactions for a metal (M) binding a ligand (L); m is the number of metals that bind the ligand and n is the number of protons that are released for this binding process.

All of these reactions listed in the above scheme can be condensed and described in the following equation:

$$\Delta H_{ITC} = m \times (\Delta H_{ML} - \Delta H_{MB}) + n \times (\Delta H_{HB} - \Delta H_{HL}) \quad \text{[equation 2]}$$

The enthalpies in equation 2 represent each individual interaction, the metal-ligand interaction, the metal-buffer interaction, the buffer-proton interaction and the proton-ligand interaction. Although many standard enthalpic values (ΔH_{HB} and ΔH_{HL}) can be found in the NIST thermodynamic table, the ΔH_{MB} value is often not listed (5). For some metals, K_{MB} , is listed, meaning that it is already accepted that there is a measurable interaction between metal and

buffer. Not accounting for the heat associated with this can lead to overestimating or underestimating ΔH_{ML} , depending on which buffer is used in the experiment. This error can be quantified and visualized when calculating the number of protons that are released or absorbed in a binding reaction, and the significance of this factor is evaluated later in this note. ΔH_{MB} can be determined by a simple titration using the ITC, but it is important to use a simple well-defined situation such as a metal, M, binding to EDTA, which has a well-established constant ΔH_{ITC} prior to the binding inflection.

Experimental

All ITC measurements were performed in the TA Instruments® standard volume nanoITC with a gold cell and carried out at 25°C while stirring at 350 rpm. The 100 mM buffer solutions of Tris, HEPES, and imidazole were adjusted to have identical ionic strength and pH ($\mu = 0.1$ M and pH = 7.4). A background titration, where CuSO_4 in each buffered solution was titrated into the same buffer and subtracted from each of the data sets. Each titration of 1.60 mM Cu^{2+} into 0.10 mM EDTA in each buffer was repeated 3 times. All data fitting was done using NanoAnalyze software and the proton released plot was created using Excel®.

Results and Analysis

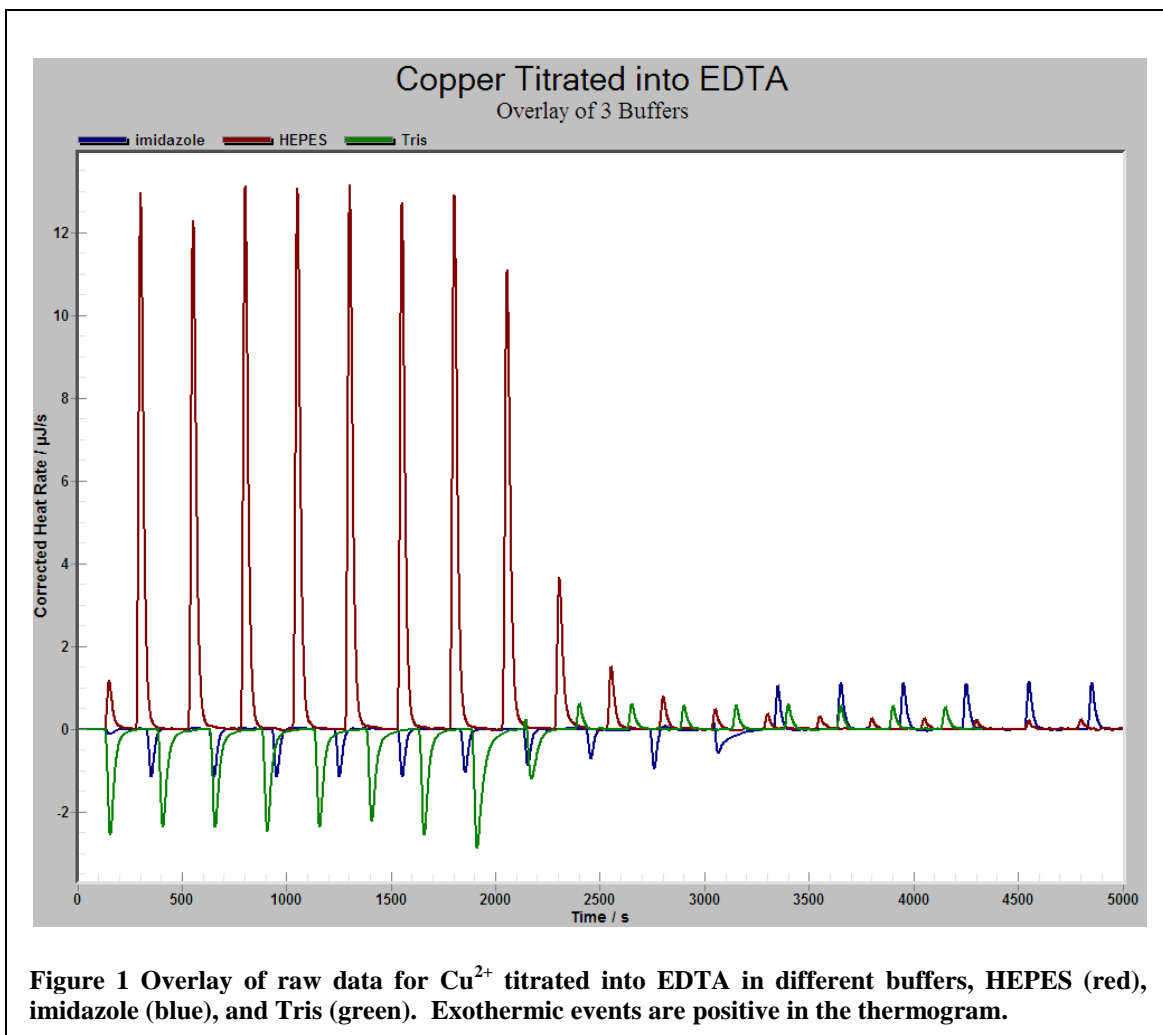
Figure 1 shows an ITC thermogram for Cu^{2+} binding to EDTA in three different buffers at pH 7.4. The particular set of reactions that are occurring in the reaction cell when Cu^{2+} is titrated into EDTA are shown in equation 3 and written out in long form in scheme 2.

