Single Injection Method software for MicroCal VP-ITC system

LABEL-FREE ANALYSIS

MICROCALORIMETRY

Introduction

The Single Injection Method (SIM) is an innovative ITC technique for use with the MicroCal VP-ITC system. SIM provides versatility and speed for many applications characterizing the molecular interactions of biomolecules.

Traditional ITC with discrete titrations has provided accurate and valuable mechanism insights into molecules that bind to one another. However, some pursuits in drug discovery and target screening require more speed than traditional ITC can currently offer. The SIM technique takes an important step forward in providing higher throughput for the applications that could not consider traditional ITC in an assay scheme. With faster stirring speed capability and the highest precision possible for a single continuous injection of ligand, the overall flexibility of ITC using SIM is much improved.

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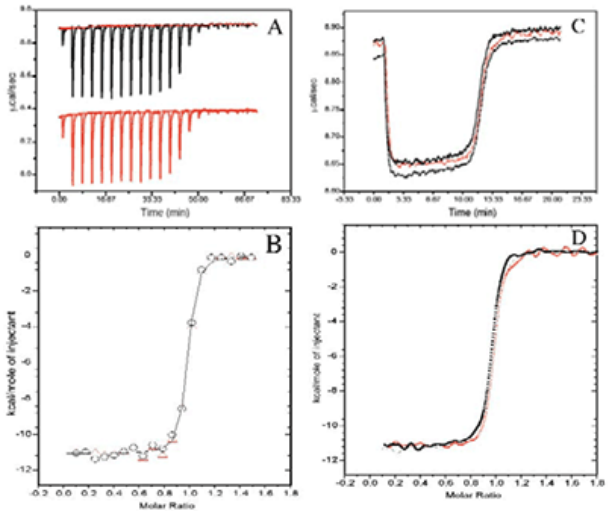

Figure 1. Comparison of single injection and multiple injection ITC data. Carbonic Anhydrase II (9.5µM) titrated with Acetozolamide (200µM) in PBS, 1% DMSO, pH 7.4. A Multiple injection ITC with 20 injections at 10µl each (first injection = 2µl), injection duration = 20 sec., injection spacing = 210 sec., stir speed = 610 rpm. Two separate experiments are shown, and the raw data are shifted on the Y axis for visibility. B Binding isotherm for 1A using Origin® 7.0 and one binding site model. C Single Injection Method ITC with one 100µl injection, injection duration = 1000 sec., total injection interval = 1200 sec., stir speed = 611 rpm. Three separate experiments are shown. D Binding isotherm for 1C using Origin® 7.0 and one binding site model. Fit parameters for 1B and 1D in Table 1.

Table 1. Comparison of VP-ITC multiple injection and SIM: Acetazolamide titrated into Carbonic Anhydrase

Injection Method	Temperature (°C)	Number of experiments	Experiment Time (min)[a]	Stoichiometry (n)	KD (M) b	ΔH (kcal mol-1)
Multiple	15 °C	3	115	0.97 ± 0.02	(13.6 ± 1.4) x 10-9	-11.1 ± 0.1
SIM	15 °C	4	65	0.98 ± 0.01	(11.7 ± 0.3) x 10-9	-11.3 ± 0.2
Multiple	35 °C	3	115	0.96 ± 0.02	(30.0 ± 6.4) x 10-9	-13.1 ± 0.5
SIM	35 °C	4	65	0.96 ± 0.03	(44.9 ± 1.8) x 10-9	-11.6 ± 0.4

[a]Experiment time includes 45 min experimental set-up (cell/syringe washing, cell/syringe filling, and final cell equilibration) and titration time.

[b] Reported as K (M^{-1}) in the software

Advantages

- **Speed:** In approximately 1/2 to 1/3 the time of traditional multi-titration ITC, a complete binding isotherm can be generated. Key binding parameters such as Stoichiometry (n), Affinity (K_D), Enthalpy (ΔH) and Entropy (ΔS) values can be accurately and quickly obtained.
- **Accuracy:** Accuracy of each thermodynamic parameter is comparable to those generated by the traditional multiple titration ITC (see table below.)
- **Versatility:** Applications include 1) rank-order binding, 2) small molecule-protein interactions or 3) drug-target interactions where higher throughput is required (e.g. Yes/No answer).

The SIM technique for ITC is optimized for use with binding models exhibiting a linear relationship (e.g. 1:1; 2:2). Capable of being run at a wide range of temperatures (15ºC to 40ºC) and with binding partners exhibiting affinities from 10^3 to 10^9 M^{-1} , the SIM can provide additional versatility.

