How to Choose an ITC Cell Volume

Introduction:

ITC is the most direct approach for determining most thermodynamic parameters in a single experiment, i.e. binding constant, stoichiometry and enthalpy of a reaction. Several ultrasensitive ITCs are available from TA Instruments that will measure binding constants from 10^3-10^{12} M^{-1}. Direct titrations will typically be useful for binding constants from 10^3 to 10^9 M^{-1}. However when measuring binding constants above 10^9 M^{-1} a competitive binding experimental design may be required. The Nano ITC instruments are designed specifically for dilute aqueous solutions of biological macromolecules. They have sample cell volumes of between 0.190 and 1.0 mL, and syringe sizes ranging from 0.05 to 0.250 mL. Biological macromolecules are sometimes available in very limited quantities only, which make lower volume ITC appealing. In the following, the choice of a proper sample volume for ITC is discussed.

Experimental Considerations:

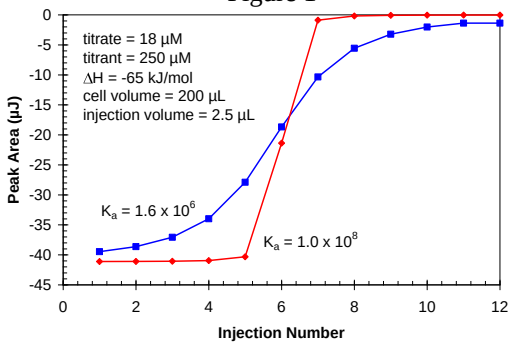
In a typical ITC experiment, a ligand solution (often a small molecule) is injected from a syringe, stepwise into the solution of the receptor molecule, while the entire system is stirred and maintained at a constant temperature. Sufficient ligand is added to saturate the receptor at the end of the experiment; for ITCs with 1 mL cells, this occurs typically over 20-25 injections in order to provide sufficient data points for the transition region that is crucial for precise determination of the binding constant. The heat evolved or absorbed by the binding reaction upon each addition of ligand is measured.

The choice of the appropriate concentration of the receptor in the cells depends on the anticipated binding constant (within a few orders of magnitude). The concentration of the ligand in the syringe is then generally chosen about 10X the concentration of the receptor assuming a 1:1 stoichiometry. In the absence of any prior binding data (for example, from UV/Vis or fluorescence experiments), a software tool is sometimes available on the ITC instruments in order to assist the researcher in this task, and which is based on several important considerations. In order to obtain a meaningful binding constant and stoichiometry for a reaction by ITC, the following relationship must be satisfied:

$$10 < K_a[M]_T < 1000 \qquad \text{(eq. 1)}$$

where $[M]_T$ is the total concentration of the macromolecule in the sample cell and K_a is the binding constant – again assuming a 1:1 stoichiometry. This requirement resides in the necessity to obtain sufficient data points in the curvature when plotting heat of reaction vs. injection number (or ratio of titrant to titrate) in order to calculate the binding constant. A calculated data set for a medium strength binding reaction ($K_a \sim 10^6$/M), obtained with 12 injections (a typical number of injections using an ITC with a small volume cell), is shown in blue in Figure 1:

Figure 1



If the relationship $10 < K_a[M]_T < 1000$ is not satisfied, the slope of the titration plot becomes very flat and thus difficult to accurately fit for low affinity reactions, or so steep for high affinity reactions such that too few data points would be obtained to get a good fit (data shown in red in Fig. 1, where the binding constant of the reaction has been modestly increased from 10^6 to 10^8/mol). In each case, the determination of K_a will be compromised.

Considerations when choosing a Standard Volume (1 mL) ITC cell or a Low Volume (0.2 mL) cell:

TA Instruments offers two state-of-the-art Nano ITC instruments. The Standard Volume Nano ITC has a 1 mL cell and utilizes one of two interchangeable injection syringes (0.1 or 0.25 mL total volume). Injection volumes are user-selectable and are generally between 5 and 10 μL. An incremental titration experiment usually involves 20-40 injections for this larger cell, providing thus a higher number of data points outlining the curvature in the data plot. With a minimum detectable heat of 0.1 μJ and a maximum measureable heat of up to 10,000 μJ, the Nano ITT SV is extremely versatile and can measure both high and low enthalpy binding reactions. Importantly, because of its large heat detection range, the Nano-ITC SV can reliably measure both weak binding reactions (which require high concentrations of reactants to satisfy equation 1) and strong binding reactions, where low concentrations of reactants are necessary and high sensitivity is required.

The Nano ITC Low Volume instrument has 0.190 mL cells and is equipped with a 0.05 mL injection syringe. The small cell volume can exhibit significantly faster equilibration and baseline stabilization times. Due to the small syringe size and low absolute heats of reaction produced, typically fewer than 20 injections are made, thus attention must be paid to the experimental design of the titration. The small number of injections may make it difficult to obtain adequate curvature in the data set, compromising the accurate determination of K_a. Curvature is less critical for the determination of stoichiometry and enthalpy, so these can generally be reliably determined. Since the cell volume is approx $1/5^{th}$ of a 1 mL cell, in theory $1/5^{th}$ of the amount of material is required per experiment. However, the loss in absolute signal amplitude must be balanced by an equal gain in sensitivity of the instrument. Data generated on the Nano ITC LV has shown that the sensitivity improvement over the Nano ITC SV is approximately double. For relatively high enthalpy reactions using a Nano ITC LV will require significantly less material than a Nano ITC SV instrument.

When choosing whether to use the Nano ITC Standard Volume or the Nano ITC Low Volume, more than just sample consumption must be considered. For relatively high enthalpy reactions with binding constants between 10^3-10^8 M^{-1}, the choice of a Nano ITC LV with its inherent lower sample volume requirements will provide high quality, reproducible ITC data. However, the savings in sample material that may be realized when using the Nano ITC LV may be out of balance for low enthalpy reactions, as higher sample concentrations may be necessary to offset the decreased sample volume in order to generate measurable heats. With most low enthalpy reactions the Nano ITC SV would be the instrument of choice for high quality, reproducible ITC data.

Summary:
TA Instruments offers a choice of the Nano ITC Standard Volume with 1.0 mL sample cells and the Nano ITC Low Volume with 190 uL sample cells. The decision to purchase an ITC instrument with specific sample cell volumes many times will depend on its performance flexibility and the primary set of applications it will be used for. When measuring medium to high binding affinities (10^3 – 10^{12} M^{-1}), especially in combination with a moderate to low molar enthalpy, the Nano ITC Standard Volume will generate the highest quality ITC data. If the anticipated heat of reaction is high and the binding constant is not too high, the Nano ITC Low Volume will require significantly less sample and still generate the highest quality ITC data. When considering a choice of either the Nano ITC Standard Volume with its outstanding overall sensitivity or the Nano ITC Low Volume with its significantly lower sample requirements and increased sensitivity, the key considerations should be the potential enthalpy range for the reactions, the binding constant possibilities and the sample availability.