

# A Simple Method for Isothermal Titration Calorimetric Data Analysis

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**Summary:** A simple method was introduced for isothermal titration calorimetric data analysis. This method was applied to the binding of monosaccharide methyl  $\alpha$ -D-mannopyranoside to concavalin A at pH 6.9 and temperature of 25°C. The dissociation binding constant (K) and the molar enthalpy of binding ( $\Delta H$ ) were 0.135 mM and -28.47 kJ/mol, respectively. These results were markedly consistent with the results obtained from previous method.

## Introduction

Calorimetry is the principal source of thermodynamic information, and it is indeed both a very versatile and sensitive technique [1]. It is a very general method due to the fact that practically all physical, chemical and biological processes are accompanied by heat exchange. So, calorimetry is one of the most powerful tools for expanding knowledge and understanding in many fields of science and technology [2,3]. The principal calorimetric techniques that have contributed are differential scanning calorimetry (DSC) and isothermal titration calorimetry (ITC) [4]. DSC experiments perturb the system under study by scanning temperature followed by recording the heat capacity of the system against temperature [4-6]. The nature and magnitude of the process that stabilize biomacromolecules and transition states for conversion of native to denatured form are investiga-

ted by DSC experiments [6-9]. The energetics of biochemical reactions or molecular interactions at constant temperature are measured by ITC [10]. Experiments are performed by titration of a reactant into a sample solution containing the other reactant(s) necessary for reaction. After each addition, the heat released or absorbed as a result of the reaction is monitored by the isothermal titration calorimeter. The total concentration of titrant is the independent variable under experimental control. Thermodynamic analysis of the observed heat effects that permits quantitative characterization of the energetic processes associated with the binding reaction [10].

ITC gives invaluable information about biomacromolecule-ligand interaction [11-13] allosteric transition [14], protein denaturation [15-

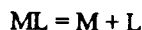
17] enzyme inhibition [18,19], quality, safety and shelf-life of materials and material stability [20-23].

Different methods have been reported for data analysis of ligand binding study by ITC [19, 24-27]. The principal of these methods is to fit the experimental data in an equation relating equilibrium constant, molar enthalpy of binding and reactants concentration. The Wiseman method [27] for data analysis has extensively used for ligand binding study by ITC and a computer program needs for using this method [28-34].

Here, a simple method is introduced for ITC data analysis. In the present study, I provide ITC data for the binding of the monosaccharide methyl  $\alpha$ -D-mannopyranoside to concavalin A at pH 6.9 and temperature of 25°C, where the protein exists as a monomer with one binding site [34]. This interaction has also been studied before, and calorimetric data analysis has been done by Wiseman method [34]. The results from two methods will be comprised.

### Results and Discussion

Consider a solution containing ligand (L), and a macromolecule ( $M_n$ ) that contains "n" sites capable of binding the ligand. If the multiple binding sites on a macromolecule are identical and independent, the ligand binding sites can be reproduced by a model system of monovalent molecules ( $M_n \rightarrow nM$ ) with the same set of dissociation equilibrium constant (K) values. Thus, the reaction under consideration can be written



$$K = [M][L]/[ML] \quad (1)$$

By titration of a solution containing "M" with a solution of ligand L, the equilibrium reaction is moved toward increasing concentration of ML complex. The heat value of reaction depends on concentration of ML complex ( $q \propto [ML]$ ). Moreover, the maximal value of heat that would be observed when all the M is present as ML, that is,  $q_{max} \propto [M]_{total}$ , or  $q_{max} \propto [M]+[ML]$ . Therefore, it can be concluded:

$$\frac{q}{q_{max}} = \frac{[ML]}{[M]+[ML]} \quad (2)$$

Because of the equilibrium assumption, [ML] can be expressed in terms of [L], [M], and K. Substituting for [ML]:

$$\frac{q}{q_{max}} = \frac{([M][L]/K)}{[M]+([M][L]/K)} \quad (3)$$

The heat equation for the simple unireactant system can be rearranged to yield the more familiar Henri-Michaelis-Menten equation

$$\frac{q}{q_{max}} = \frac{[L]}{K + [L]} \quad (4)$$

The value of  $q_{max}$  will be equal to the molar enthalpy of binding ( $\Delta H$ ), if the values of q are calculated per mole of single-site protein (M). It is assumed all the "M" available in the sample vessel is present as ML for this calculation. However, at very high concentrations of ligand, this assumption can be correct. In the other word, titration of a solution containing "M" with a ligand solution leads to the increasing value of q, and finally, to attain the  $\Delta H$  value at high concentration of ligand. Thus, equation 4 will lead to:

$$\frac{q}{\Delta H} = \frac{[L]}{K + [L]} \quad (5)$$

which rearranges to linear forms

$$\frac{1}{q} = \frac{K}{\Delta H} \frac{1}{[L]} + \frac{1}{\Delta H} \quad (6)$$

or

$$\frac{q}{[L]} = \frac{\Delta H}{K} - \frac{1}{K} q$$

Thus, the plot of  $1/q$  versus  $1/[L]$  gives dissociation equilibrium constant (K) and molar enthalpy of binding ( $\Delta H$ ). According to equation 6,  $\Delta H$  is obtained from Y-intercept as a q value, when the concentration of ligand is infinite, and the value of K can be obtained from the slope.

