TAM: Thermal Activity Monitor

Basic Theory &
Applications Training
2015

Agenda – Basic TAM Course

- Introduction to calorimetric principles and TAM instruments
- Calibration
- Overview of TAM Assistant
- Experimental part: Calibration
- Sample preparation and experimental considerations
- Experimental part: Setting up an experiment
- Data handling and generating reports

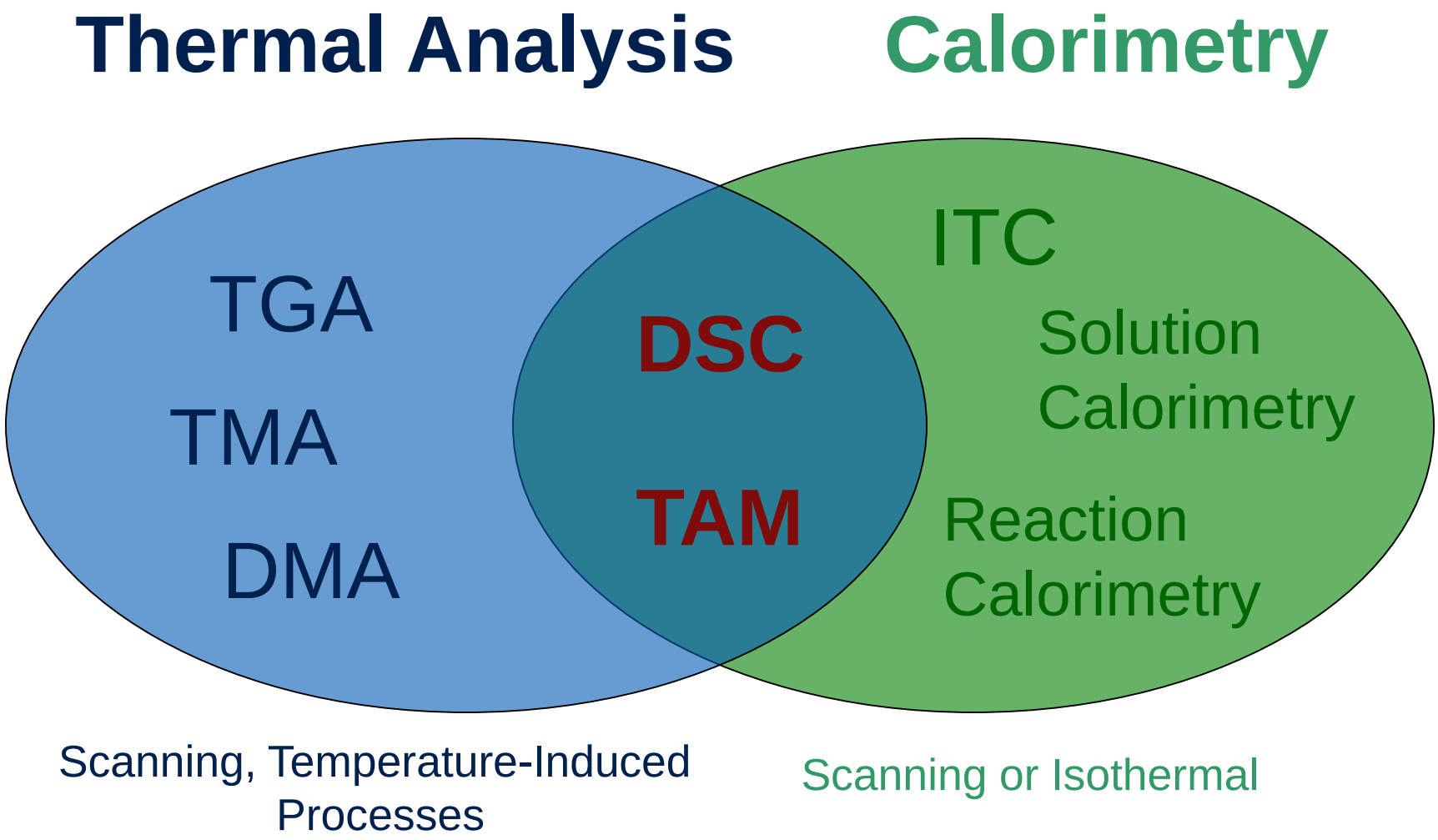
Thermal Measurements

- Virtually all chemical and physical processes result in either heat production or heat absorption.
- Calorimetry quantifies the amount and rate of heat release in terms of heat flow, heat and heat capacity.
- Calorimetry is a non-specific technique making it ideal for studying almost all kinds of physical and chemical processes in life sciences, materials sciences and within pharmaceuticals.

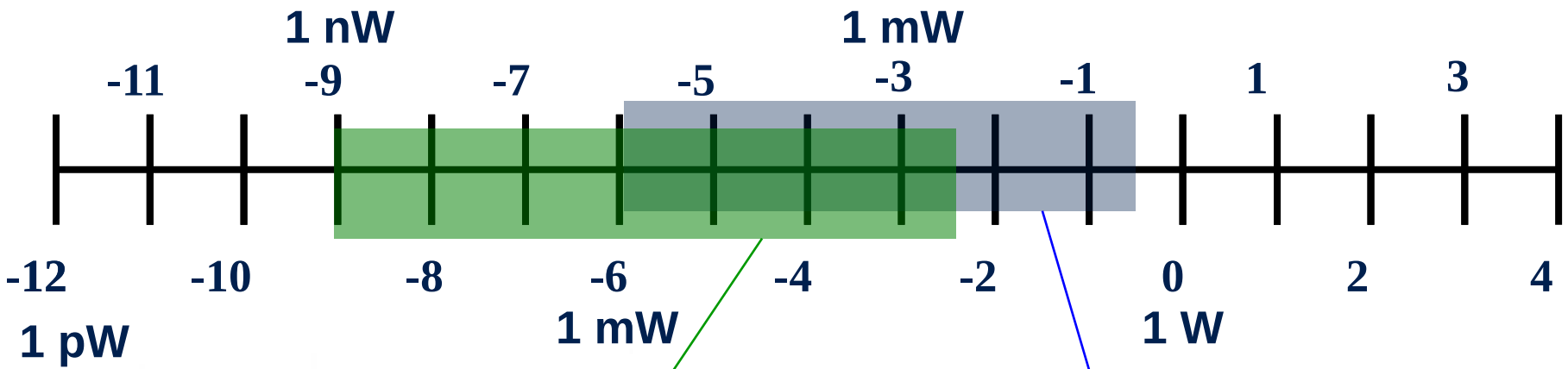
Thermal Analysis and Calorimetry

- Thermal analysis
  - The change of a property as function of temperature
  - DSC (scanning mode), TGA, DMA, TMA (slow scanning mode)
- Calorimetry
  - The measurement of heat properties, *i.e*. heat flow, heat and heat capacity as function of time and temperature

Thermal Analysis versus Calorimetry



Calorimetric Range



Specific Sensitivity – a Comparison

	Sensitivity	Sample amount	Specific sensitivity
Nanocalorimeter	10 nW	5 g	2 nW/g
DSC	200 nW (0.2 mW)	10 mg	20 µW/g

Difference in specific sensitivity X 10,000

TAM versus DSC

- Complementary techniques
- High sensitivity in the range - nW vs. µW
- High specific sensitivity - g vs. mg
- Measurements in hours vs. minutes
- Absolute heat capacity determinations – < 0.2 % vs. 1-5 %
- Most TAM experiments are done isothermally

INSTRUMENTATION

TAM – Thermal Activity Monitor

Represents a range of products used for calorimetric measurements

TAM IV

TAM III

SolCal

TAM Air

General Features of the TAM

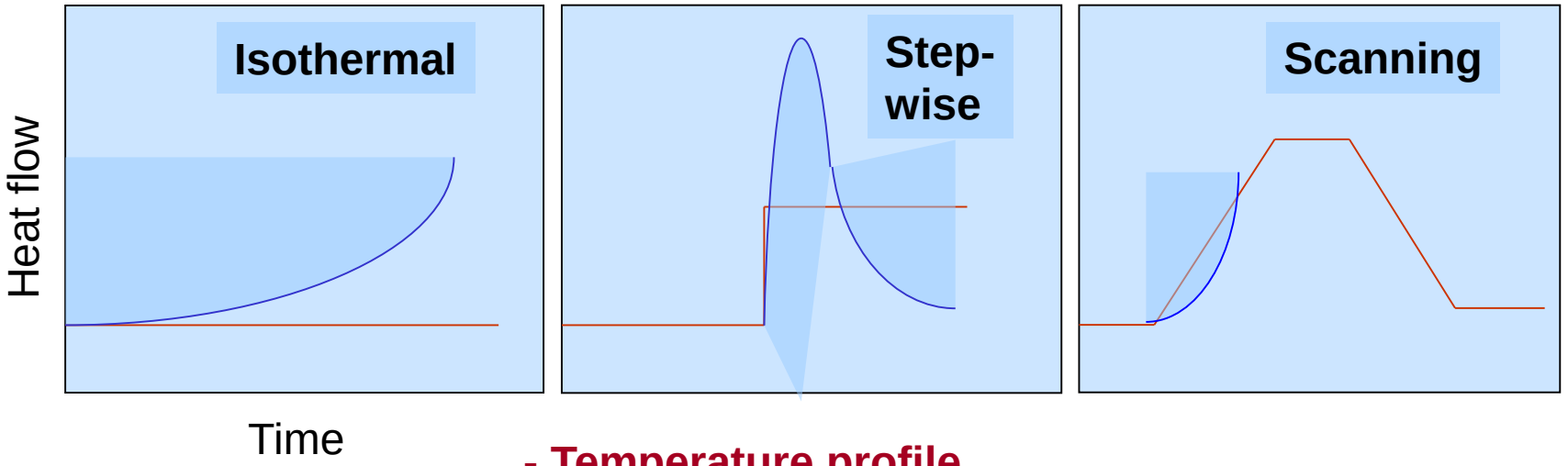
- Thermostat
- Calorimeters (4mL, 20mL, 125mL)
- Sample handling (Ampoules)
- Accessories
- Software

TAM – Isothermal and Scanning Thermostat

- Temperature range: 4° – 150 °C (TAM III is 15° – 150 °C)
  - Isothermal and slow scanning (2 °C/h)
  - Temperature stability: < 0.1 mK/24 h
  - Temperature accuracy: ±0.1°C
  - Temperature precision: < ±0.1 mK
- Multi functional calorimeters and accessories
  - Different measuring modes
- High sample throughput
  - Up to 48 individual twin heat flow calorimeters.
- Outstanding sensitivity and long-term stability (μW-nW)

TAM Temperature Modes

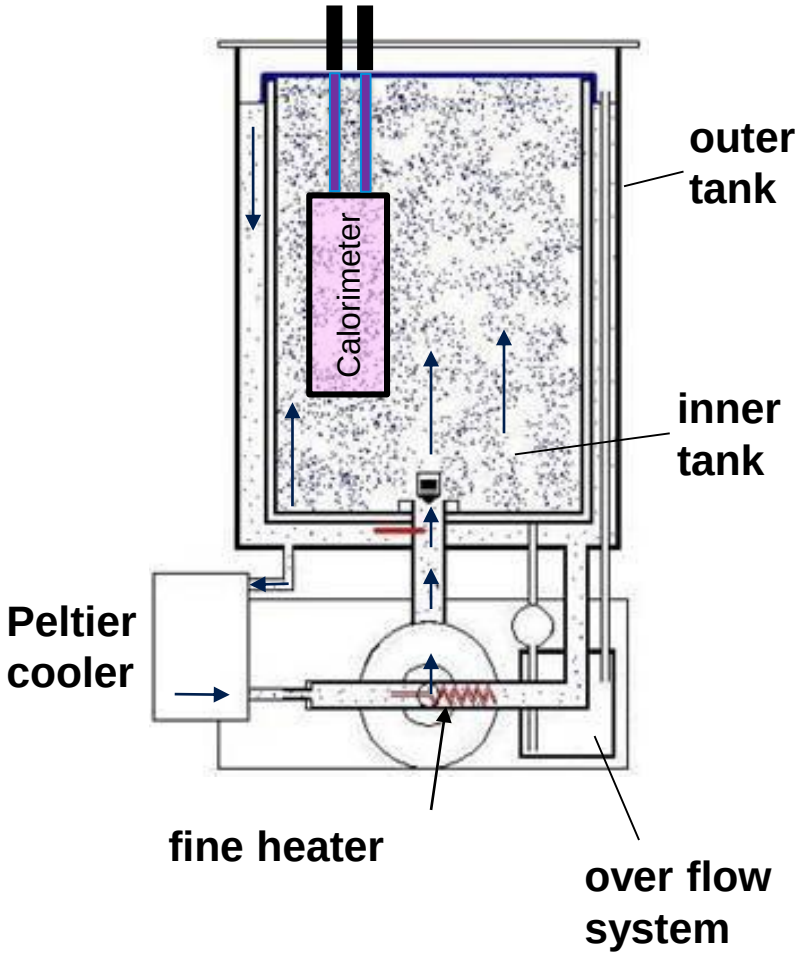
- Isothermal
- Step-wise scanning
- Slow scanning (max ±2 °C/hr)



- Temperature profile
- Example heat flow data

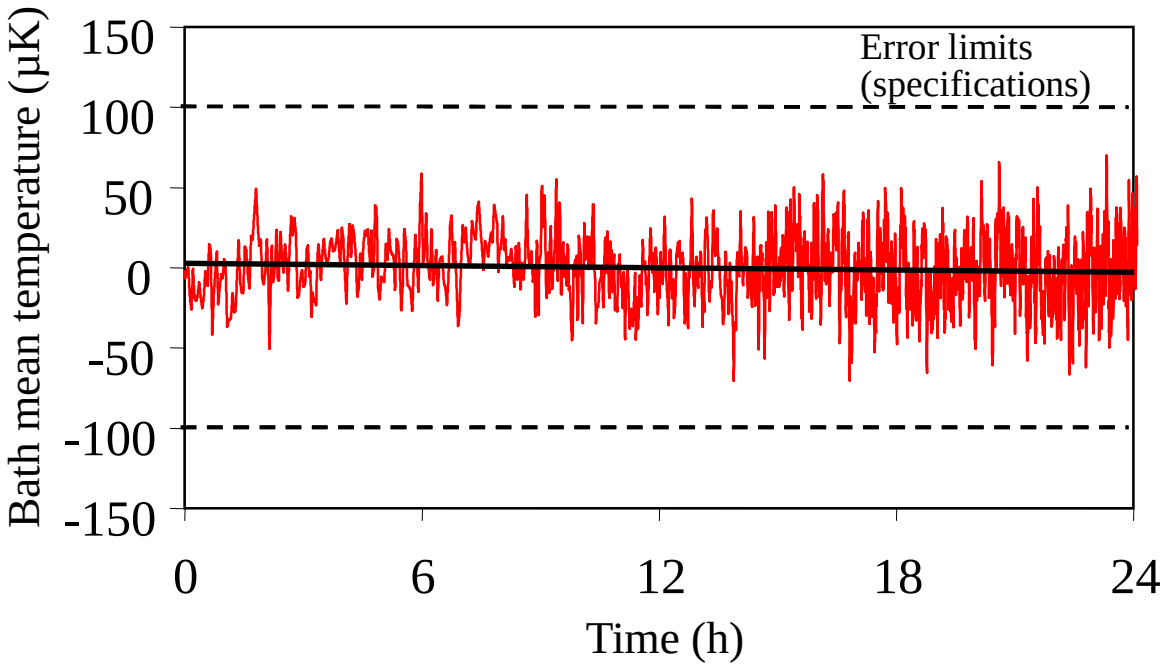
TAM Thermostat

An oil based liquid bath system for a continuously circulated heat sink medium that prevents any thermal event in a test sample or from the room environment from altering the constant temperature bath



TAM IV Thermostat Features

- A temperature regulation system utilizing state-of-the-art electronic thermistor sensors to constantly adjust the heating, cooling and uniform oil flow speed for a temperature drift over 24 hours that is less than ±100 µ°C



Multi-Functional Calorimeters of the TAM III/IV

- TAM is a flexible system which can be configured for a variety of applications.

- Additional functions or increased measuring capacity is easily obtained by adding:
  - Calorimeters
  - Sample handling systems (ampoules)
  - Accessories or micro-reaction systems

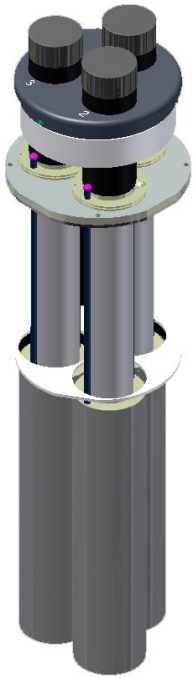
The Flexibility of the TAM

Sample size

Nanocalorimeter

Multicalorimeter (4 mL)

Microcalorimeter



Multicalorimeter (20 mL)

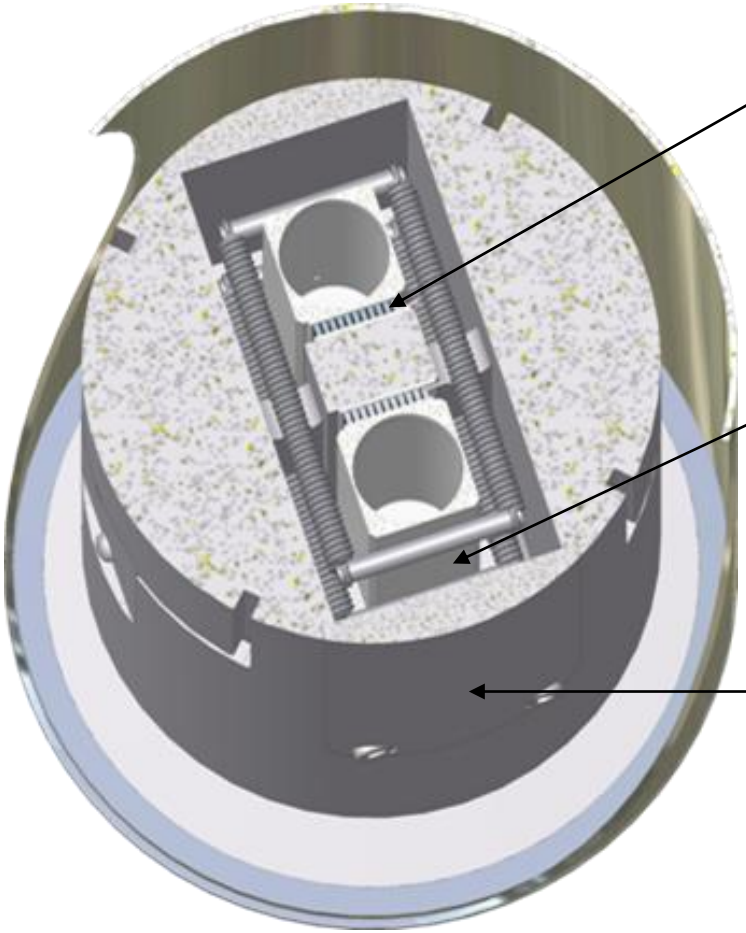
Macrocalorimeter

Absolute Sensitivity

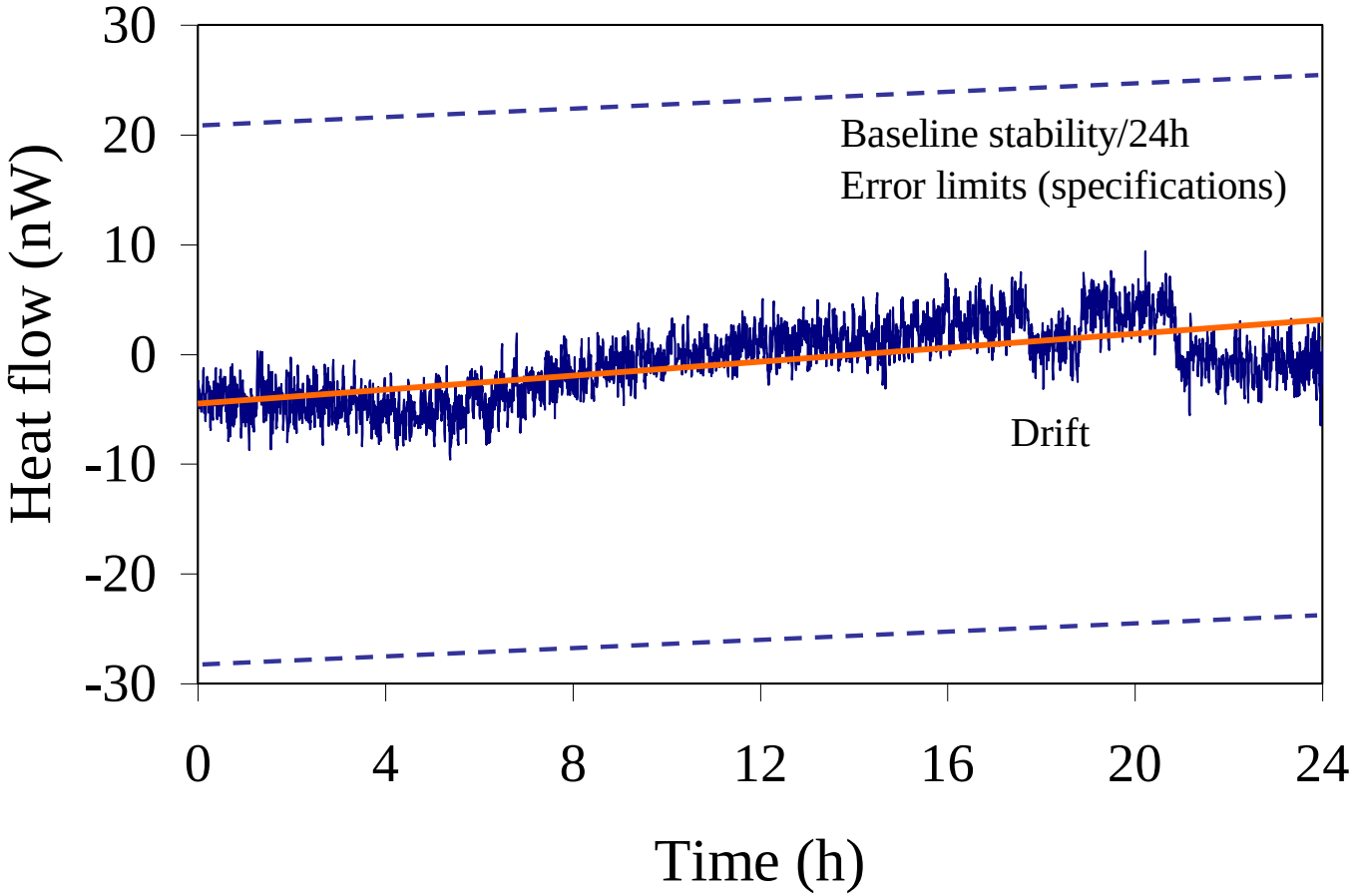
Nanocalorimeter

- Highest sensitivity twin channel calorimeter
  - Heat flow (or flux)
    - **high sensitivity, longer time scales**
  - Dynamic heat flow
    - Considers the thermal inertia of a calorimeter
  - Power compensation **(high resolution, shorter time scales)**
    - Referred to as '**Feedback**' in software
    - A constant electric power is supplied to both sample and reference calorimeters continuously.
    - Time constant of calorimeter significantly smaller than heat flow mode.
- Reference accessible by user
  - Up to 4 mL total volume ampoules
- Use with 1 or 4 mL Micro Reaction System(s)
  - Only choice for high sensitivity isothermal titration calorimetry (ITC)

Detection System of the Nanocalorimeter



Nanocalorimeter – Heat Flow Stability over 24hr



The absolute temperature in this measurement was 35.0˚C

Microcalorimeter

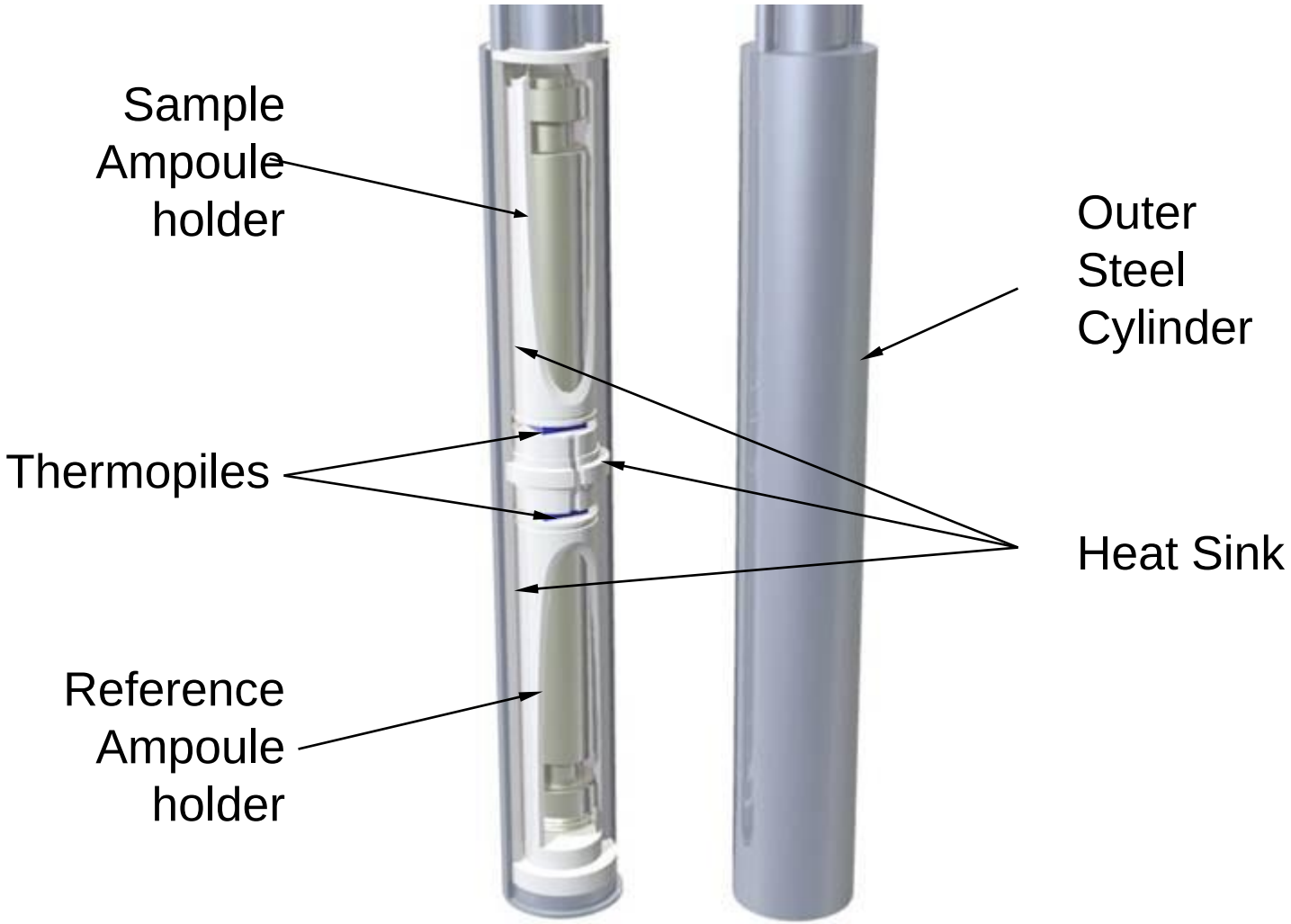
- Highest specific sensitivity twin channel calorimeter
  - Heat flow (or flux)
    - **high sensitivity, longer time scales**
  - Dynamic heat flow
    - Considers the thermal inertia of a calorimeter
- Reference accessible by user
  - 20 mL total volume ampoules
  - For large samples or increased gas phase
- Used with 20 mL Micro Reaction System(s)
  - Only choice for Micro Solution Ampoule

Minicalorimeter (4 and 20 mL versions)

- High Throughput twin channel calorimeter
  - Heat flow (or flux)
  - Dynamic heat flow
- Reference NOT accessible by user
  - Must determine type of plug to be inserted to minicalorimeter for best heat capacity balance and performance.
  - 4mL size best for temperature ramp
  - 20 mL size for vacuum/pressure ampoule

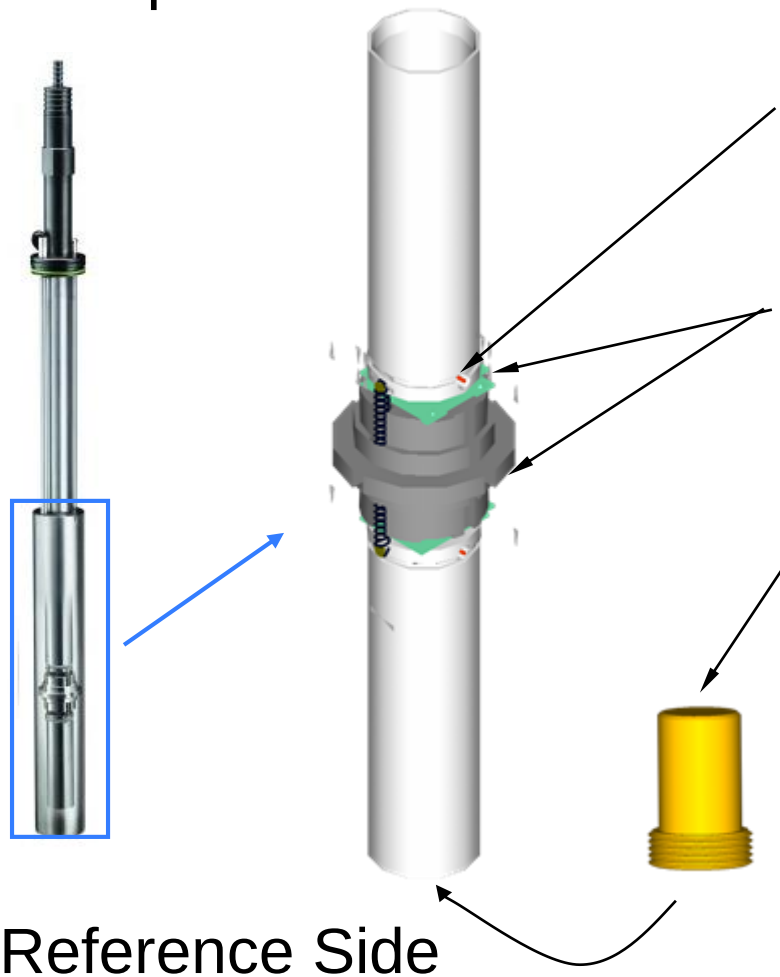
4mL minicalorimeter attached to its computer interface

Minicalorimeter (4 mL)



Minicalorimeter Measuring Assembly

Sample Side



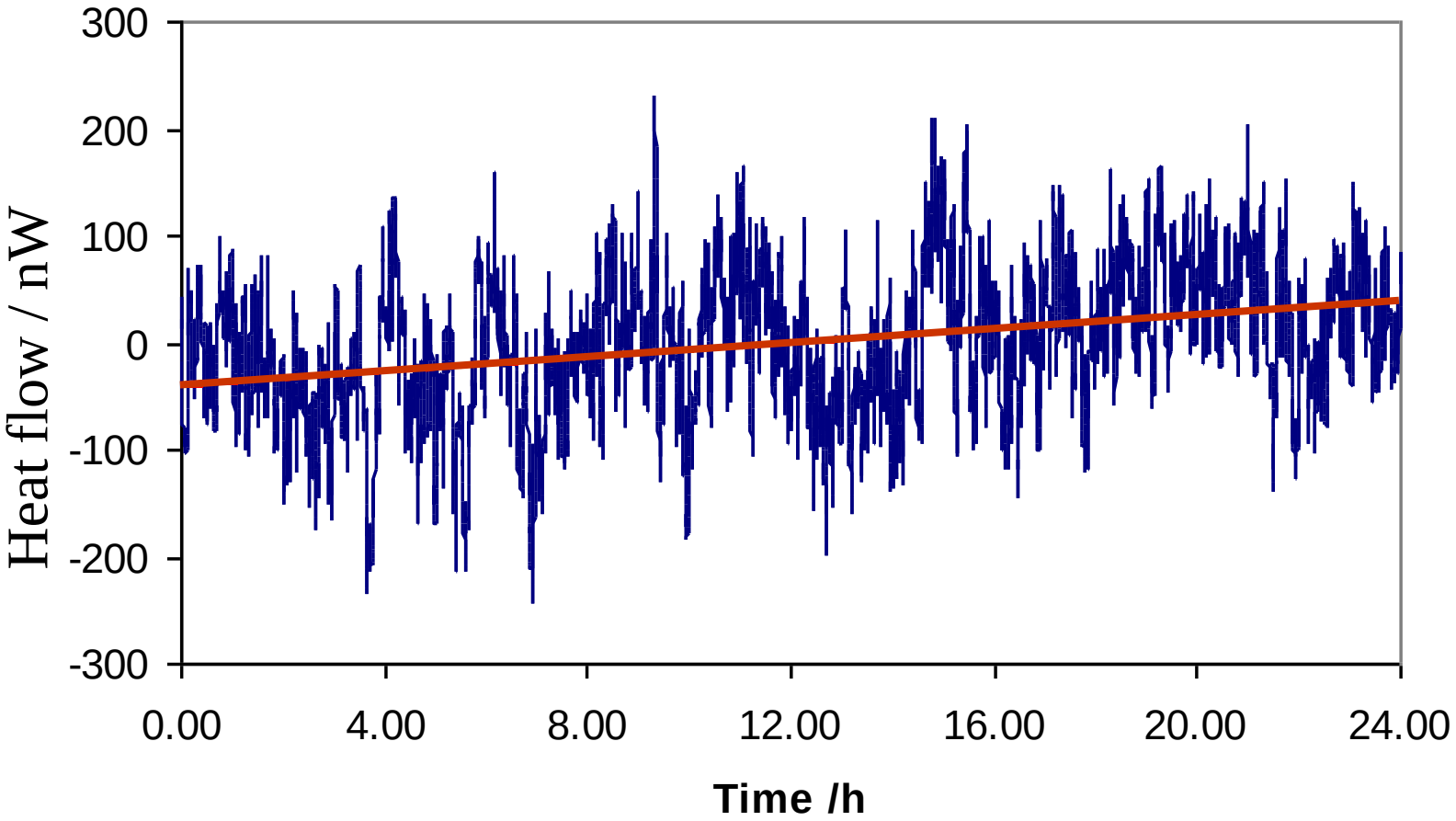
Calibration Heater

Thermopiles (one each side)

Reference plug is permanently inserted and must be selected based on the type of ampoule being used.

Reference Side

Minicalorimeter (unbalanced) - Baseline Stability

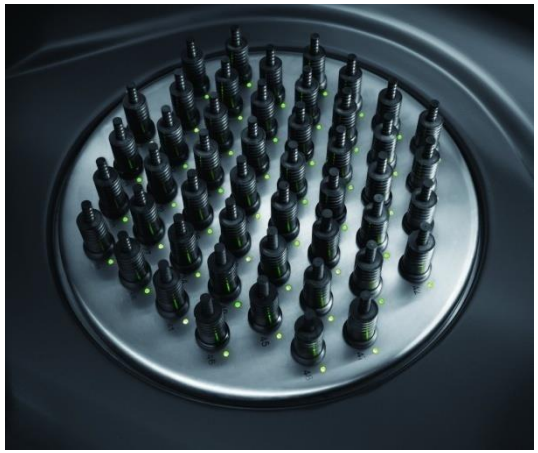


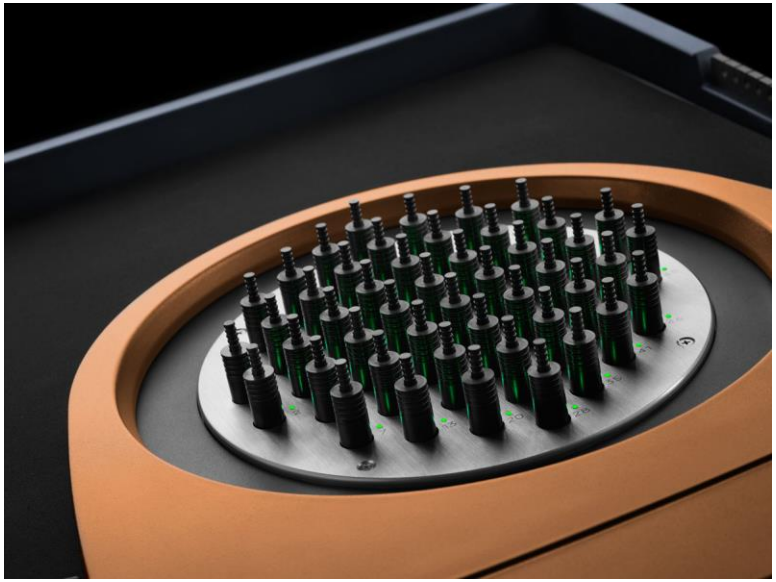
The absolute temperature in this measurement was 50.0 ˚C. Note: must acknowledge that the calorimeter is not balanced due to the permanent reference plug.

Multicalorimeter (4 mL)

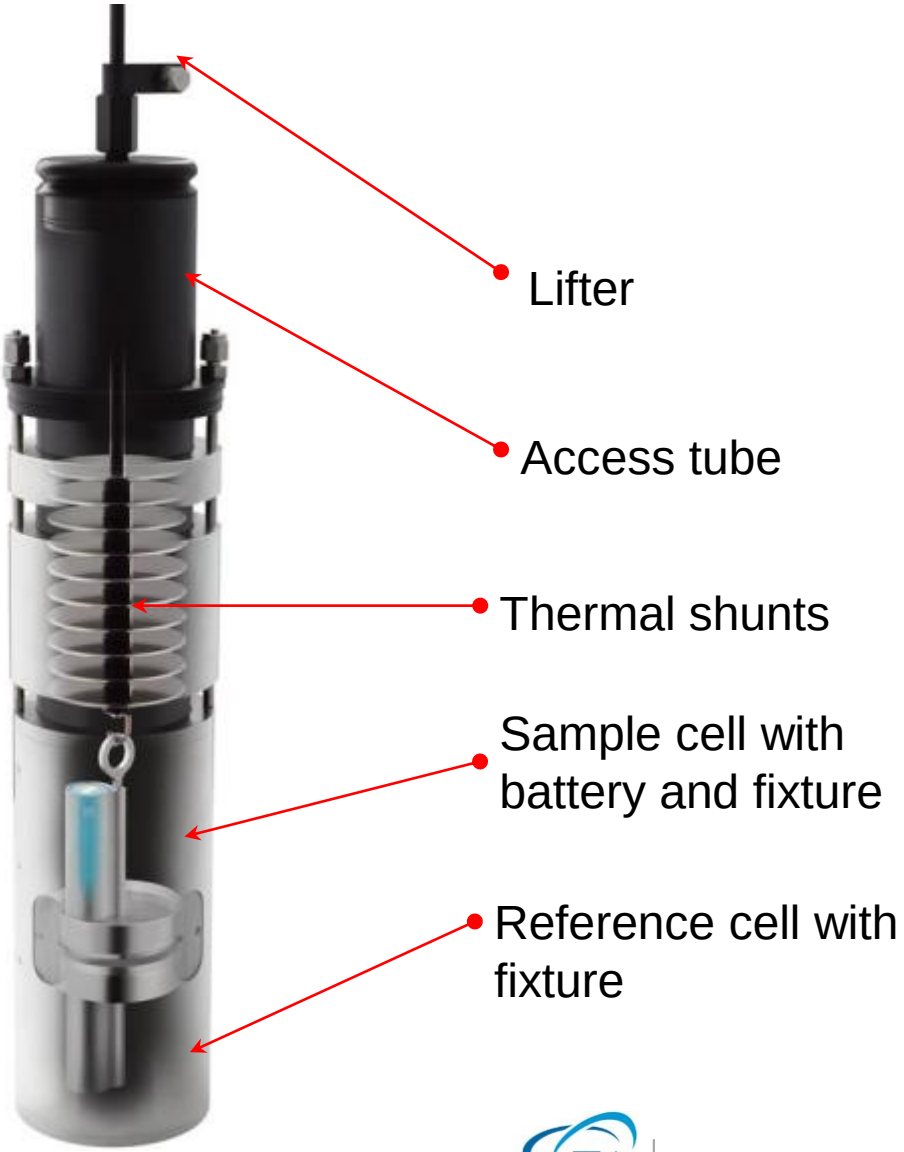
- For increased measuring capacity and productivity of TAM
- Consists of six minicalorimeters (4 mL)
  - Also 20 mL multicalorimeters that consist of 3 – 20 mL minicalorimeters (not shown)
- Four multicalorimeters can be used with a TAM thermostat to provide 24 simultaneous measurements or alternatively the TAM 48...

Minicalorimeters (4 mL) in TAM 48





Macrocalorimeter



Calorimeter Theory

Definitions

- Rate of heat production
  - The rate of heat produced (exothermic) or consumed (endothermic) by the sample

- Rate of heat exchange
  - The rate of heat flow between the sample and the surrounding

Note: During Steady State conditions these properties are equal

Exothermic reactions → Positive Heat flow signal
Endothermic reactions → Negative Heat flow signal

The heat flow is proportional to the rate of the reaction

Rate Equation in Terms of Heat Flow

$$\frac{dC}{dt} = k \cdot f(c) \qquad \left[\frac{mol}{m^3 s}\right]$$

$$\frac{dQ}{dt} = \frac{dC}{dt}\Delta H \qquad \left[\frac{J}{m^3 s}\right] = \left[\frac{mol}{m^3 s}\frac{J}{mol}\right]$$

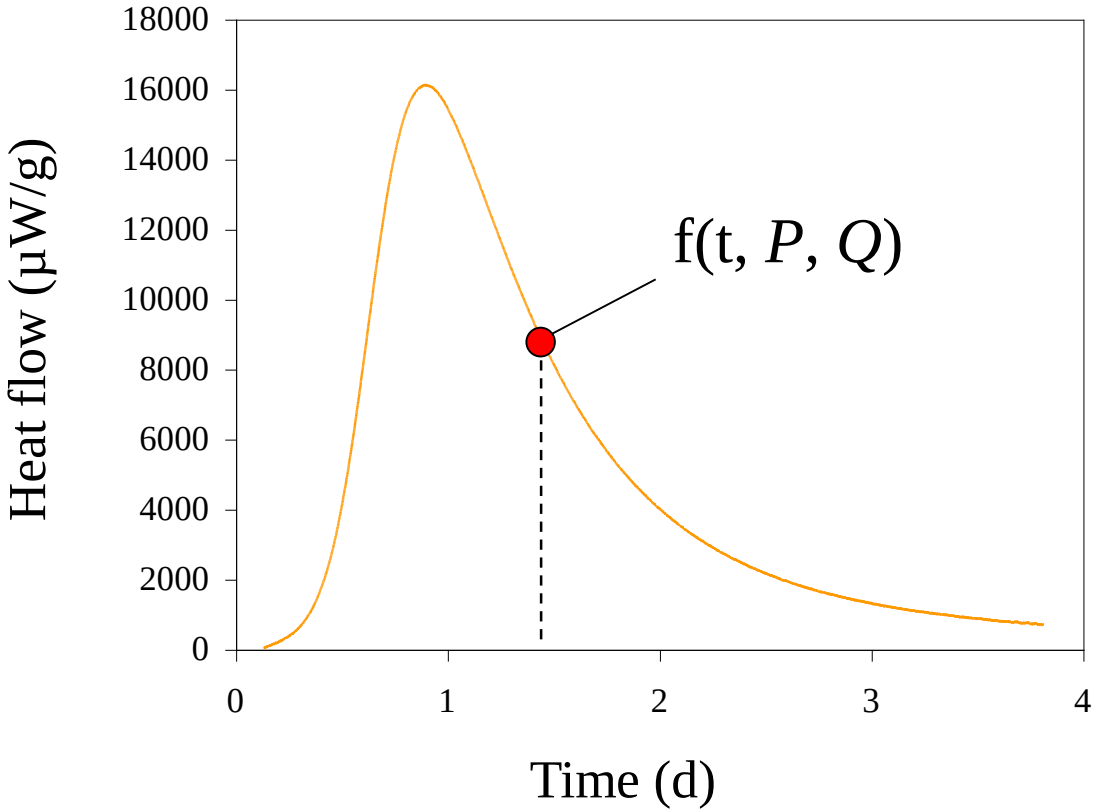
$$\frac{dQ}{dt} = \Delta H \cdot k \cdot f(c)$$ = Heat flow signal from TAM

Enthalpy → Thermodynamic Information

Reaction rate → Kinetic Information

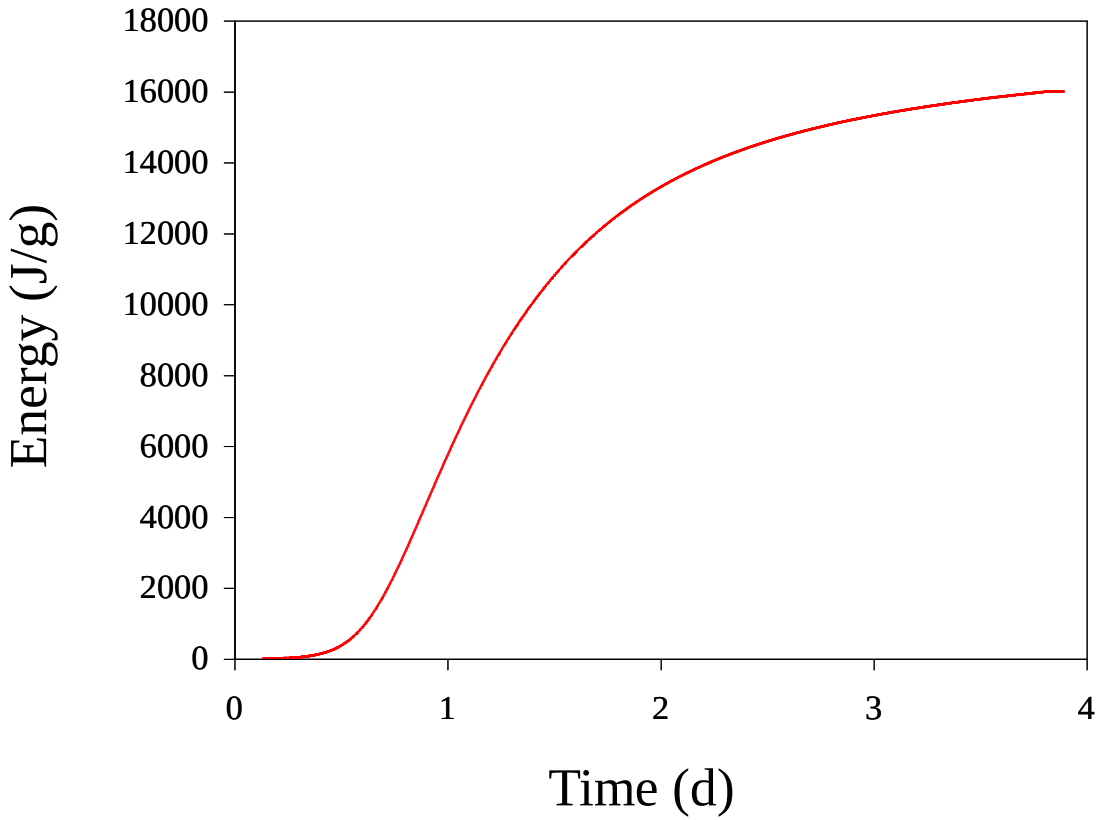
Concentration → Analytical Information

Heat Flow versus Time



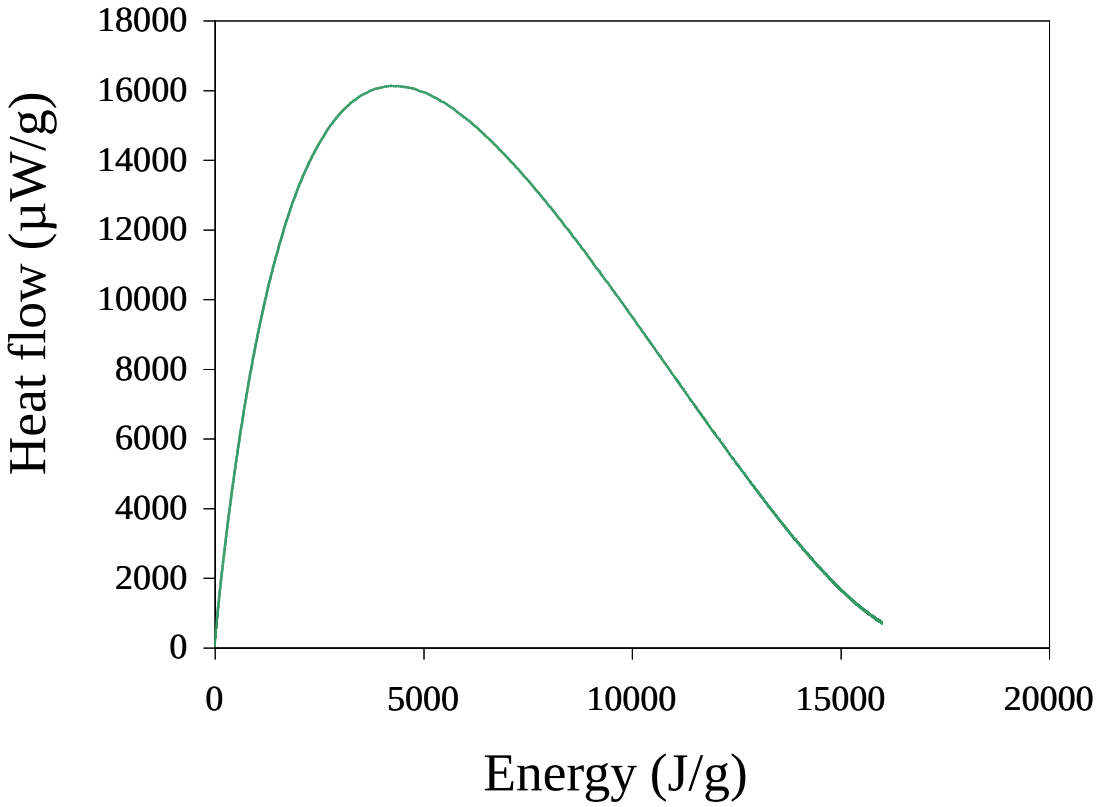
Shows how the reaction rate varies with time.

