Experimental Considerations

Estimation of required concentrations

(as per MicroCal WebPage: http://www.microcalorimetry.com/tips/Default.htm)

The following equation is a good approximation of the concentration of the material in the sample cell (i.e. an enzyme):

Ka * C = 10 to 50

Where Ka is the binding (association) constant expressed as a positive power of 10 (i.e., 10) and C is the molar concentration of the binding component in the sample cell. If Ka is unknown, a best guess will have to suffice.

The molar concentration of the binding material in the syringe (i.e. an enzyme inhibitor) is estimated as follows:

100 µl syringe: 20 * n * C
250 µl syringe: 7 * n * C

Where "n" is the stoichiometry of the reaction and C is the molar concentration of the binding material in the sample cell.

These are "rules of thumb" and not absolute. However, if Ka and n are unknown, best guess and perhaps a range finding experiment should quickly allow the investigator to determine the correct experimental conditions.

As an example, assume we have an enzyme in the cell with a binding constant of 10. The concentration of enzyme should therefore be 10 * C = 10 to 50 or a concentration of between 10 to 5x10 molar. Assuming a stoichiometry of 1, the concentration of inhibitor in the 100 µl syringe should be 20-fold higher than the concentration of the enzyme in the cell (20 x 1 x 10 or 2 x 10 molar, in the case of a 1 µ molar enzyme solution). If it were desirable to use the 250 µl syringe, the concentration of inhibitor in this syringe should be 7 times the concentration of enzyme in the cell.

The ITC instrument in Biophysical Resource has a 250 µL titration syringe.