$$\Delta H_{ITC} = \Delta H_{M-EDTA} + n_3 \Delta H_{H-Buffer} - \Delta H_{M-Buffer} - n_1 \Delta H_{H-EDTA} - n_2 \Delta H_{H_2-EDTA} \quad \text{[equation 2]}$$

Protons released, n_1 , n_2 and n_3 , take into consideration the percentage of each species in solution, where $n_3 = n_1 + n_2$, which depends on the pK_a 's of EDTA.

<i>Reaction</i>	<i>n</i>	<i>ΔH (kJ/mol)</i>
$\text{Cu}^{2+} + \text{EDTA}^{4-} \rightleftharpoons \text{CuEDTA}^{2-}$	1	-34 (5)
$\text{EDTAH}^{3-} \rightleftharpoons \text{EDTA}^{4-} + \text{H}^+$	0.95	-23 (5)
$\text{EDTAH}_2^{2-} \rightleftharpoons \text{EDTA}^{4-} + 2\text{H}^+$	0.05	-40 (5)
$\text{CuBuffer}^{2+} \rightleftharpoons \text{Cu}^{2+} + \text{Buffer}$	1	?
$\text{HBuffer}^+ \rightleftharpoons \text{H}^+ + \text{Buffer}$	1.05	NIST(5)