Examples in the above table demonstrate a significant savings in titration time using SIM. Intelligent experimental design with equivalent equilibration times, comparable wash/rinse cycles and cell/syringe fill times has the potential of resulting in up to a 2-2.5 times increase in throughput over standard multiple injection ITC runs.

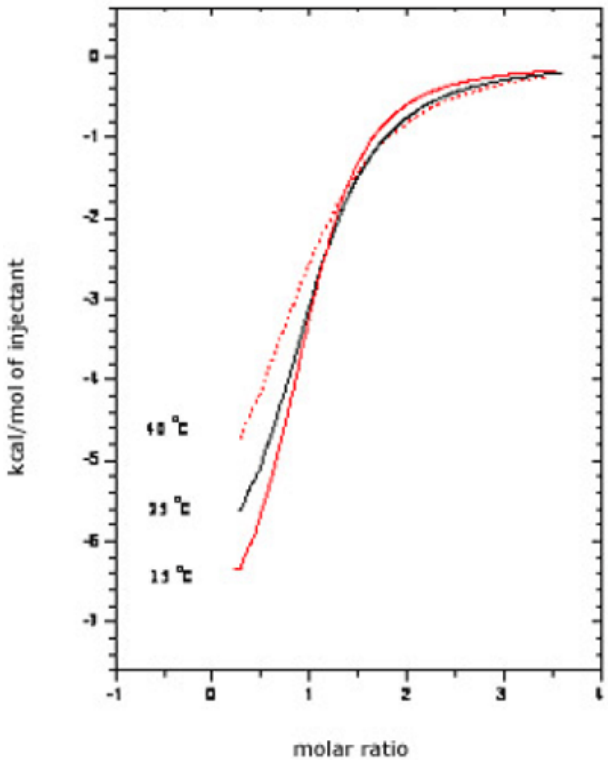

Figure 2. Binding of barium chloride to 18-crown-6 in MicroCal VP-ITC, using single injection method. 18-crown-6 solution (0.98 mM) in deionized water was in MicroCal VP-ITC cell and equilibrated to indicated temperature. 18-crown-6 was titrated with 1 injection of barium chloride (50.36 mM). Injection volume of 100 µl. Injection duration 1000 sec, total injection interval 1200 sec, stirring speed 611 sec. Binding isotherms were generated using Origin 7.0 for SIM. Binding isotherms were fit with Origin 7.0 for ITC using one set of sites model. Fit parameters are in Table 2 below.

Table 2. 18-crown-6 titration with barium chloride, at 15, 25 and 40° C, with MicroCal VP-ITC. Comparison of multiple injection ITC and SIM.

Injection Method	Temperature (°C)	Number of experiments	Experiment Time (min)[a]	Stoichiometry (n)	K_D (M) [b]	ΔH (kcal mol^{-1})
Multiple	15°C	1	153.5	1.07	11.07×10^{-5}	-7.63
SIM	15°C	2	65	1.02 ± 0.01	$(12.56 \pm 0.95) \times 10^{-5}$	-7.69 ± 0.1
Multiple	25°C	1	153.5	1.01	17.76×10^{-5}	-7.15
SIM	25°C	2	65	1.04 ± 0.01	$(18.51 \pm 0.72) \times 10^{-5}$	-7.38 ± 0.01
Multiple	40°C	2	153.5	0.94 ± 0.03	$(31.67 \pm 2.0) \times 10^{-5}$	-7.03 ± 0.26
SIM	40°C	2	65	1.02 ± 0.01	$(28.68 \pm 0.82) \times 10^{-5}$	-6.69 ± 0.22

Experiments and data analysis performed as described in the figure legend above.

[a]Experiment time includes 45 min experimental set-up (cell/syringe washing, cell/syringe filling, and final cell equilibration) and titration time.

[b] Reported as K (M^{-1}) in the software

The precision of the SIM measurements is sample dependent. The determinations shown in the table above made on identical samples are comparable for SIM and multiple injection methods. More demanding samples may result in a slightly higher error rate in the binding constant due to unpredictable influences such as noisy baselines. For applications requiring maximum precision, the multiple injection ITC method will continue to be the gold standard for measuring molecular interactions. For screening applications or initial exploratory binding characterization experiments, SIM will provide additional versatility and speed.

Hardware and software upgrades may be required to use SIM on your system. For older MicroCal VP-ITCs, this is the perfect way to tune-up your trusted calorimetry work horse. For newer Microcal VP-ITCs only a software upgrade may be required. Contact your local Malvern office for upgrade details.

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