The raw data obtained from isothermal titration microcalorimetry of concavalin A with methyl  $\alpha$ -D-mannopyranoside was shown in Fig. 1.

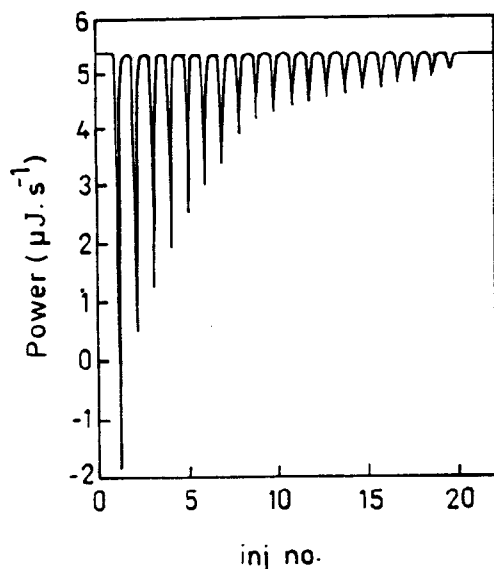


Fig. 1: Raw calorimetric titration data for 20 automatic cumulative injections, each of 20  $\mu\text{l}$ , of methyl  $\alpha\text{-D}$ -mannopyranoside solution, 50 mM, into the sample cell containing 2 ml concavalin A solution at a concentration of 0.04 mM. The experiment carried out at 6.9 pH, phosphate buffer 50 mM, and constant temperature 25°C.

Saturation of ligand binding due to the increase concentration of ligand is observed from the decrease area under the calorimetric signals from an injection to another injection in Fig. 1. Fig. 2a is showing the heat of each injection and Fig. 2b is showing the heat of related to each total concentration of ligand. The total concentration of ligand is much more than total concentration of concavalin A. So, it can be assumed that the total and free concentrations of ligand are approximately equal.

The plot of  $1/q$  versus  $1/[L]$  was shown in Fig. 3. The values of  $K$  and  $\Delta H$  obtained from slope and Y-intercept, respectively, are:

$$K = 0.147 \text{ mM} \quad \Delta H^\circ = -28.61 \text{ kJ/mole}$$

The results from Wiseman method has been reported as belows [34].

$$K = 0.132 \text{ mM} \quad \Delta H^\circ = -28.45 \text{ kJ/mol}$$

The differences between results obtained by two methods are related to the assumption of

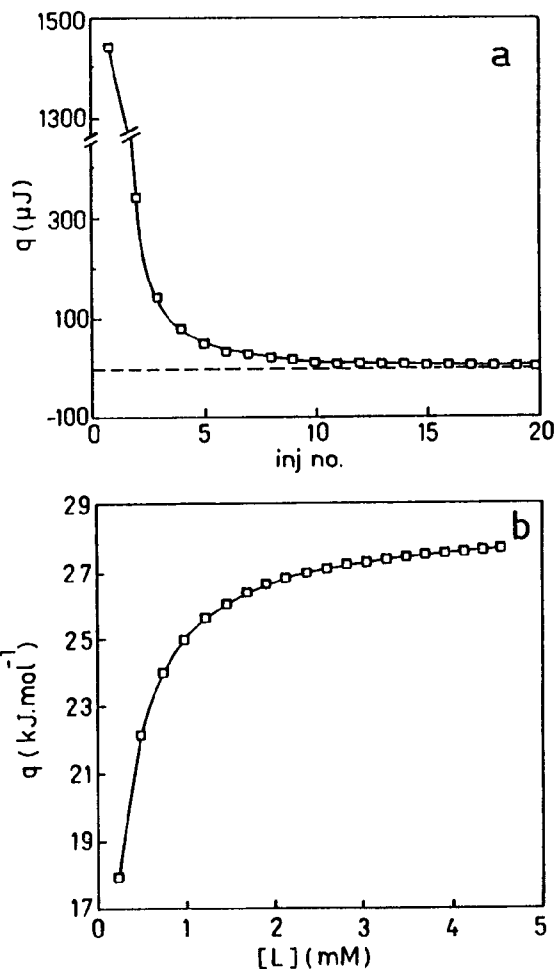


Fig. 2: (a) The heat of methyl  $\alpha\text{-D}$ -mannopyranoside binding on concavalin A for 20 automatic cumulative injections, each of 20  $\mu\text{l}$ , of methyl  $\alpha\text{-D}$ -mannopyranoside solution (50 mM), into the sample cell containing 2 ml concavalin A solution (0.04 mM). (b) The heat of binding versus total concentration of methyl  $\alpha\text{-D}$ -mannopyranoside, calculated from fig. 2a.