Energy versus Time



Shows how the extent of reaction varies with time.

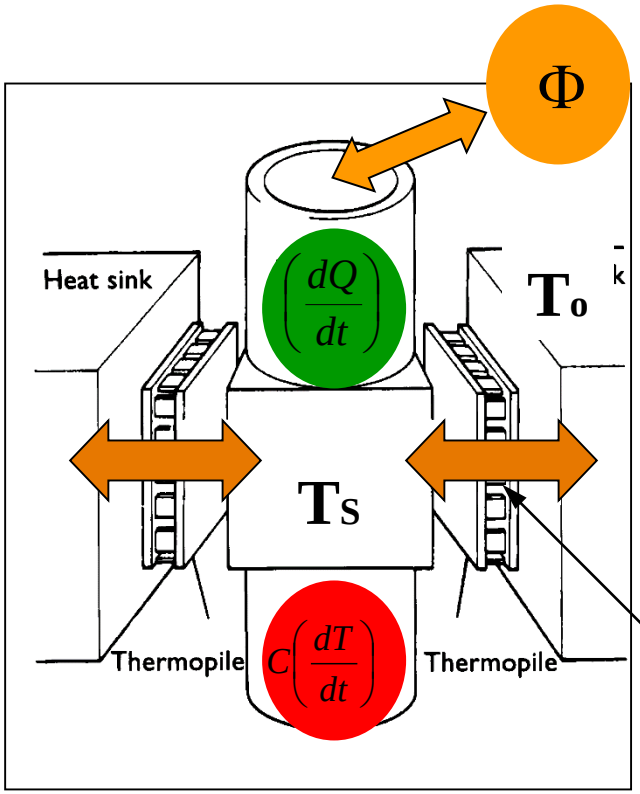
Heat Flow versus Energy



Shows how the reaction rate varies with the extent of reaction

(ex. 1 order → *Q* proportional to *P* with rate const., k, as proportionality constant)

Calorimetric Unit



General Heat Balance Equation

$$\frac{dQ}{dt} = \Phi + C\left(\frac{dT}{dt}\right)$$

Rate of Heat Production = **Rate of Heat Exchange** + **Rate of Heat Accumulation**

The measured property

After calibration the following holds:

Rate of Heat Production (dQ/dt) = **Heat flow Monitored by TAM**

The Heat Balance Equation (in terms of temperature)

$$\frac{dQ}{dt} = \Phi + C\frac{dT}{dt}$$

Single calorimeter

$$\Phi = k\left(T - T_o\right)$$

(Newton's cooling law)

Note: dQ/dt depends on the surrounding temperature.

k = Heat Conductance = C/τ

$$\frac{dQ}{dt} = k(T - T_o) + C\frac{dT}{dt}$$

The temperature can be monitored by a thermopile as is the case in TAM or by using thermistors, as is the case of the Solution Calorimeter (SolCal).

The Heat Balance Equation (in terms of voltage)

$$\frac{dQ}{dt} = \Phi + C\frac{dT}{dt}$$

Substitute Newton's cooling law and potential (or voltage) terms [Seebeck]

$$\Phi = k\left(T - T_o\right) = \frac{k}{g}V \quad \Rightarrow \quad T = \frac{U}{g} + T_o \quad \Rightarrow \quad \frac{dT}{dt} = \frac{1}{\mathrm{g}}\frac{dU}{dt}$$

$$\frac{dQ}{dt} = \frac{k}{g}V + \frac{C}{g}\frac{dV}{dt} = \frac{k}{g}\left(V + \frac{C}{k}\frac{dV}{dt}\right) = \varepsilon\left(V + \tau\frac{dV}{dt}\right)$$

Tian Equation

$$\frac{dQ}{dt} = \varepsilon\left(V + \tau\frac{dV}{dt}\right)$$

$g = \text{Seebeck coefficient (V/K)}$

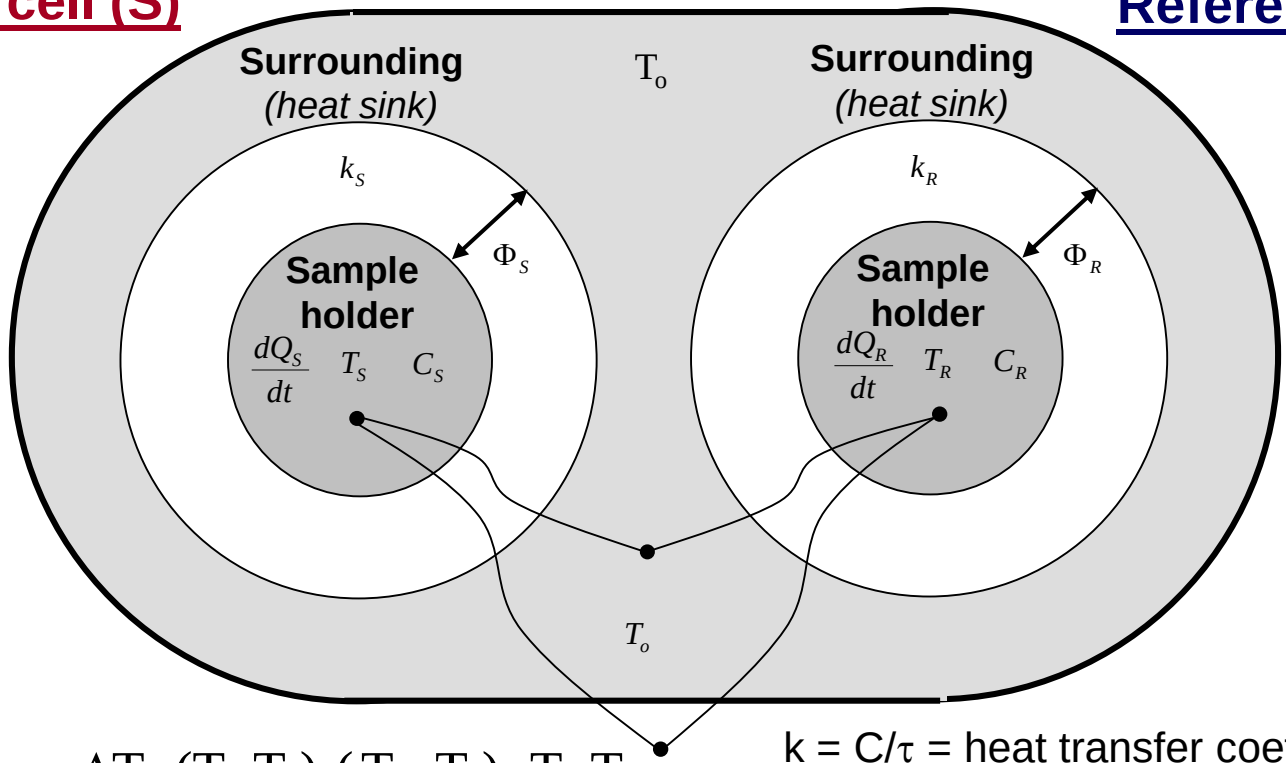
$\varepsilon = \text{calibration constant (W/V)}$

$\tau = \text{time constant (s)}$

Twin Channel Calorimeter (as used in TAM)

Sample cell (S)

Reference cell (R)



$$\Delta T=(T_s-T_o)-(T_R-T_o)=T_s-T_R$$

k = C/τ = heat transfer coefficient between the sample and the surrounding or heat conductance

τ = time constant

With a twin system the noise will be reduced!

The Heat Balance Equation (Twin System)

Sample side

$$\frac{dQ_S}{dt} = k_S(T_S - T_o) + C_S\frac{dT_S}{dt}$$

Reference side

$$\frac{dQ_R}{dt} = 0 = k_R(T_R - T_o) + C_R\frac{dT_R}{dt}$$

Subtraction gives

$$\frac{dQ_S}{dt} = k_S(T_S - T_o) + C_S\frac{dT_S}{dt} - k_R(T_R - T_o) - C_R\frac{dT_R}{dt}$$

The Heat Balance Equation (Twin System)

$$\frac{dQ_S}{dt} = k(T_S - T_R) + C\frac{d(T_S - T_R)}{dt}$$

Assumptions:

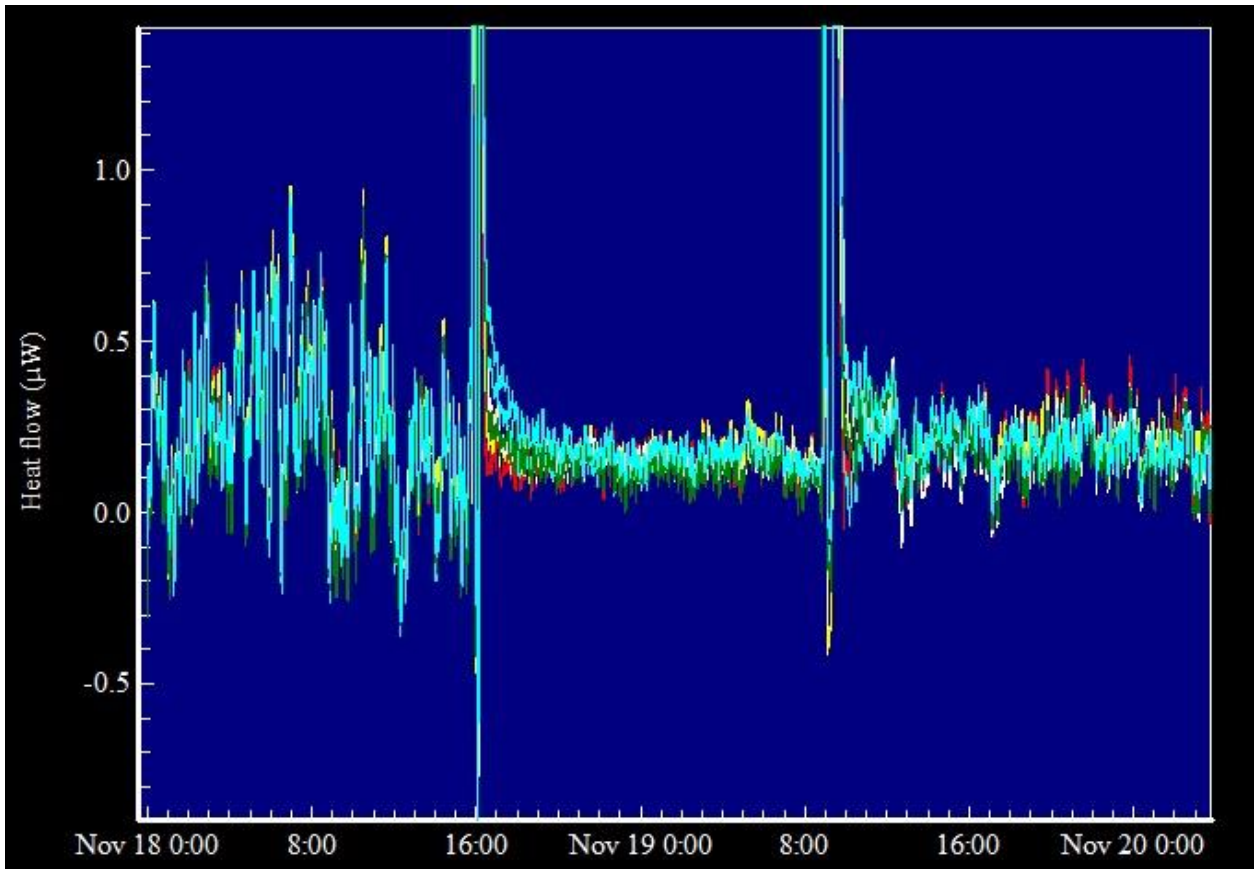
$$k = k_R = k_S$$

$$C = C_R = C_S$$

Qseries DSC does not make this assumption in (T4 mode). Q20 and 29XX DSC and most other commercial DSCs also make this assumption. High heat capacity and the thermal stability within the calorimeters aid in the acceptance of the assumptions for the TAM.

Heat Capacity Balance versus Noise Level

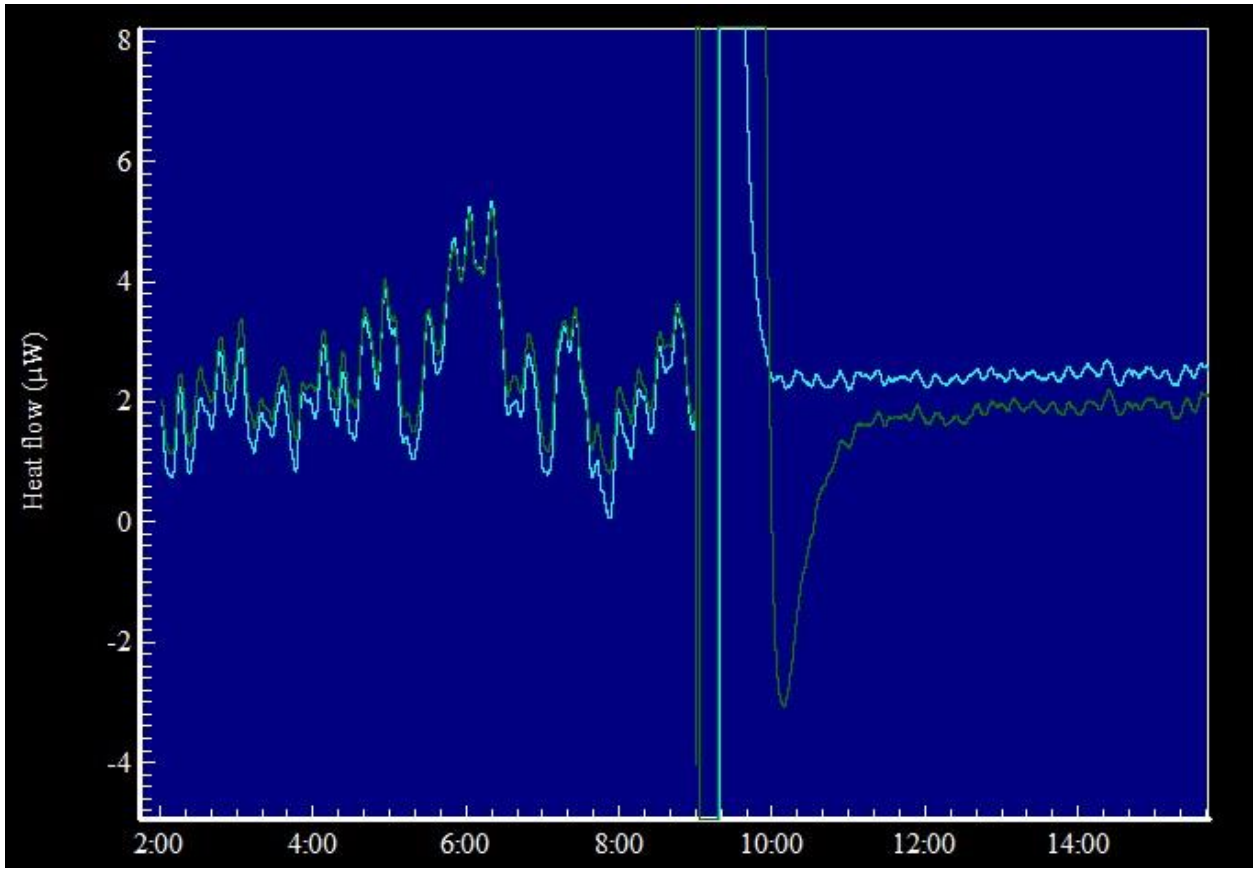
4 mL
Mini
calorimeter



Empty (unbalanced) | Balanced | High side within 20% of balance

Heat Capacity Balance versus Noise Level

20 mL
Mini
calorimeter



Empty
(unbalanced)

Balanced

Choosing a Reference

Sample side = Micro Solution Ampoule + 16 mL solvent (τ = 240 s)

Reference side = SS circlip ampoule with thick lid + 16 mL solvent (τ = 240 s)

Empty 20 mL calorimeter ($\tau \approx 60$ s)

Heat capacity (C) of common materials:	
Water	4.18 J/K•g
Sand (Quartz)	0.8 J/K•g
Glass	0.84 J/K•g
Stainless Steel	0.47 J/K•g
Aluminum	0.90 J/K•g

Inserted in 4 mL Calorimeter: τ(s)	
Empty	96
SS circlip	205
SS screw cap	202
Glass circlip	151
RH Perf./Titr.	206
1 g Water	41-44
1 g Glass beads	5-10

(measured with TAM 2277 at 25 °C)

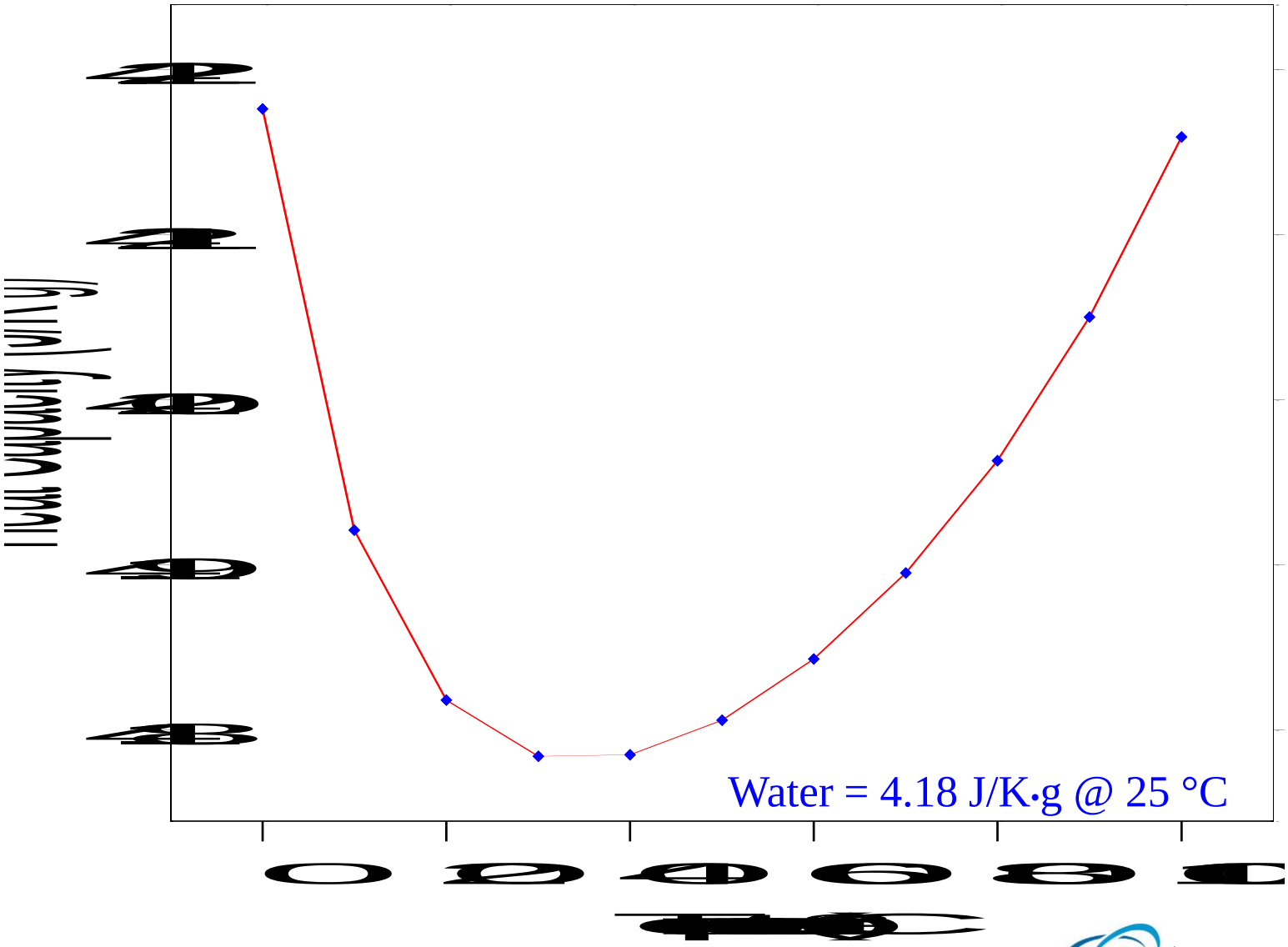
*Use GPT to measure τ.

Balance heat capacity (C) or time constant (τ)*

$$\tau = C/k$$

See also EN 008

Heat Capacity of Water Versus Temperature



Choosing a Reference – 4 mL Minicalorimeters

$$\Delta = 100 \cdot \frac{C_{p,ri} - 0.24 - C_{p,a} - C_{p,s} \cdot m}{C_{p,ri} + 7.18}$$

Minicalorimeters:

$C_{p,ri}$, $C_{p,a}$ and $C_{p,s}$ are heat capacities of reference insert, ampoule and the substance
Heat capacity of the ampoule holder (reference side) = 7.42
Difference in heat capacity of the ampoule holders sample and reference side = 0.24
Mass (m) of substance used can be optimised so that Δ approaches zero.
Maximum deviation for which the signal will lie within specifications is ±20%.

Choosing a Reference – 4 mL Minicalorimeters

	$C_{p,ri}$ /J K^{-1}	Optimized for:
Ref. insert 1/4	6.89	3-ml glass vial with 2g organic solid material
Ref. insert 2	19.18	4-ml Stainless steel ampoule with 2 ml of an aqueous solution
Ref. insert 3	8.43	4-ml glass vial with 2g organic solid material
Ref. insert 5	3.67	3-ml glass vial with 150 mg organic solid material
Ref. insert 6	15.89	4-ml Stainless steel ampoule with 2g organic solid material.
Ref. insert 7	9.24	3-ml glass vial with 3g organic solid material
Ref. insert 8	10.73	4-ml glass vial with 2.8g organic solid material

Values for the minicalorimeter balance equation shown on the previous slide.

Product no	$C_{p,a}$ /J K^{-1}	**Description**
2509-51	2.34	5 ml Heat Seal Glass Ampoule
95.53.1015	4.32	3 ml Disposable Glass Ampoule
24.20.0400	5.84	4 ml Disposable Glass Ampoule
2502-40	11.9	4 ml St.Steel Ampoule circlip cap
2277-301	11.5	4 ml St.Steel Ampoule threaded cap
3320	10.8	4 ml St.Steel Ampoule threaded cap

Choosing a Reference – 4 mL Minicalorimeters

Please refer to Table 1 that lists the total Cp of all 8 models of the 20 mL minicalorimeters. For sample Cp calculations, utilize Table 2 for a list of some common sample materials.

4mL minicalorimeter						
		Disposable	Disposable			
		3mL glass	4mL glass	4mL steel	4mL steel	4mL Hastelloy
		95.53.1015	24.20.0401	2277-301	3320-1	3320
	Ampoule Cp (J/K)	4.3	5.8	11.9	10.5	9.2
Reference Number	Total Cp (J/K)	Sample Cp (J/K)				
X = 1	6.9	2.6 ± 1.4	1.1 ± 1.4			
X = 2	19.2	14.9 ± 3.8	13.4 ± 3.8	7.3 ± 3.8	8.7 ± 3.8	10.0 ± 3.8
X = 3	8.5	4.2 ± 1.7	2.7 ± 1.7			
X = 4	6.9	2.6 ± 1.4	1.1 ± 1.4			
X = 5	3.7					
X = 6	15.9	11.6 ± 3.2	10.1 ± 3.2	4.0 ± 3.2	5.4 ± 3.2	6.7 ± 3.2
X = 7	9.3	5.0 ± 1.9	3.5 ± 1.9			
X = 8	10.8	6.5 ± 2.2	5.0 ± 2.2		0.3 ± 2.2	1.6 ± 2.2

Example 1: Sample of gunpowder loaded into a calorimeter with reference number 6 (15.9 J/K).

Using a stainless steel threaded ampoule (2277-301) with Cp of 11.9 J/K the remaining heat capacity to balance the calorimeter is 4 J/K. Knowing the sample is gun powder that has a Cp of 1.28 J/g·K one can calculate that approximately 3.1 g ± 2.5 g of gun powder must be loaded into the ampoule to best balance the calorimeter to within 20% of the total Cp.

Some common heat capacities		Cp, J K^{-1} g^{-1}
Liquids		
	Water	4,18
	Ethanol	2,43
	Propanol	2,40
	Benzene	1,73
	Toluene	1,71
	Pentane	2,33
	Heptane	2,24
	DMSO	1,93
Solids	Ethylene glycol	2,5
	Inorganic:	
	NaCl	0,86
	Quartz (SiO_2)	0,76
	Organic:	
	Lactose	1,22
	Glycine	1,32
	Urea	1,55
	Glucose	1,24
	Salicylic acid	1,16
	Gun powder	1,28

Table 2: List of Sample Cp

Choosing a Reference – 20 mL Minicalorimeters

Please refer to Table 1 that lists the total Cp of all 8 models of the 20 mL minicalorimeters. For sample Cp calculations, utilize Table 2 for a list of some common sample materials.

20 mL minicalorimeter					
			Stainless	Stainless	Glass
			Thread o-ring	High Pressure	Disposable
			3440-1	3348-1	24.60.2001
	Ampoule Cp (J/K)		34	53	15
Reference Number	Total Cp (J/K)		Sample Cp (J/K)		
X = 1	29				14 ± 5.8
X = 2	37		3.0 ± 7.4		22 ± 7.4
X = 3	46		12 ± 9.2		31 ± 9.2
X = 4	53		19 ± 10.6		38 ± 10.6
X = 5	57		23 ± 11.4	4 ± 11.4	42 ± 11.4
X = 6	62		28 ± 12.4	9 ± 12.4	47 ± 12.4
X = 7	66		32 ± 13.2	13 ± 13.2	51 ± 13.2
X = 8	71		37 ± 14.2	17 ± 14.2	56 ± 14.2

Example 1: Sample of gunpowder loaded into a calorimeter with reference number 6 (62 J/K).

Using a stainless steel threaded ampoule (3440) with Cp of 34 J/K the remaining heat capacity to balance the calorimeter is 28 J/K. Knowing the sample is gun powder that has a Cp of 1.28 J/g·K one can calculate that approximately 22 g ± 9.6 g of gun powder must be loaded into the ampoule to best balance the calorimeter to within 20% of the total Cp.

Some common heat capacities		
		Cp, J K^{-1} g^{-1}
Liquids		
	Water	4,18
	Ethanol	2,43
	Propanol	2,40
	Benzene	1,73
	Toluene	1,71
	Pentane	2,33
	Heptane	2,24
	DMSO	1,93
Solids	Ethylene glycol	2,5
	Inorganic:	
	NaCl	0,86
	Quartz (SiO_2)	0,76
	Organic:	
	Lactose	1,22
	Glycine	1,32
	Urea	1,55
	Glucose	1,24
	Salicylic acid	1,16
	Gun powder	1,28

Table 2: List of Sample Cp

When to Calibrate

Calibration is suggested if...

- TAM has been switched OFF
- Temperature has been changed
- Change experimental conditions

  - The time constants of calorimeter are increased when an ampoule or accessory are loaded.

    - Choose correct reference and perform calibration with the accessory in measuring position.

  - Routinely at regular intervals due to ageing of the semi-conductor thermopiles (e.g. once every third month)

Calibration of TAM IV

- The calorimeters of TAM IV have been calibrated at 6 different temperatures so as to diminish the influence of temperature on the users calibration results.
- When the user makes a calibration, the results are compared with that obtained from the “factory calibration” and deviation is calculated.
- The deviation is represented by a unit-less calibration constant (called the gain constant in TAM Assistant) and should be close to unity (normally 0.95-1.06).

Purpose of Calibration

- To ensure that the displayed heat flow corresponds to the true heat flow caused by the sample
  - Conversion of measured voltage to heat flow
  - Account for any heat losses

- Calibration of TAM is performed using inbuilt calibration heaters.
  - The inbuilt calibration can be validated using external calibration heaters or chemical test reactions.

Two Types of Calibrations

- Heat flow calibration – for ‘slow’ processes
- Dynamic calibration - for ‘fast’ processes

Static or Dynamic Calibration

- Any calibration makes use of an internal electrical heater (i.e. precision resistor) with known calibration power applied. The voltage monitored by the thermopiles is proportional to the heat flow through the module.
  - Calibration is needed in order to convert the voltage to the corresponding rate of heat production.
    - For slow processes a static calibration can be performed. The displayed heat flow corresponds to the rate of heat production.
    - For fast processes, *i.e.* when the response of the reaction is less or in the range of the time constant of the system, a dynamic calibration should be used. The thermal inertia of the calorimetric system is taken into consideration. The displayed heat flow always corresponds to the rate of heat production.
      - ITC experiments: utilize Feedback mode or dynamic calibration

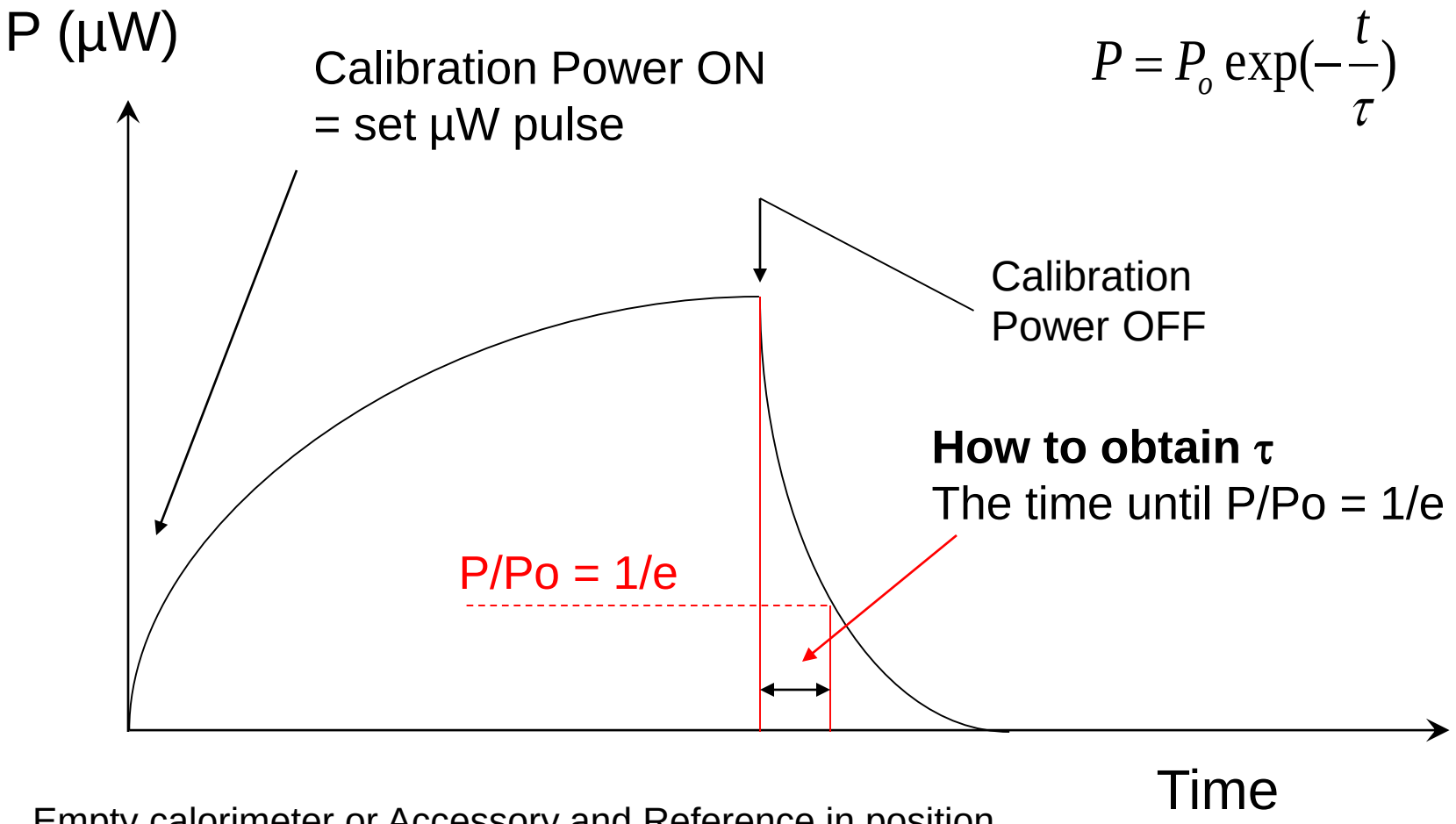
Calibration Conditions

- Ampoule Experiments
  - Empty ampoule lifters (or empty ampoules) should be in position in both sample and reference side
- RH Perfusion Experiments
  - The empty RH Perfusion ampoule should be in measuring position to consider heat loss effects through the ampoule
  - A reference ampoule should be in position
  - Calibration can be performed with or without gas flow since the cooling effect can be negligible below 40 ºC. Humidity should be set to initial RH.
- Titration Ampoule Experiments
  - The titration ampoule filled with the solvent should be in measuring position to consider heat loss effects through the ampoule
  - A reference ampoule should be in position
  - Optional: Stirrer ON
    - This can account for any shift in frictional heat flow from the stirrer. Although the baseline sections of the experiments provide this correction.

Static Calibration - Pulse

Exponential heat exchange

$$P = P_o \exp(-\frac{t}{\tau})$$



Empty calorimeter or Accessory and Reference in position.

Heat Flow Calibration Procedure

- Ensure that the heat flow baseline is stable
- Apply settings:
  - Pulse calibration and integration(~0.5-1 h)
  - Steady state calibration (2-3 h)
    - Not for minicalorimeters
- Start the calibration in TAM Assistant
  - Keep a running record of the calibration constants

Dynamic Mode

- **Heat flow data** will not reflect the true response of the sample for reactions with elapsed times less than 10 min. For reactions where the slope of the heat flow time curve (ϕ) is changing rapidly a dynamic correction can be applied to obtain the true response of the sample (P) using the following formula (*Tian's equation*)

$$P = \phi + \tau \frac{\mathrm{d}\phi}{\mathrm{d}t}$$

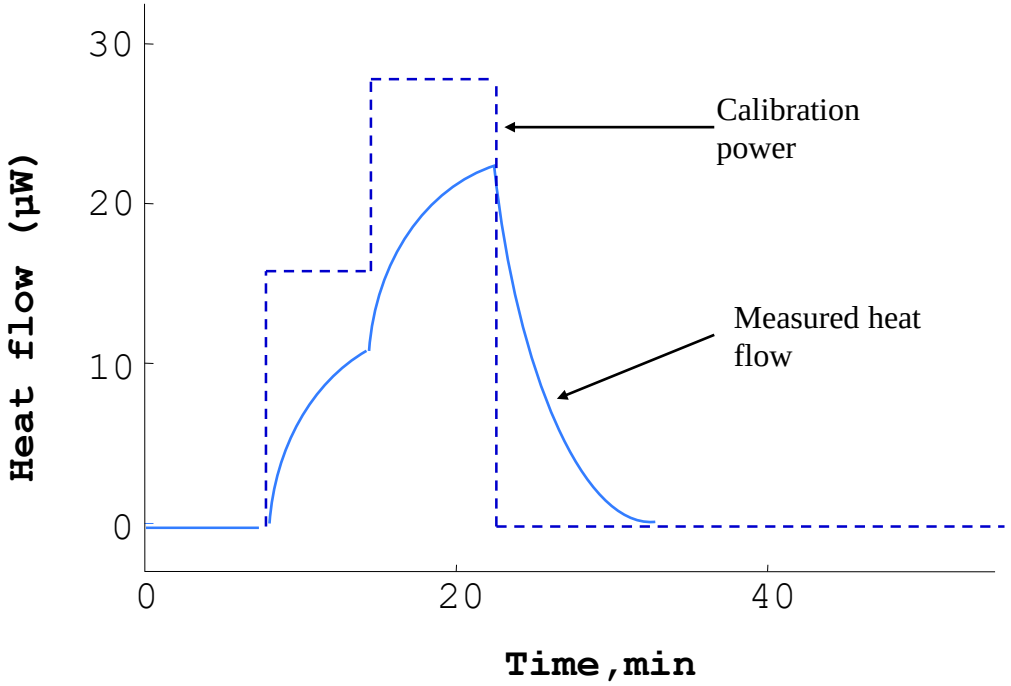
- **Dynamically corrected data** represents the true data of the sample and has been calculated from Heat flow data using the information about time constants obtained from a dynamic calibration.
  - The TAM Assistant software contains functions for considering the effects of the thermal inertia (dynamic mode). TAM Assistant uses *two* time constants rather than one to get a better precision in the correction (*cf*. Taylor expansion).

$$P = \phi + (\tau_1 + \tau_2)\frac{d\phi}{dt} + \tau_1 \cdot \tau_2 \frac{d\phi}{dt^2}$$

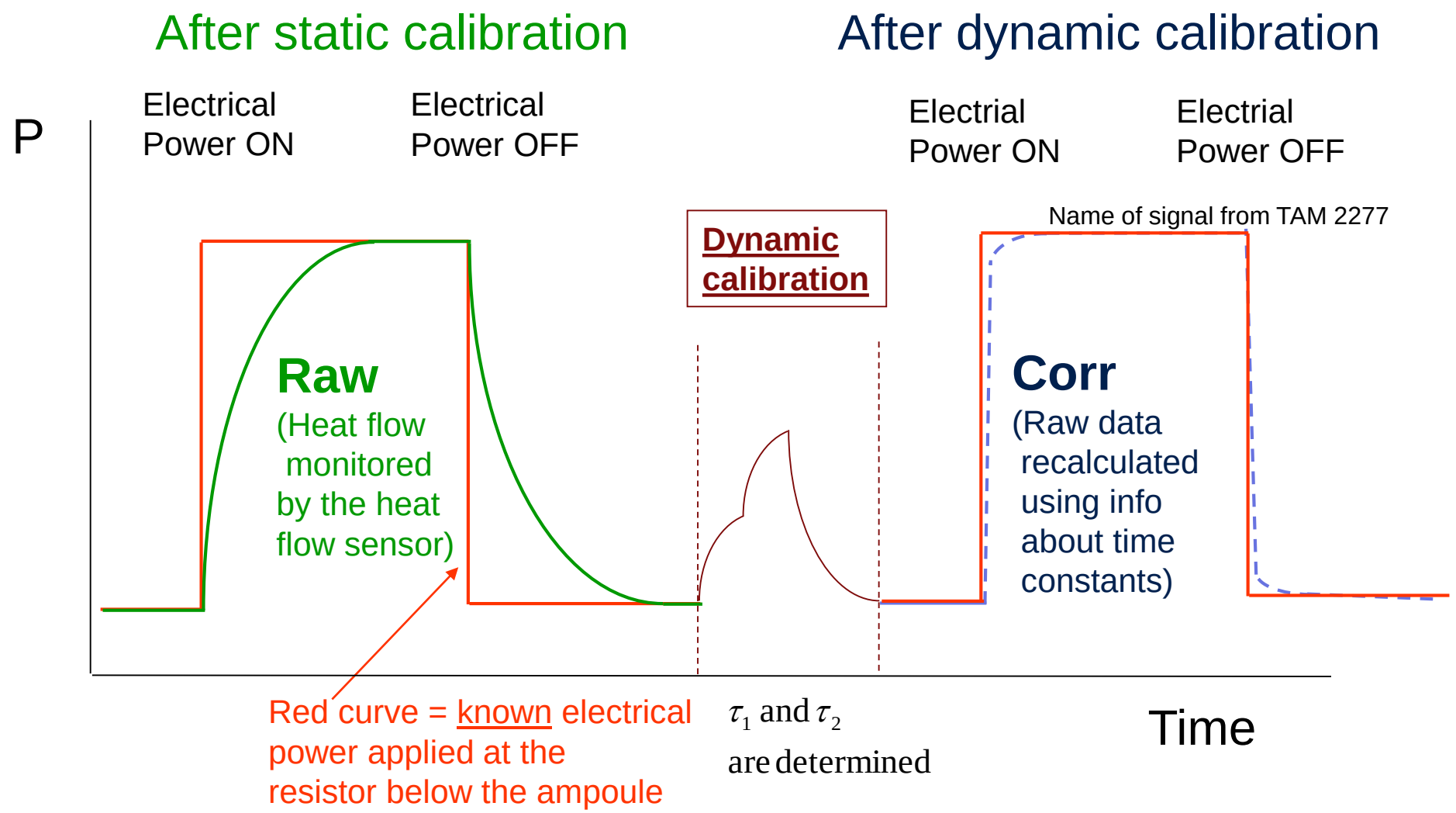
τ_1 and τ_2 are time constants obtained from dynamic calibration

Dynamic Calibration

- Dynamic calibration refer to calibration under non-steady state conditions
- A known electrical calibration power is applied in two steps and the dynamic of the curvature is analyzed in terms of time constants.
- Dynamic calibration should always be performed if the response time of a process is less than 15 min



Calibration Results Example



Dynamic Calibration Procedure

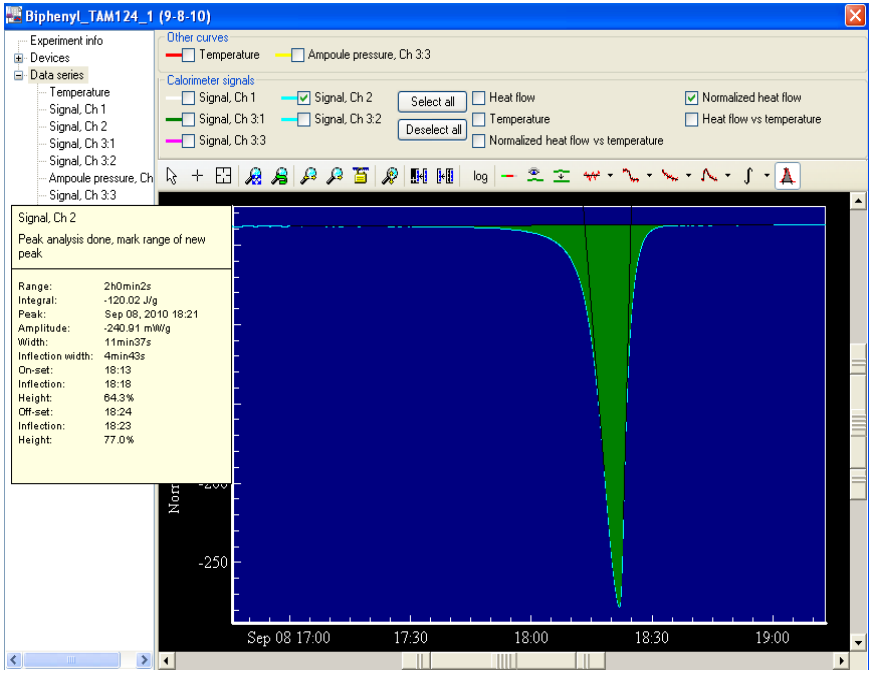
- Set measuring principle to “Dynamic mode”
- Introduce the ampoule with sample and/or reference
- Wait until the heat flow signal is stable.
- Start the calibration: there are two options
  - Time-constant calibration
  - Full dynamic calibration
- After 30-60 min the dynamic calibration is completed

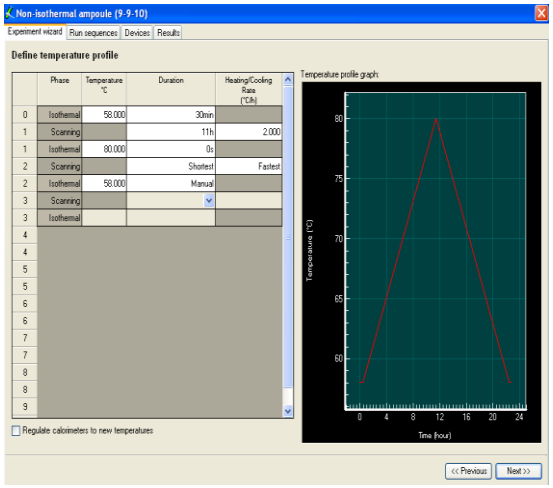
General Performance Test (GPT)

- General Performance Test
  - A test to evaluate the performance of a calorimetric system, i.e. thermostat with a calorimetric unit.
  - Recommended to perform GPT near ambient temperature
    - 20 - 40°C
- Calculated parameters (for A and B side)
  - Time constants and difference between A and B side.
  - Drift, Fluctuation and Error over 24 hrs
  - Short term noise
- Method: GPT experimental wizard (Validation folder)
- Evaluation: GPT analysis
  - The analysis function gives a report with a Yes or No answer as to whether the calorimeters are within specifications

Calorimeter Enthalpy Validation

- Non-isothermal experimental wizard.
  - Ramp 2°C/hr from 58-80 °C
- Accurately weigh biphenyl in TAM ampoule (single use only).
- Utilize peak analysis button on tool bar.
- Theoretical = 121.41 J/g
  - Note that endothermic enthaply is calculated as a negative value in TAM Assistant and the negative sign should be neglected.





