

binding



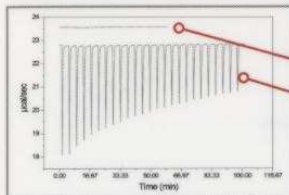
stability



kinetics



Controls / Buffers

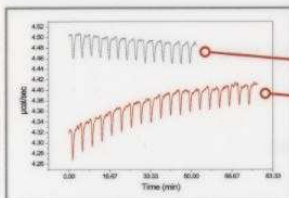


Situation 8

Buffer into Buffer
Ligand into Buffer

Observation
Unmatched buffers

Solution
Match ligand solution and buffer
- e.g. check pH

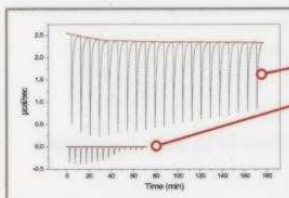


Situation 9

Ligand into Buffer
Ligand into Macromolecule

Observation
No heat of binding

Solution
Do again at different temp. (ΔT 5-10°C).
If still no heat then there is either no binding or very weak binding ($< [M_{tot}]$)

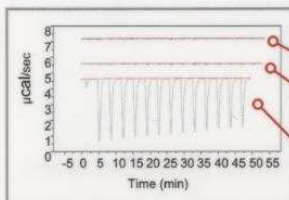


Situation 10

Before dialysis
After dialysis

Observation
Buffer mismatch

Solution
Dialyse if possible.



Situation 11

Matched Buffer
Slight Buffer mismatch
Mismatched Buffer -DMSO in Ligand

Observation
DMSO in Ligand

Solution
Add DMSO to protein to match

Can't find your problem? Please contact your local MicroCal team

Ultrasensitive Calorimetry for the life Sciences



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MicroCal, LLC

"A Troubleshooting Guide for Isothermal Titration Calorimetry"

A guide for tracking down and solving the most common problems encountered with running samples on your VP-ITC or AutoITC.

These are possible problems but do not cover everything. If your problem is not addressed in this guide, please contact your local MicroCal Customer Service Team:

Tel: +44 (0) 1908 576330

Fax: +44 (0) 1908 576339

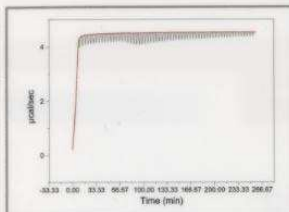
Email: scientificsupport@microcal.eu.com

Please keep this guide with your ITC.

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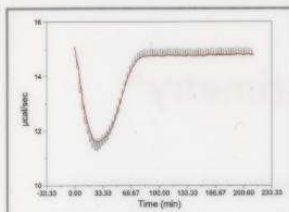
Common Problems



Situation 1

Observation
Erroneous Baseline

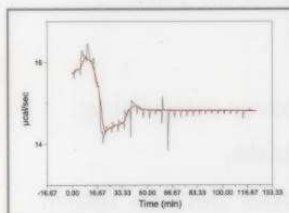
Solution
Clean cells thoroughly



Situation 2

Observation
Erroneous Baseline

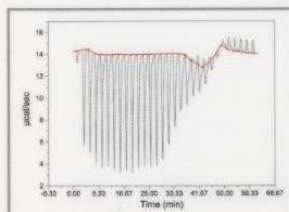
Solution
Clean cells thoroughly



Situation 3

Observation
Erroneous Baseline

Solution
Clean cells thoroughly



Situation 4

Observation
Apparent baseline change

Solution
Increase time between injections

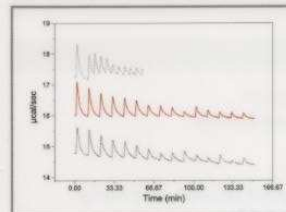
binding



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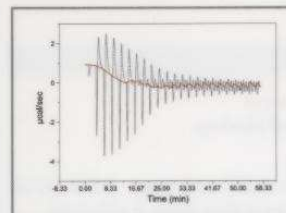
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Situation 5

Observation
Power not returning to baseline

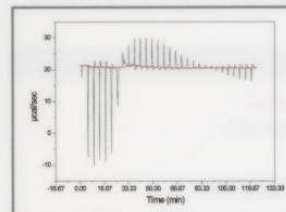
Solution
Increase time between injections



Situation 6

Observation
Oscillating signal, power below zero

Solution
Set reference offset higher
- check for sample aggregation



Situation 7

Observation
Multiple effects, power below zero
and multiple binding events

Solution
Increase reference offset and then fit to data to multiple
binding site model

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