equating total and free concentration of ligand. It is expected the results from our method could be better by considering only the last 10 injections (see the insert linear plot in Fig. 3), which the above assumption would be more correct. The results from considering only last 10 injections are as below:

$$K = 0.135 \text{ mM} \quad \Delta H^\circ = -28.47 \text{ kJ/mol}$$

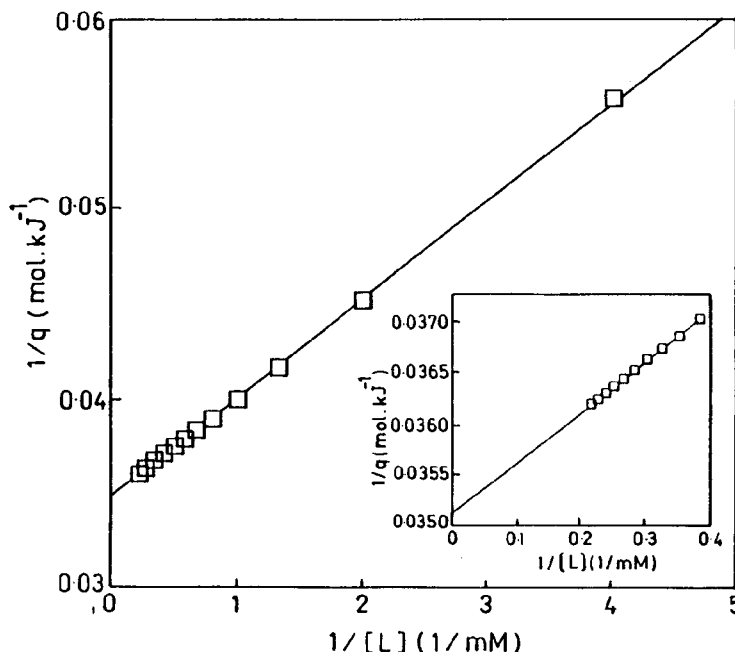


Fig. 3: The inverse heat of methyl  $\alpha$ -D-mannopyranoside binding on concavalin A versus inverse total concentration of methyl  $\alpha$ -D-mannopyranoside, according to equation (6), at 6.9 pH and temperature of 25°C. In the insert, a linear plot is shown for the last 10 injections.

These results are closer to the results of Wiseman method [34].

The conformity of dissociation binding constants ( $K$ ) and the molar enthalpies of binding ( $\Delta H$ ) obtained from two methods supports our method and the assumption.

#### Experimental

Methyl  $\alpha$ -D-mannopyranoside was purchased from Aldrich Chemical Co. Concavalin A (type IV) was purchased from Sigma Chemical Co. Solutions were made in double-distilled water. Phosphate solution, at 50 mM concentration, 6.9 pH, was used as a buffer.

The isothermal titration calorimetric experiments were performed with the 4-channel commercial microcalorimetric system, Thermal Activity Monitor 2277, Thermometric, Sweden. Each channel is a twin heat-conduction calorimeter where the heat-flow sensor is a semiconducting thermopile (multi-junction thermocouple plates) positioned between the vessel holders and the

surrounding heat sink. The insertion vessel was made from stainless steel. Methyl  $\alpha$ -D-mannopyranoside solution (50 mM) was injected by use of a Hamilton syringe into the calorimetric stirred titration vessel, which contained 2 ml concavalin A (0.04 mM) in phosphate buffer (50 mM) 6.9 pH at constant temperature of  $25.000 \pm 0.005^\circ\text{C}$ . Thin (0.15 mm inner diameter) stainless steel hypodermic needles, permanently fixed to the syringe, reached directly into the calorimetric vessel. Injection of methyl  $\alpha$ -D-mannopyranoside solution into the perfusion vessel was repeated 20 times, and each injection included 0.020 ml reagent. The software of "Thermometric Digitam 3 program" calculated the heat of each injection. The heat of each injection is related to the binding of methyl  $\alpha$ -D-mannopyranoside to concavalin A, dilution of methyl  $\alpha$ -D-mannopyranoside and dilution of concavalin A. The heat of dilution of the methyl  $\alpha$ -D-mannopyranoside solution was measured as described above except concavalin A was excluded. The enthalpy of methyl  $\alpha$ -D-mannopyranoside dilution was subtracted from the enthalpy of interaction between methyl  $\alpha$ -D-mannopyranoside and

cocavalin A. The enthalpy of dilution of concavalin A is negligible. The calorimeter was frequently calibrated electrically during the course of the study.

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