Ampoules and Accessories

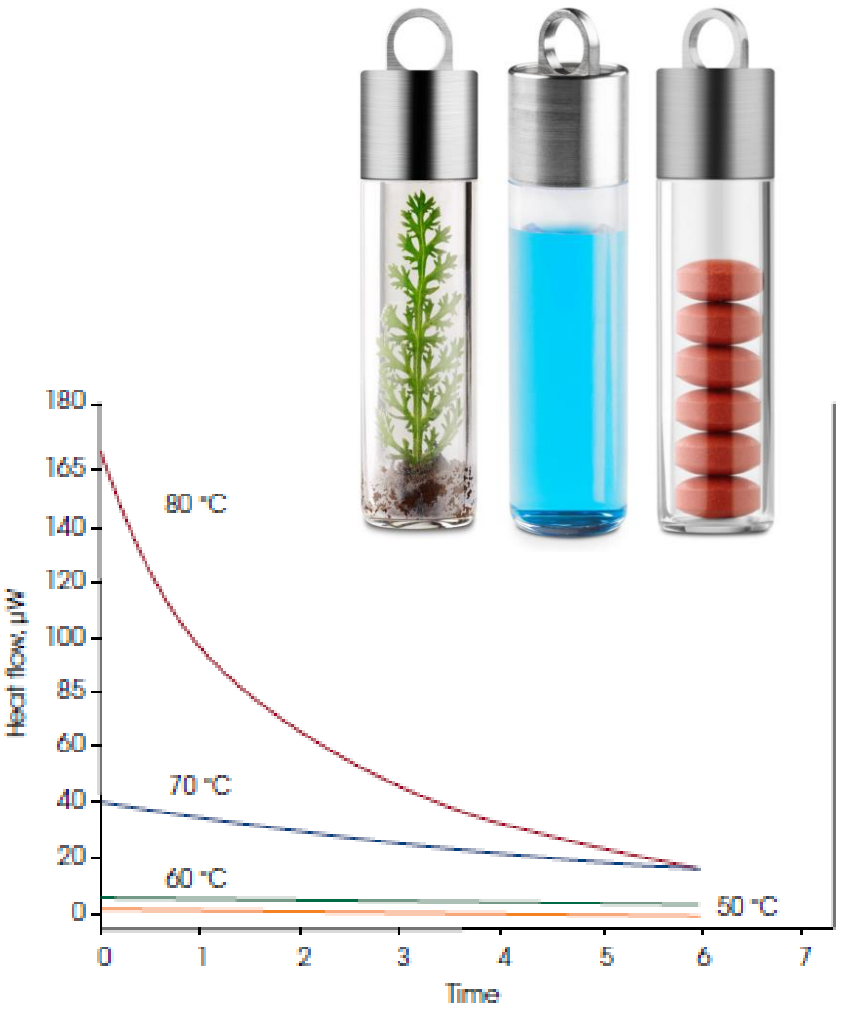
TAM IV – sample handling system

The TAM IV offers a complete array of ampoules in two basic types; open and closed.

- Open ampoules are part of the micro reaction system for the direct manipulation or modification of the sample or its surroundings during the experiment. (examples right)
- Closed, also referred to as static, ampoules contain the specimen in a static fashion: no manipulation of the sample is performed during the measurement. (examples below)

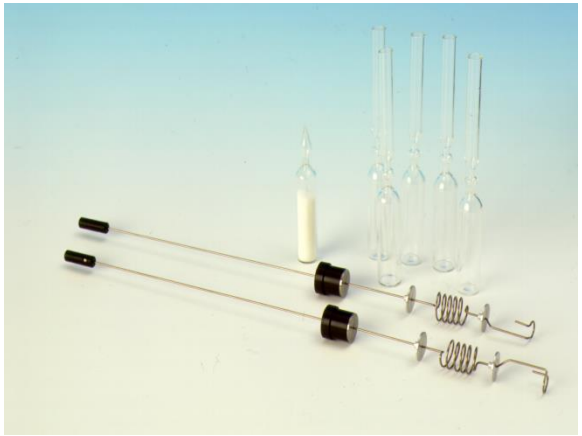
Static measurements

- Stability
- Compatibility
- Reaction kinetics
- Amorphicity
- Polymorphism
- Curing
- Safety assessment
- Microorganism growth
- Etc...



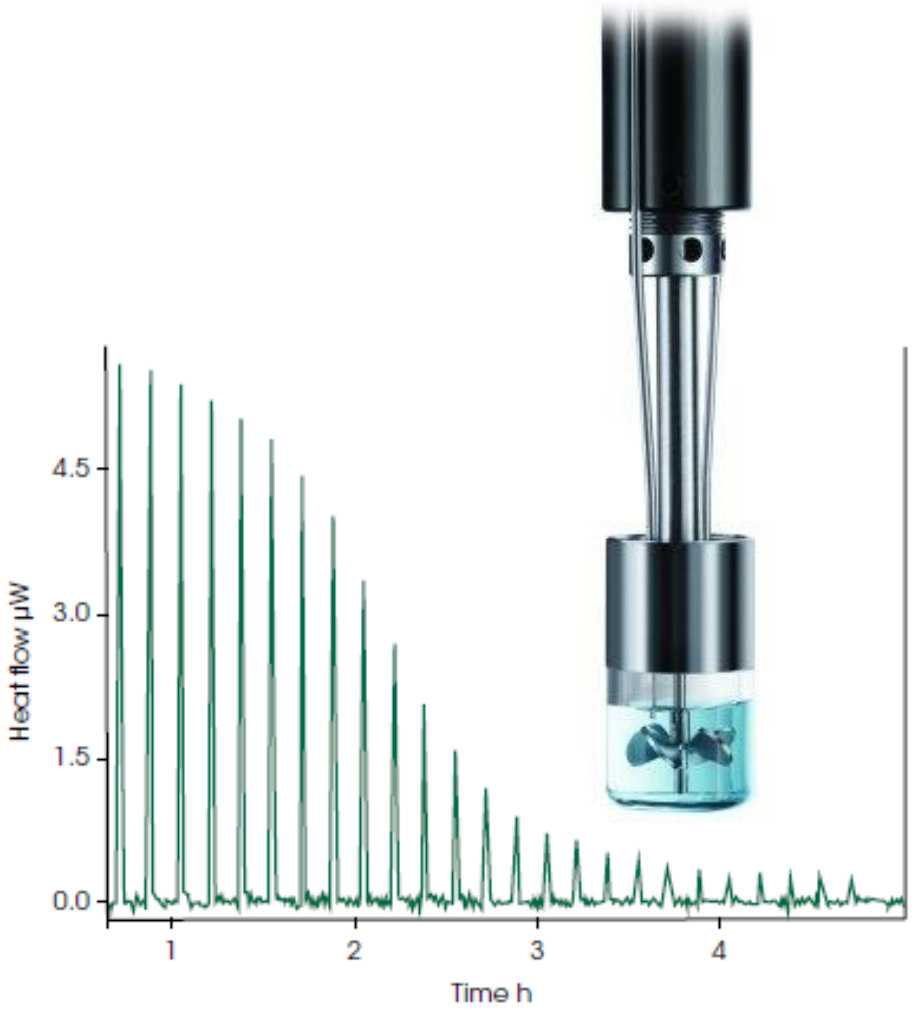
Closed, Sealed or Static Ampoules

- Disposable glass ampoules
  - 3, 4, and 20 mL
- Glass heat seal ampoules
  - 5 mL
- Stainless steel and hastelloy (pH<4)
  - Threaded with Teflon™ or o-ring seal
  - 4 and 20 mL
- Circlip cap ampoules in stainless steel, hastelloy, or glass
  - 1, 4 and 20 mL



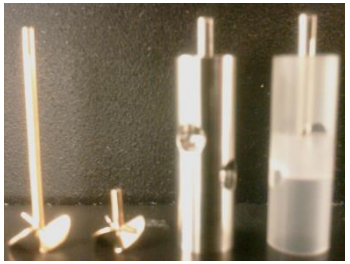
Titration setup – Possibilities for adding and mixing

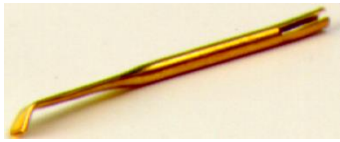
- Binding affinity, stoichiometry and thermodynamics
- Complex formation
- Cmc determinations
- Enzyme kinetics
- Mixing enthalpies
- Dissolution kinetics
- Absorption
- Reaction kinetics
- Swelling
- Drug effect on living cells
- Etc...



Titration Ampoule

- For isothermal titration calorimetry (ITC)
  - Refers to repeated injections of a substrate into a solvent in order to study ligand binding or other molecular interactions.
- Dedicated software control and analysis functions makes the evaluation of ligand binding simple and straight forward.
- Gold propeller or turbine stirrers available
- Stainless steel, glass or hastelloy
  - 1 (shown), 4 or 20 mL sizes available

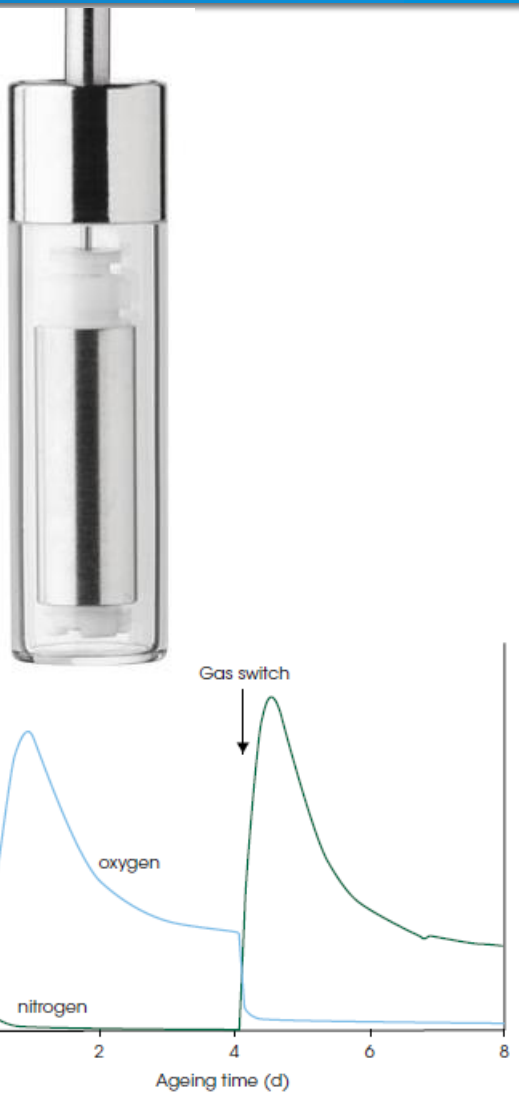




Gas or liquid perfusion

- Stability
- Compatibility
- Absorption
- Reaction kinetics
- Oxidation
- Safety assessments
- Hydration
- Swelling
- Metabolism of living small animals
- Etc...

Could add mixing & injection possibilities



Matrix Cartridge

- Stainless steel hollow tube, with Teflon mesh at each end, capped by Teflon ends.
  - Use with 4 mL perfusion ampoules
  - Attach to the central shaft forcing liquid through the “column” then around and out.
  - Mesh has 105 µm openings
- Useful for making sure that the perfusion liquid flows through the material to be tested, rather than just over the top of the material bed.

Glass Plate Holder

- Frame made in stainless steel that can hold two glass plates
  - Use with 4 or 20 mL perfusion or titration ampoules
  - Attach to the central shaft
  - Can be stirred or rotated
- Useful for studying biological activity and for studying immobilized samples

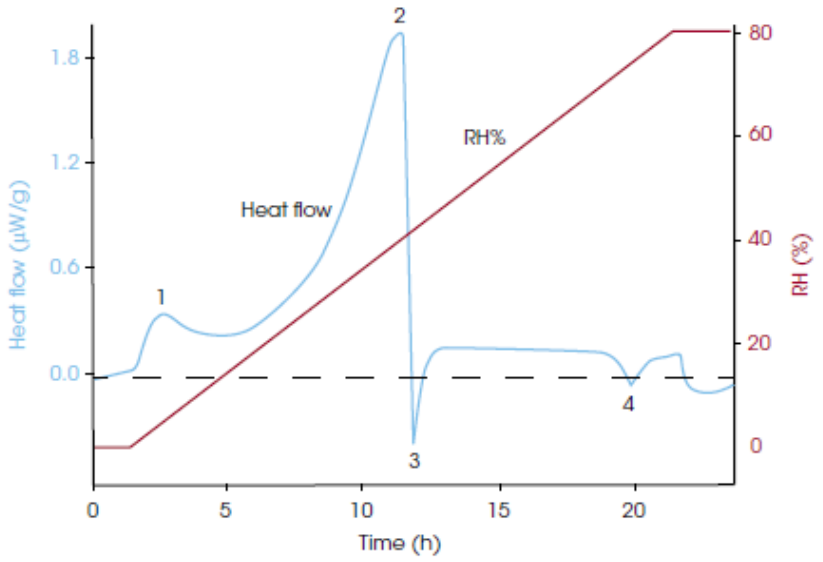
Perfusion Insert

- Attaches to the central shaft and allows gas to flow to the bottom of the reaction vessel.
  - Use with 4 mL perfusion MRS
  - Attach to the central shaft – 3 holes in the bottom of the shaft
  - Typically placed in reaction vessel before sample is placed in the vessel.
- Useful for making sure gas flows through the sample rather than just over the top of the sample bed.

Relative humidity perfusion

- Amorphicity assessments
- Polymorphism
- Stability
- Compatibility
- Absorption
- Reaction kinetics
- Safety assessments
- Hydration
- Swelling
- Etc...

Equally applicable for other type of solvents and vapour pressures



Verify the Humidifying System

- One Point calibration with Zero
  - Zero adjustment using an empty RH Perfusion ampoule (0% RH)
  - A salt solution with a known RH is loaded into the ampoule and heat flow is adjusted to zero to calculate error in RH.
  - The difference between the expected RH and calculated RH defines the error
- Two Point Calibration
  - Zero adjustment with a saturated salt solution in the ampoule
  - The first salt solution is replaced with a second salt solution and the RH adjusted to zero to calculate error in RH.
  - The difference between the expected RH and adjusted RH defines the error

Suitable Salt Solutions

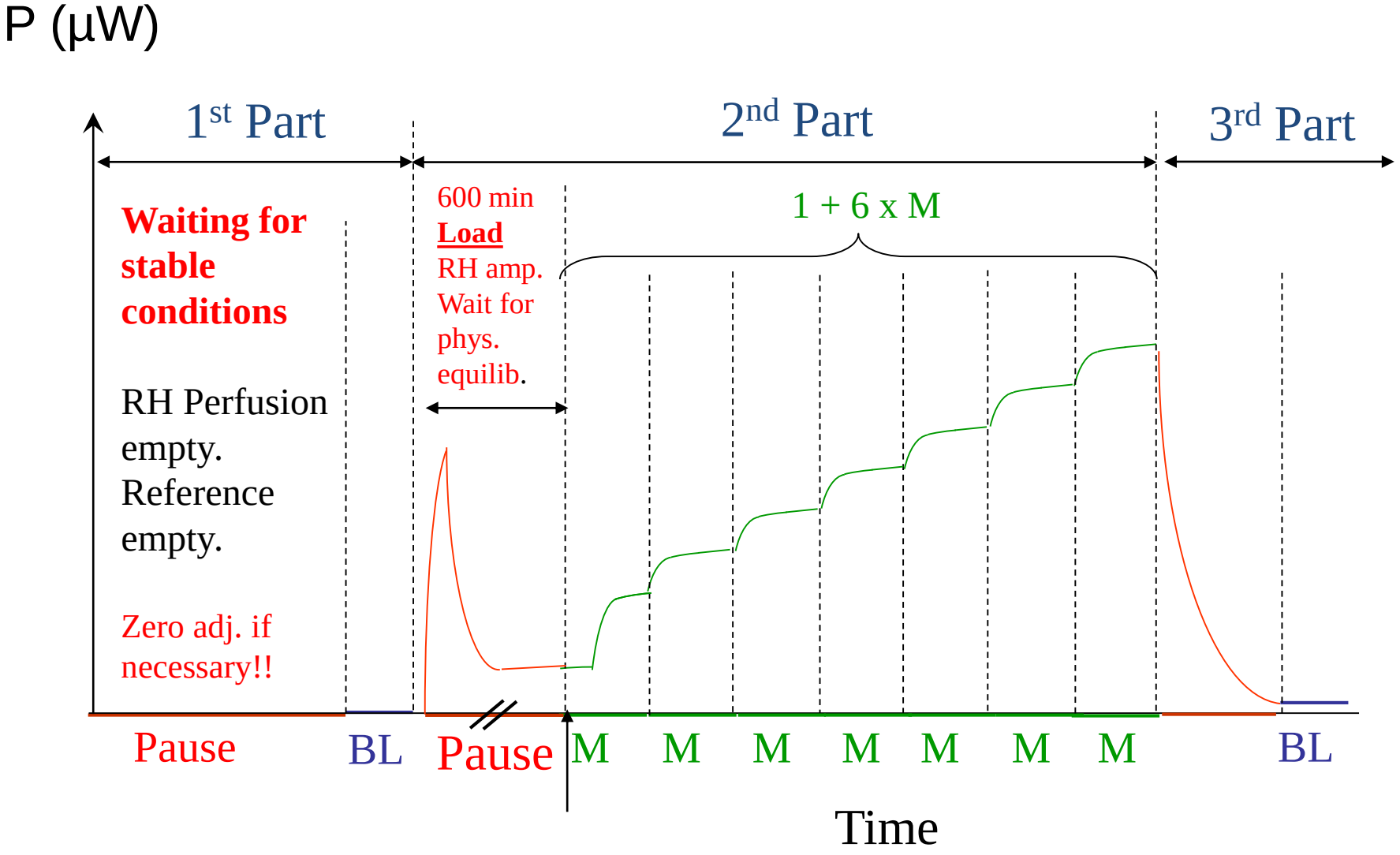
Relative humidity (%)

Salt	Temperature (°C)		
	25	35	45
LiCl	11.3	11.2	11.2
CH_3COOK	21.6	21.6	21.5
$MgCl_2$	32.8	32.0	31.1
NaI	38.2	37.4	31.0
$Mg(NO_3)_2$	52.8	50.0	47.1
NaBr	57.5	54.0	52.0
$CuCl_2$	68.0	69.4	70.1
NaCl	75.3	74.8	74.7
KCl	84.3	82.9	81.7
KNO_3	93.7	90.8	87.0

*From H. Nyqvist, *Int. J. Pharm. Tech. & Prod. Mfr* ., **4** (2) 47-48 (1983)

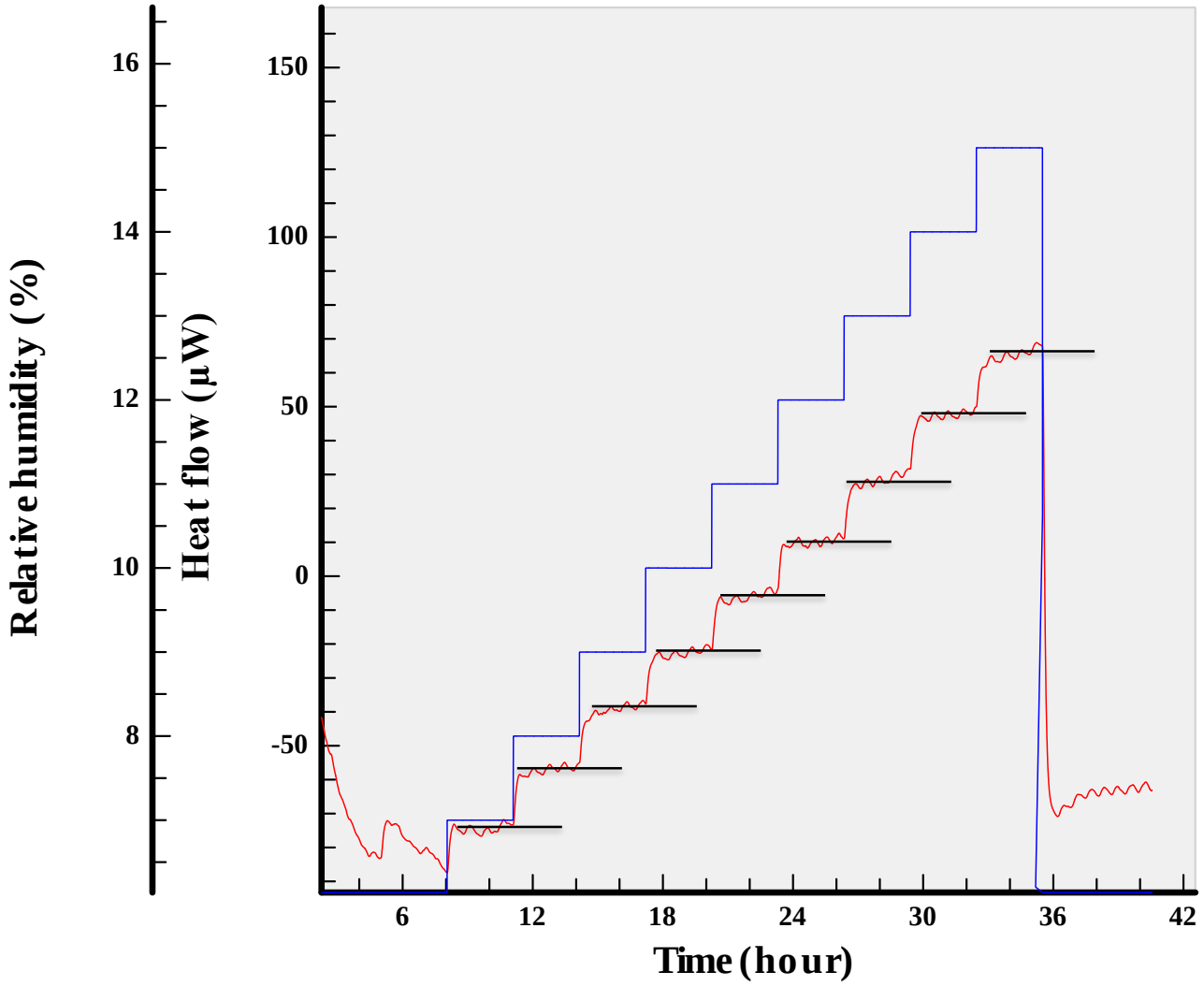
The relative humidity obtained at other temperatures and some other salt solutions can also be found in this paper.

RH Perfusion Calibration – Method I



LiCl at 25°C; RH from 5-15% in 1% steps

RH_{set} (%)	P_{uncorr} /uW
6	-79.1
7	-74.7
8	-57
9	-39.1
10	-22.7
11	-6.28
12	10
13	28.4
14	47.5
15	65.3



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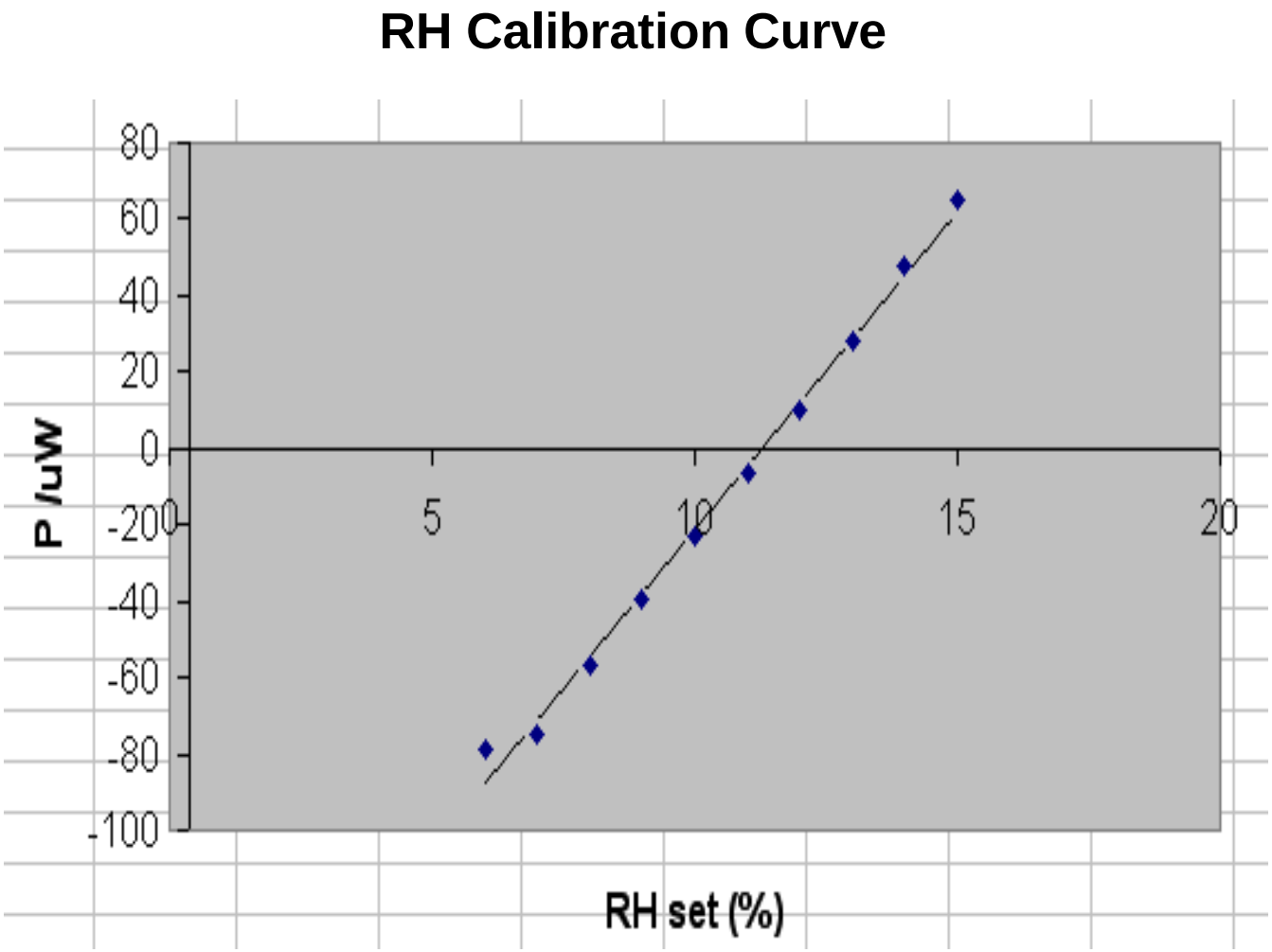
13	28.4
14	47.5
15	65.3



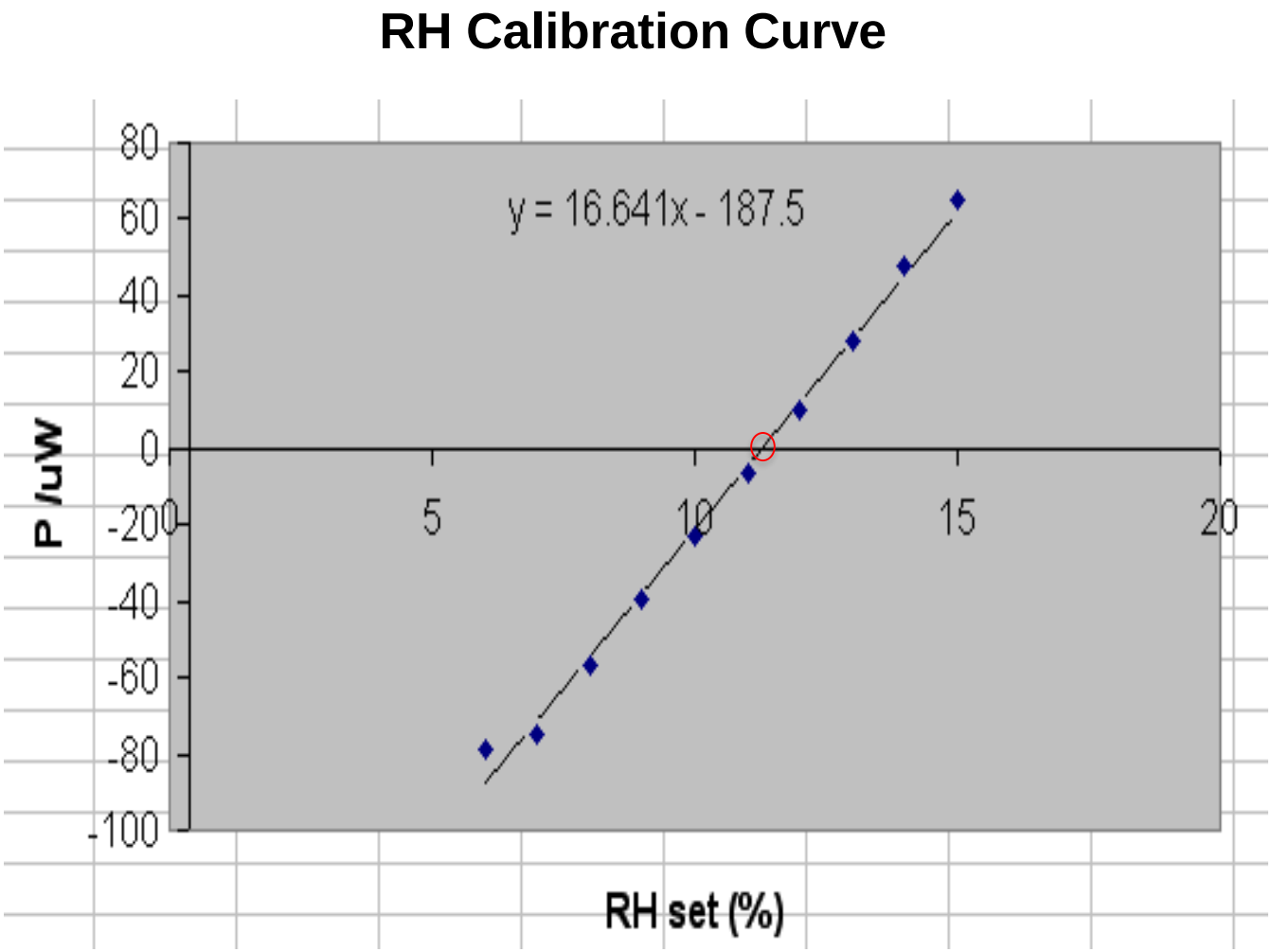
LiCl at 25°C; RH from 5-15% in 1% steps

Solve for y = 0

187.5 / 16.641
= 11.27%

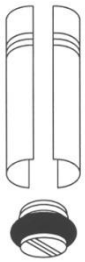
LiCl at 25°C
Should be 11.3%

Correction factor
= 0.03%



Dissolution

- Heat of dissolution
- Heat of wetting
- Amorphicity
- Polymorphisms
- Dissolution kinetics
- Etc...

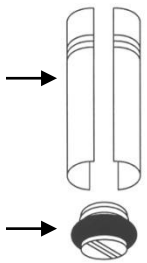


Microsolution ampoule
(solid sample)

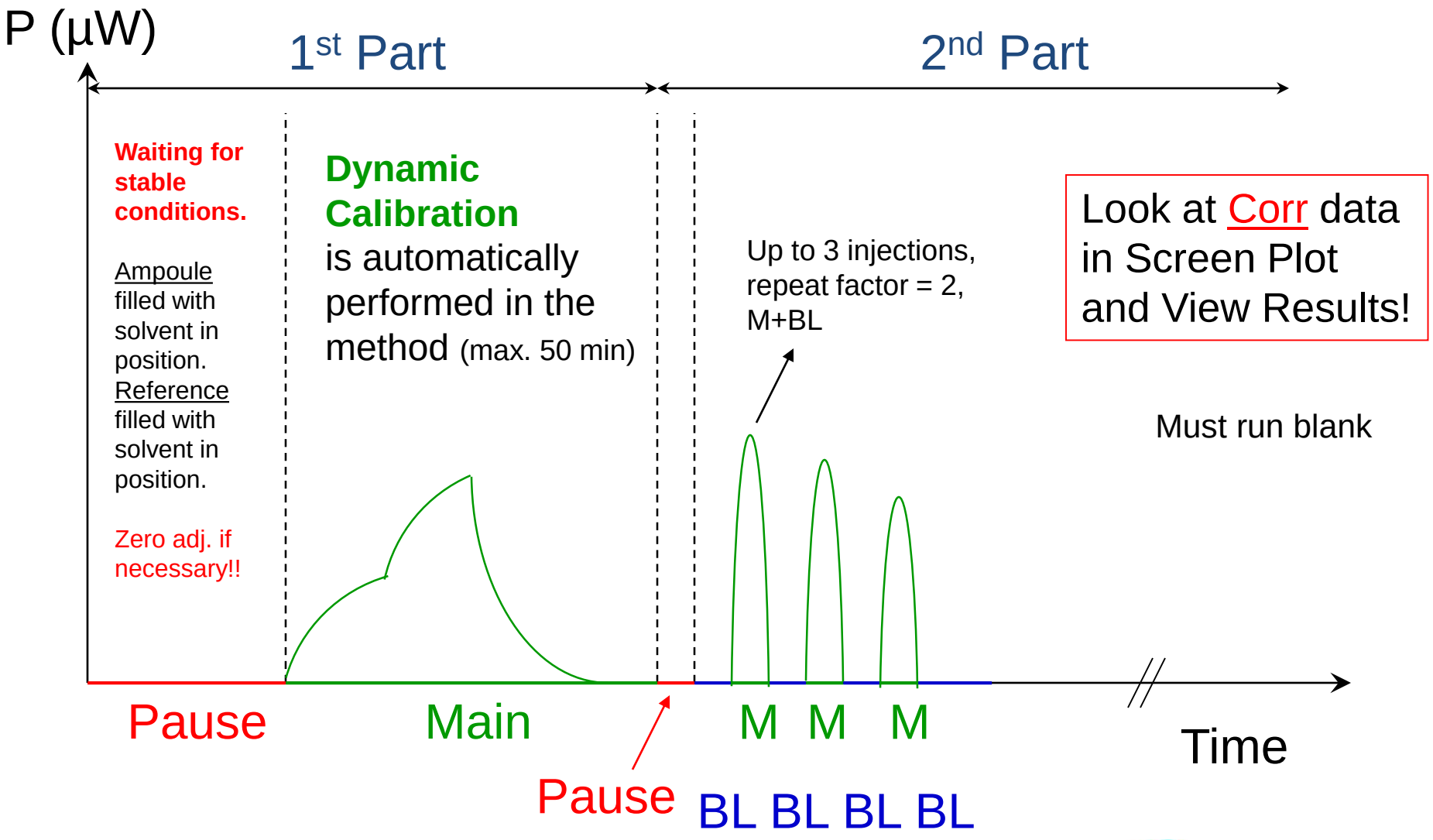
Solution Calorimeter
(solid or liquid samples)

Micro Solution Ampoule

- Designed for monitoring dissolution
  - Available in 20 mL volume only and used with a Microcalorimeter
  - Up to three repeated injections of a solid sample
    - Sample size 1-50 mg



Micro Solution Calorimeter Experiment



Precision Solution Calorimeter

Crushing ampoule (1 mL)
in stirrer

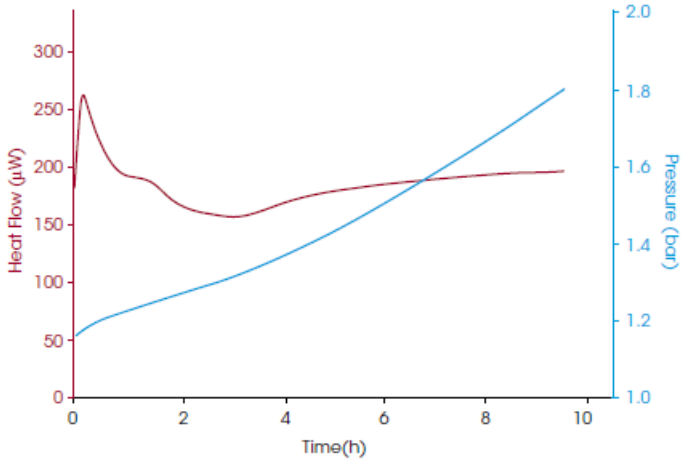
Reaction vessel with
solvent (100 or 25 mL)

Sapphire tip

Simultanoues pressure and heat flow measurements

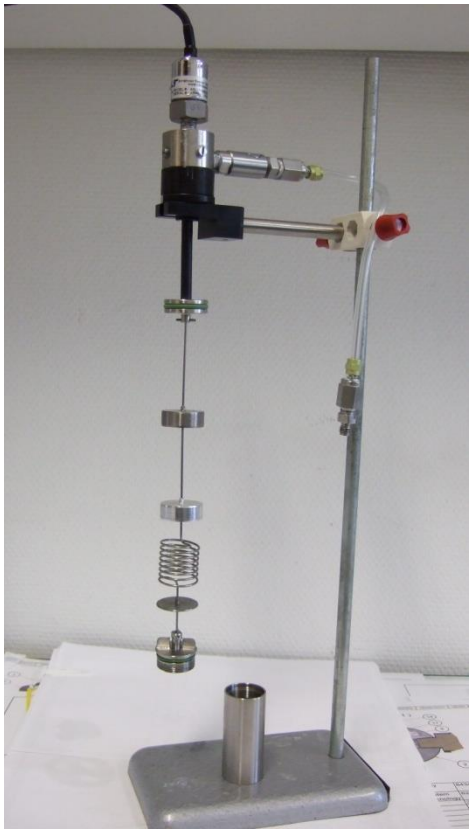
Vacuum / Pressure ampoule

- Gas producing reactions
- Safety assessment
- Absorption
- Etc...



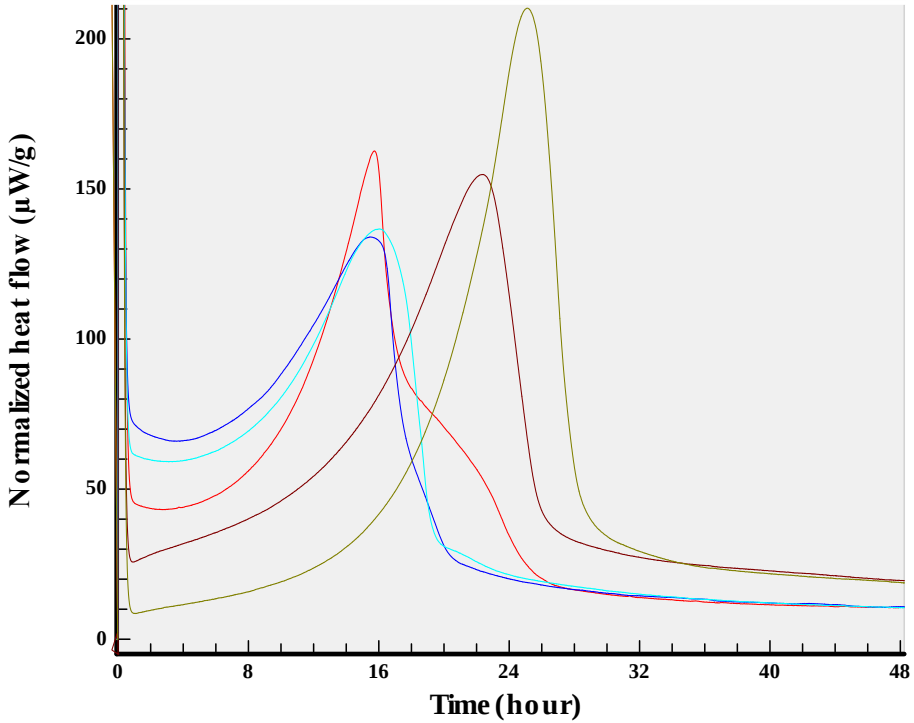
Vacuum / Pressure Ampoule

- Designed for Vacuum Thermal Stability (VTS) test
  - STANAG 4582 and Military standard 1751A
  - Available in 4 and 20 mL
  - Up to 10 bar pressure
  - 10-100 mTorr vacuum



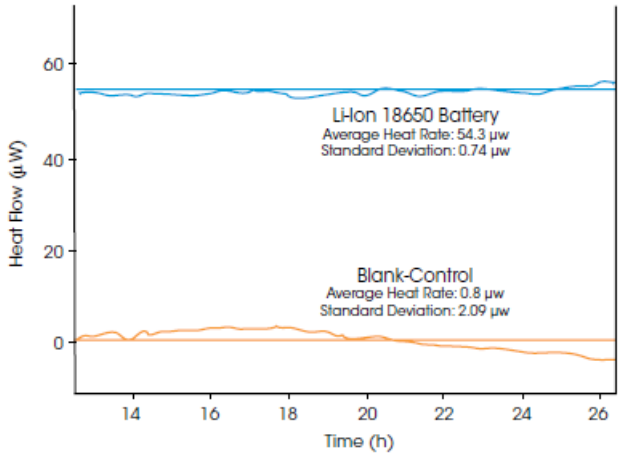
Large and heterogenous samples

- Batteries
- Environmental science
- Food applications



Battery testing

- Only method that directly measures the occurrence of non-current producing reactions under load
- Very sensitive method to assess self-discharge, sometimes the only method
- Non-destructive
- Simple



Macrocalorimetry Accessories

- P/N 604226.901 Macrocalorimeter Ampoule Lifter (Qty 1)
- P/N 604227.901 Macrocalorimeter Battery Lifter (Qty.1)
- P/N 604334.901 125 mL Glass Ampoule (Qty. 20)
- P/N 604328.901 Start-up Kit 125 mL Glass Ampoule
- P/N 604331.901 Lifting Hook for 125 mL Glass Ampoule (Qty 1)
- P/N 604329.901 18650 Battery Adapter Kit
- P/N 604353.901 C-Cell Battery Adapter Kit
- P/N 604352.901 D-Cell Battery Adapter Kit

125 mL Glass Ampoule

18650 Battery Adapter

Battery Fixtures

- Currently available fixtures for the Macrocalorimeter are for C- and D-cell regular batteries and 18650 Li-ion batteries.
  - To optimise the thermal contact between the battery and the heat flow detector
  - To make sure the position of the battery in the calorimeter is reproducible
  - To facilitate the insertion of the battery into the calorimeter
  - To avoid short-circuiting the battery and facilitate connections to the battery for load measurements

Accessories

- New accessory interface will control up to eight independent accessories
  - Mass flow controllers
  - Peristaltic pumps
  - Syringes pumps and stirrer control
  - New Voltage In/Out module

This module can supply or measure voltages for up to three independent probes/sources. This can be accessories such as a user-configured pH-probe or a light source.

Voltage I/O module

- This module can supply or measure voltages for up to three independent probes/sources.