Scheme 2. Set of coupled reactions involved in Cu^{2+} binding to EDTA



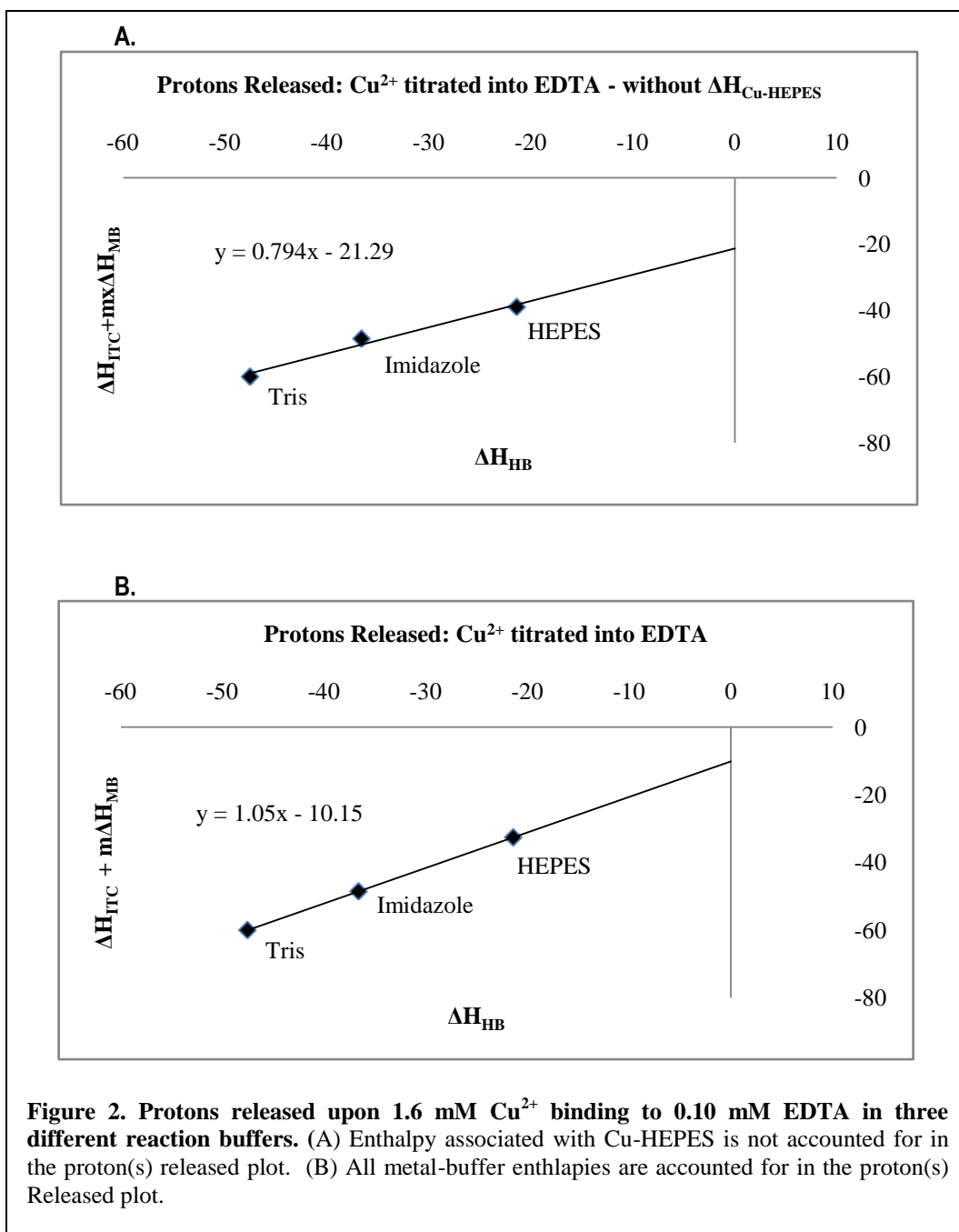
As seen in the Scheme 2, protons are displaced from EDTA when Cu^{2+} binds. To experimentally determine the number of protons being displaced, ITC data is collected in at least two buffers under identical conditions. Rearrangement of equation 2 into equation 4 and plotting $\Delta H_{\text{ITC}} + \Delta H_{\text{Cu-Buffer}}$ versus $\Delta H_{\text{H-Buffer}}$ gives a linear relationship with a slope of n_3 , total protons released. At pH 7.4, 1.05 H^+ should be released from EDTA upon Cu^{2+} binding this chelator.

$$\Delta H_{\text{ITC}} + \Delta H_{\text{M-Buffer}} = n_3 * \Delta H_{\text{H-Buffer}} + (\Delta H_{\text{M-EDTA}} n_1 * \Delta H_{\text{H-EDTA}} - n_2 * \Delta H_{\text{H}_2\text{-EDTA}}) \quad \text{[equation 4]}$$

It is common for a colorimetric change to be seen when Cu^{2+} is coordinated to a ligand and this color change is seen when Cu^{2+} is prepared in Tris or imidazole buffers. A colorimetric change is not seen when Cu^{2+} is dissolved in HEPES buffer and may be assumed that HEPES does not interact with the metal. However, this assumption that HEPES does not interact with Cu^{2+} appears to be incorrect as the enthalpy for Cu^{2+} binding to EDTA stated in the literature does not match the enthalpy collected using ITC. The HEPES- Cu^{2+} interaction is further validated when the number of protons is calculated and compared to the expected protons released upon binding. If the calculated

$\Delta H_{\text{Cu-HEPES}}$ is not accounted for when calculating the number of protons involved in this binding reaction, the value is underestimated by the slope (0.80 H^+) of the plot shown in Figure 2A. When $\Delta H_{\text{Cu-HEPES}}$ is taken into consideration, the number of experimental protons released, 1.05 H^+ , equals the theoretical number of protons released in the experiment.

The calculated enthalpies for each buffer are: $\Delta H_{\text{Cu-HEPES}} = 6.4 \text{ kJ/mol}$, $\Delta H_{\text{Cu-Tris}} = -71 \text{ kJ/mol}$, and $\Delta H_{\text{Cu-imidazole}} = -54 \text{ kJ/mol}$. These values can then be applied to experiments that involve Cu^{2+} binding any other type of ligand and independent values of buffer contributions can be reported.



Conclusion

ITC is an excellent technique for the direct measurement of metal-binding systems. When the system is set-up correctly and properly analyzed useful information can come from these studies. Although this study focuses on metal and ligand interactions, these same principles can be applied to other buffer dependent chemical events. When condition independent thermodynamics of a system are known, one can predict how the system will respond to changes in its environment whether the change is introduced through the presence of another protein, metal, or other species that are involved in the equilibrium state of the system.

References

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