Input
0 to 15 V (1 amp max)

<u>User defined Probes</u>
pH

Turbidity

Dissolved O_2

UV-Vis detection

Ionic strength

Measuring voltage
battery testing

Output
0 to 14 V

<u>Uses</u>
Activating pumps

Switch selection
solvent lines

Activating a light in the
sample chamber
UV or visible

TAM Assistant

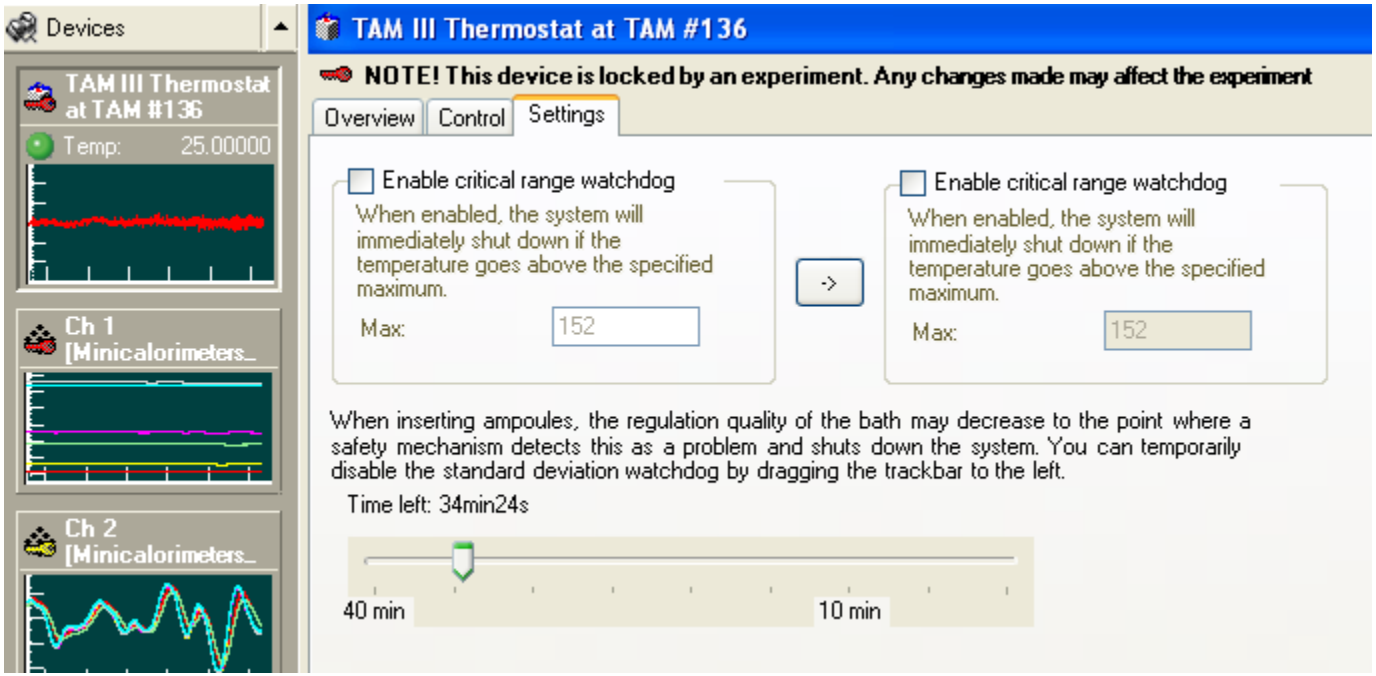
Dedicated software for control of TAM III, TAM IV, or TAM Air for data collection, data analysis, and report creation.

TAM Assistant Allows You to:

- Control devices
- Run experiments
- View and edit results
- Perform analysis and calculations
- Create and edit reports
- 21 CFR 11 compliant version available

Temperature Watchdog

- When loading a cold ampoule into a warm thermostat it may be necessary to deactivate the watchdog temporarily.



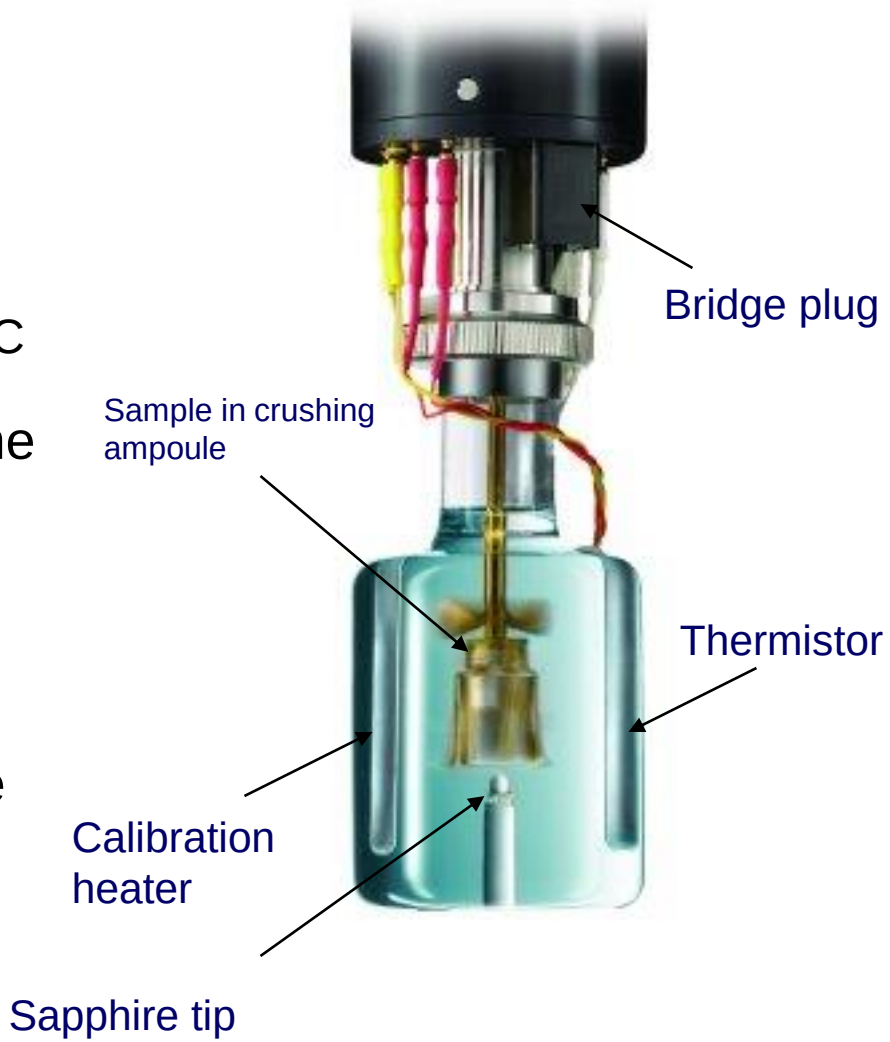
Precision Solution Calorimeter (SolCal)

Precision Solution Calorimeter

- Semi-adiabatic - some of the heat formed will be exchanged with the surroundings.
  - Surrrounding is an air jacket held isothermal by the TAM thermostat
- The temperature of the sample will change during the experiment.
- The temperature of the solution is measured by means of a thermistor.
- Heat of dissolution
  - amorphicity

Precision Solution Calorimeter

- Solvent volume of 25 or 100 mL
  - Available with titration configuration
- Temperature range 15-90 °C
  - Default temperatures 25, 35, and 45 °C
- Crushing ampoules with sample volume (solid or liquid) up to 1.1 mL
- Highest accuracy and versatility in sample concentration
- Separate SolCal software for complete experimental control, data acquisition, data analysis and reports



Heat Balance Equation - SolCal

$$-\frac{dQ}{dt}-\frac{dQ_F}{dt}=C\cdot\frac{dT}{dt}+k\cdot\left(T-T_s\right) \quad (1)$$

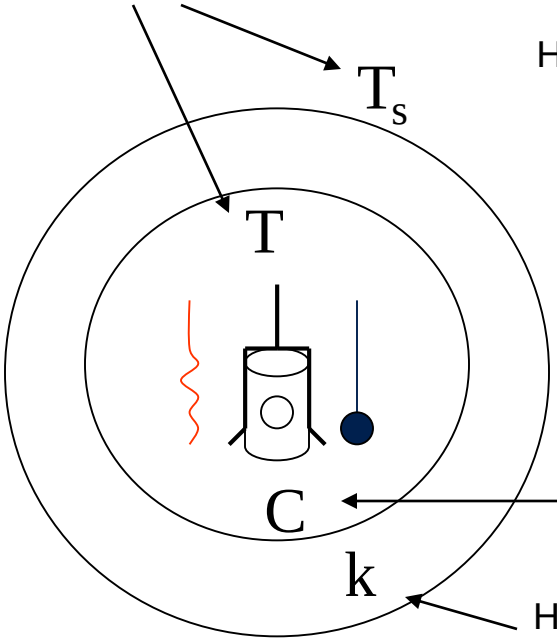
Heat of Solution

Stirring, heating from termistor

Heat accumulated by the system

Heat exchanged with surrounding

Temperature of surrounding and in calorimeter

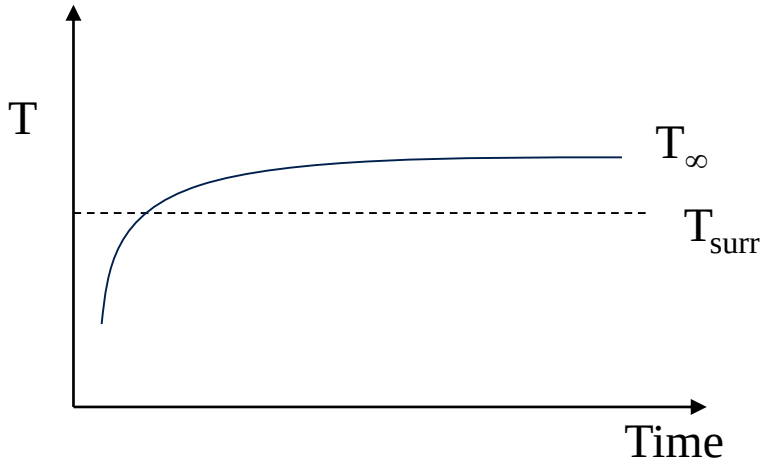


Heat Capacity

Heat exchange coefficient

Refer to Ch. 2 Precision Solution Calorimeter Instruction Manual

Heat Balance at Baseline



$$-\frac{dQ_F}{dt} = C \cdot \frac{dT}{dt} + k \cdot \left(T - T_s\right) \qquad (2)$$

At t_∞ $\qquad C \cdot \frac{dT}{dt} \to 0$

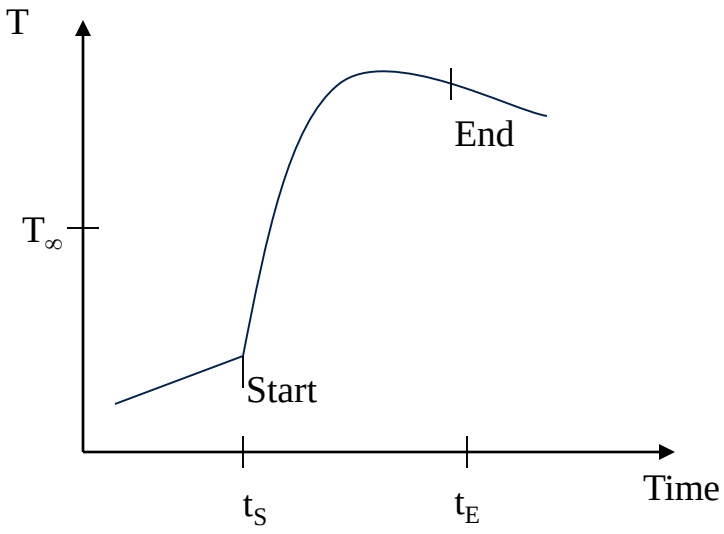
$$-\frac{dQ_F}{dt} = k \cdot \left(T_\infty - T_s\right) \qquad (3)$$

Substitution of eq. 3 into eq.1 gives

$$-\frac{dQ}{dt} = C \cdot \frac{dT}{dt} - k \cdot \left(T - T_\infty\right) \qquad (4)$$

$\frac{dQ_F}{dt}$ is eliminated

Heat of Solution



$$-\frac{dQ}{dt} = C \cdot \left(\frac{dT}{dt} - \frac{1}{\tau} \cdot (T - T_\infty) \right) \qquad (4b)$$

where $\tau = \frac{C}{k}$

Integration of 4b

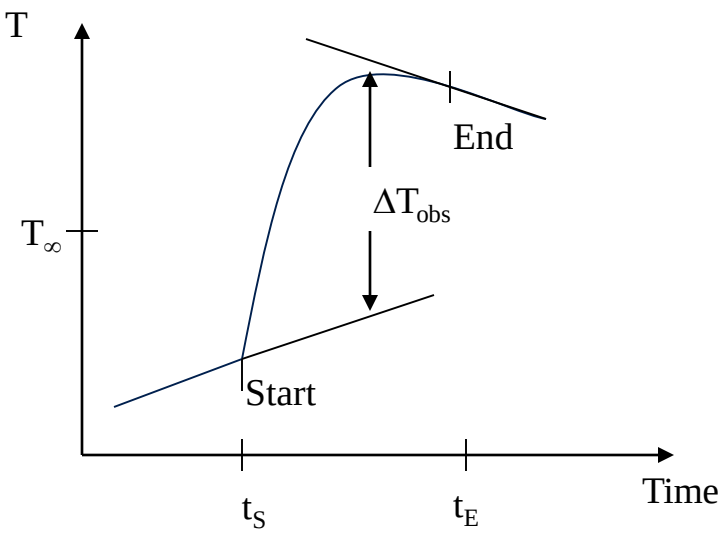
Heat of Solution = $-Q = \int_S^E -\frac{dQ}{dt} \cdot dt$

$$-Q = C\left(\Delta T_{obs} + \frac{1}{\tau} \cdot \int_S^E (T - T_\infty) \cdot dt \right)$$

$$\Delta T_{obs} = T_{End} - T_{Start}$$

$$-Q = C \cdot \Delta T_{corr}$$

Heat of Solution



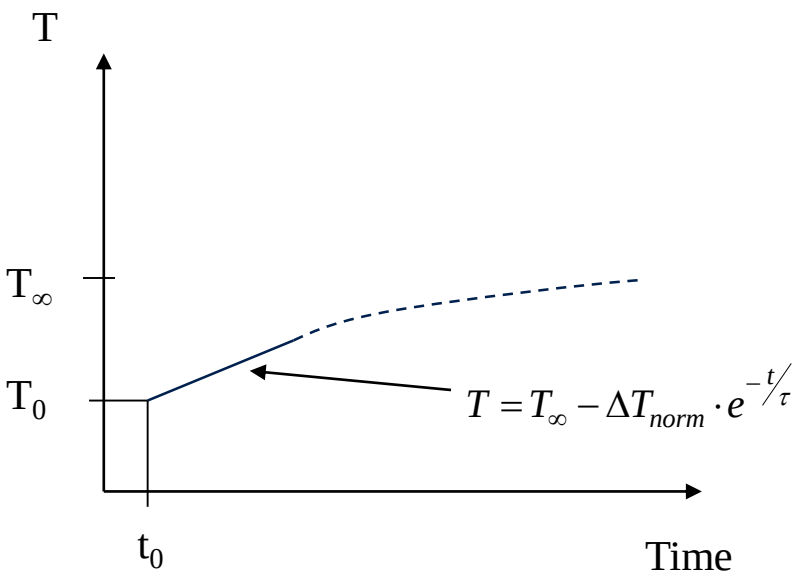
$$-Q = C \cdot \Delta T_{corr}$$

$$\Delta T_{corr} = \Delta T_{obs} - \Delta T_{adj}$$

$$\Delta T_{adj} = \int_S^E \frac{1}{\tau} \cdot (T - T_\infty) \cdot dt$$

In order to calculate ΔT_{adj}, t and T_∞ have to be obtained

Baseline



$$0 = C \cdot \frac{dT}{dt} + k \cdot \left(T - T_\infty\right) \quad (5)$$

by differentiation

$$T = T_\infty - \Delta T_{norm} \cdot e^{-t/\tau} \quad (6)$$

where

$$\Delta T_{norm} = T_0 - T_\infty \quad (7)$$

The exponential temperature function for the baseline gives us

T_∞, τ

Break Experiment

	Electrical Calibration	Break Experiment
Input	Q_{Cal}	?
Output	ΔT_{obs}^{Cal}	ΔT_{obs}^{Break}
	-	-
Baseline Analysis, T_∞, t	ΔT_{adj}^{Cal}	ΔT_{adj}^{Break}
	ΔT_{corr}^{Cal}	ΔT_{corr}^{Break}

$$C = \frac{Q_{Cal}}{\Delta T_{corr}^{Cal}} \mapsto Q_{reaction} = C \cdot \Delta T_{corr}^{Break}$$

Isothermal versus Adiabatic Calorimetry

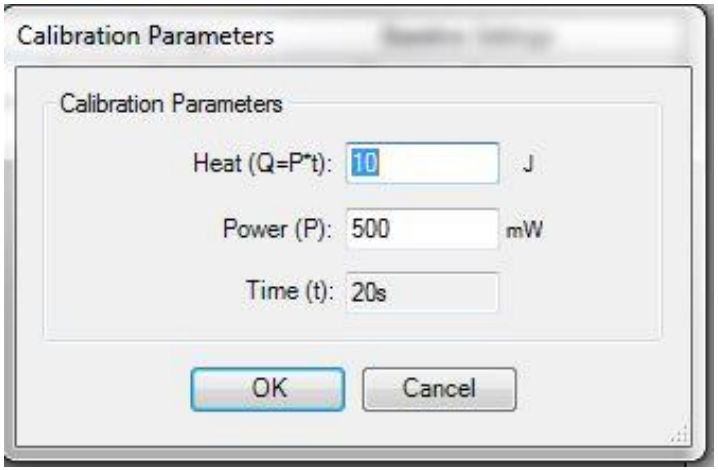
- Isothermal calorimeters directly measure the rate of heat production, which is proportional to the overall reaction rate. The heat capacity of the sample may be unknown.
- Adiabatic calorimeters measure the change in temperature of a sample, which is used to calculate the heat produced. The heat capacity of the system must be known.
- Isothermal calorimeters are very stable and need not be calibrated more than a few times a year. In contrast, adiabatic calorimeters must be calibrated often and are typically done before each run.
- The temperature in an isothermal calorimeter doesn't increase to unrealistic temperature whereas the final temperature in adiabatic calorimeters can be very high.
- Semi-adiabatic calorimeters are a better choice for faster reactions, but isothermal calorimeters exhibit long term stability.
- Isothermal calorimetric experiments are easy to perform.

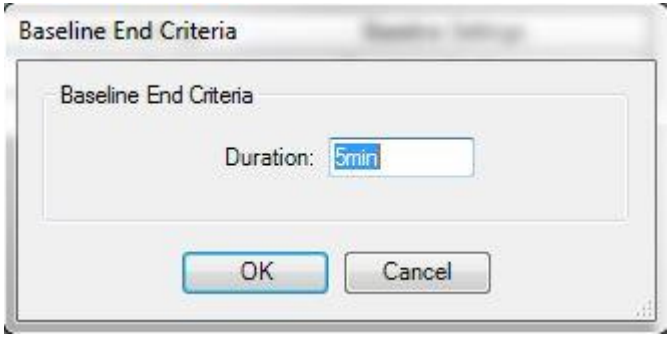
Verify Performance of SolCal

- 100 mL water
- 3 - 50 J electronic calibrations
- C_p of system = 445 ±8 J/K
- Average of three within 0.2 J/K
- $\tau = C/k \cong 8.200$ ks

Use 10 J calibrations for 25 mL vessel

C_p estimated at 115-118 J/K

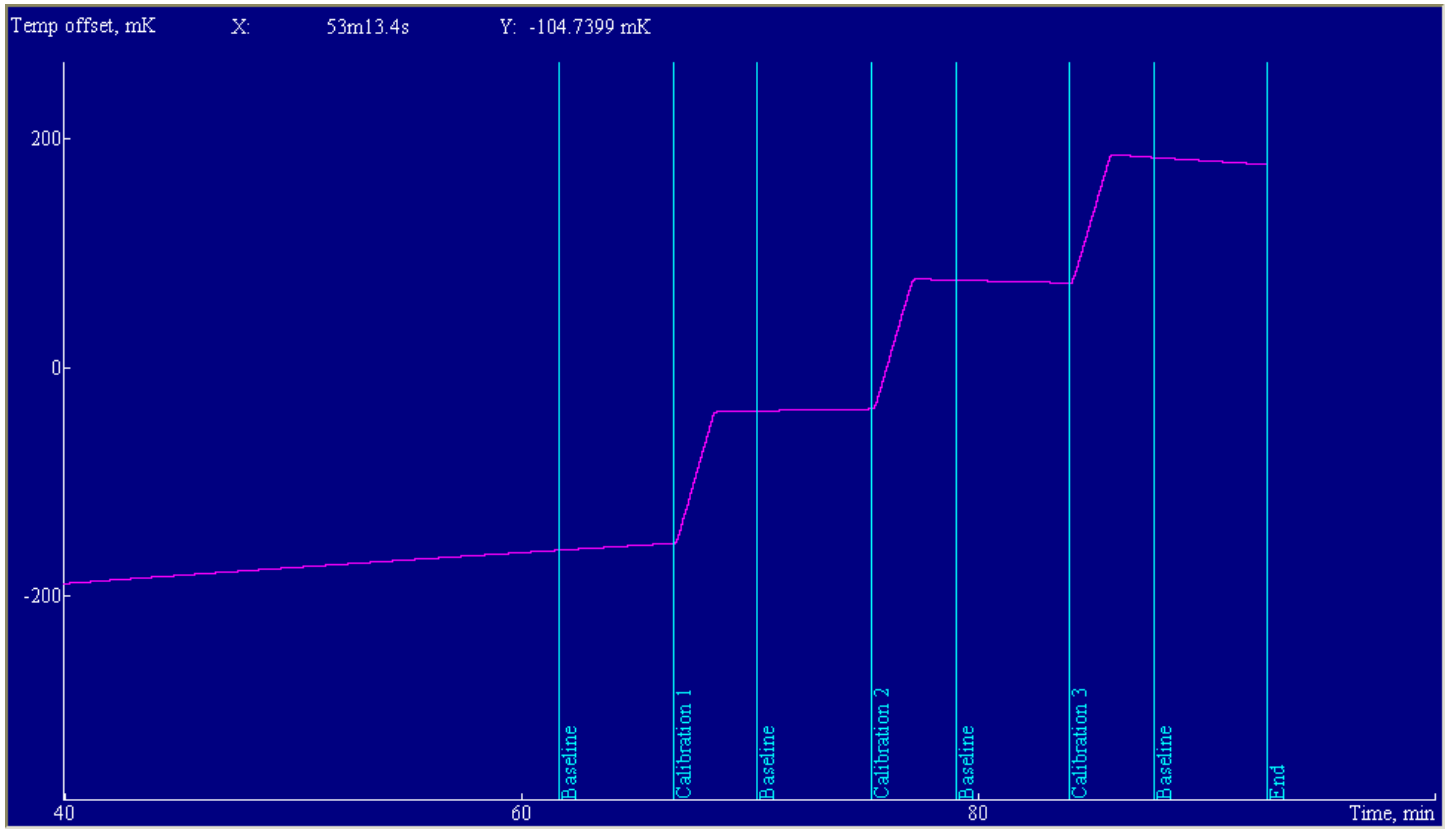




Verify Performance of SolCal

Calculate heat capacity of water and vessel.

SYSTEM/Check Exponential Fit less than 10 μK.



Chemical Calibration

- Exothermic heat reaction
  - Dissolution of 0.5 g TRIS in 0.1 M HCl
    At 25 ºC $\Delta_R H^o$ = -29.75 ±0.02 kJ/mol

- Endothermic heat reaction
  - Dissolution of 0.5 g TRIS in 0.05 M NaOH
    At 25 ºC $\Delta_R H^o$ = +17.19 ±0.02 kJ/mol

TRIS - molecular weight: 121.137 g/mol, Density: 1.35 g/cm^3, ΔC_p: 124 JK^{-1}mol^{-1}

- Endothermic heat reaction
  - Dissolution of KCl in water
    At 25 ºC $\Delta_R H^o$ = +17.58 ±0.02 kJ/mol

Example Report – SolCal experiment (page 1)

Calculation Results

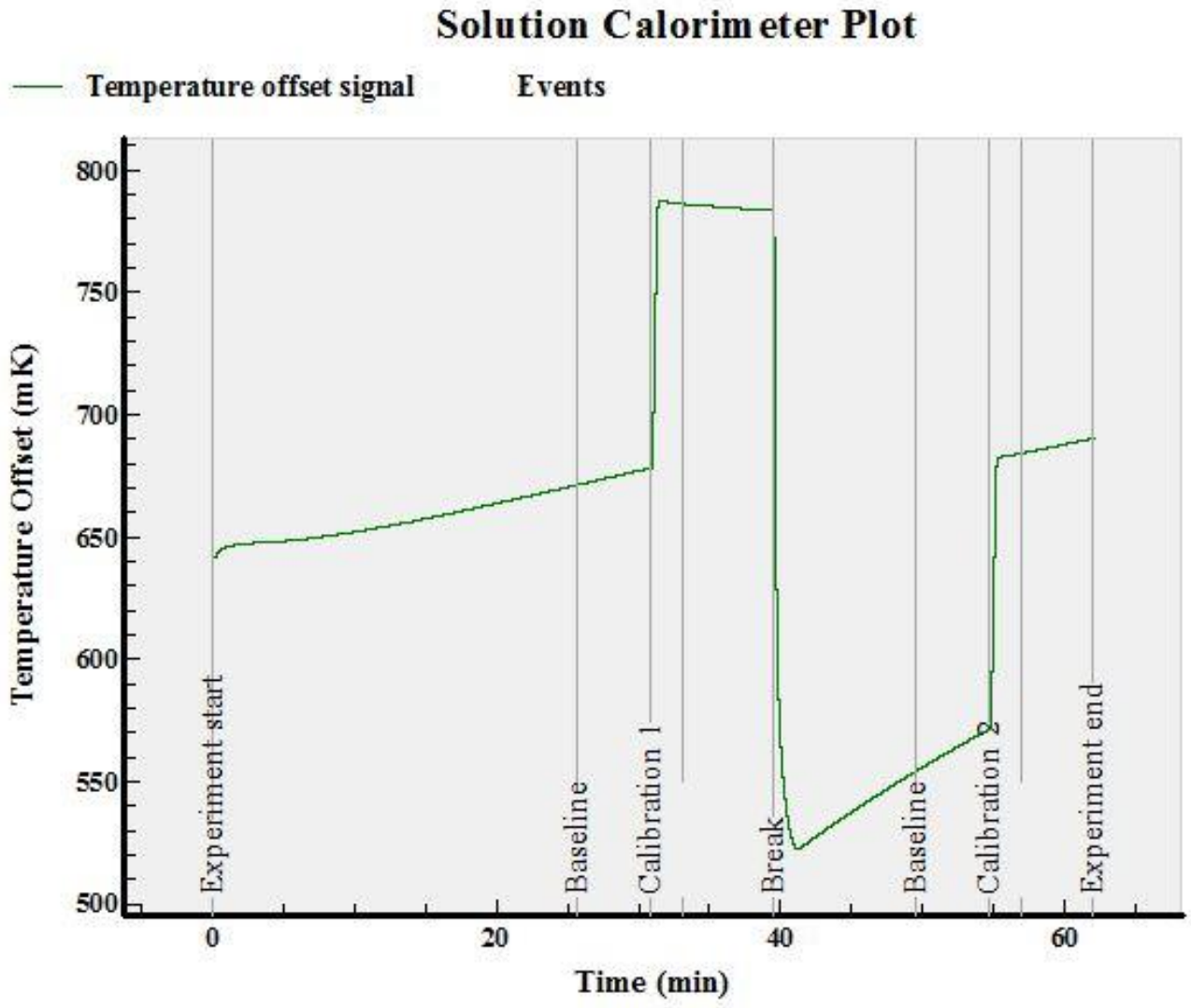
Calibration model: Individual fits
Break model: Dynamics of calibrations

Calibration(s) before break:		Calibration(s) after break	
$C_{average}$	119.264 J/K	$C_{average}$	118.876 J/K
Δ^{T}corr	570.614 µK	Δ^{T}corr	-289.431 µK
$Q_{reaction}$	-68.054 mJ	$Q_{reaction}$	34.406 mJ
Δ^{H}reaction		Δ^{H}reaction	
$^{T}t_{end}$	25.558 °C	$^{T}t_{start}$	25.521 °C
T_{inf}offset	957.442 mK	T_{inf}offset	970.789 mK
C/k	3.441 ks	C/k	3.469 ks

Δ^{C}_{p}

Cal#	Qcalibration	Initial T_{offset}	ΔT	ΔT_{corr}	C J/K
1	11.925 J	270.324 mK	122.653 mK	99.99 mK	119.264 J/K
Break					
2	11.926 J	597.285 mK	110.928 mK	100.319 mK	118.876 J/K

Example Report – SolCal experiment (page 1)



Example Calculation for KCl Experiment

Before and After Break

Molality of KCl = m = 0.00465 mol/0.1 kg = 0.0465 mol/kg

$\Delta H_{sol(KCl)}$ (25 °C) from Equation 1 = 235.032 J/g

ΔC_p from Equation 2 = -158.85 J/mol·K

M_{KCl} = 74.5513 g/mol

Corrected Mass of KCl = 346.602 mg = 0.00465 mol

Mass of Water = 100.0 mL = 0.1 kg

Assume: ρ_{water} = 1.00 kg/dm^3

$\Delta H_{sol(KCl)}(T)$ from Equation 3:

Before break - T = 24.95 °C - $\Delta H_{sol(KCl)}(T)$ = 235.14 J/g

After break - T = 25.127 °C - $\Delta H_{sol(KCl)}(T)$ = 234.76 J/g

Error Calculation:

Before break - $\Delta H_{sol(KCl)}(T)$ = 234.77 J/g (from report)

Error = [(234.77 - 235.14)/235.14]×100 = **-0.16%**

After break - $\Delta H_{sol(KCl)}(T)$ = 234.78 J/g (from report)

Error = [(234.78 - 234.76)/234.76]×100 = **0.001%**

Comments on KCl Experiment

- Usually calculation using the calibration before or after the break give slightly different results of ΔH. One reason is hydrodynamic stirring changes after breaking the ampoule and may result in different estimations of t_∞ and τ, which are essential for calculation of the calibration constant. Secondly, the system may not have achieved steady-state when the first calibration begins (check standard deviation). For this reason the calculation that utilizes the calibration after break is typically a better representation.
- Corrections for evaporation and condensation effects when breaking the ampoule can be taken as negligible in this case.
- For additional sources of error, see reference by I. Wadso in the Appendix of the Precision Solution Calorimeter Instruction Manual.

Calculation – Heat of Solution for KCl

Concentration Dependence

Conditions:

Molecular weight: 74.5513 g/mol (KCl: NIST 1655)

Temperature = 25 °C = 298.15 K

KCl Molality = m_{KCl} = 0.05551 - 0.15 mol/kg

Deviation from NIST certificate using the formula below should be less than ±0.3 % when concentrations are in the given range. Concentrations outside this range should be regarded as an extrapolation and may result in an increase in error.

$$\Delta H_{sol(KCl)} = A \cdot m_{KCl}^{3} + B \cdot m_{KCl}^{2} + C \cdot m_{KCl} + D \quad [1]$$

Definitions: $\Delta H_{sol(KCl)}$ in J/g

A = 203.7205 J/g·kg^3·mol^3 B = -144.7988 J/ g·kg^2·mol^2

C = 31.5119 J/ g·kg·mol D = 233.8599 J/g

Calculation – Heat of Solution for KCl

Temperature Dependence

Semi-adiabatic calorimeters by definition measure change in temperature. Therefore, the heat of solution should be calculated at the temperature to which the dissolution process is measured by using the change in heat capacity (ΔC_p).

$$\Delta C_p = (-114.1 + 28.95 \cdot m^{1/2} + 6.7 \cdot m)^{*} - 51.30 \quad [2]$$

$$\Delta H_{sol(KCl)}(T) = \Delta H_{sol(KCl)}\,(25\ ^\circ C) + \Delta C_p\,(T - 25.00)/M_{KCl} \quad [3]$$

*Apparent C_p of solution

Definitions:

$\Delta H_{sol(KCl)}$ in J/g C_p of crystalline KCl = 51.30 J/ mol·K

ΔC_p in J/mol·K M_{KCl} = 74.5513 g/mol

Example of Bouyancy Correction

$W_v = W_a + (V\rho_{air} - V_{cw}\rho_{air})$

Substitute…

$V = W_v/\rho_{KCl}$

Then…

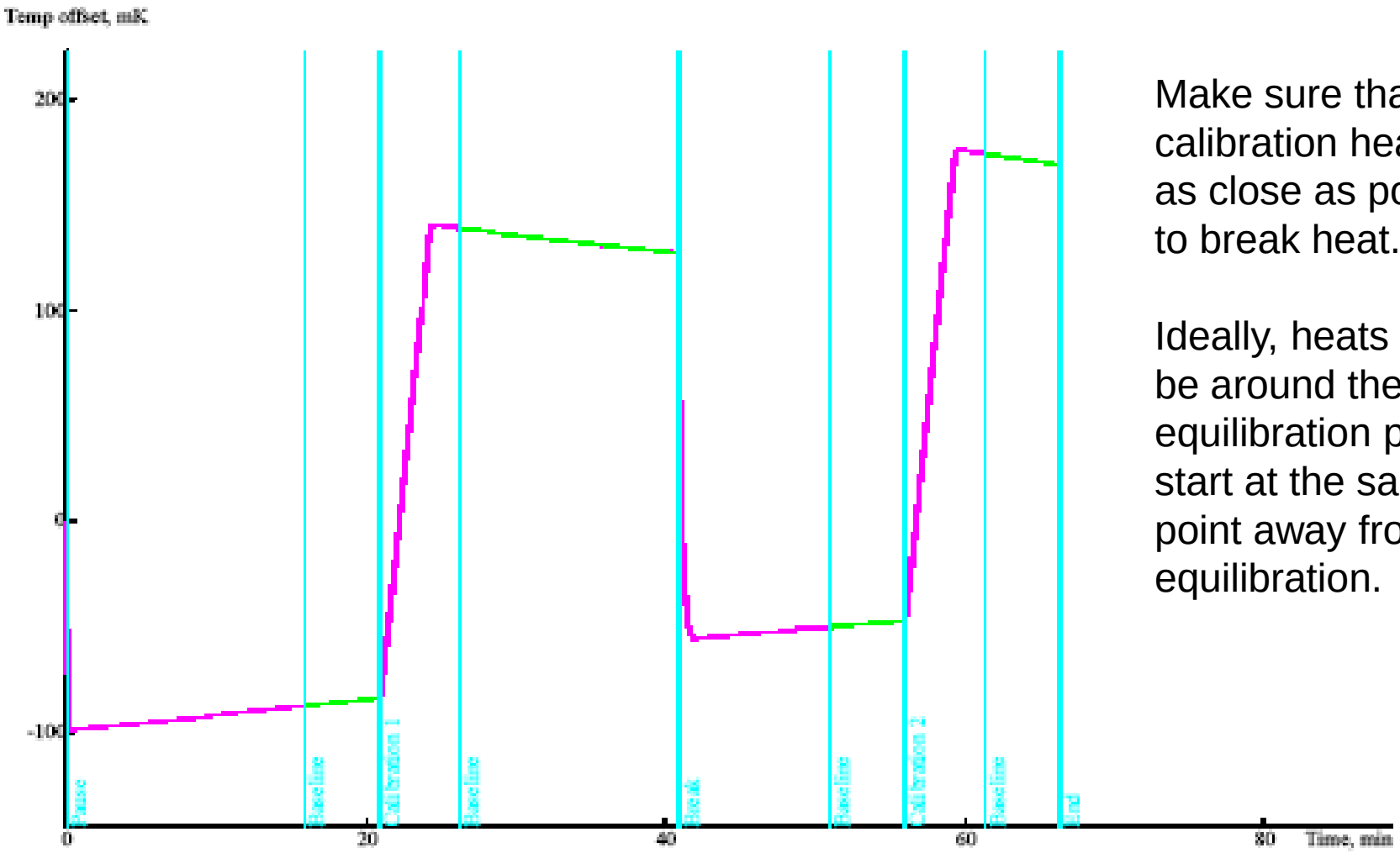
Definitions:
Weight of KCl in air = W_a
Weight of KCl in vacuum = W_v
Volume of KCl = V
Volume of Counter weights = V_{cw}

$$W_v = W_a \times \frac{1 - \rho_a/\rho_{cw}}{1 - \rho_a/\rho_{KCl}} = W_a \times \left[1 + \rho_a\left(1/\rho_{KCl} - 1/\rho_{cw}\right)\right] \cong W_a \times (1.000455)$$

Assume:
Ambient Temperature = 22 ± 1 °C
Ambient RH = 35 ± 15%
Ambient Pressure = 750 ± 10 mm Hg
Density of Air = ρ_{air} = 0.0012 g/cm^3
Density of KCl = ρ_{KCl} = 1.98 g/cm^3
Density of Counter Weights = ρ_{cw} = 7.95 g/cm^3 (e.g. $\rho_{brass} \cong$ 7.8 g/cm^3, $\rho_{ss} \cong$ 8.0 – 8.4 g/cm^3)

Concentration of KCl and Temperature are also important for calculation of ΔH!

Ideal experimental tips

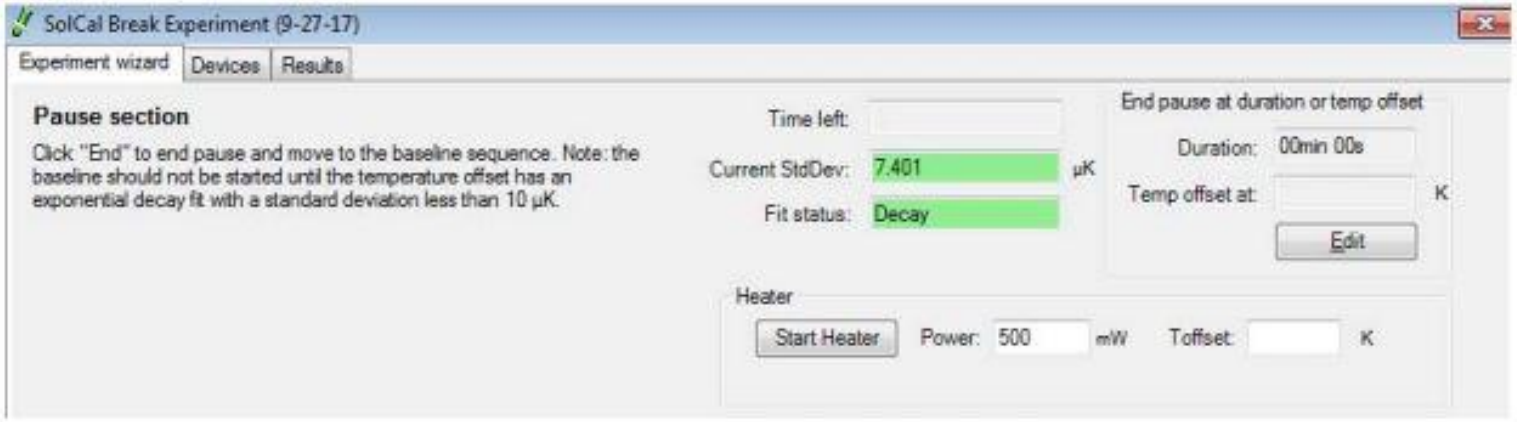


Make sure that calibration heats are as close as possible to break heat.

Ideally, heats should be around the equilibration point or start at the same point away from equilibration.

Ideal experimental tips

Ideal experimental tips

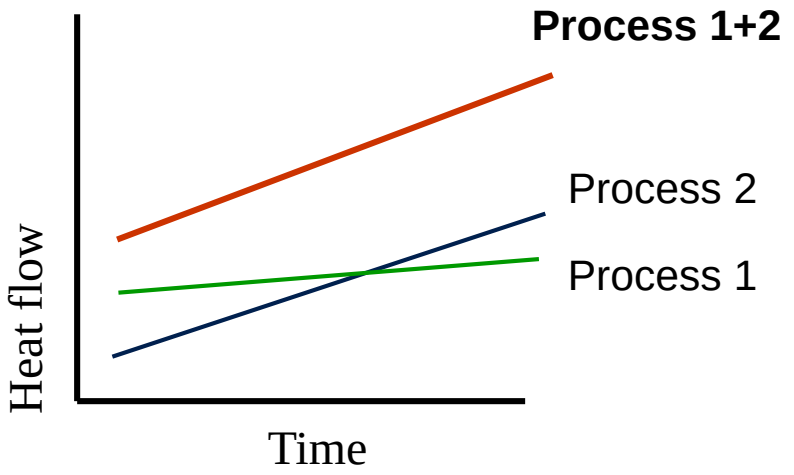


Wait until the standard deviation of the baseline is less than 10 uK and that the fit status is in Decay before starting the run.

Experimental Considerations

TAM is a Non-Specific Technique

- TAM is sensitive to all physical and chemical processes associated with a heat flow. Thus, the monitored heat flow may contain contributions from several processes.
- Individual contributions may be distinguished by varying the experimental conditions.
- Consider a blank experiment



Designing an Experiment

- Choice of sample handling system
- Handling of ampoules
- Sample considerations
- What to use as reference

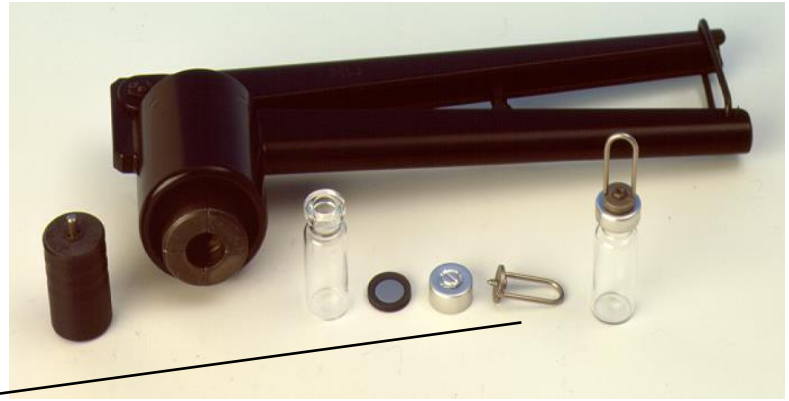
Sample Handling Systems

- Closed or sealed (static) Ampoules
- Open ampoules - Micro Reaction System
- Micro Solution Ampoule

Tools Required - Disposable Glass Ampoules

- Crimping tool
  - Used to seal the cap on the glass ampoule
- Adjustment tool (not shown)
  - Adjust the dimension of the caps when in position
- Centring tool
  - Used to make a mark for the lifter eyelet

- Ampoules and Caps
  - Aluminum, Butyl rubber and a Teflon gasket
  - May introduce initial disturbances!
- Lifting eyelets

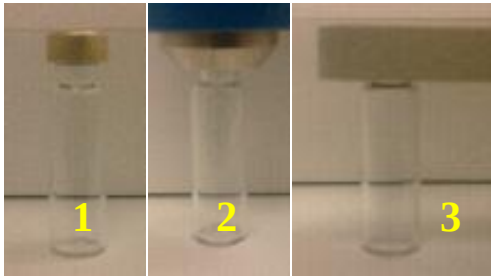


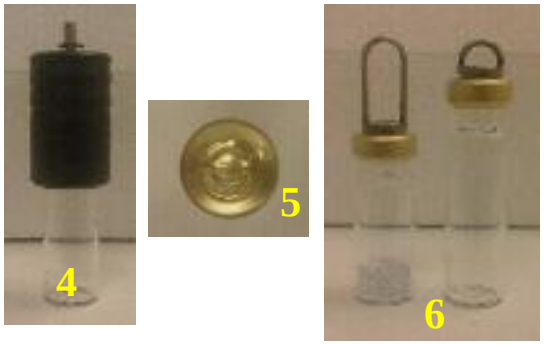
3mL lifting eyelets shown here

Sealing Disposable Glass Ampoules (3 & 4 mL)

Sealing the Ampoule:

- Weigh ampoule and sample. Place an aluminum cap and seal onto a clean ampoule rim (Figure 1).
- Crimp the cap with the tool provided. Rotate the crimping tool 90° and crimp again (Figure 2). Verify the seal by trying to rotate the cap.
- Align the cap with the PEEK alignment tool. Rotate the alignment tool 90° and crimp again (Figure 3). Verify the seal again by trying to rotate the cap.
- Use centering tool to make an indention in the cap (Figure 4). This indention will be a guide for the lifting eyelet (Figure 5).
- Thread the lifting eyelet into the cap (Figure 6).
- Make sure to wipe off all sample and fingerprints from the outside of the ampoule before loading into the calorimeter.
- Do not to throw away the lifting eyelet after the experiment is completed.



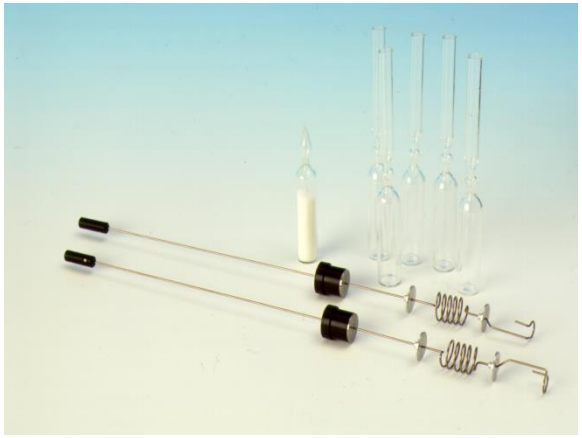


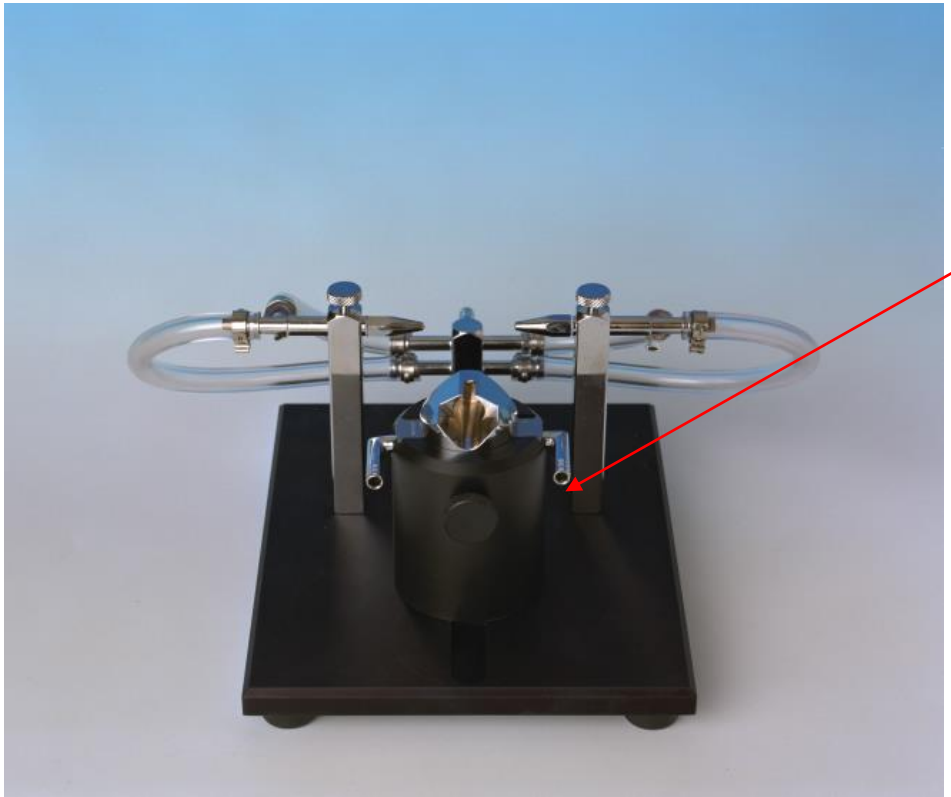
Disturbances with Disposable Glass Ampoules

- These ampoules may be associated with a disturbance in the 1-5 µW-range during the first 10 hours due to:
  - the sealing procedure introducing stress and subsequent relaxation phenomena
  - sorption/desorption phenomena
- Can be minimized by
  - pre-storing the ampoules and lids at the operating temperature for 24 hours
  - Preparing the sample and the reference ampoule at the same time (use a new reference for each measurement)
- Always consider the risk for interaction between the sample and the ampoule
  - Steel ⇔ peroxides, HCl
  - Basic solvents ⇔ glass
  - Pressure build up

Heat Seal Ampoules

- Completely sealed
- Heat seal ampoules of glass
- Special ampoule lifters required





Water circulation inlet/outlet

Note: precautions should be taken to protect the sample towards the heat during the sealing procedure

Threaded Cap Ampoules

- Stainless steel
  - resistance towards corrosion
  - should not be used for solvent with pH < 4
- Hastelloy
  - Improved resistance towards corrosion and acids
  - excellent for use with organic solvents
- The cap is sealed with a disposable Teflon disc and/or o-ring (not shown)
- Stable for most applications
- Stands pressures up to at least 2 bar

Circlip Cap Ampoules

- Stainless steel
  - resistance towards corrosion
  - should not be used for solvent with pH < 4
- Hastelloy
  - Improved resistance towards corrosion and acids
  - excellent for use with organic solvents
- Glass with stainless steel or Hastelloy collar
- O-sealing made in Nitrile, EPDM, Viton® or Kalrez ®
- Stands 8 bar pressure (precautions must be made)

Equilibrium

- Thermal equilibrium
  - Within 60 min after loading
    - depends on sample size – 20mL ampoules may take longer to fully equilibrate
- Physical equilibrium
  - Depends on the sample and the pre-history
  - Might depend on the ampoule itself
- Chemical equilibrium
  - Slow/fast reactions

Pre-history of the Sample

- The sample should be stored under controlled conditions for at least 24 hours before a measurement
  - relative humidity
  - temperature
  - atmosphere (e.g. nitrogen, air, oxygen)
- The time to reach physical equilibrium must be considered

Sample Geometry and Surface Area

- Powder (small particle size)
  - Chemical processes will occur homogeneously in the sample
- Bulk samples (large particle size)
  - May show a heterogeneous response
    - diffusion limited oxidation
    - pressure build-up by volatiles formed
- Try powder, films, granules or, specimens with different thickness. The influence of geometry can be studied using different particle size with the same amount of sample.
  - If the specific heat flow (µW/g) is the same for two different sizes, this effect is not important. Otherwise it must be considered.

Sample Amount

- The response in heat flow may be dependent on the amount of samples (different bed volumes) in the ampoule
  - If the specific heat flow (µW/g) is the same for different amounts of samples either there is possibly a layering (or caking) effect or the effect is not important (heat flow/g consistent).

Kinetic Evaluation

- Be sure the response in heat flow reflects the kinetics of the process of interest
- In many case the first 5-10 hours should be excluded because of a non physical equilibrium
  - other process(es) contribute to the heat flow
    - Examples: Evaporation from hygrostat and adsorption on the walls of the ampoule

Choice of Reference Materials

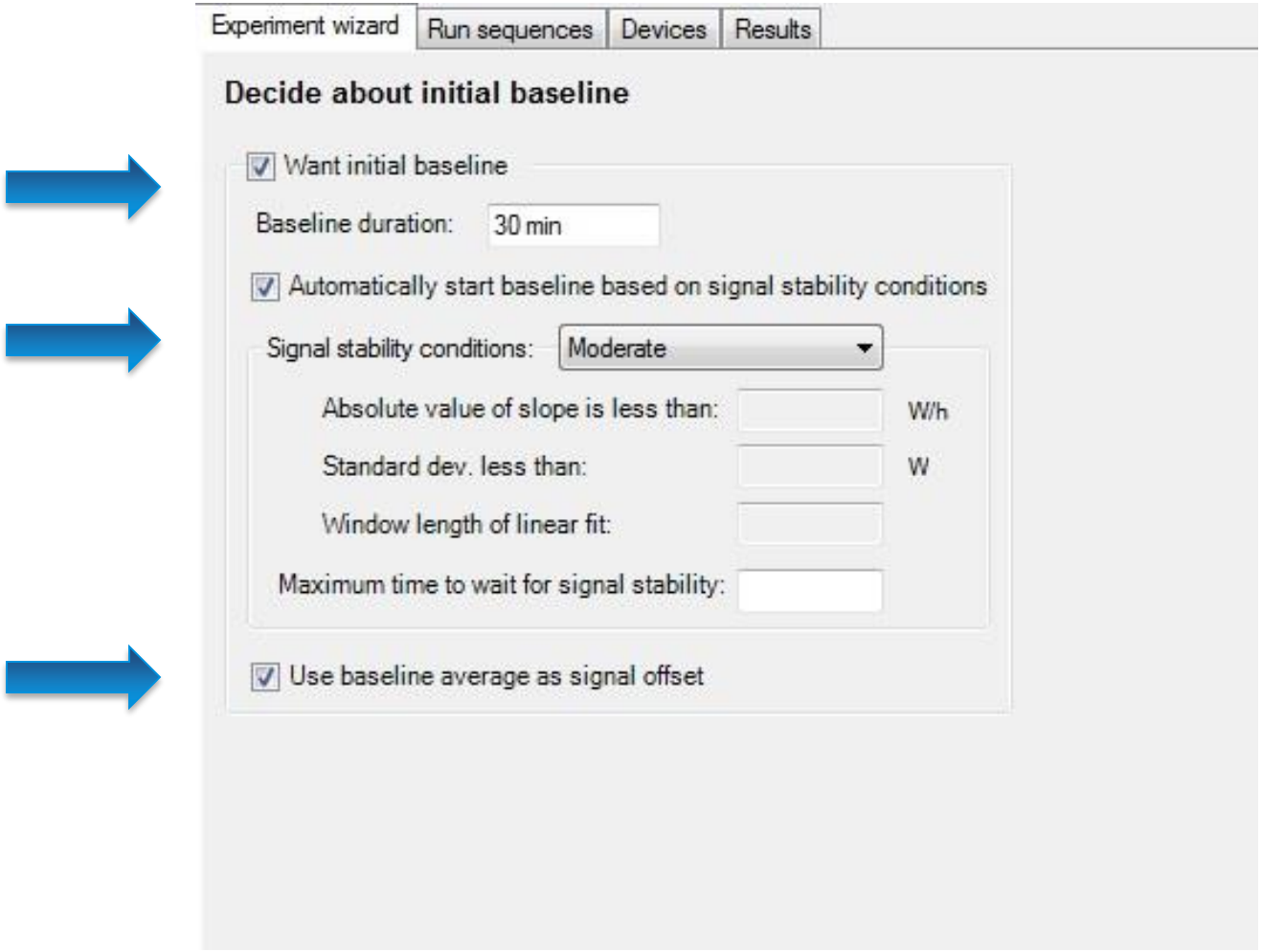
- A reference material is used to balance the heat capacity of the sample and the reference ampoule.
- With a good balance in heat capacity the short-term noise will be reduced. However, if the system is not well-balanced the average heat flow values is not affected.
- A proper balancing of the ampoules is needed when the response in heat flow is low, e.g. during titration experiments.
- Example of reference materials: sand, glass pearls, water

Baseline collection

- When looking at absolute energies or comparing two curves, it is essential that you have a baseline that is set to zero. There should be no heat being produced which means an empty calorimeter and empty reference.

- Collecting a good baseline means:
  - Waiting until the baseline is stable before starting collection
    - Set “Signal Stability Conditions” and let the instrument wait until the signal is stable
    - Set a “Maximum time to wait” if time is critical

Baseline collection

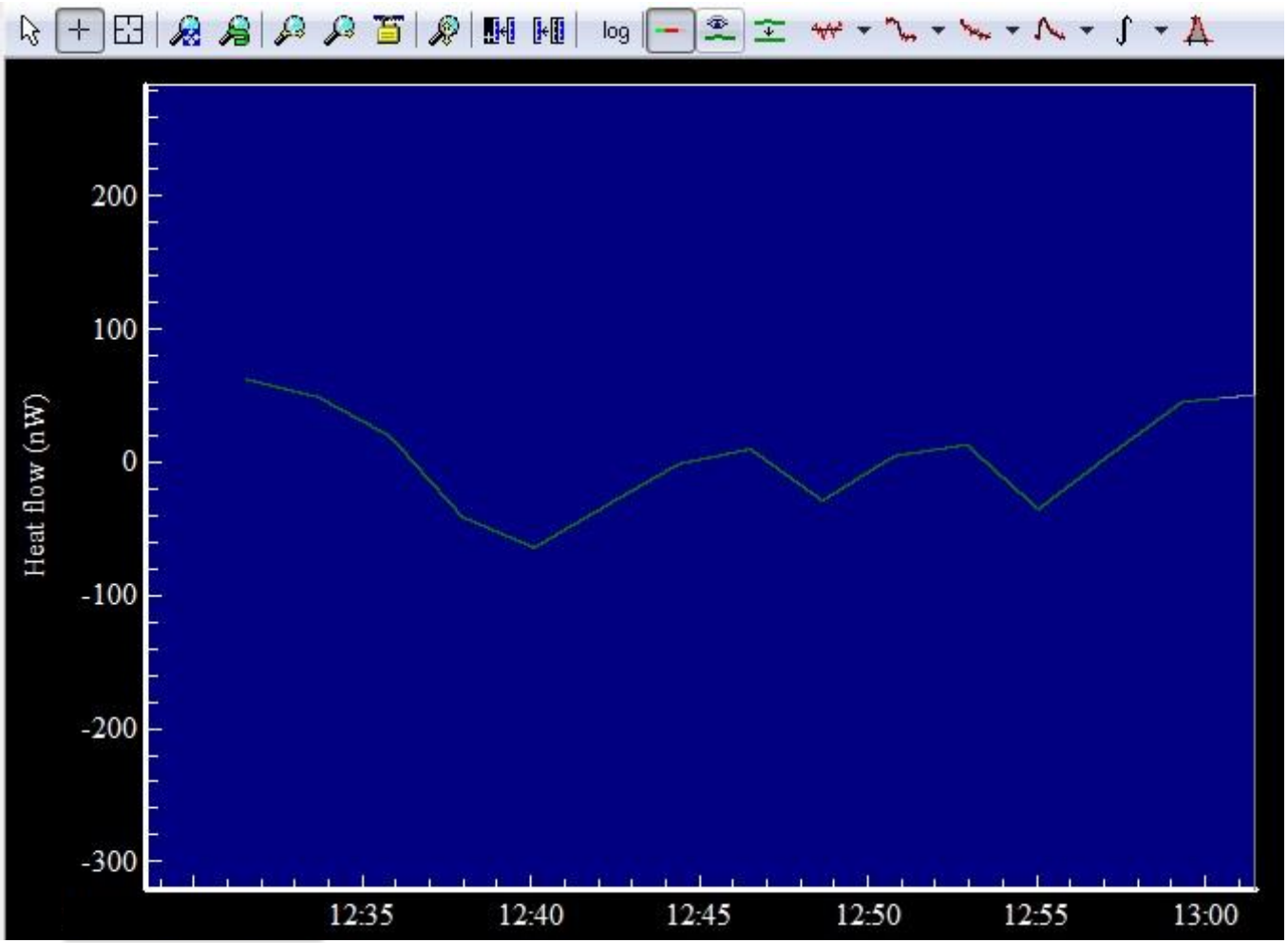


Baseline collection

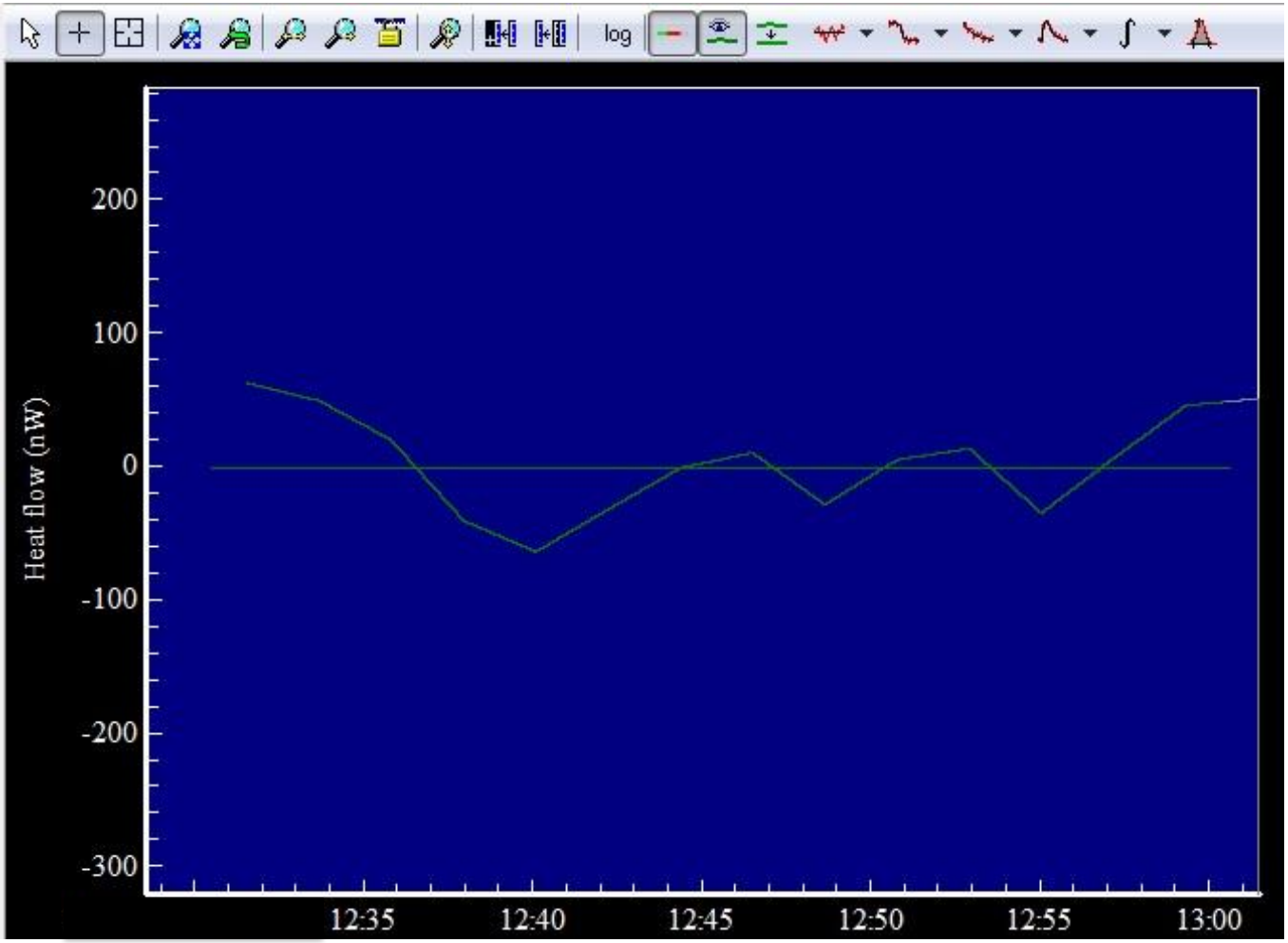
- When looking at absolute energies or comparing two curves, it is essential that you have a baseline that is set to zero. There should be no heat being produced which means an empty calorimeter and empty reference.

- Collecting a good baseline means:
  - Waiting until the baseline is stable before starting collection
    - Set “Signal Stability Conditions” and let the instrument wait until the signal is stable
    - Set a “Maximum time to wait” if time is critical
  - Collecting enough baseline to determine the zero point
    - 30 min is the default.
    - Make sure you have enough time to capture peaks and valleys to get a good average
  - Not disturbing the instrument while the baseline is being collected
    - Due to the data reduction, it is wise to wait a few minutes after the baseline has finished to disturb the calorimeter by inserting the samples.

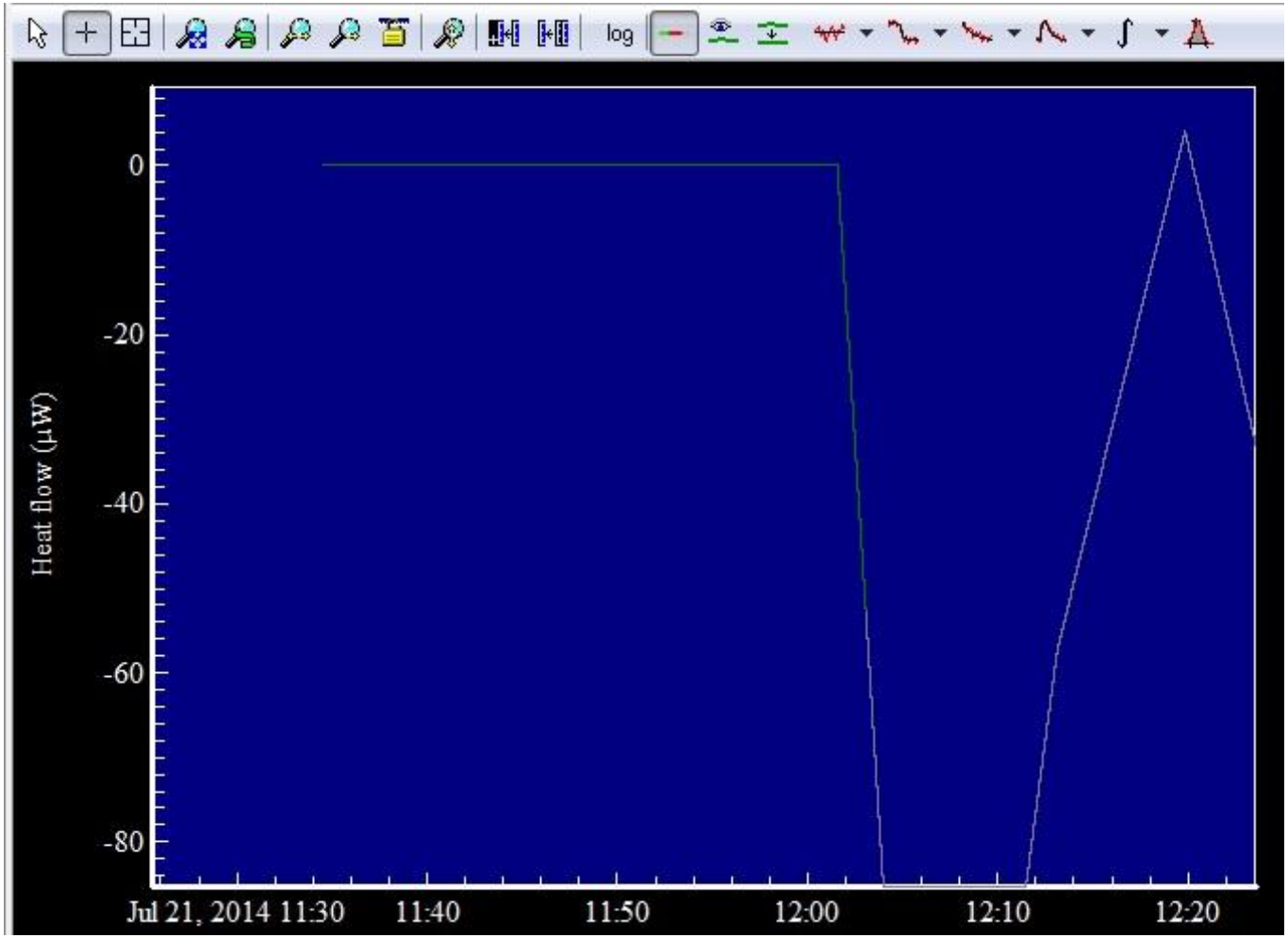
Baseline collection



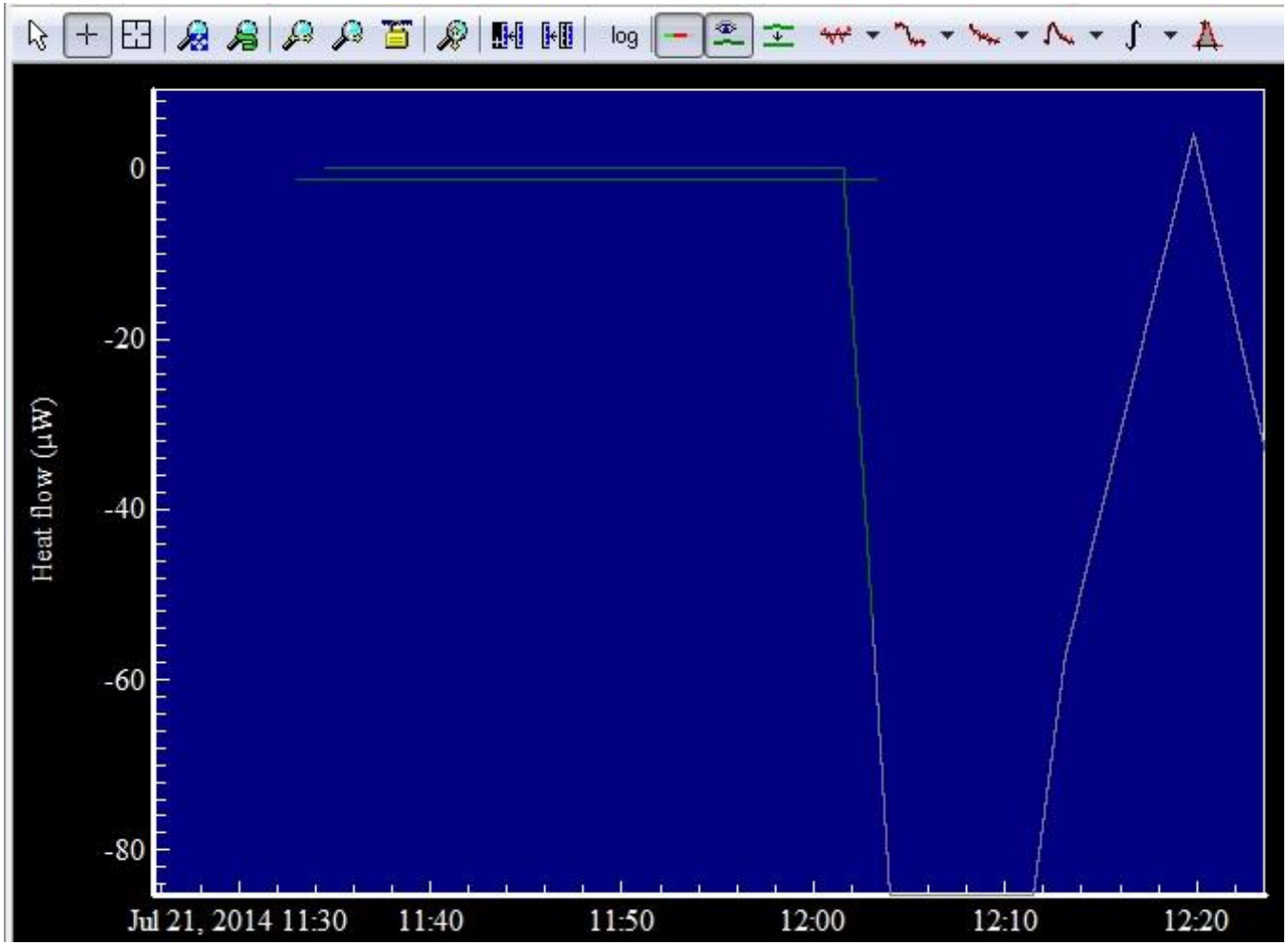
Baseline collection



Baseline problems

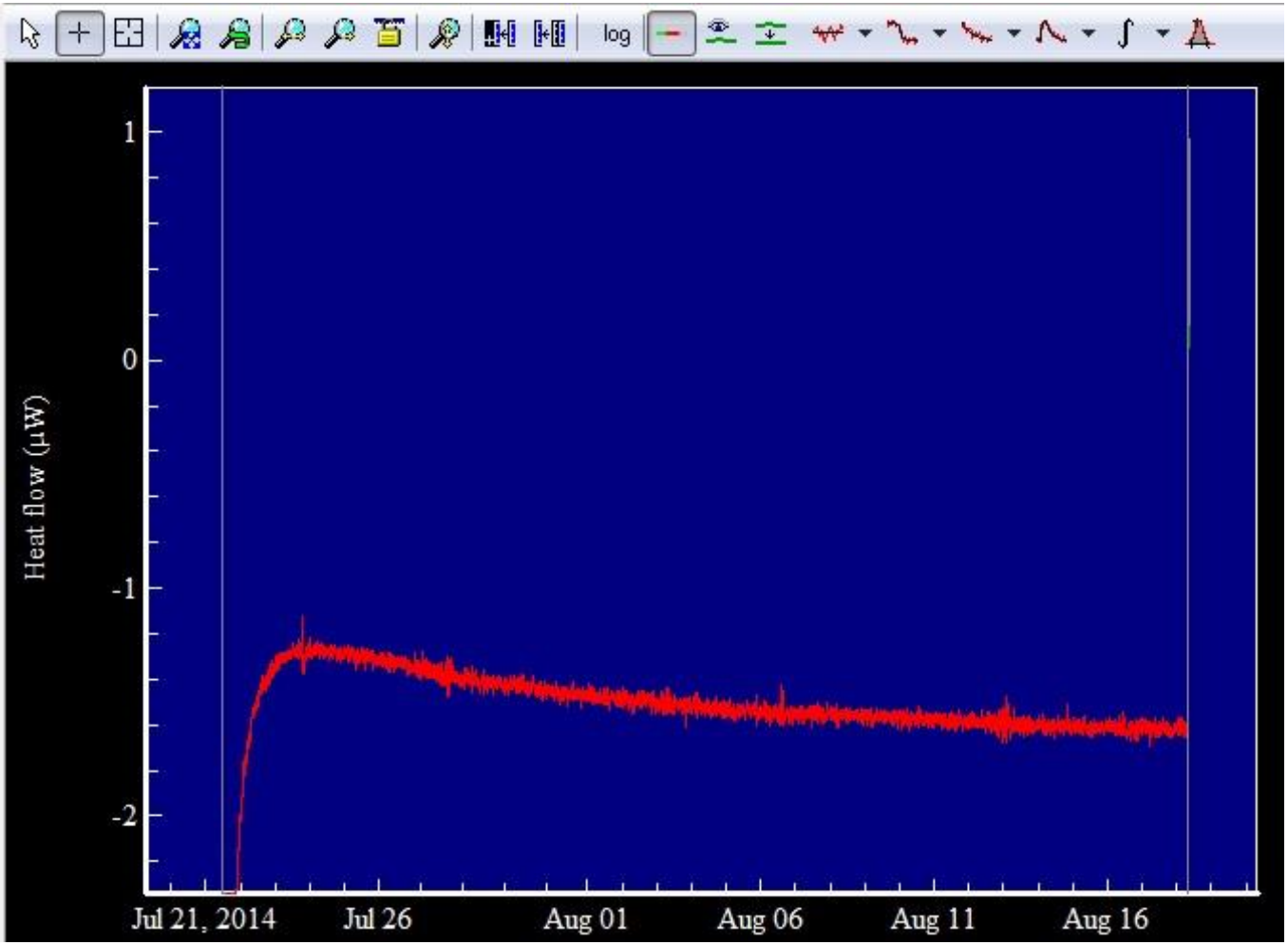


Baseline problems

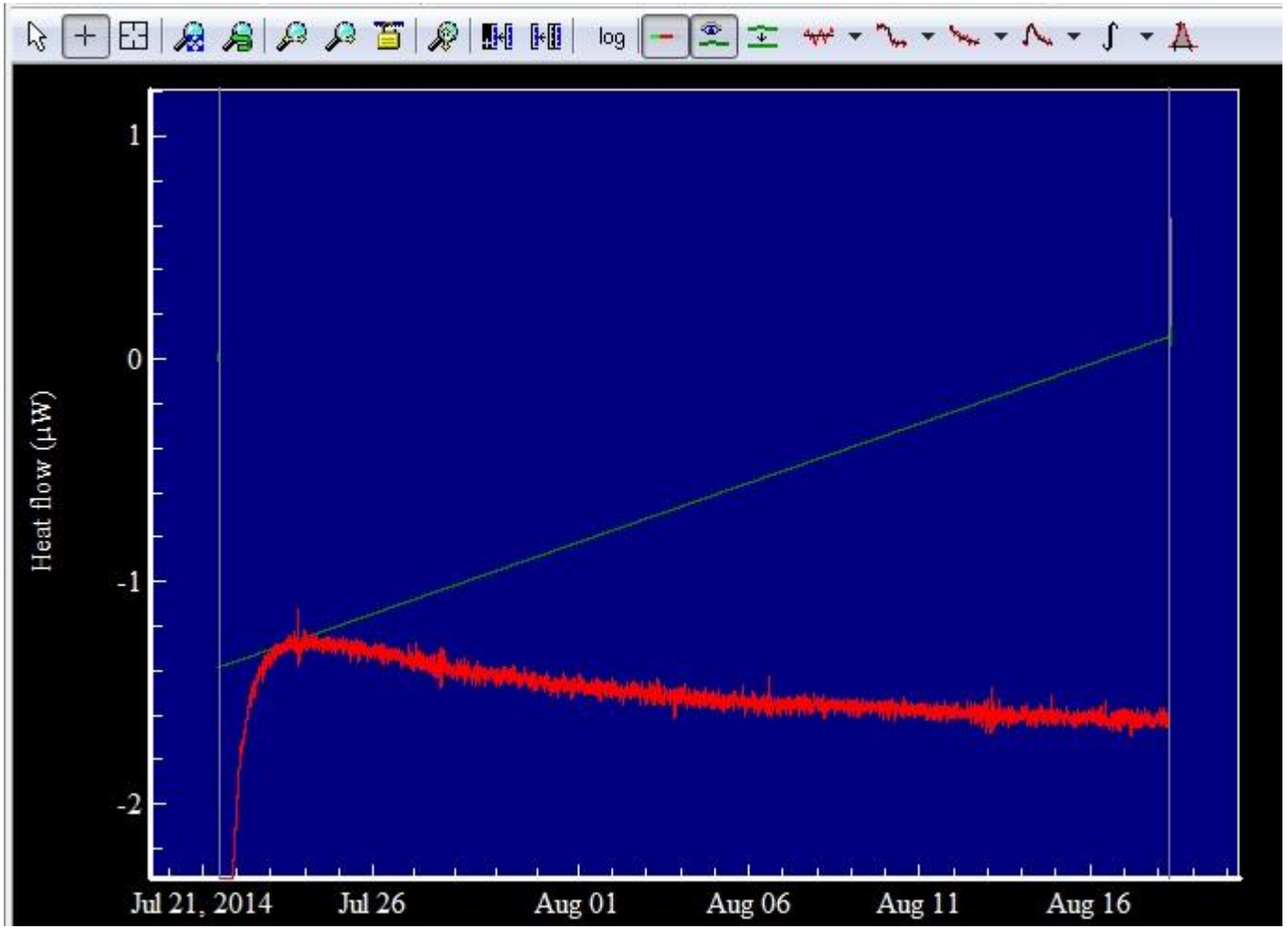


Baseline problems

Baseline problems

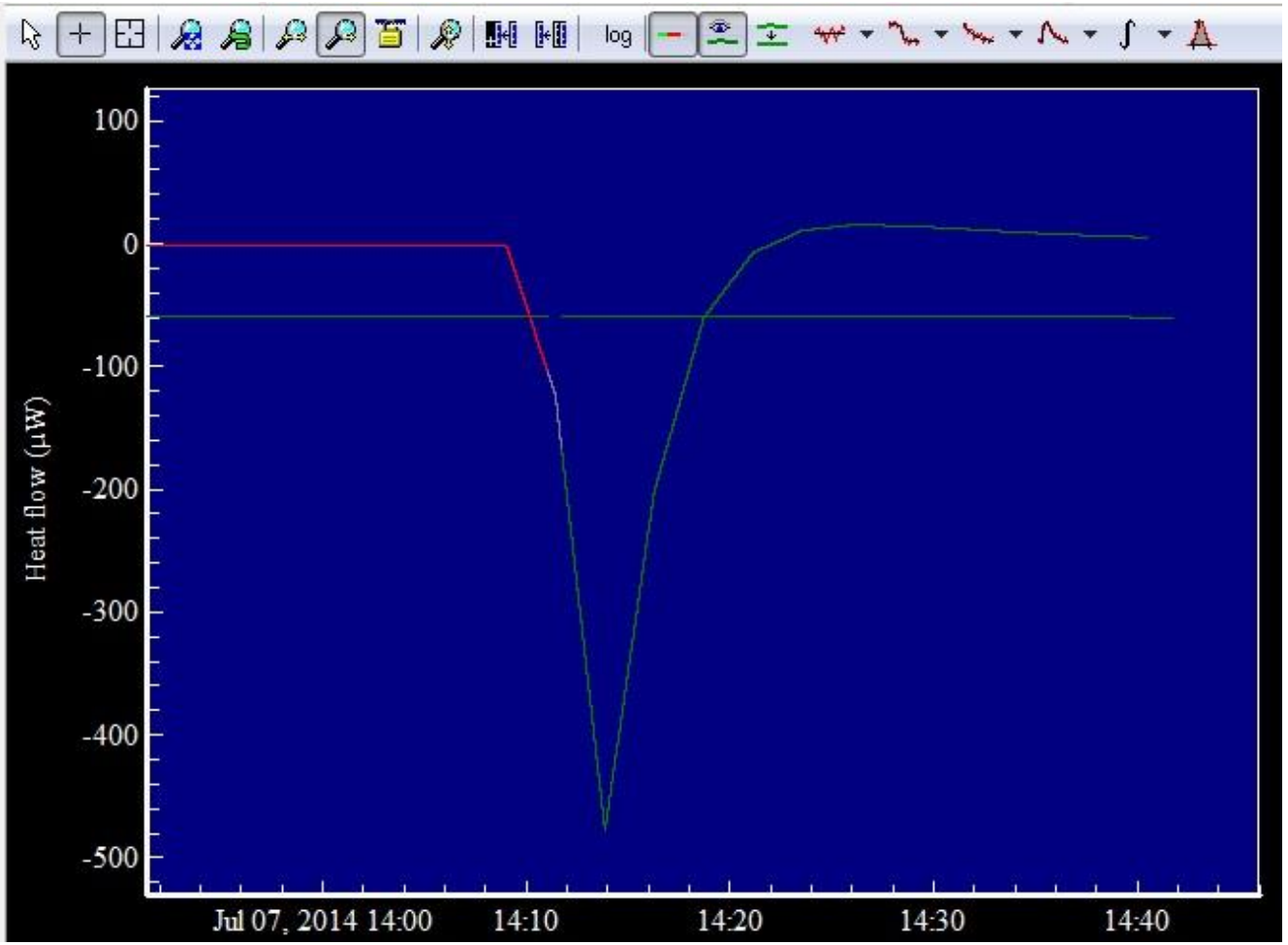


Baseline problems



Baseline problems

Baseline problems



Baseline problems

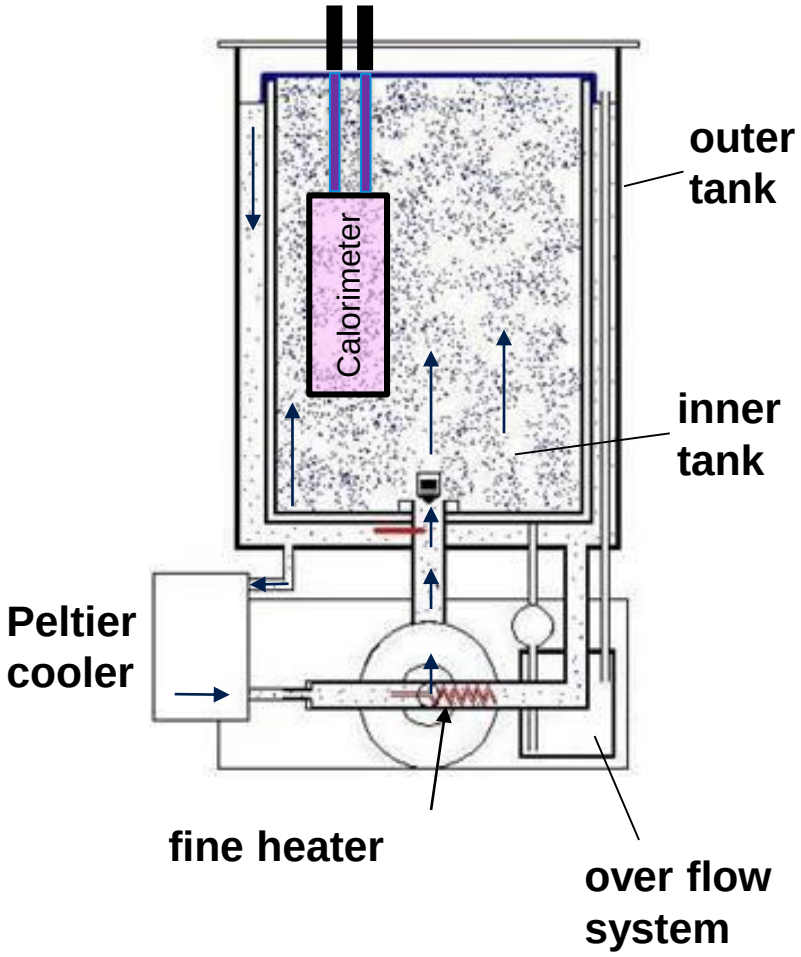
Baseline collection

- When looking at absolute energies or comparing two curves, it is essential that you have a baseline that is set to zero. There should be no heat being produced which means an empty calorimeter and empty reference.

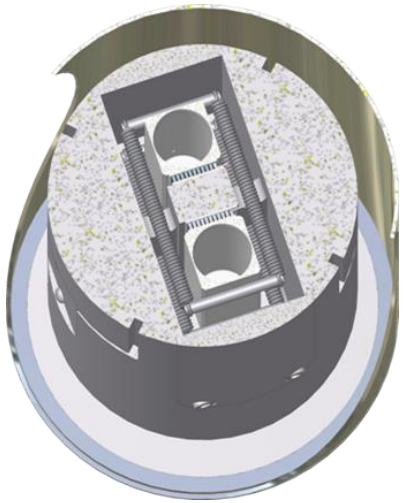
- Collecting a good baseline means:
  - Waiting until the baseline is stable before starting collection
    - Set “Signal Stability Conditions” and let the instrument wait until the signal is stable
    - Set a “Maximum time to wait” if time is critical
  - Collecting enough baseline to determine the zero point
    - 30 min is the default.
    - Make sure you have enough time to capture peaks and valleys to get a good average
  - Not disturbing the instrument while the baseline is being collected
    - Due to the data reduction, it is wise to wait a few minutes after the baseline has finished to disturb the calorimeter by inserting the samples.

TAM Thermostat

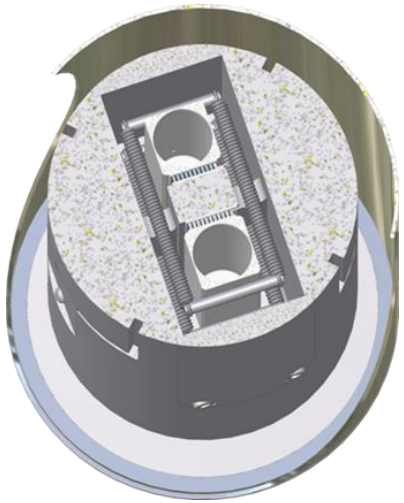
An oil based liquid bath system for a continuously circulated heat sink medium that prevents any thermal event in a test sample or from the room environment from altering the constant temperature bath



Calorimeters



Calorimeters



Wait 15-20 min for sample
to equilibrate with the oil bath

Calorimeters

The move into the measurement area causes a disturbance.

Instrument waits 45 min for signal to be correct.

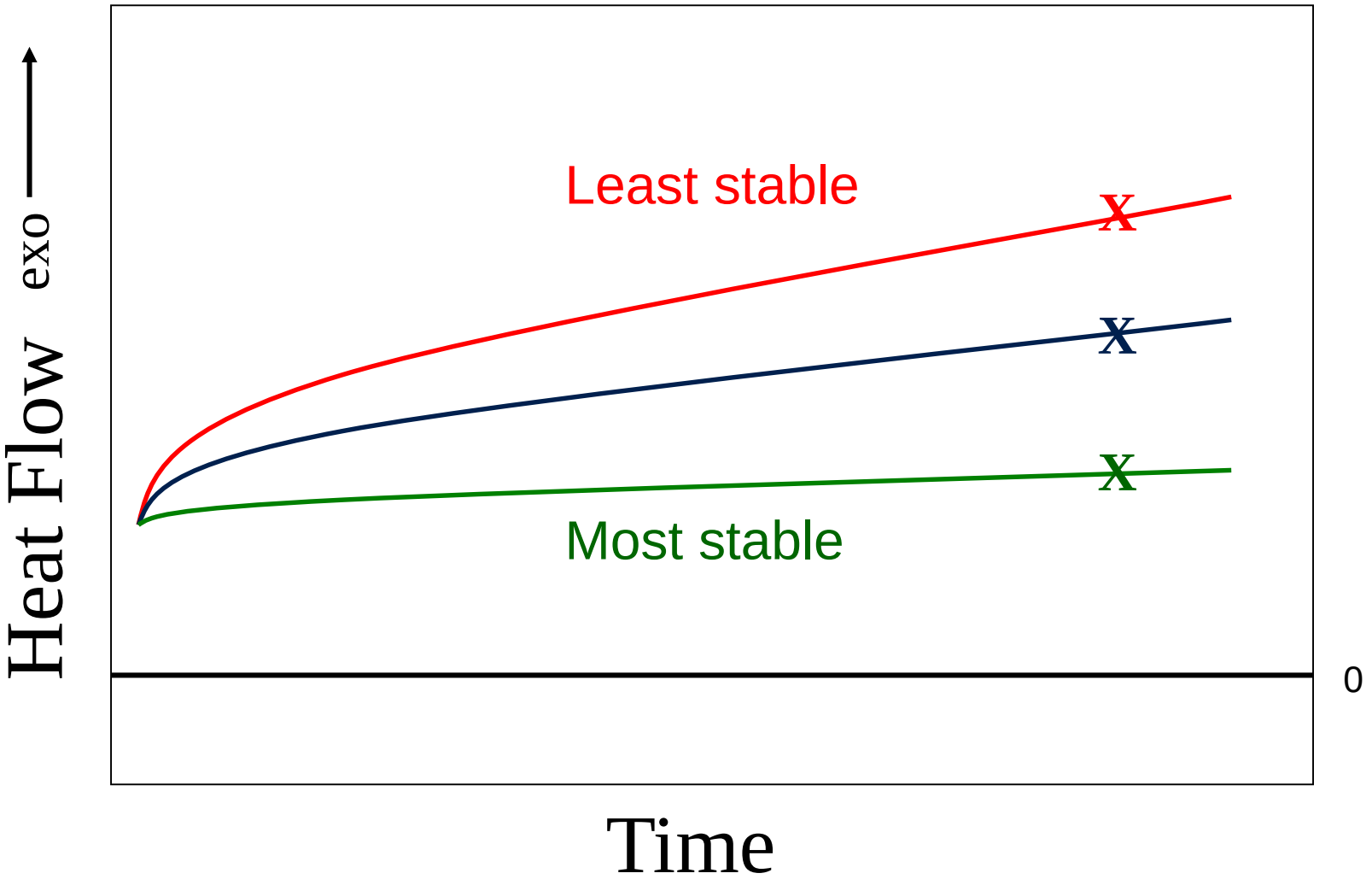
TAM Applications

Pharmaceuticals
Life Science
Material Science

TAM Applications

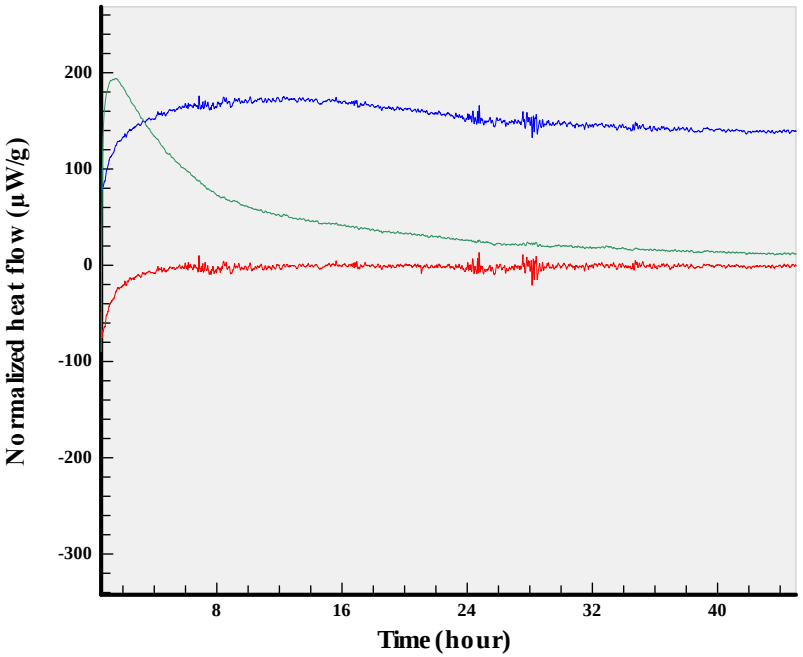
Stability

TAM Stability Testing

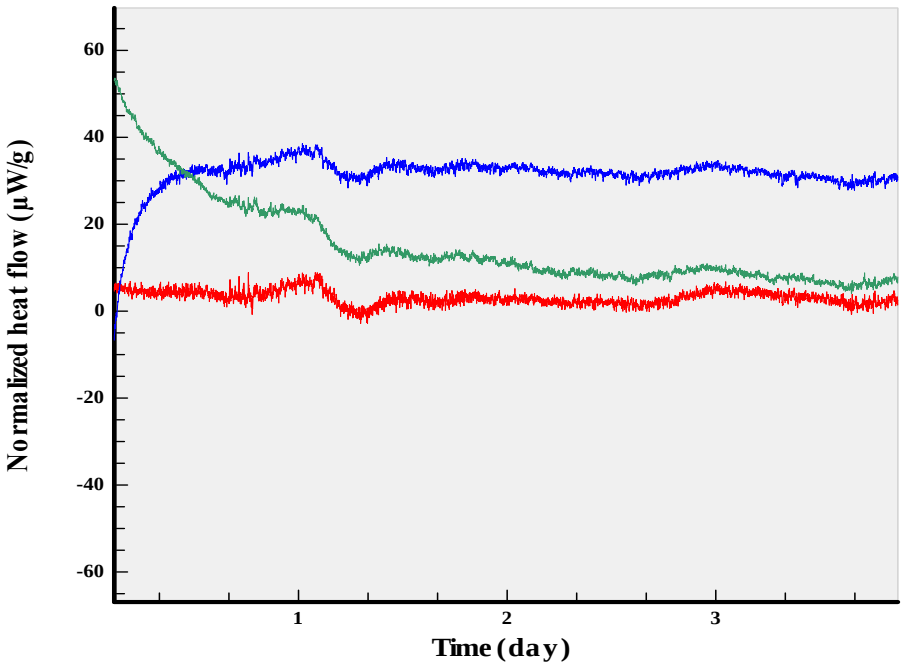


TAM Stability Testing

40°C



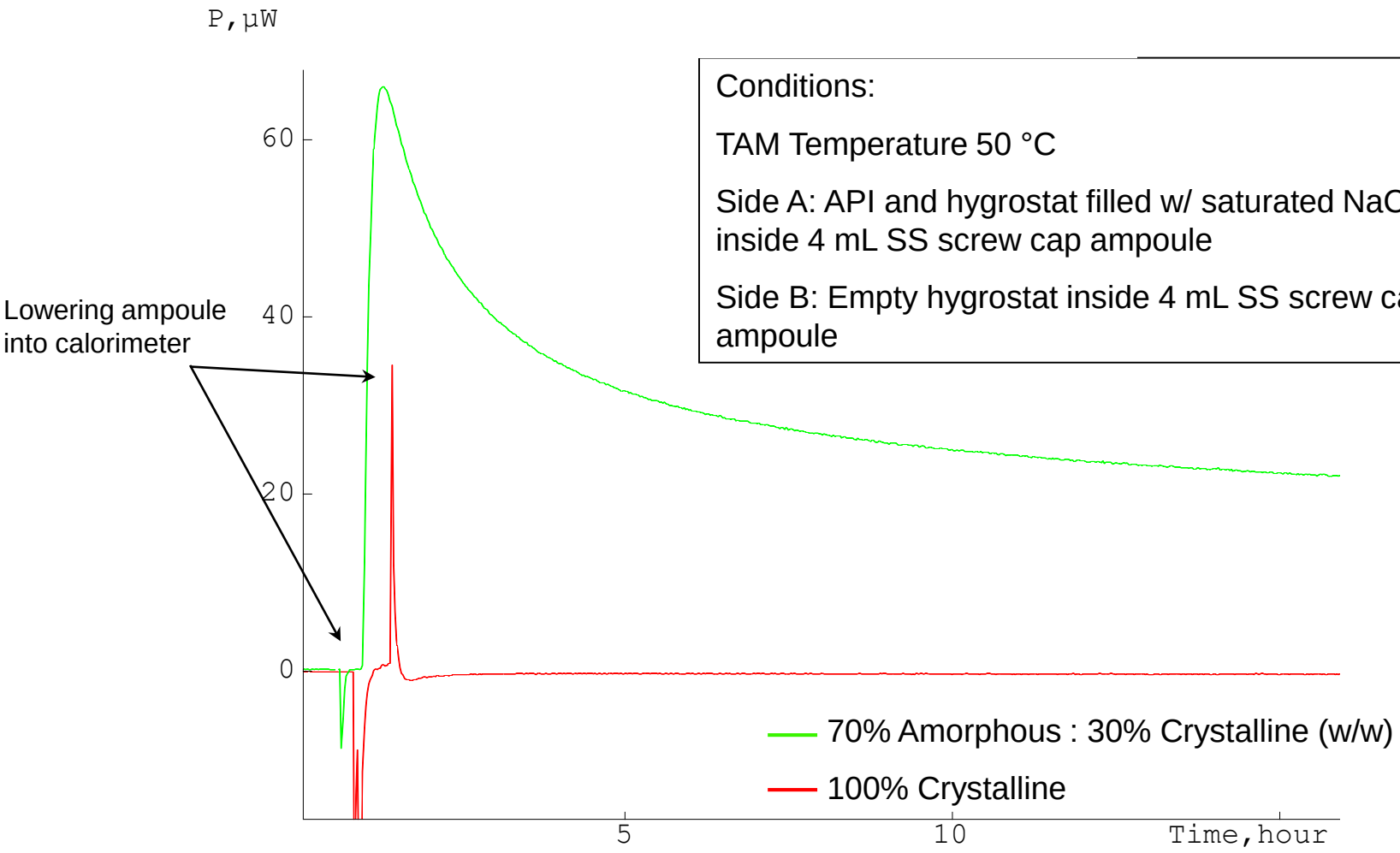
30°C



Three different lots of the same material at two different temperatures.

Active Pharmaceutical Ingredient Stability

(~75% Relative Humidity)



Conditions:

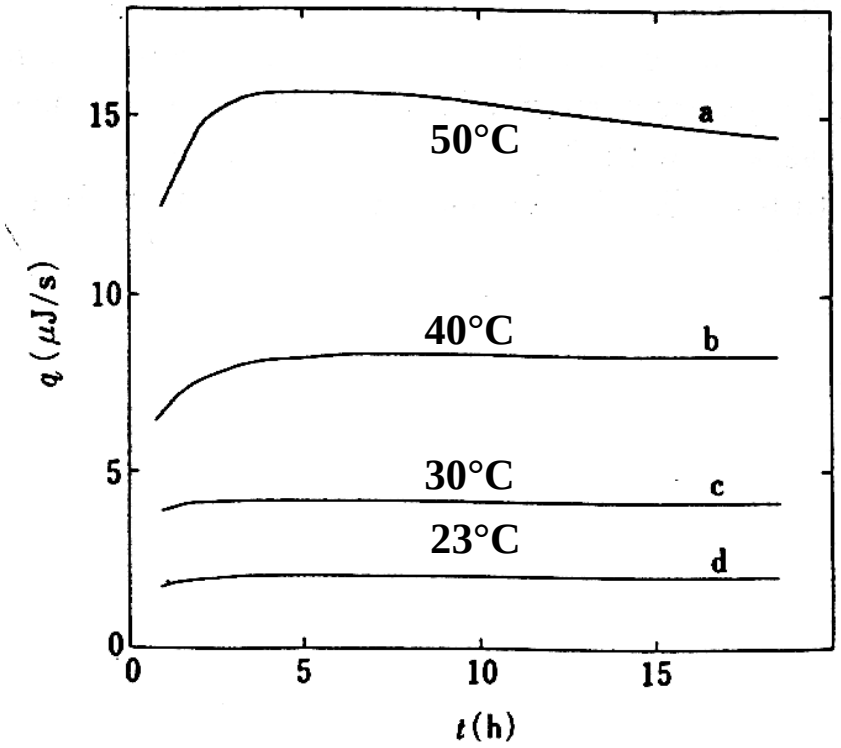
TAM Temperature 50 °C

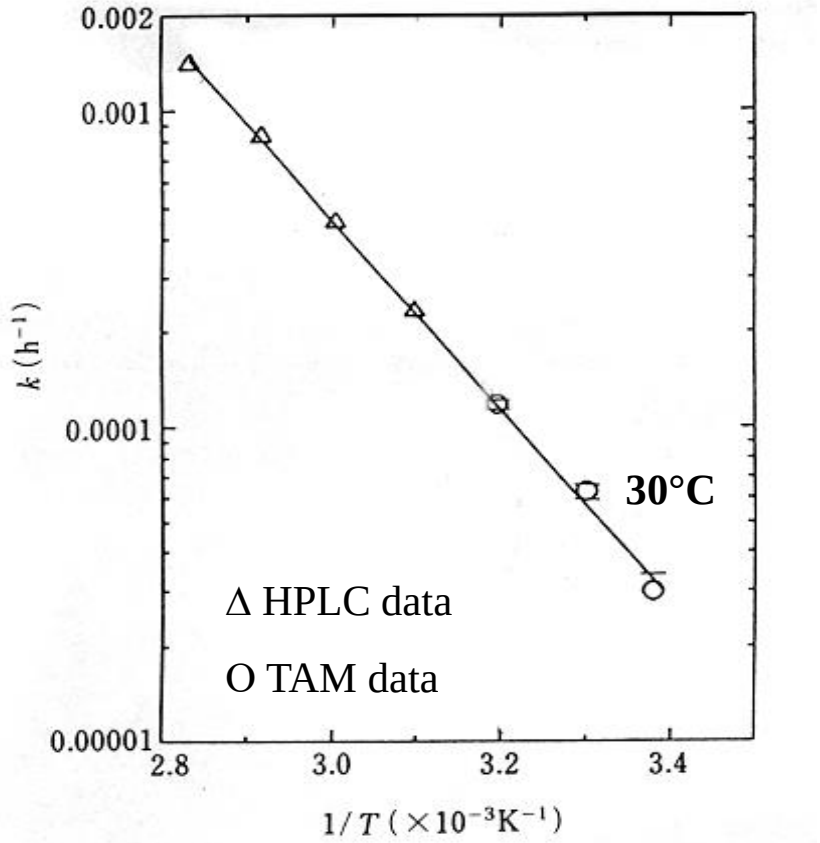
Side A: API and hygrostat filled w/ saturated NaCl inside 4 mL SS screw cap ampoule

Side B: Empty hygrostat inside 4 mL SS screw cap ampoule

Stability with TAM and Compared with HPLC

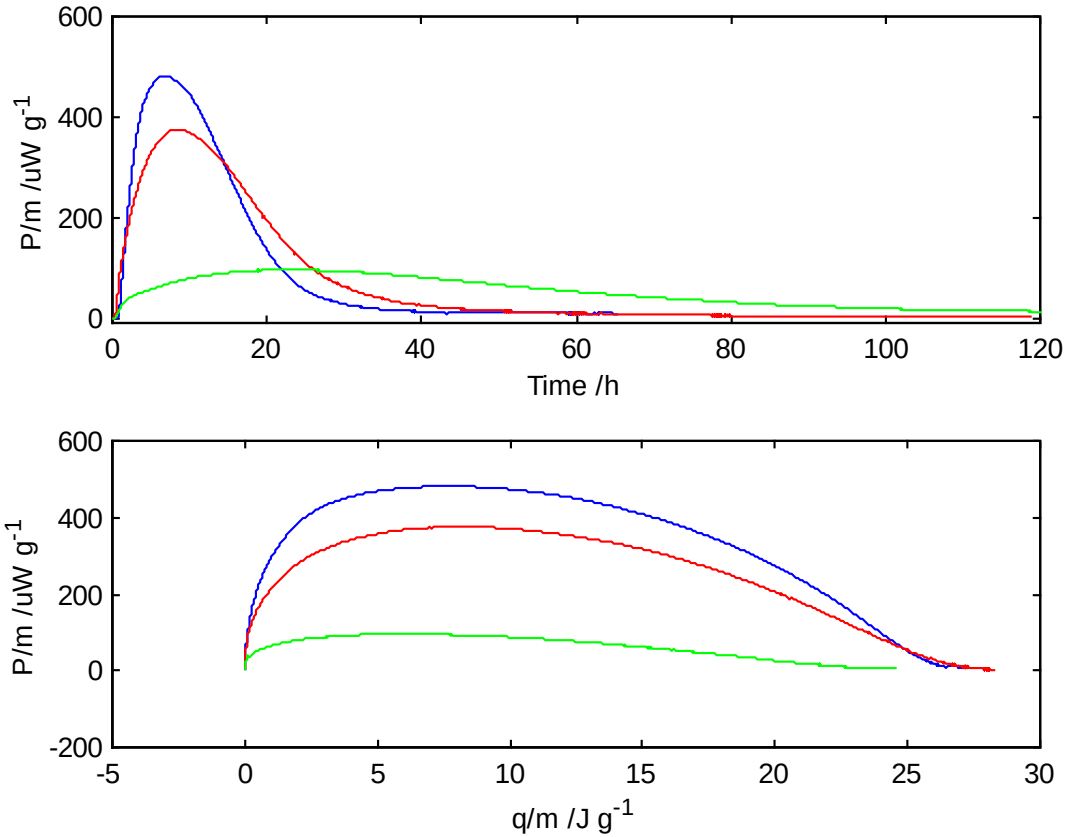
Oxidation of Meclofenoxate Hydrochloride Containing *dl*-a-Tocopherol





Otsuka T., Yoshioka S., Aso Y. and Terao T., *Chem. Pharm. Bull.*, **42**(1) 1994

Hydrate Formation in Ethinyl Estradiol



Measuring temperature: 45°C

- Blue trace: 100 %RH
- Red trace: 95 % RH
- Green trace: 88 %RH

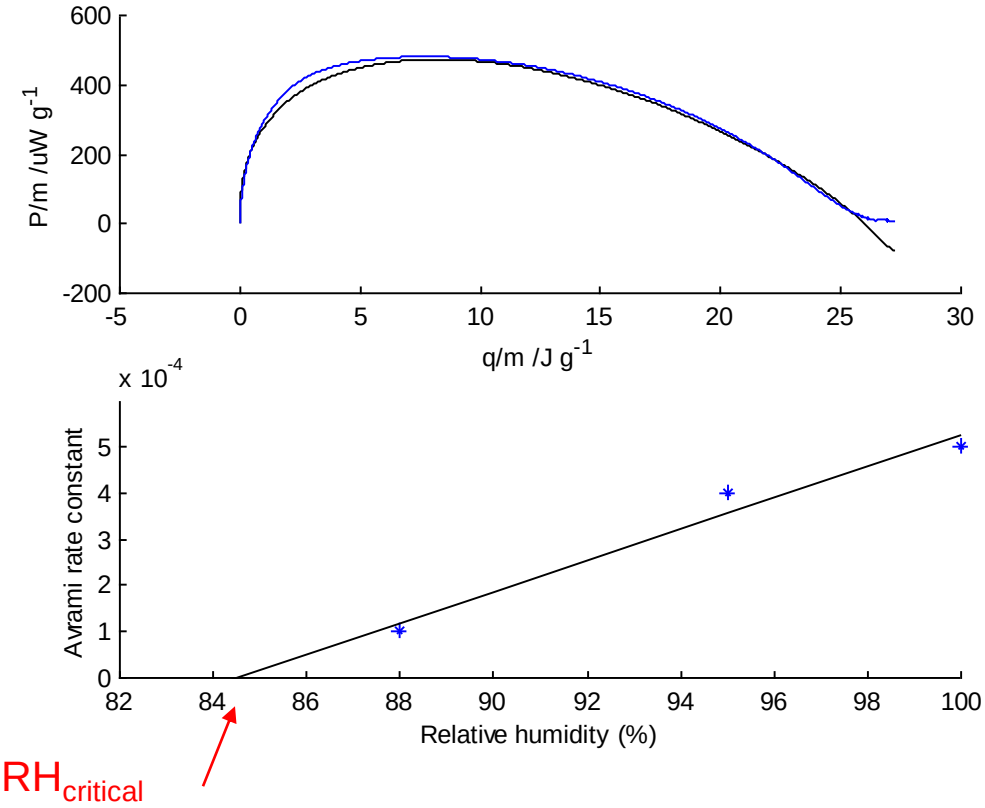
Rate equation:

$$P = k(T) \cdot f(q/\Delta H)$$

Avrami's Model

$$P = k(T) \cdot f(q/\Delta H)$$

$$f(q/\Delta H) = 2(1-q/\Delta H) \cdot [-\ln(1-q/\Delta H)]^{1/2}$$



- Blue trace: experimental data
- Black trace: fitting equation ($k=0.0005\ s^{-1}$)
- Rate constant as a function of relative humidity

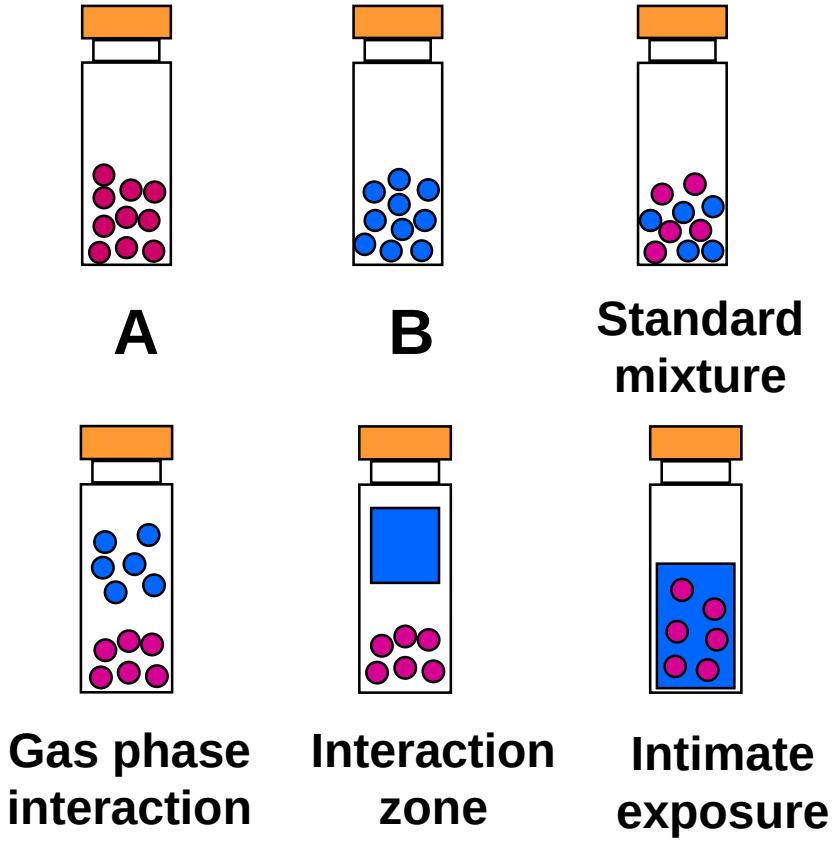
TAM Applications

Compatibility Testing

Compatibility Measurements

Basic sample set, 2-component test

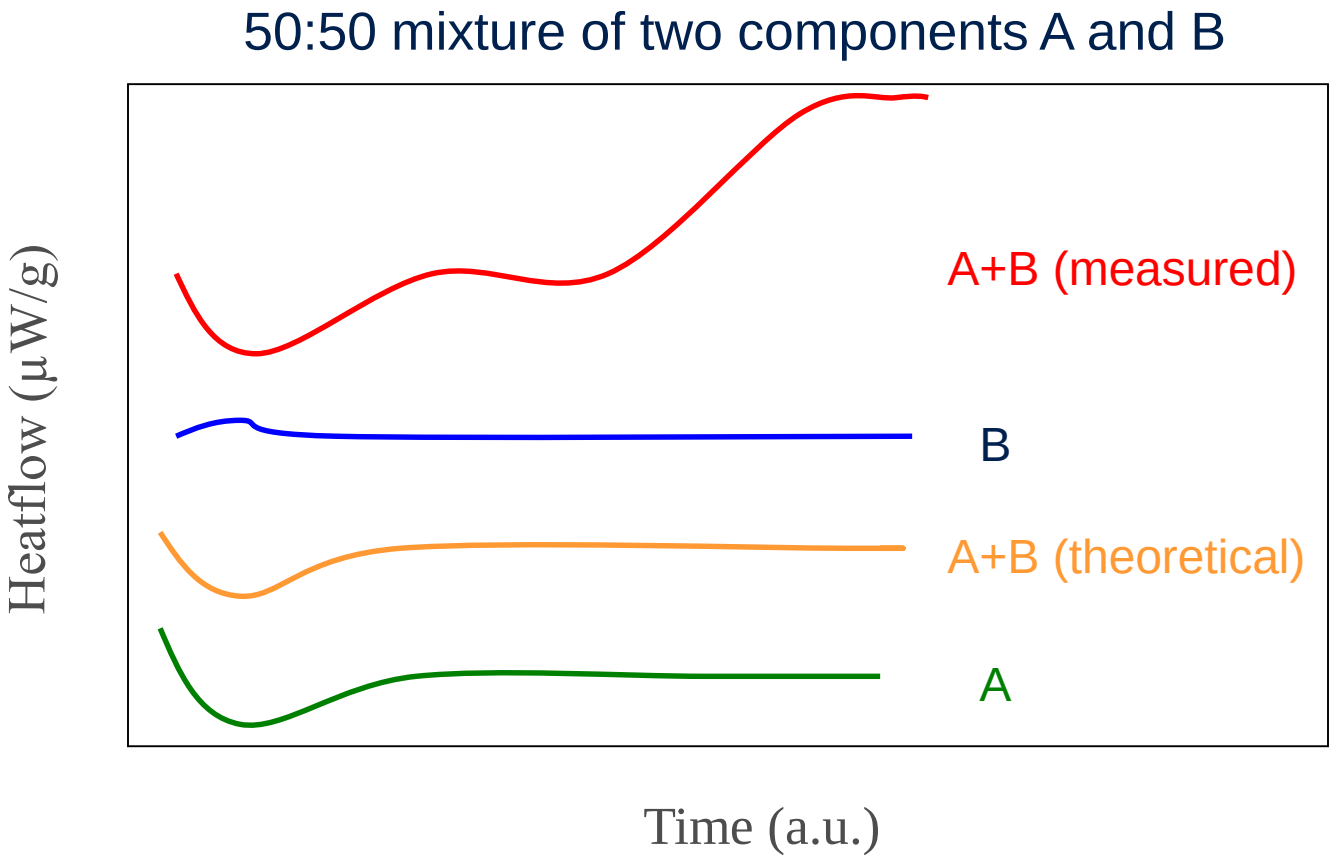
Additional mixture samples for special tests



The term compatibility refers to a mutual physical or chemical interaction between two or more components of a mixture, which leads to a change in the mixture or component properties.

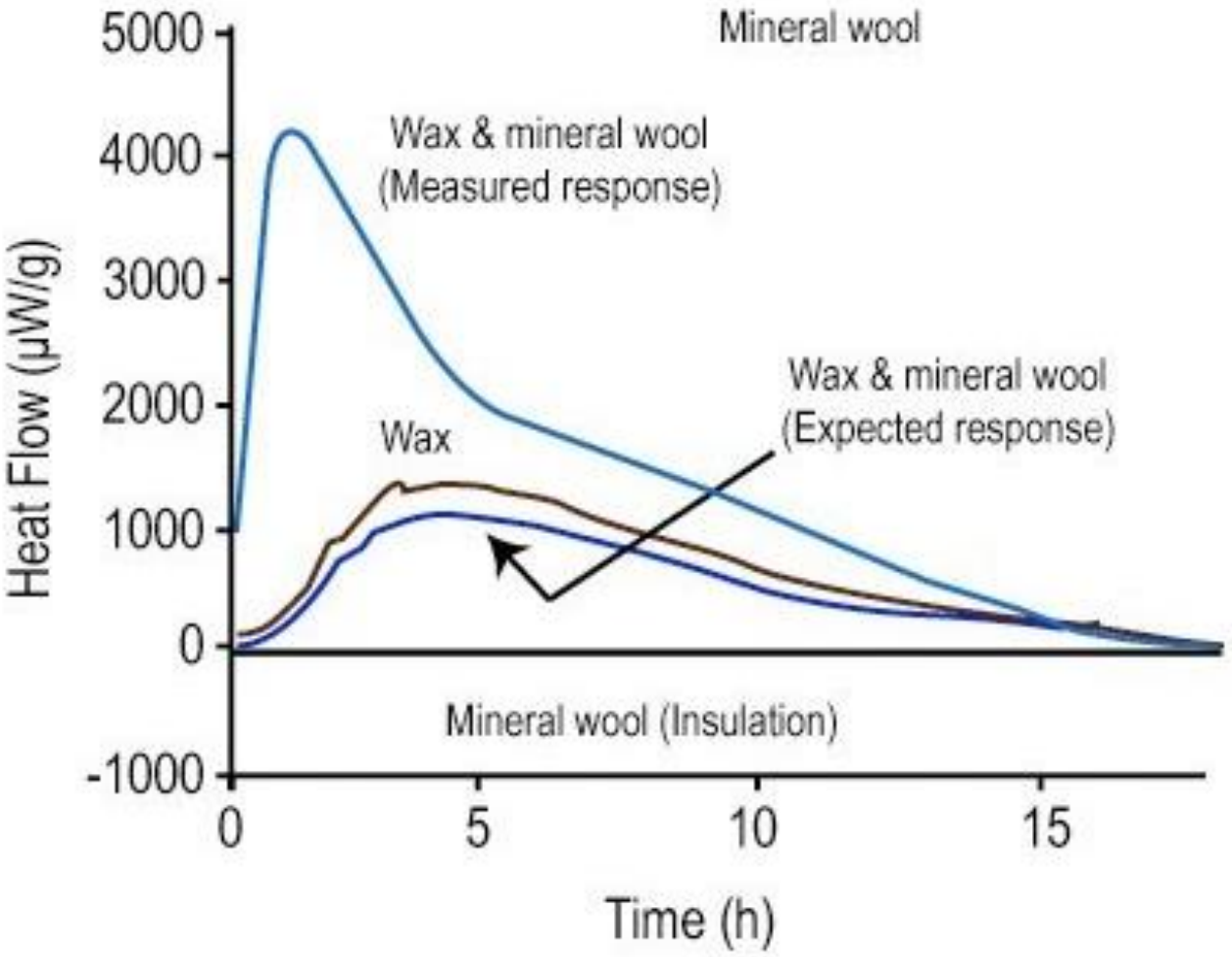
By Lars-Gunnar Svensson, Celsius Materials CMK, Karlskoga, Sweden

Evalutation of Compatibility Measurements



If the heat flow curve of **A+B (measured)** differs from **A+B (expected)**, this is an indication that the materials affect each other or are incompatible.

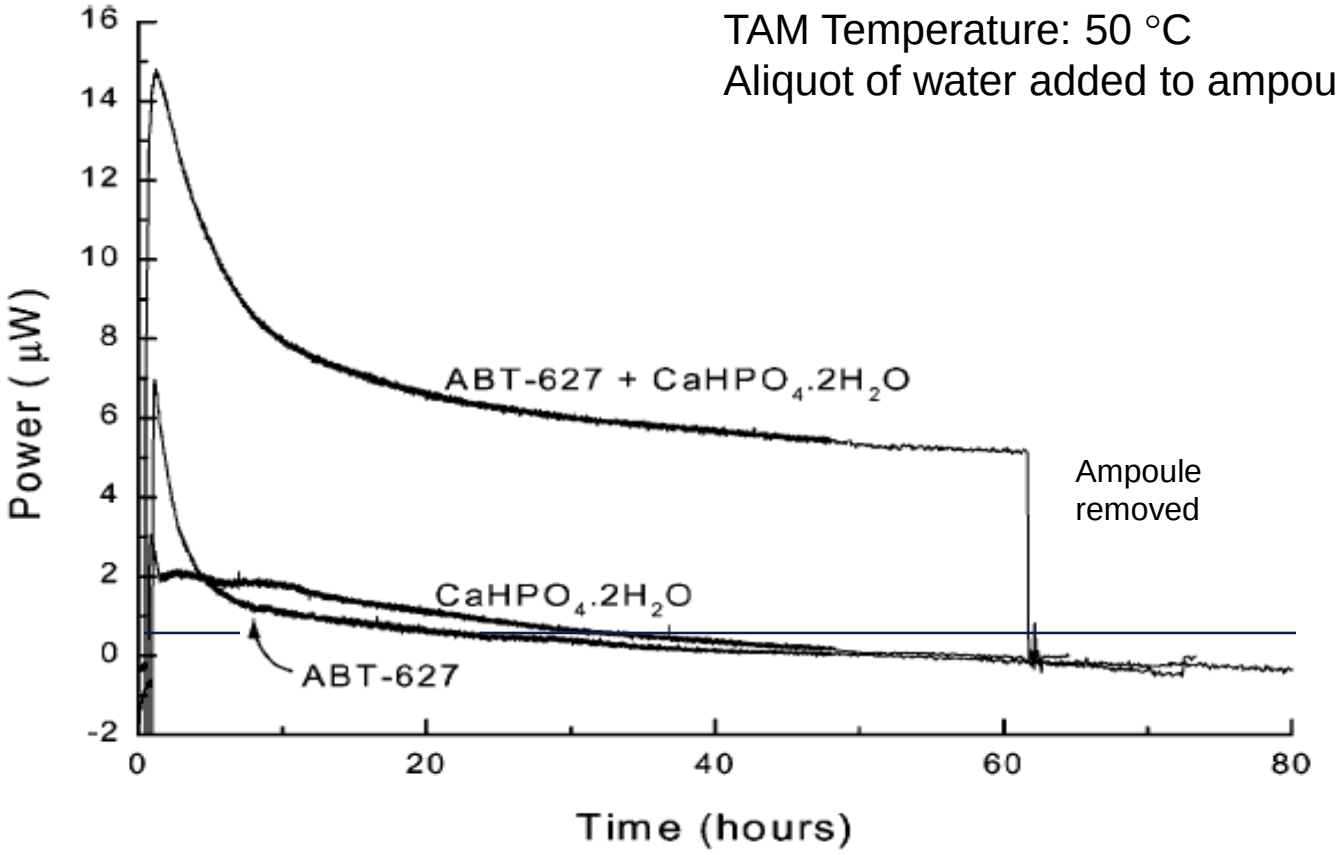
Compatibility Between Wax and Mineral Wool



Data provided by Svensson, *Bodycote Materials AB*, Sweden (2003)

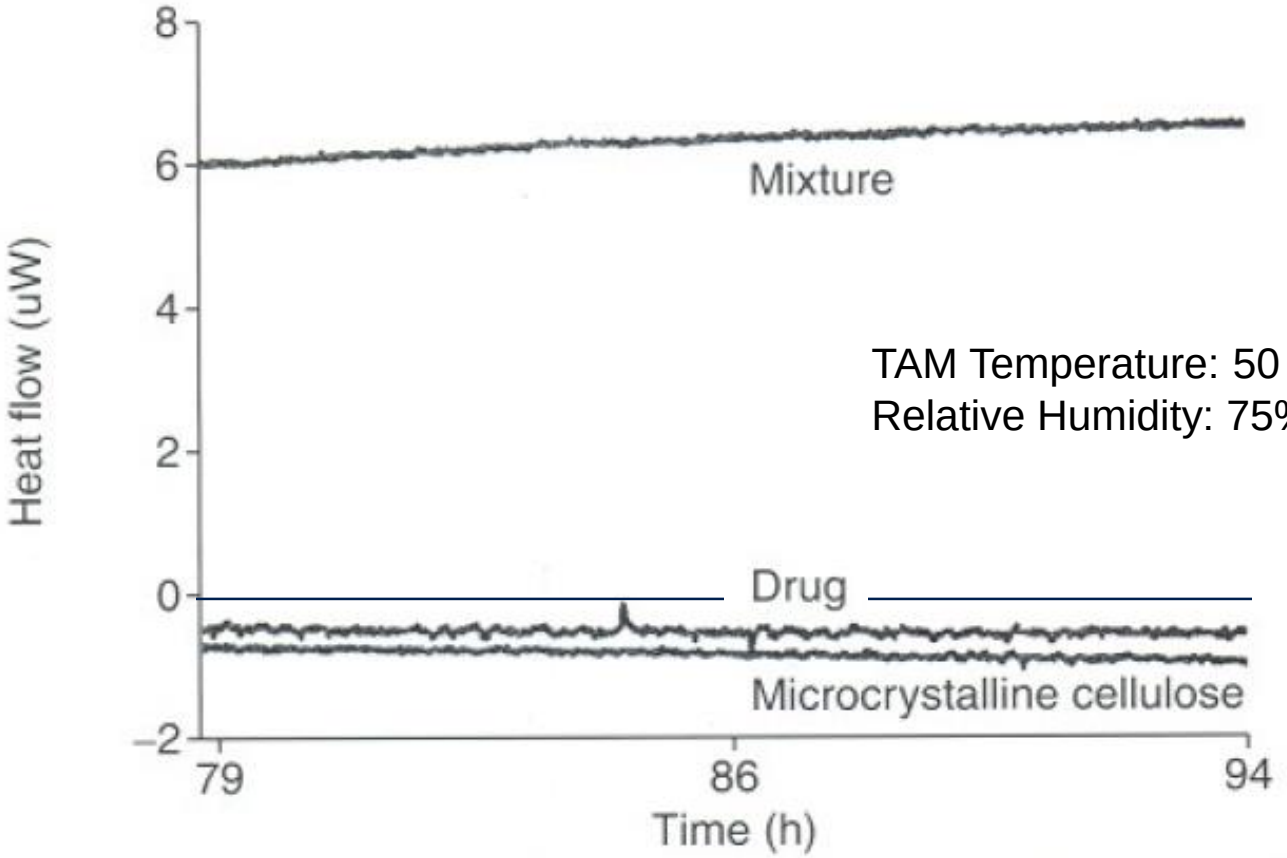
Compatibility Experiment with TAM

TAM Temperature: 50 °C
Aliquot of water added to ampoule



Schmitt, E.A.; Peck, K.; Sun Y.; Geoffroy, J-M. *Thermochim ACTA*, **380,** 175-183 (2001)

Compatibility Experiment with TAM



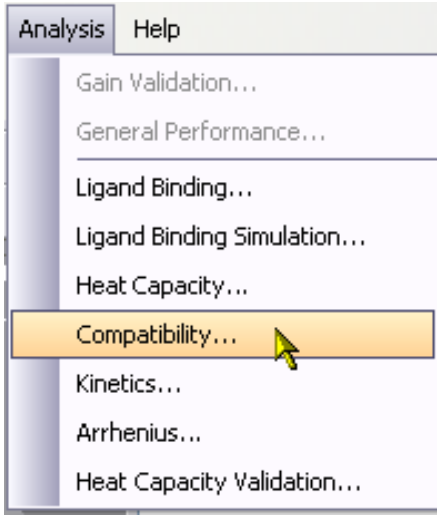
TAM Temperature: 50 °C
Relative Humidity: 75%

Phipps, M.A.; Mackin, L.A. *Pharm. Sci. Tech. Today*, **3**(1), 9-17 (2000)

TAM Assistant Analysis (1 of 2)

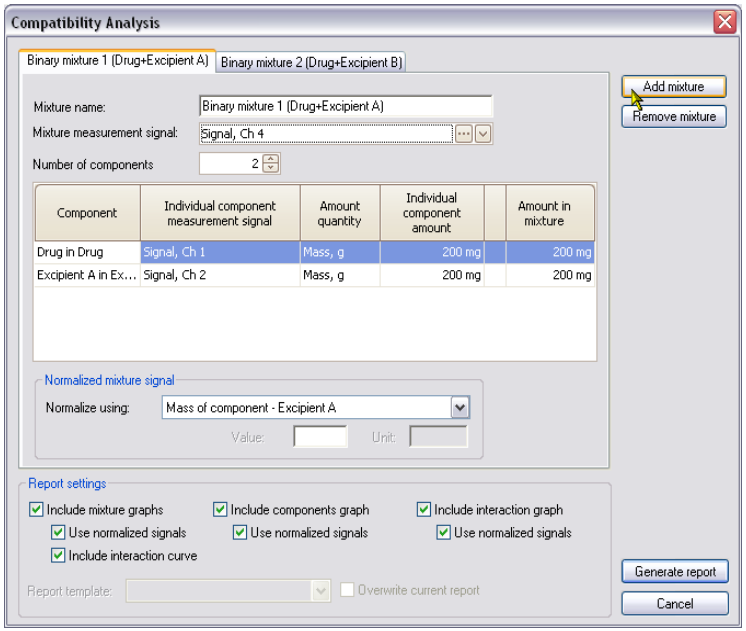
- Data must be collected with the mass of individual components entered.

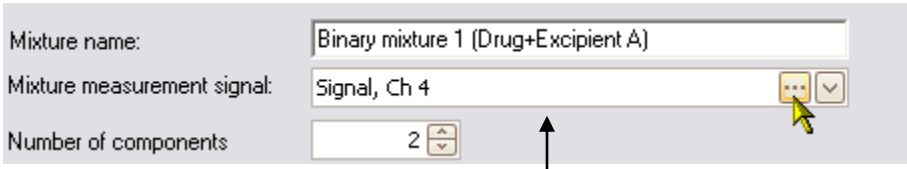
1. Open data file
2. Click Analysis/Compatibility
3. Add Mixture and select Results file (.rslt)
4. Change the time scale (if required)
   - Select ··· button to the right “Mixture measurement signal” field
5. Generate Report
   - Copy plot and information to alternative program for presentation (if required)

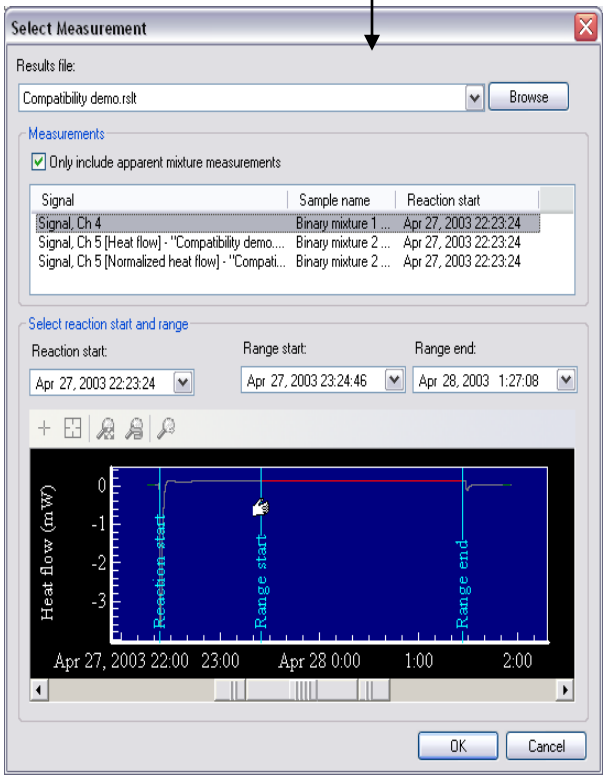


TAM Assistant Analysis (2 of 2)

Step 3







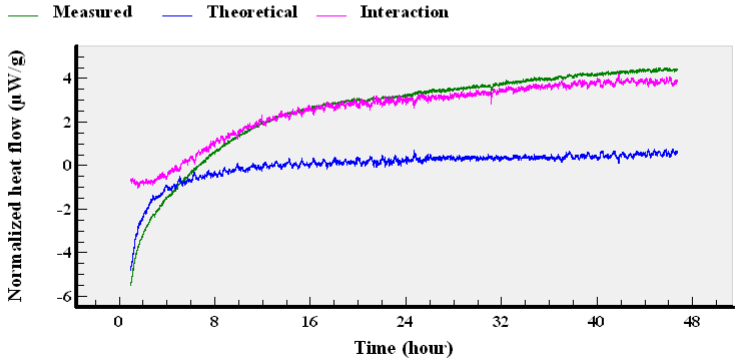
Step 4

ASA with Mg-Stearate and Sucrose

Lab Assistant Analysis Report
Compatibility

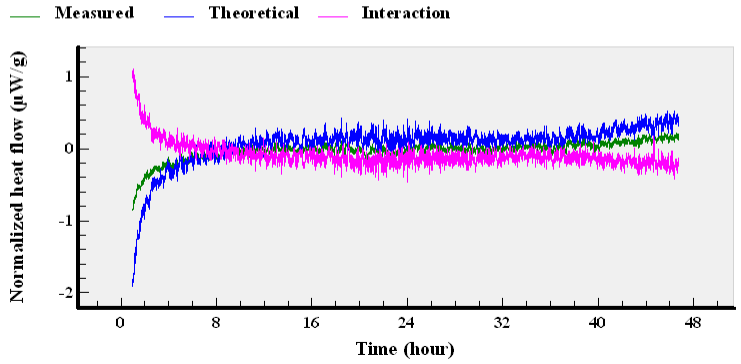
(ASA+Mg-stearate)

Mass, ASA:	500 mg
Mass, Mg-stearate:	446.54 mg
Measurement signal:	Data series.Signal, Ch 2:3 [Heat flow]
	ASA + sucrose (5-1-07).rslt
Reaction start:	May 01, 2007 16:57:49
Interaction integral:	420.14 mJ/g
Interaction average heatflow:	2.5504 µW/g
Interaction error:	2.8817 µW/g



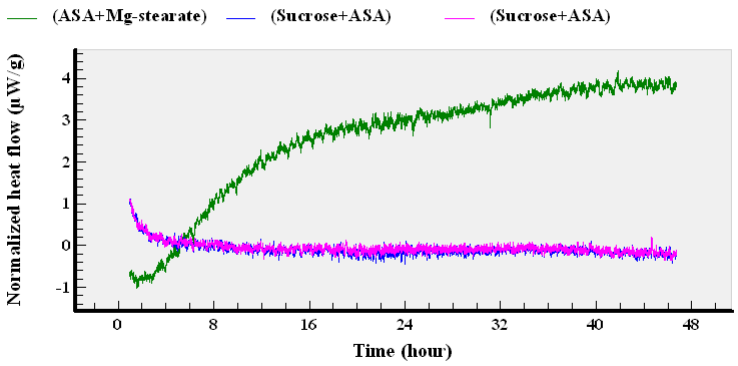
(Sucrose+ASA)

Mass, Sucrose:	497.42 mg
Mass, ASA:	502.65 mg
Measurement signal:	Data series.Signal, Ch 2:5 [Heat flow]
	ASA + sucrose (5-1-07).rslt
Reaction start:	May 01, 2007 16:57:49
Interaction integral:	-15.335 mJ/g
Interaction average heatflow:	-93.086 nW/g
Interaction error:	188.72 nW/g

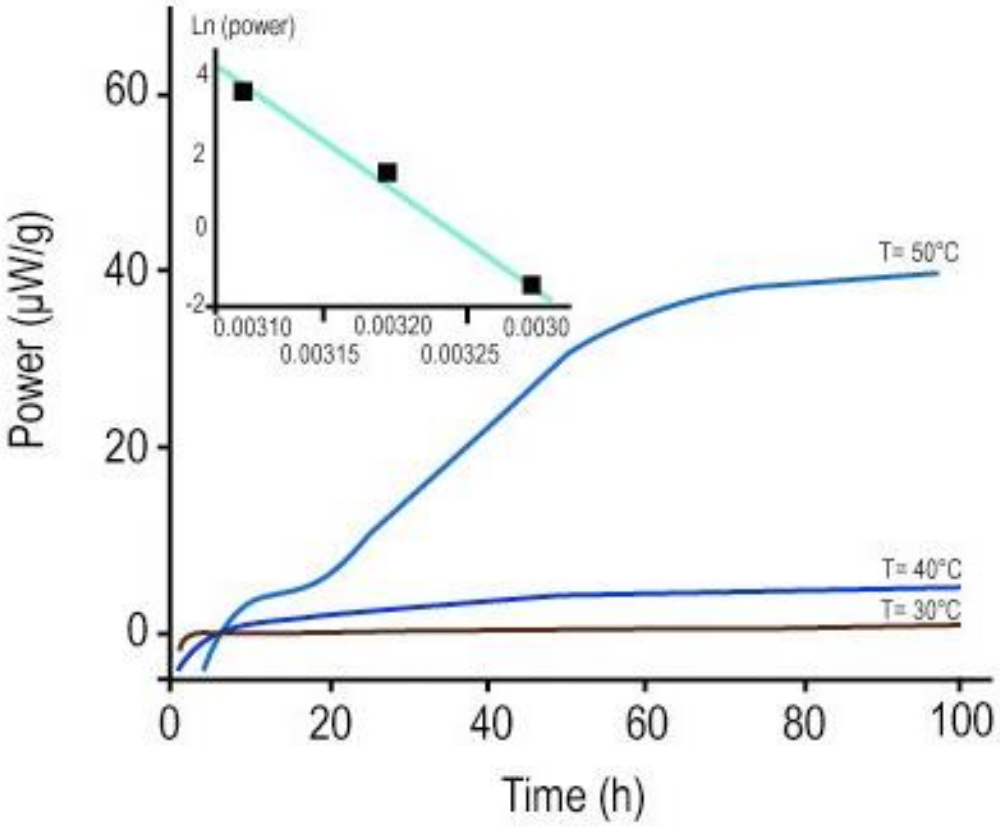


TAM III at 40 °C

Interaction graph



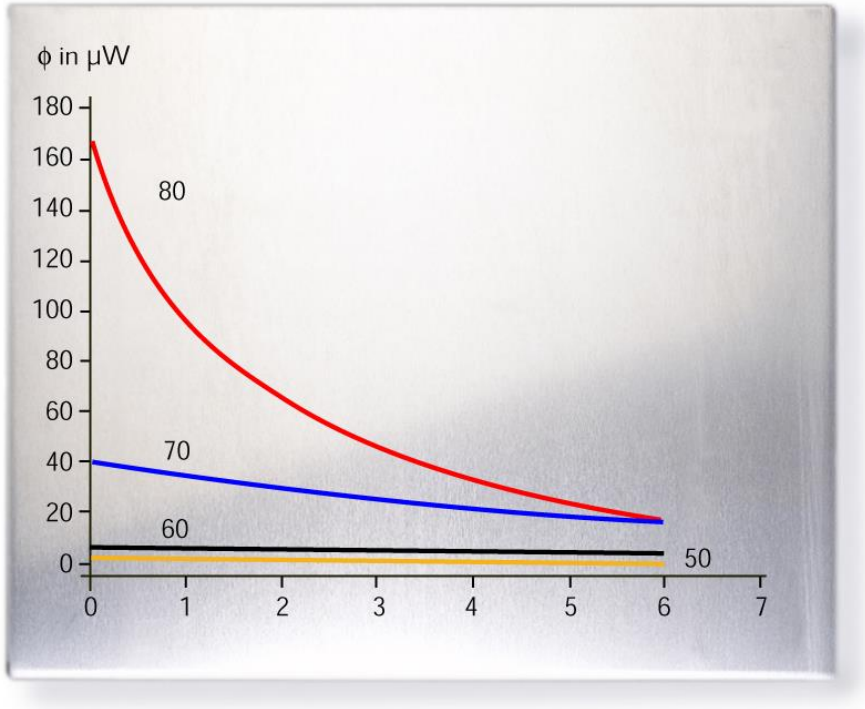
Amine-Lactose Interactions



One approach to perform excipient compatibility screening is to add water to the powder mixture. The graph shows the response of an amine-lactose interaction at different temperatures with 20% water added.

Schmitt, Peck, Sun & Geoffroy, *Thermochim. Acta*, 380, 175-183, (2001).

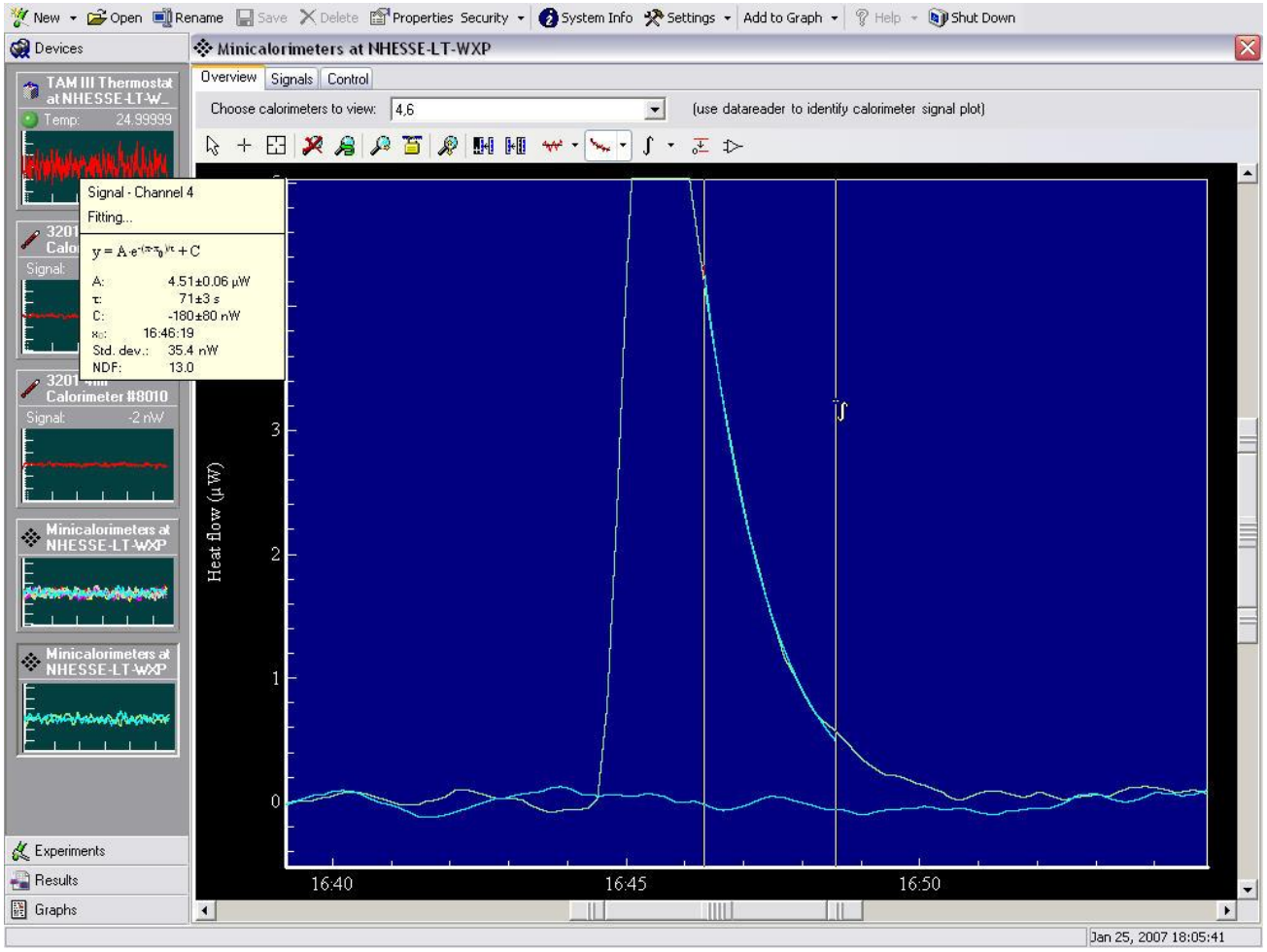
Drug - Excipient Stability



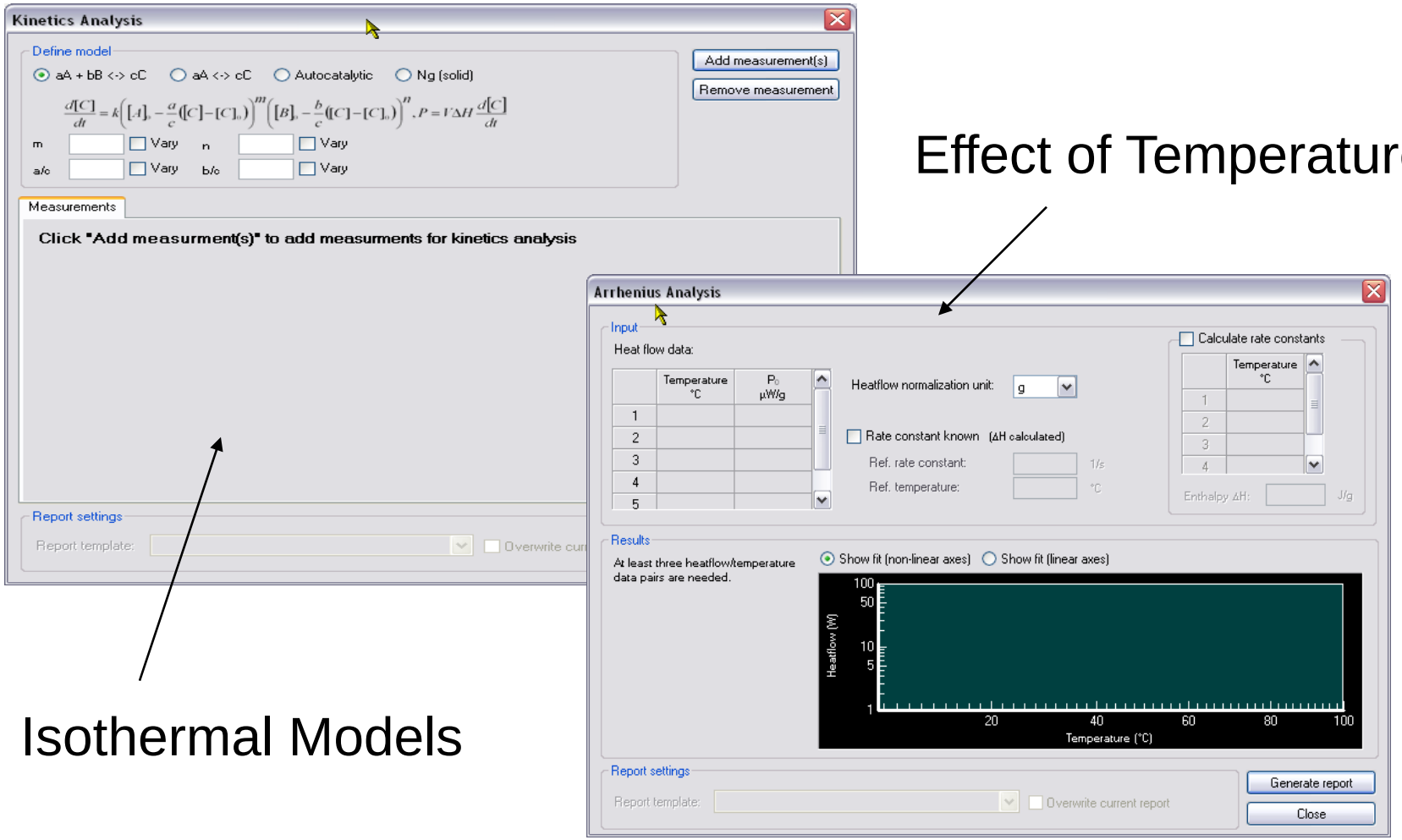
Another approach to perform excipient compatibility screening is to compress (make a tablet) with the ingredients. The figure shows the same compressed mixture at several different temperatures and clearly the rate of reaction increases as the temperature increases.

Selzer, Radau & Kreuter, *Int. J. Pharm*., 171, 227-241, (1998).

Linear and Exponential Fitting



Kinetic Analysis with TAM Assistant



Effect of Temperature

Isothermal Models

TAM Applications

Amorphicity and Polymorphic Studies

Definition of Crystallinity

- The degree of crystallinity is a measure of crystal imperfection
- Imperfections increase the energy (enthalpy) of the crystal
- The enthalpy increase of a crystal relative to a reference crystal of high crystallinity can be measured by calorimetry, either as *heat of solution* or *heat of crystallization*.

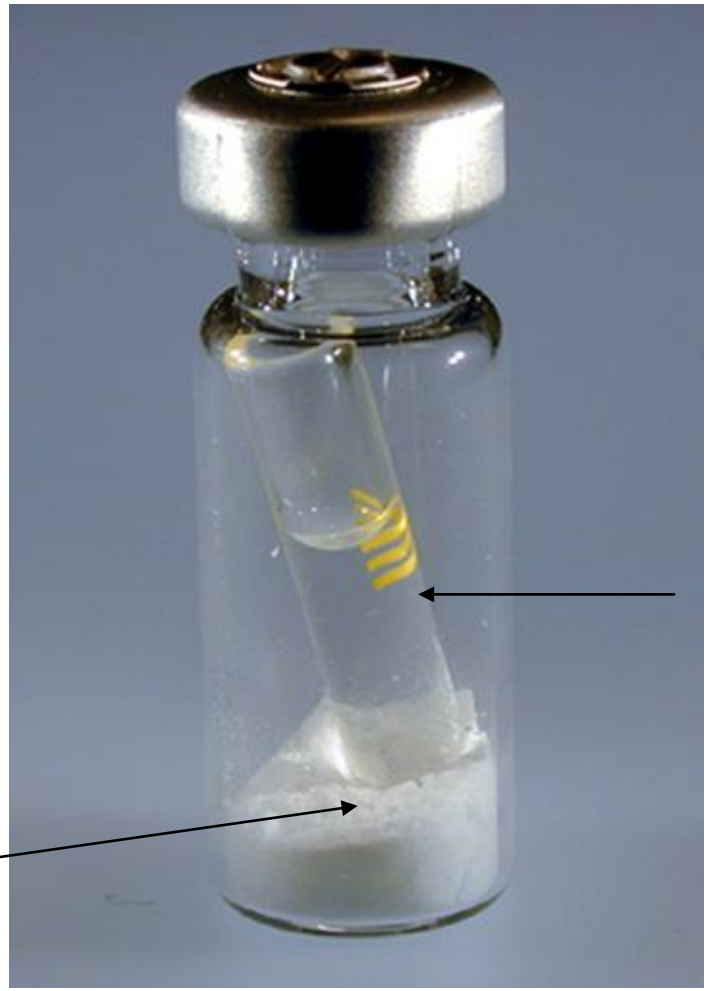
Relevance of Crystallinity

- The presence of imperfections (amorphicity) in a crystal affect relevant *properties*.
- Properties affected are: *chemical stability*, *solubility*, *bioavailability*, *surface energy*.
- To have a material well characterized it is very important to have a good control over these key properties.

Characterize Amorphicity with TAM

1. The Microhygrostat Method
2. The Controlled RH Perfusion method
3. The SolCal Method

The Microhygrostat Method



Solid sample

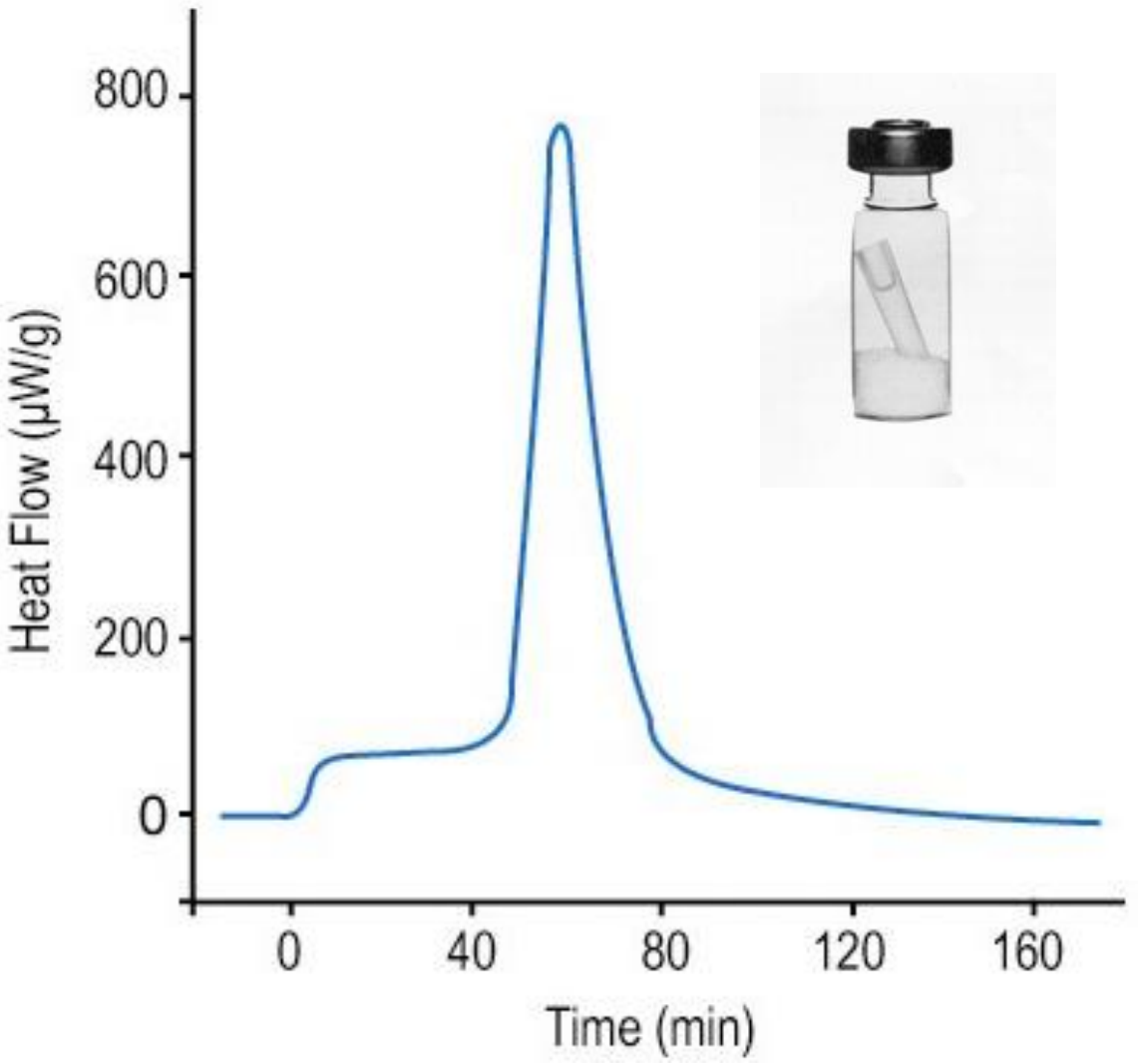
Microhygrostat:

Glass tube with pure solvent or a solvent saturated by a salt (e.g. sat. NaCl (aq))

Developed independently by:

Angberg, Uppsala University and Byström, Astra Zeneca (1992)

Moisture Induced Crystallization

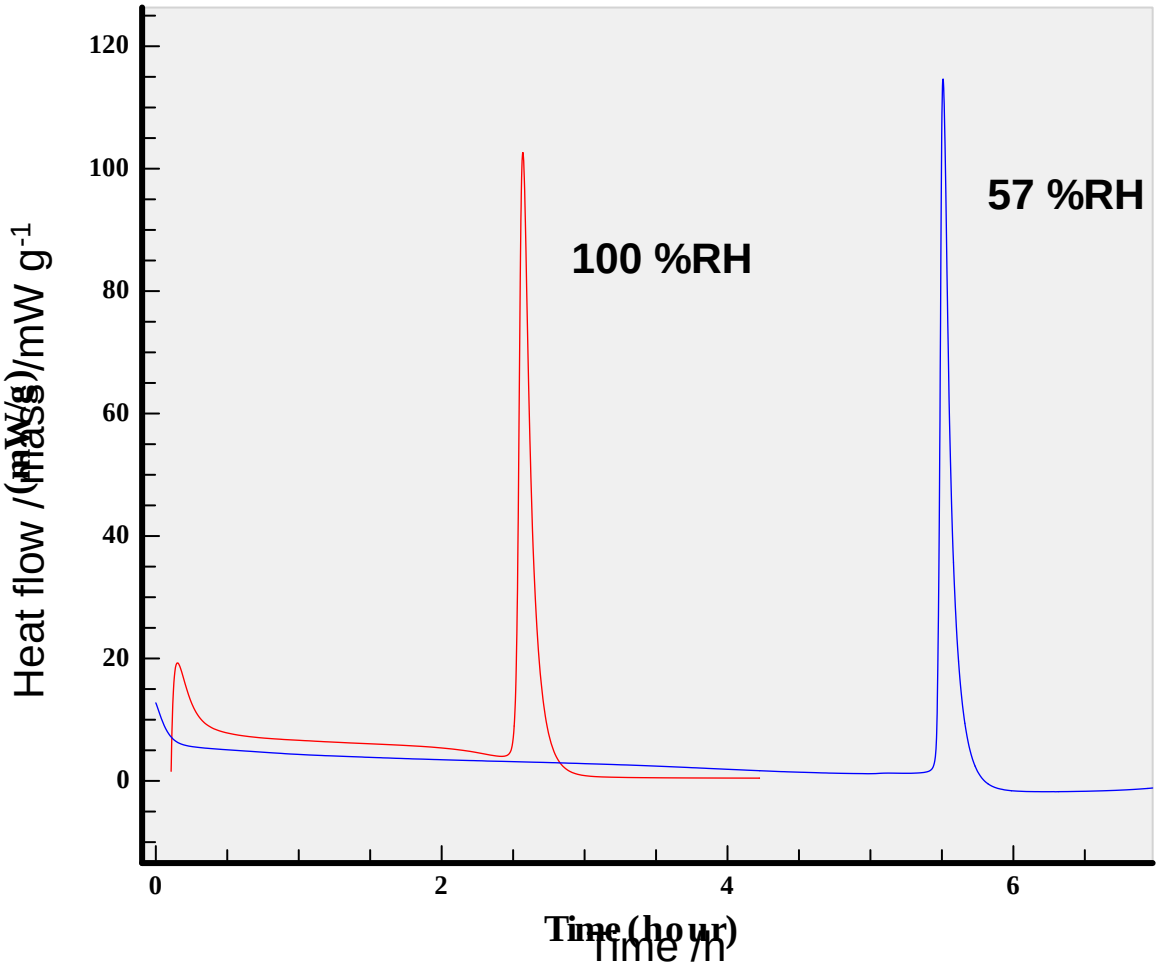


Bystrom, *Thermometric Application Note* 22004

The induction time depends on:

- The vapor activity
- The temperature
- The sample size
- Presence of crystals or seeds

Recrystallization as a Function of Relative Humidity



- Amorphous lactose
- Exp temperature: 25°C
- Sample mass: ≈ 30 mg
- Microhygrostat: Pure water (100 %RH) and sat. aq. NaBr (57 %RH)

The Controlled RH Perfusion Ampoule

a) inlet of dry nitrogen

b) dry flow line

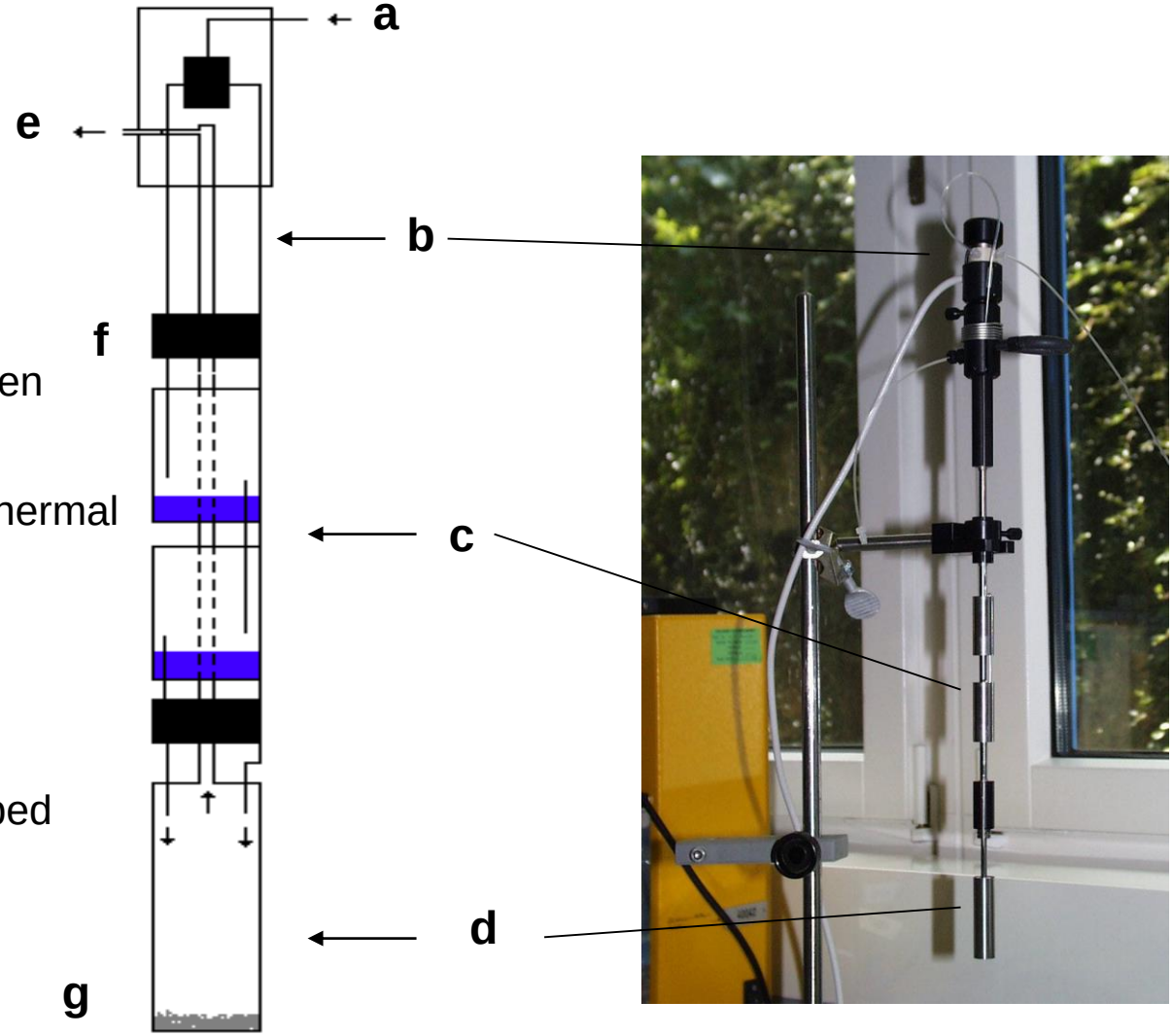
c) humidifiers and thermal equilibrators

d) sample ampoule

e) outlet

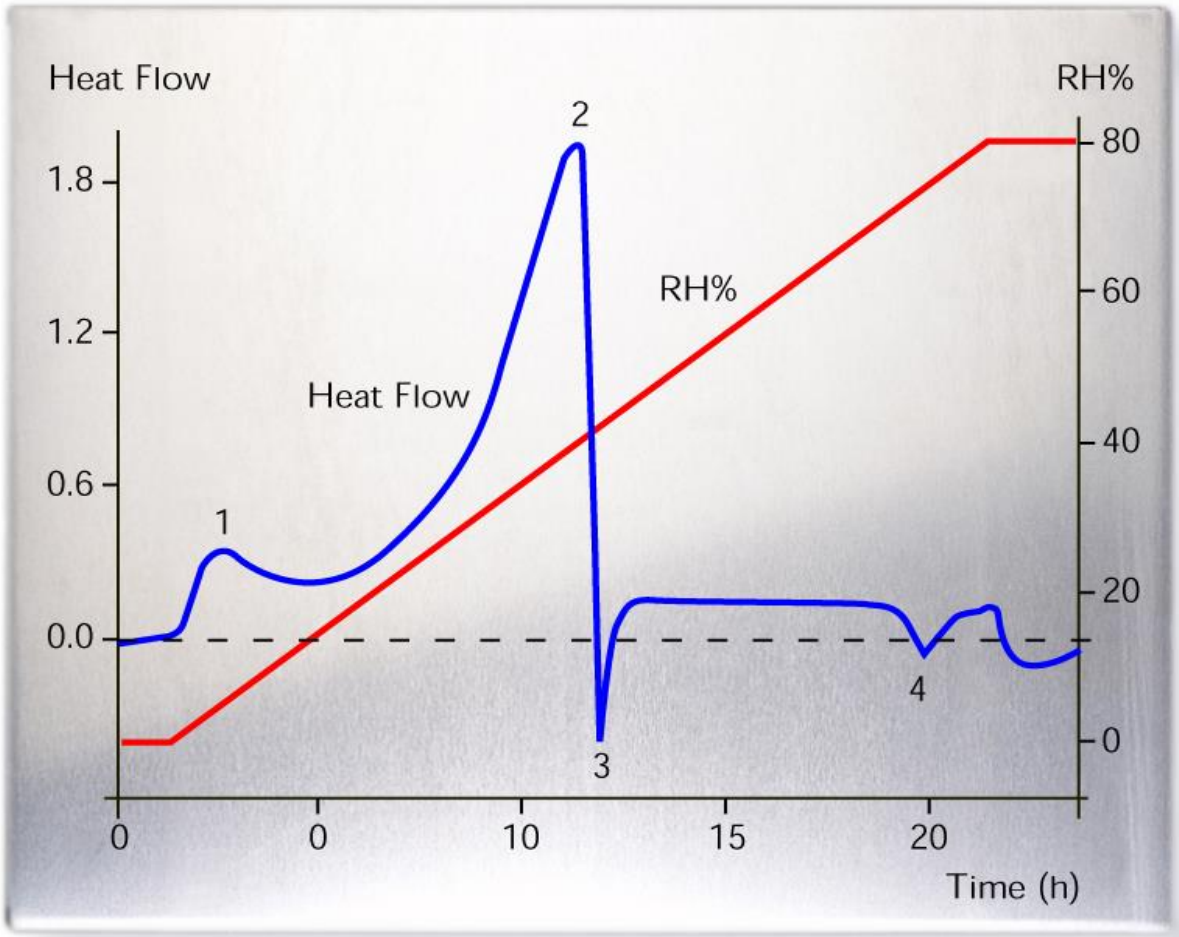
f) wet flow line

g) sample powder bed



Moisture Induced Crystallization

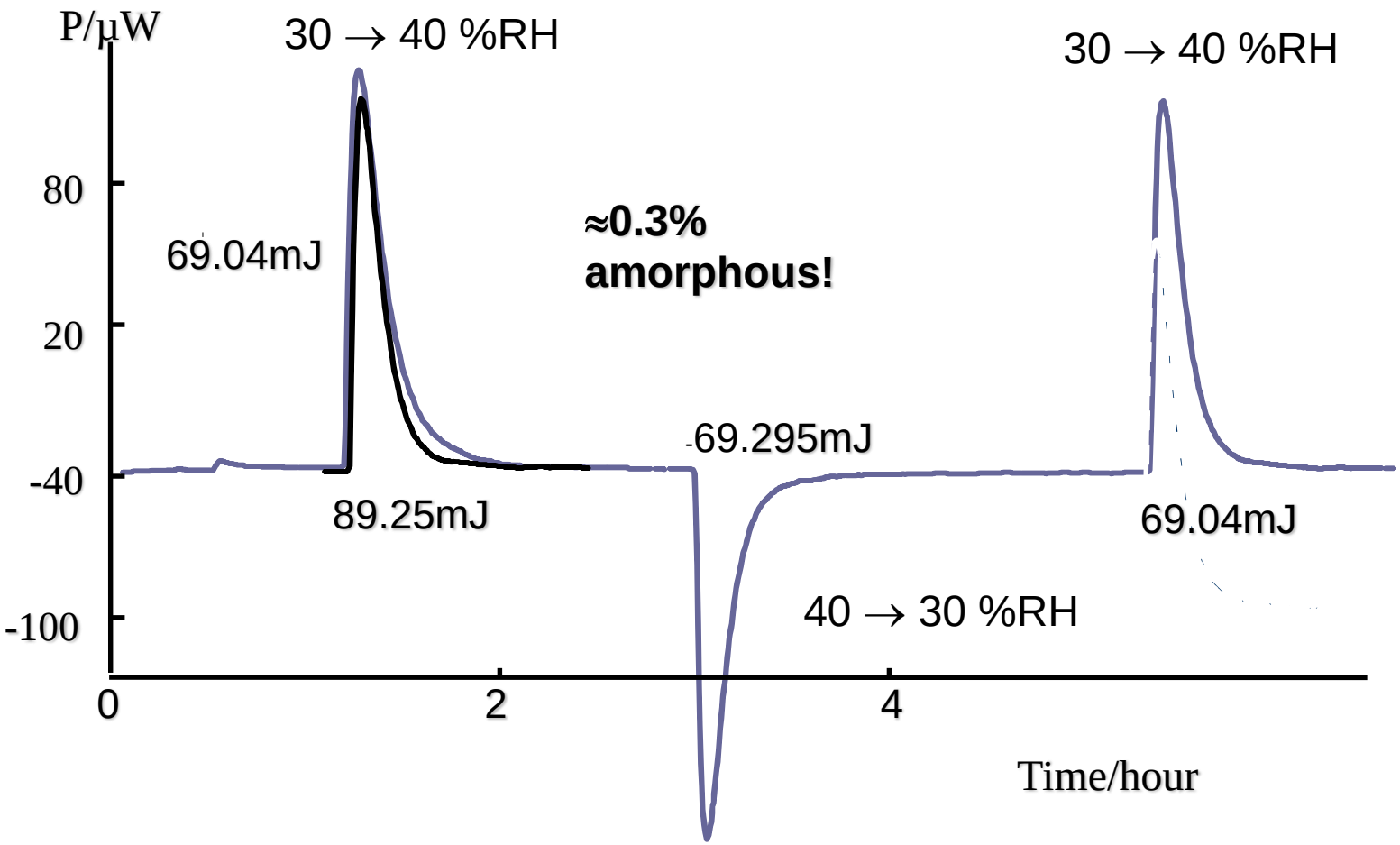
RH ramp



L.E. Briggner (Astra-Zeneca), *Thermometric Application Note* 22022

Moisture Induced Crystallization

Highly crystalline lactose



L.E. Briggner, AstraZeneca, (2002)

See also: L. Mackin, et. al. *Int. J. Pharm.* (2002), **231**, 227-236

The Solution Calorimetry Method

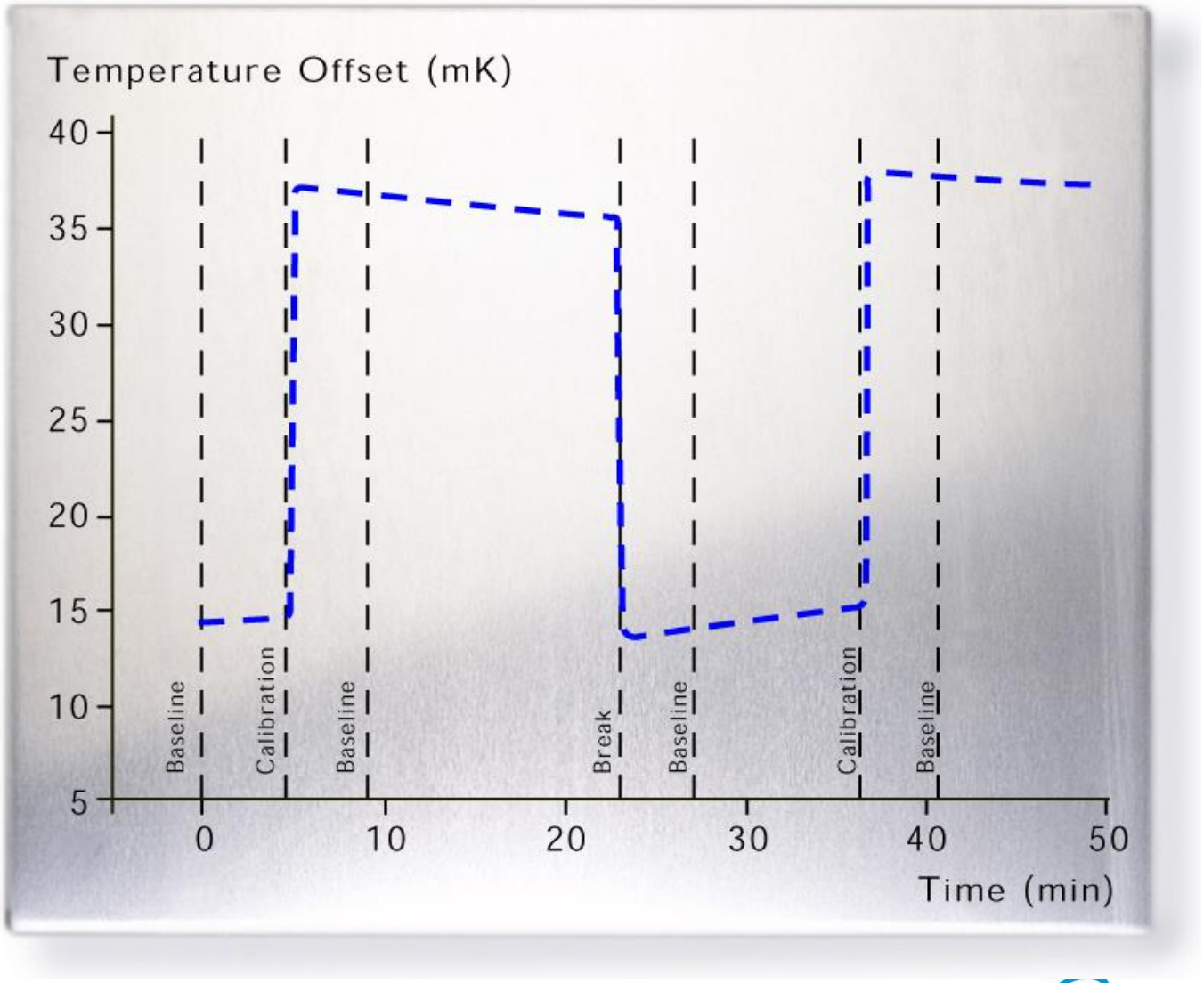
SolCal = Precision Solution Calorimeter

Crushing ampoule (1 mL) in stirrer

Reaction vessel with solvent (100 or 25 mL)

Sapphire tip

Dissolution of Lactose

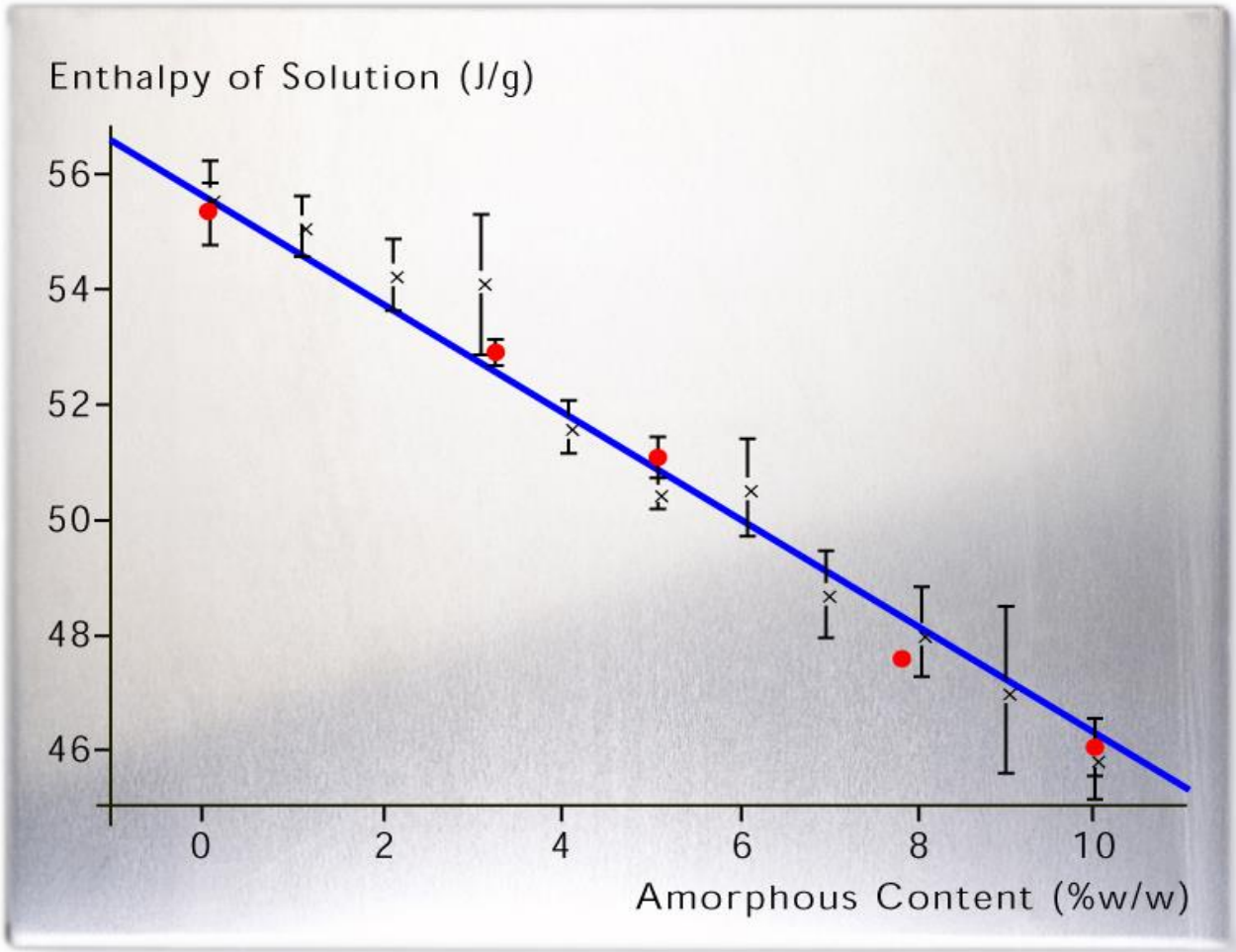


Heat of Solution for Different Lactose Forms

Type	Heat of solution (J /300 mg)	Amorphicity content (*)
Monohydrate	43.89	≈ 0 %
Spray dried	-49.93	100 %
Mixture 1	32.91	11.7 %
Mixture 2	41.42	2.7 %

(*) degree of crystallinity = 100-% amorphicity content

Standard Curve <10%



Hogan & Buckton, *Int. J. Pharm*., 207, 57-64, (2000)

Summary – Amorphicity & Crystallinity

- Measure enthalpy of crystallization (and sorption effects)
- 10 to 500 mg (typically 50-150)
- High sensitivity (towards 0.1%)
- High reproducibility
- Easy to adapt method after substance
- Hydrophobic or hydrophilic substances
- Calibration important (standard curve)
- Timescale 1-5 hours

Benefits of TAM Over Other Techniques

Sensitivity

Detection limits below 1%, towards 0.1% is possible

Results are highly reproducible

Versatility

Adapt method to substance (different solvent vapors, sample sizes and operating temperatures)

Sample throughput

The sample throughput attainable with TAM IV and TAM 48 can not be achieved by any other method today.

Polymorphism Introduction

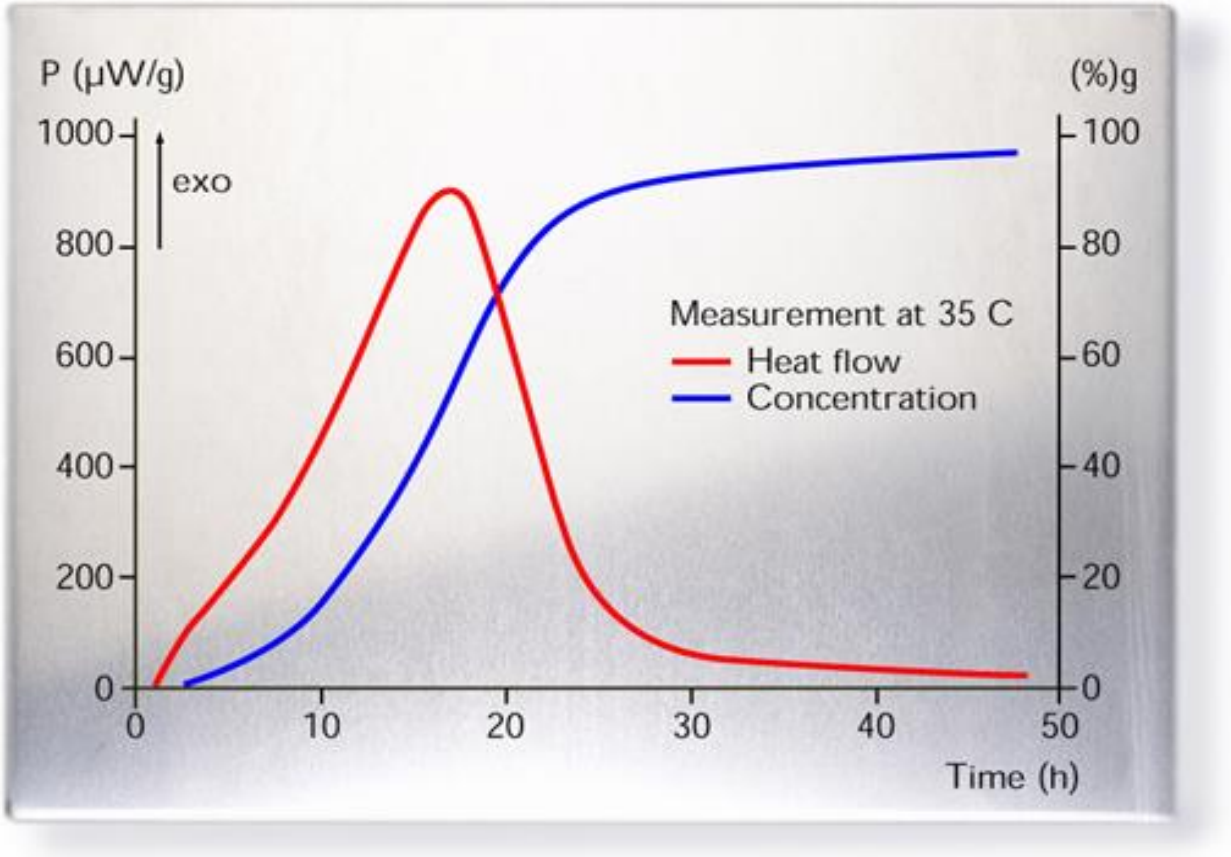
The study of ***Polymorphic behaviour*** of drugs and excipients is an important part of preformulation work because it affects:

- bioavailability mediated via dissolution
- solid state reactions (stability)
- hygroscopicity
- mechanical stability
- compactability
- batch and source variation

Main Issues

- Relative stability of polymorphic pairs
- Equilibrium transition temperatures
- Assesment of stability (meta-stable or stable)
- Kinetics of polymorphic transitions

α→ β Transformation of Tripalmitin



Hongisto, Lehto & Laine, *Thermochim. Acta,* 276, 229-242, (1996).

α→ β Transformation of Tripalmitin

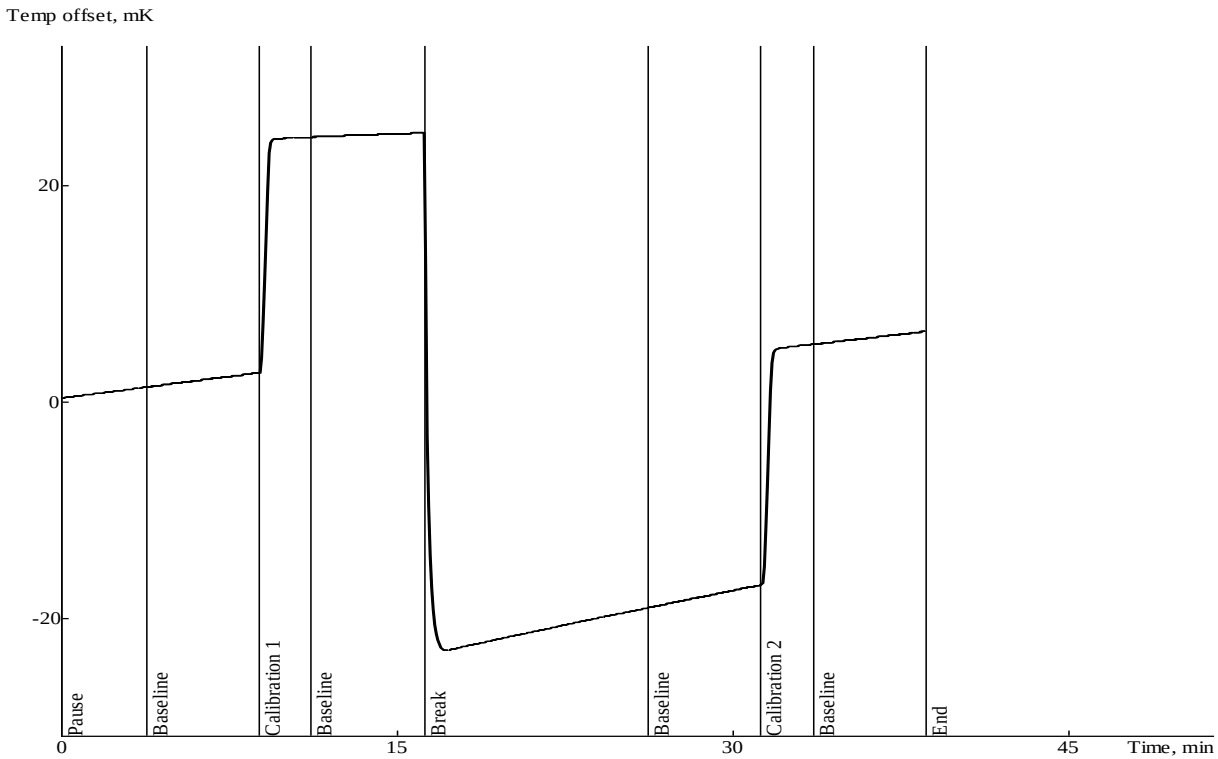
Temperature/ C	$t_{1/2}$/h	ΔH_r/Jg^{-1}
25	-	-
30	305	45.5
33	50.9	45.5
35	16.3	45.7

Note: the results were obtained below the DSC sensitivity level

The enthalpy does not change with temperature indicating that the same transition has been observed at all the different temperature.

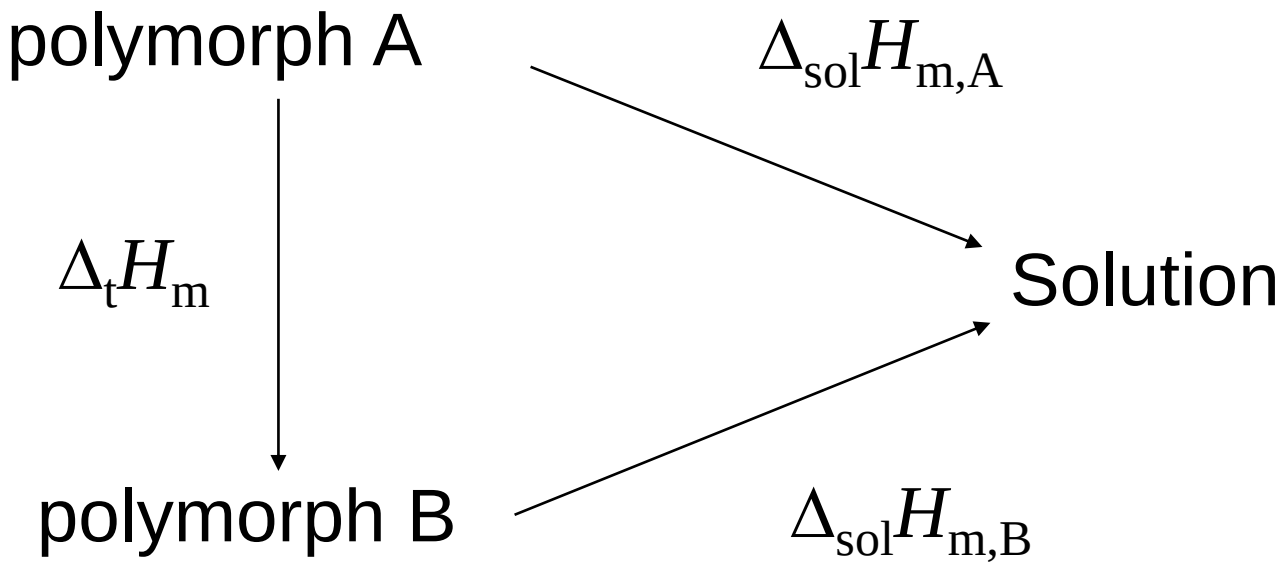
Hongisto, Lehto & Laine, *Thermochim. Acta,* 276, 229-242, (1996).

Solution Calorimetry



T = 45 °C

Heat of Solution



$$\Delta_tH_{m,A\text{ to }B} = \Delta_{sol}H_{m,A} - \Delta_{sol}H_{m,B}$$

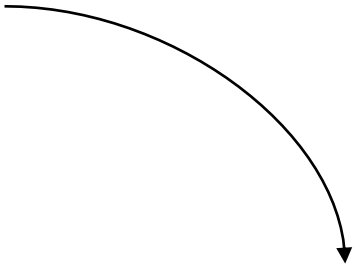
Which Direction? A → B or B → A?

Answer: most likely is the direction for which $\Delta_t H_m < 0$ (exothermic)

- $\Delta_t H_m = \Delta_{sol} H_{m,A} - \Delta_{sol} H_{m,B} < 0$
  - A more stable than B
- $\Delta_t H_m = \Delta_{sol} H_{m,A} - \Delta_{sol} H_{m,B} > 0$
  - B more stable than A

Transition Temperature of a Polymorphic Pair (A and B)

Transition enthalpy from SolCal
+ solubility of form A and B



The transition temperature, T_{trans}, between form A and B

References:

Chong-Hui Gu and David J. W. Grant. (2001) *Estimating the relative stability of polymorphs and hydrates from heats of solution and solubility data*. J. Pharm. Sci. 90 (9).

Koji Urakami et al. (2002) *A novel method for estimation of transition temperature for polymorphic pairs in pharmaceuticals using heat of solution and solubility data*. Chem. Pharm. Bull. 50 (2).

Results with 4 drug compounds

Drug	Crystal form	$\Delta_{trans}H$	Solubility (25C)	T_{trans} /C	
		(kJ mol-1)	(mg ml-1)	Measured value	Literature value
Seratrodast	Form I	6.05	0.543	84.9	83.4
	Form II		0.817		
Acetazolamine	Form A	2.02	2.04	72.1	78.4
	Form B		2.28		
Carbamazepine	Form I	-2.93	11.56	77.6	73
	Form III		9.68		
Indomethacin	Form α	-1.13	0.576	534.3	-
	Form γ		0.432		

The estimated T_{trans} for **indomethacine** indicates monotropic relationship. This is also supported by DSC data interpreted with the "Heat of fusion rule" (Burger & Ramberger, 1982). $\Delta_{fus}H_{\gamma} > \Delta_{fus}H_{\alpha}$, $T_{fus,\gamma} > T_{fus,\alpha}$

Data from : Koji Urakami et al. (2002) *A novel method for estimation of transition temperature for polymorphic pairs in pharmaceuticals using heat of solution and solubility data*. Chem. Pharm. Bull. 50 (2).

Polymorphic Transformations

Polymorphic transformations can occur via two distinct mechanisms:

Directly via molecular rearrangements in the dry state

”slow process”

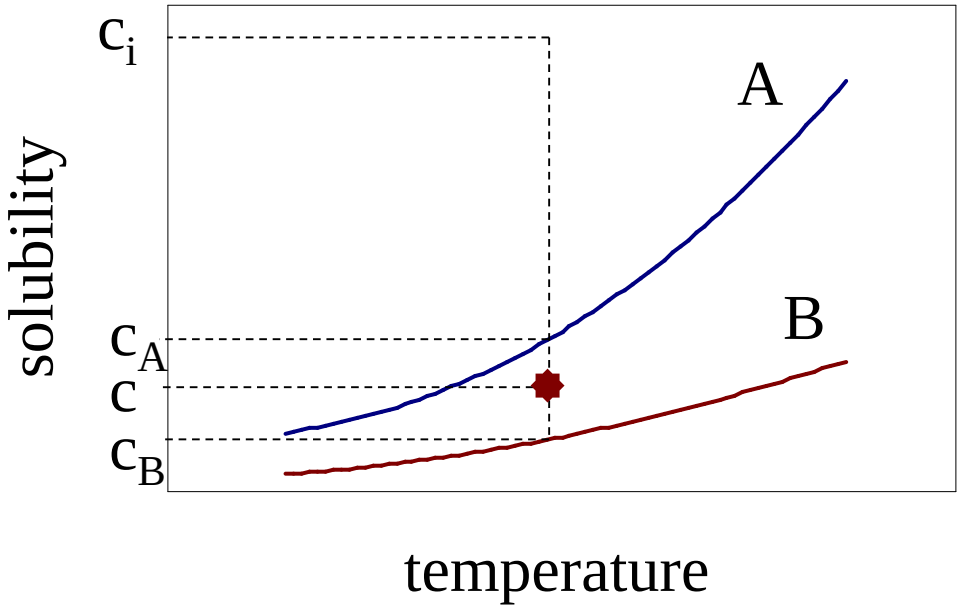
or

Via a solvent phase like a solvent- mediated polymorphic transformation (smpt)

”can be made to run fast”

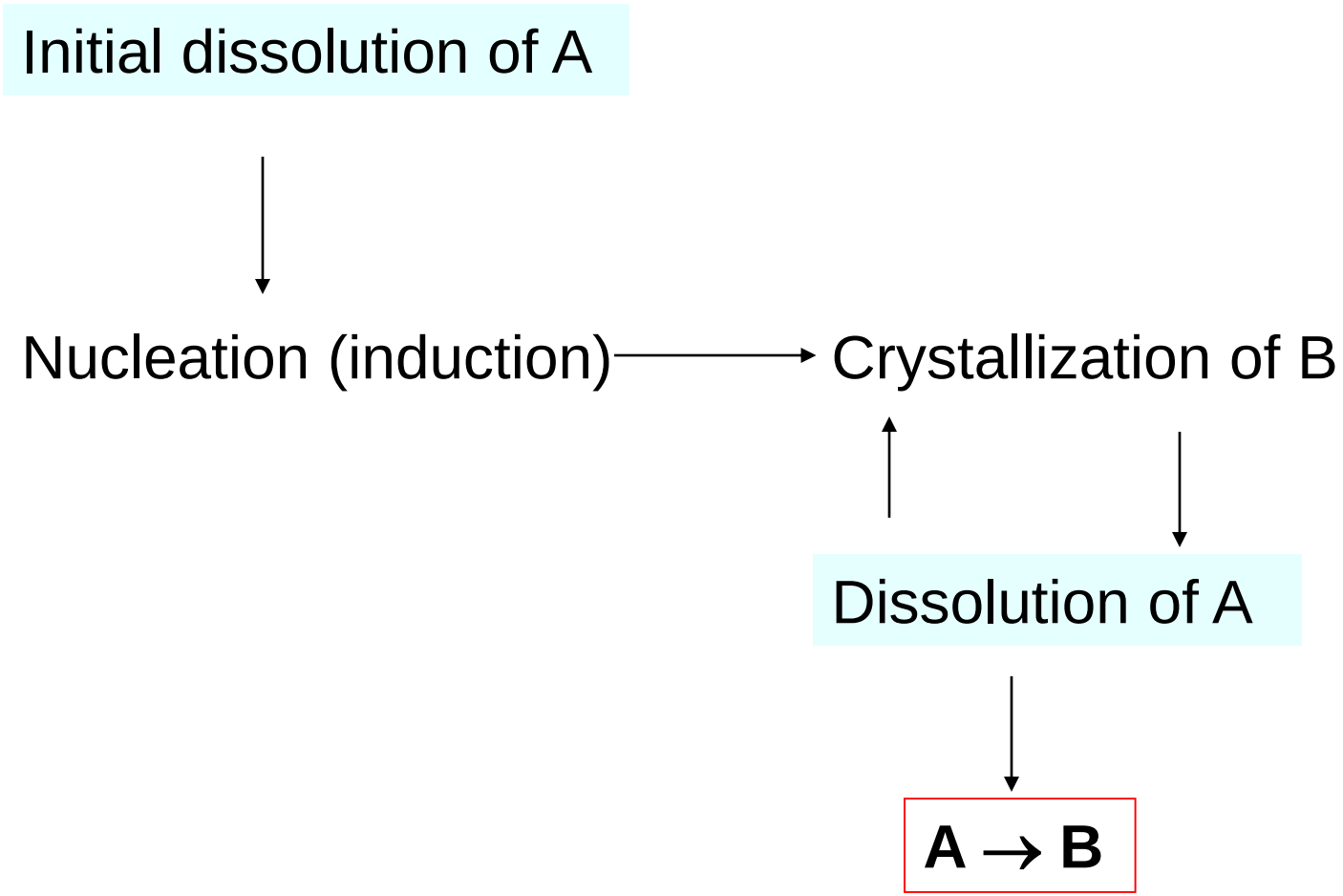
Crystallization from a Super-Saturated Solution

Typical solubility curves for a polymorphic pair



Typically the most soluble polymorph is not the most stable form (Form A)

The Solvent Mediated Process



SMPT with Microcalorimetry

Solvent mediated polymorphic transformation = SMPT

The Heat Flow: $$P = \left|\frac{dm_A}{dt}\right| \Delta_D H_m + \left|\frac{dm_B}{dt}\right| \Delta_C H_m$$

The enthalpy change: $$\Delta_t H_m = \Delta_D H_m + \Delta_C H_m$$

(D = dissolution, C=crystallization)

Water Slurries of a Meta-Stable Drug Compound

Solvent mediated polymorphic transformation = SMPT

Slurries with pure drug were prepared directly into 3-ml glass vials serving as vessels for the calorimeter

Operating temperature: 45°C.

Approximately 70 mg drug and 2 g water

Peter Johansson (2002) J&J-PRI, Switzerland

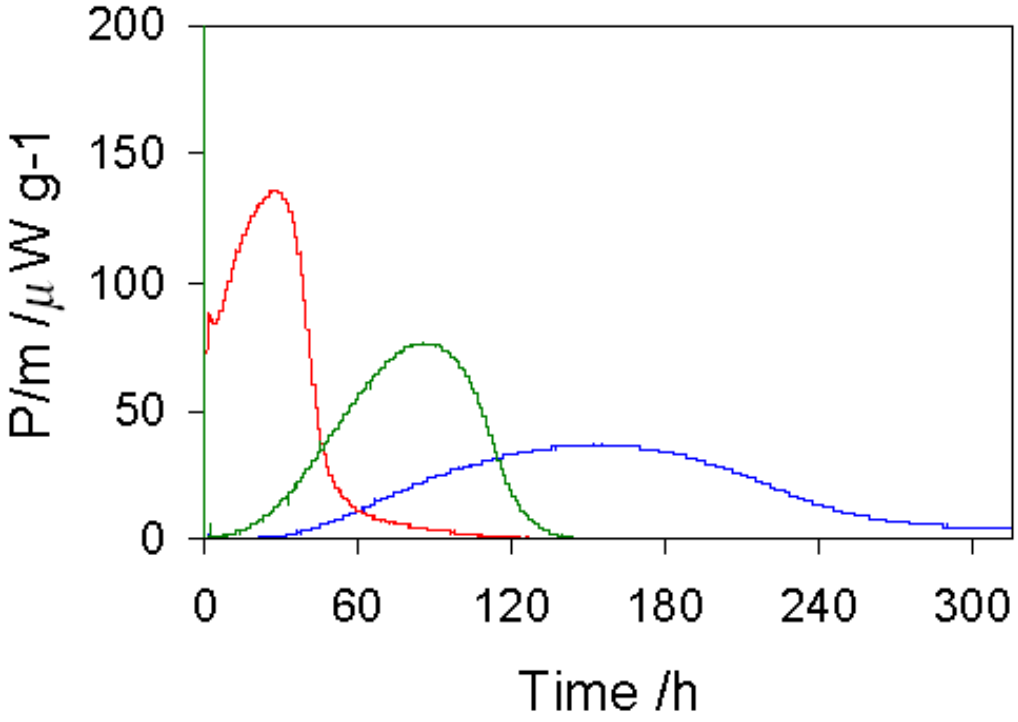
Results with Three Meta-Stable Drug Lots

Results from water slurries with pure drug at 45 °C – variation in particle size investigated.

$\Delta_t H_m$ = -20.1 J g^{-1}

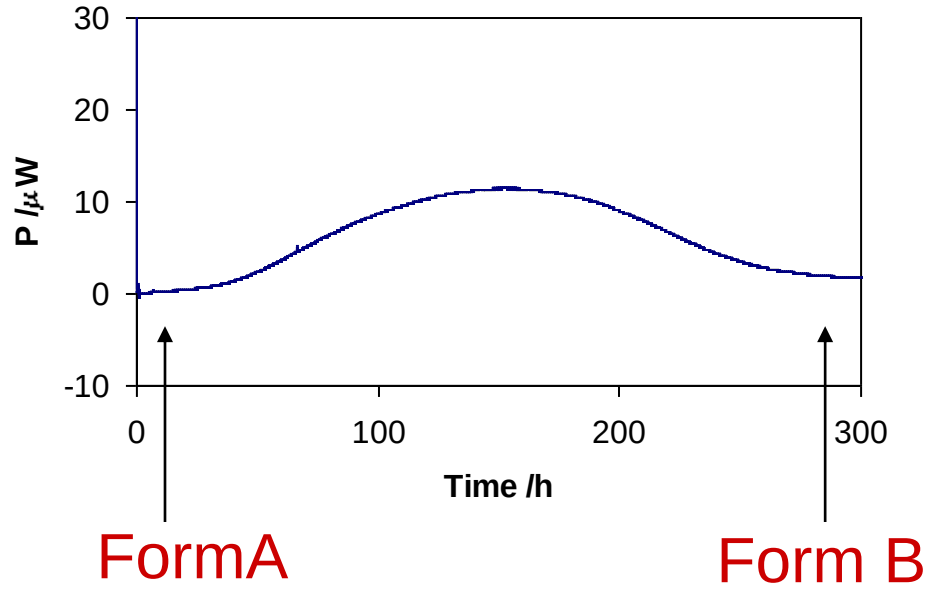
(std dev = 1.2 J g^{-1})

n = 6



Blue trace: lot A (largest particle size)
Green trace: lot B
Red trace: lot C (micronized)

Before and After



TAM is non destructive. After measurement is complete additional analysis can be made on the same sample.

TAM Applications

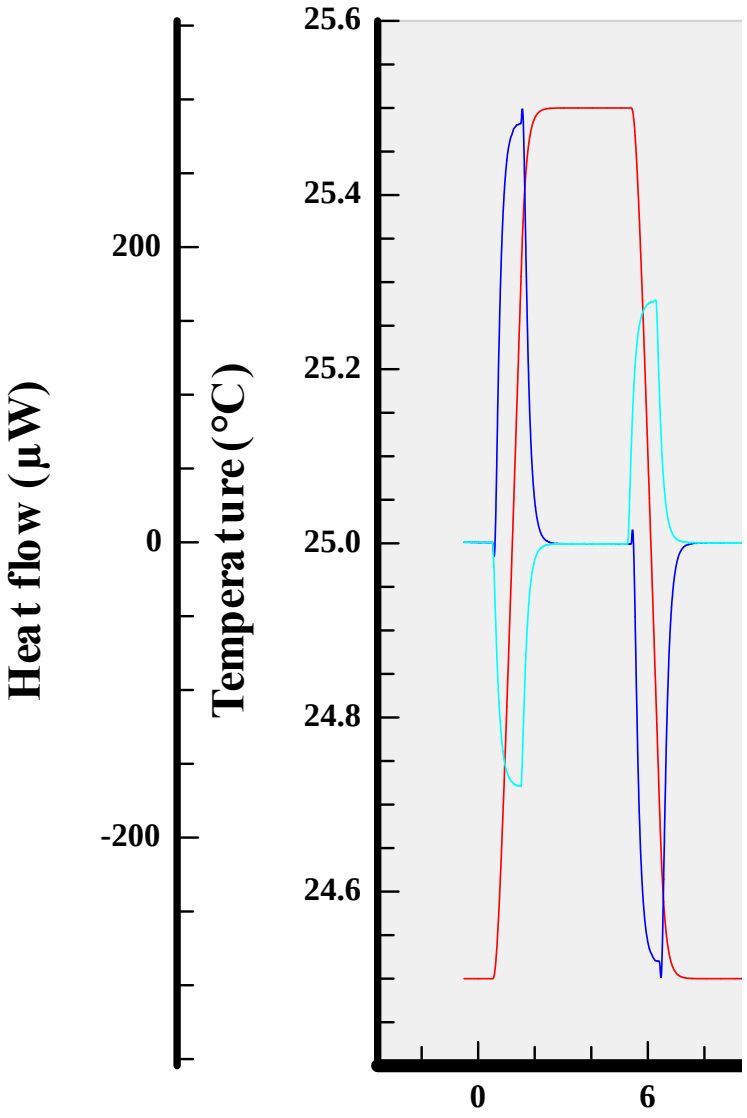
Temperature Scanning

Applications of Slow Scanning

- Polymorphism
- Crystal Hydrates
- Heat Capacity

- Melting Behavior
- Glass Transitions

Heat Capacity Determination



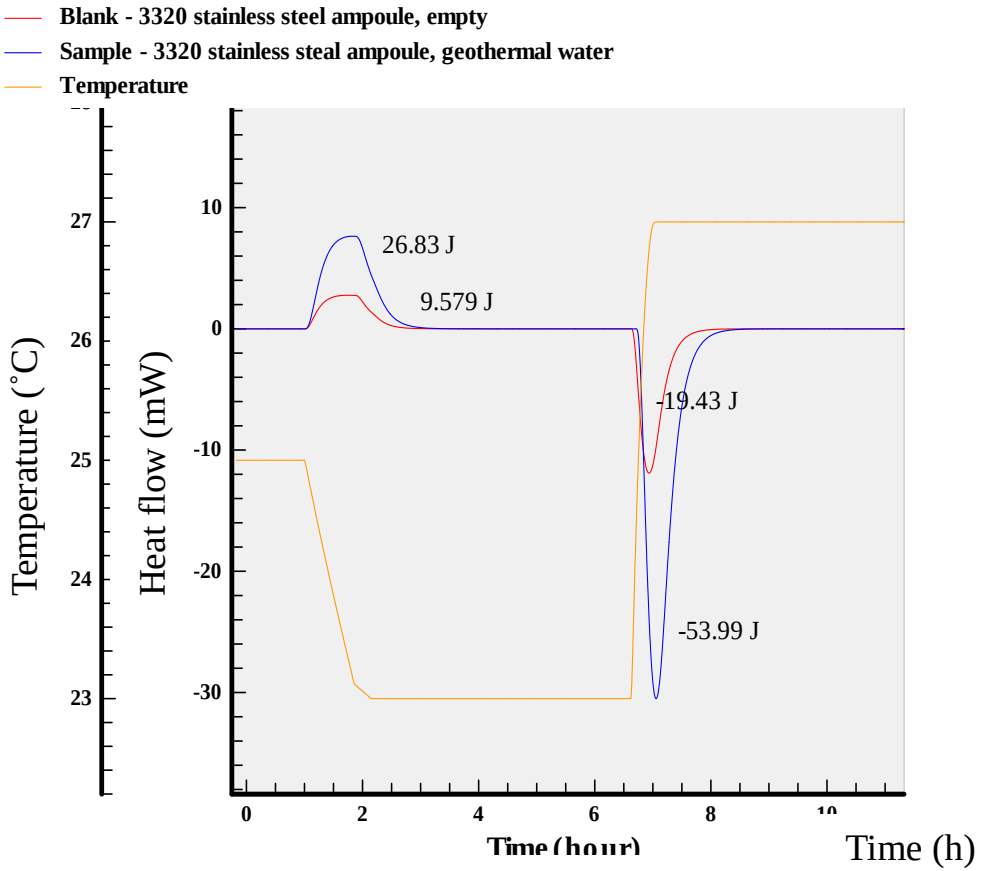
Method:

1. Insert empty ampoule in sample side
2. Change the thermostat temperature by typically 1°C (blue curve)
3. Restore the original temperature
4. Fill ampoule with sample
5. Repeat the temperature change with the same limits (cyan curve)
6. Calculate Cp from the energy difference between the curves and the sample weight

- For best accuracy apply calibration substance with known Cp

Heat Capacity Determination with TAM III

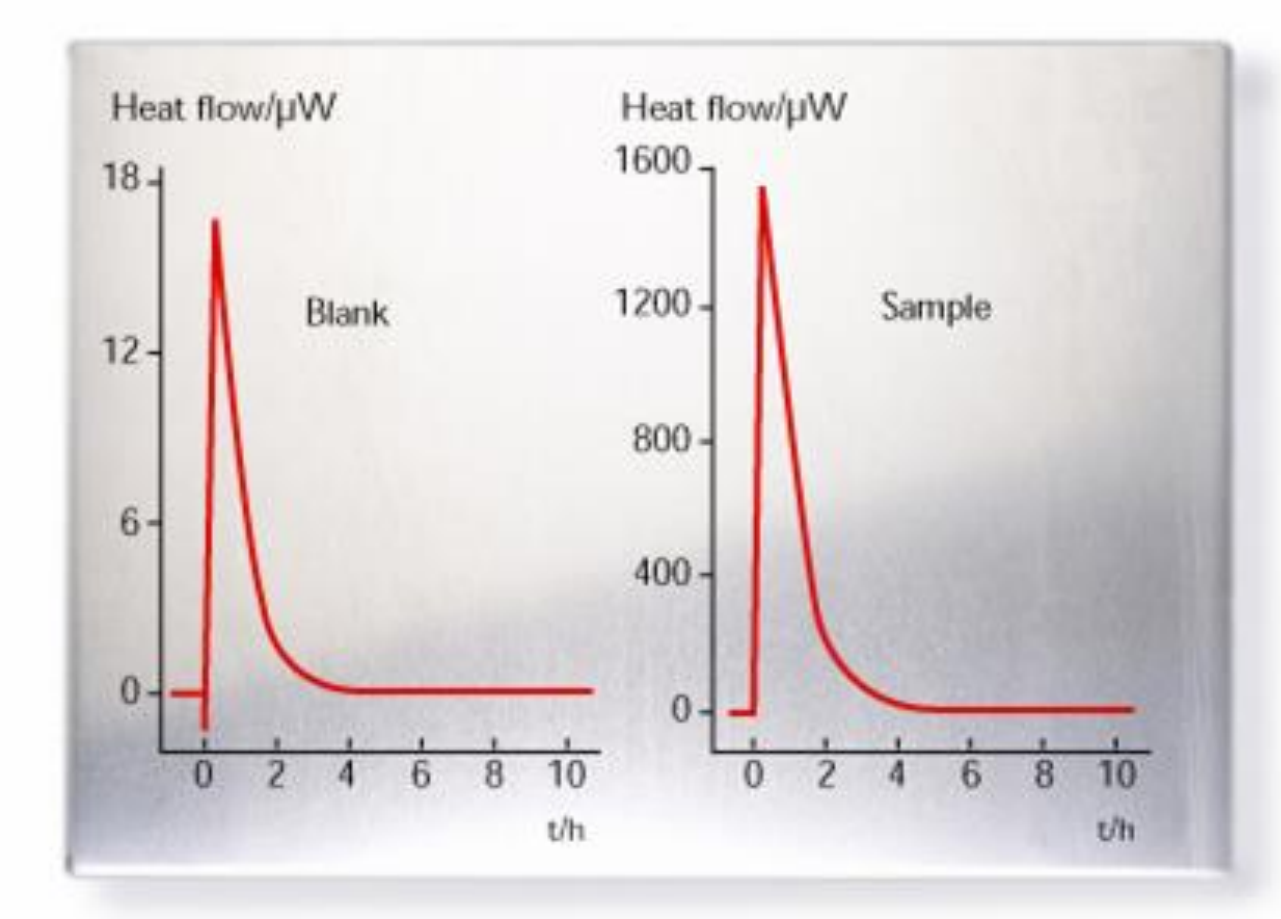
The Geolgically heated water is a strong electrolyte with a heat capacity almost 20% lower than that of pure water.



Results

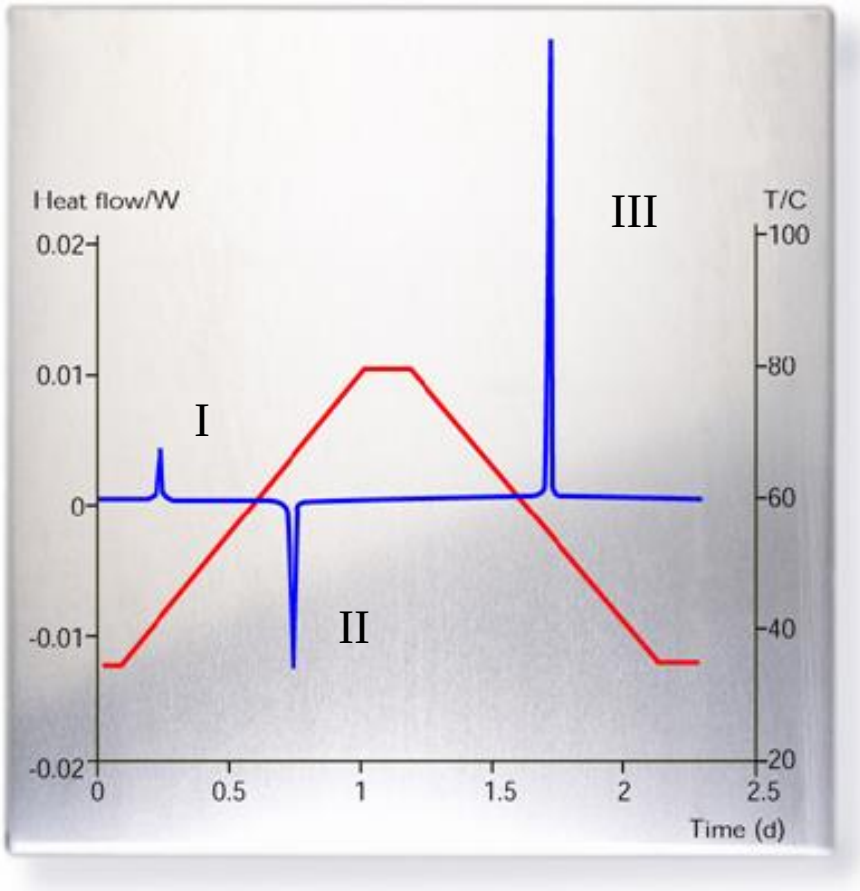
	mass /g	ΔT /C	corr. factor	C_p /J $K^{-1}g^{-1}$
peak 1	2.2767	2	0.9125	3.457
peak 2	2.2767	4	0.9125	3.463
mean				3.460

Heat Capacity Using the Step Isothermal



Lehto, Laine, Ylianttila, Hyysalo and, Jokela, *J. Therm. Anal*., Vol. 53, 685-695 (1998)

α → β Transformation of Tripalmitin



On heating

I. α → β transformation at 40°C (exo)

II. Melting of β form at 65°C (endo)

On cooling

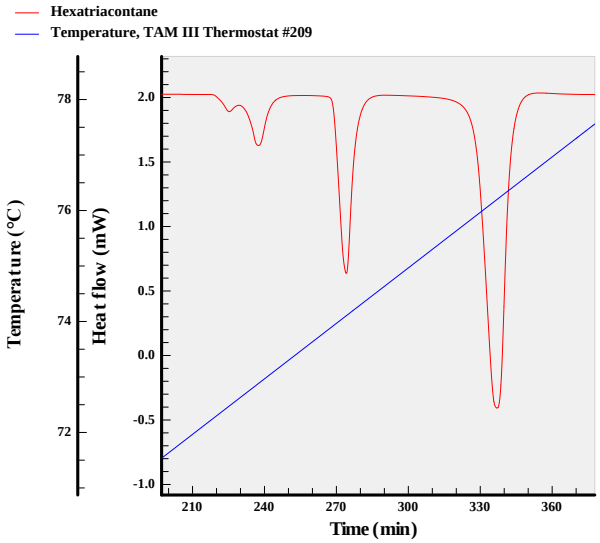
III. Crystallisation of β form (exo)

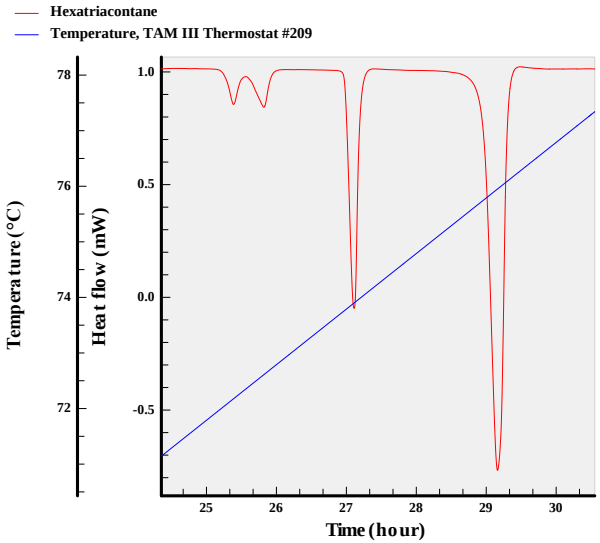
The results indicates that the α → β transformation is irreversible.

Note: Sharps peaks due to the slow scanning rate.

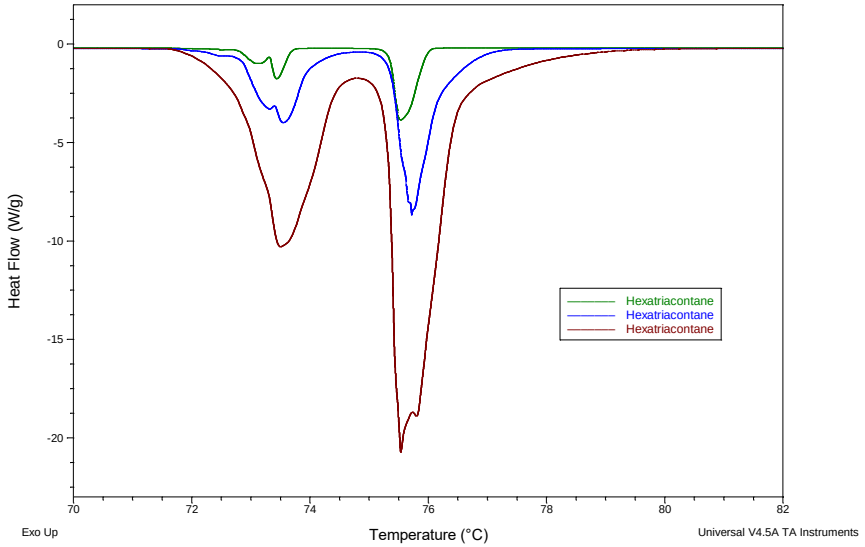
Thermometric Application Laboratory, *internal results* (2003)

Hexatriacontane - Function of Scanning Rate





- TAM – 2 °C/hr (top)
- TAM – 1 °C/hr (bottom)
- QDSC – 0.5, 2.0, and 10 °C/min



Hexatriacontane - Function of Scanning Rate

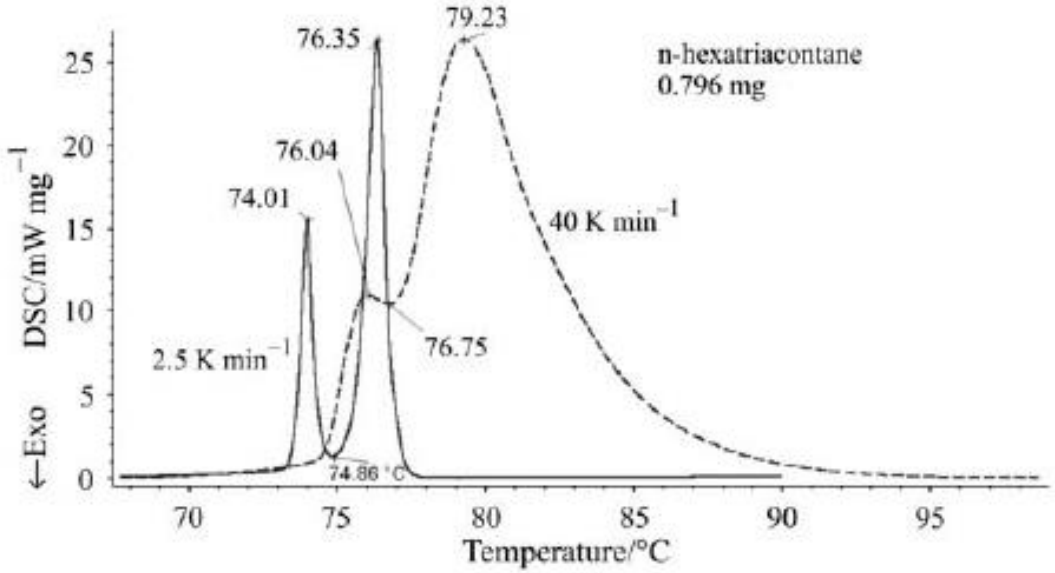


Fig. 7 *n*-hexatriacontane: Improvement of DSC resolution (uncorrected curves, relative scaling at *Y*-axis) through reduction of heating rate

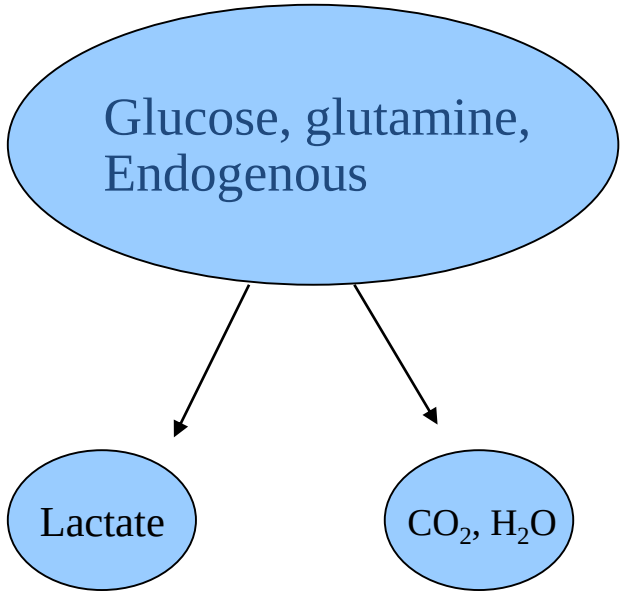
Marti et al (2004) J. Thermal Analysis Calorim **77**

TAM Applications

Life Sciences

Other Methods used as Bioassays

- For the metabolic response: failed to give results for many systems
- The drug effect has to be specified prior to analysis (e.g. cell growth)
- One assay has to be used to assess each biological response – many methods



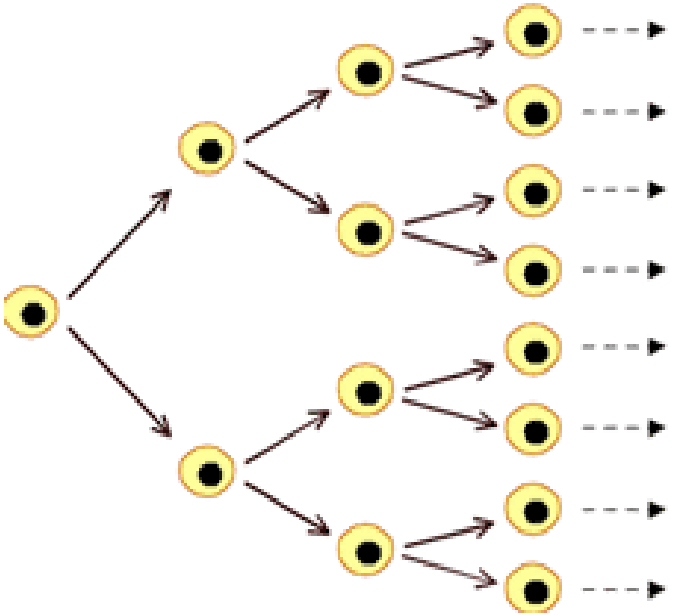
Both ways give heat

All changes in metabolism are measured by microcalorimetry

If something is happening calorimetry will tell you!

Rapid detection of Mycobacteria in culture

- A typical bacteria will produce heat at a rate of about 2 pW
- Detection is achived by their replication
- With a calorimetric detections limit of 200 nW, only 100 000 growing mycobacteria are required for detection
- Thus the calorimeter will detect a infection well before either a colony is visible or all oxygen is consumed (as in BACTEC 960 MGIT).

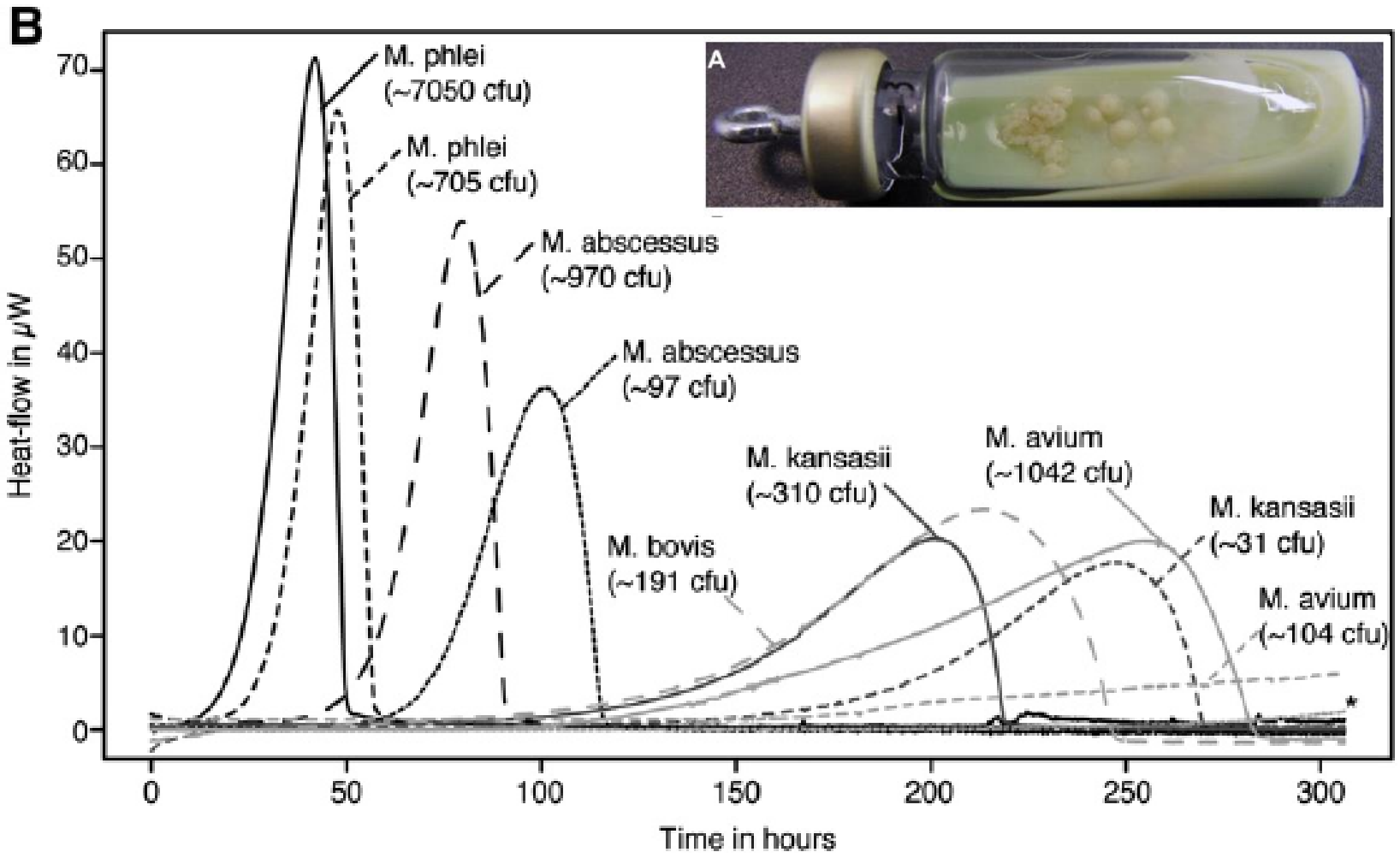


Rapid detection of Mycobacteria in culture

- Tuberculosis (TB) is a major concern for public heath
  - TB kills about 1.5 million people worldwide each year
  - An additional 0.2 million people per year die from HIV-associated TB
  - Also, other mycobacterial infections are emerging and their incidence increasing.
- Culture-based detection techniques is the gold standard.
- In many developing countries, simple solid culture media are used, and detection is achieved by visual inspection. Detection is very slow, since one must wait until a visible colony appears.
- Detection of mycobacterial growth using liquid culture is currently achieved using a radiolabelled substrate (e.g. BACTEC 12) or fluorescent indicators sensitive to oxygen concentration (e.g. BACTEC 960 MGIT). However, such indicators are expensive additions to culture media.

From: O. Braissant*, D. Wirz, B. Göpfert, A.U. Daniels
Tuberculosis (2009), doi:10.1016/j.tube.2009.11.001

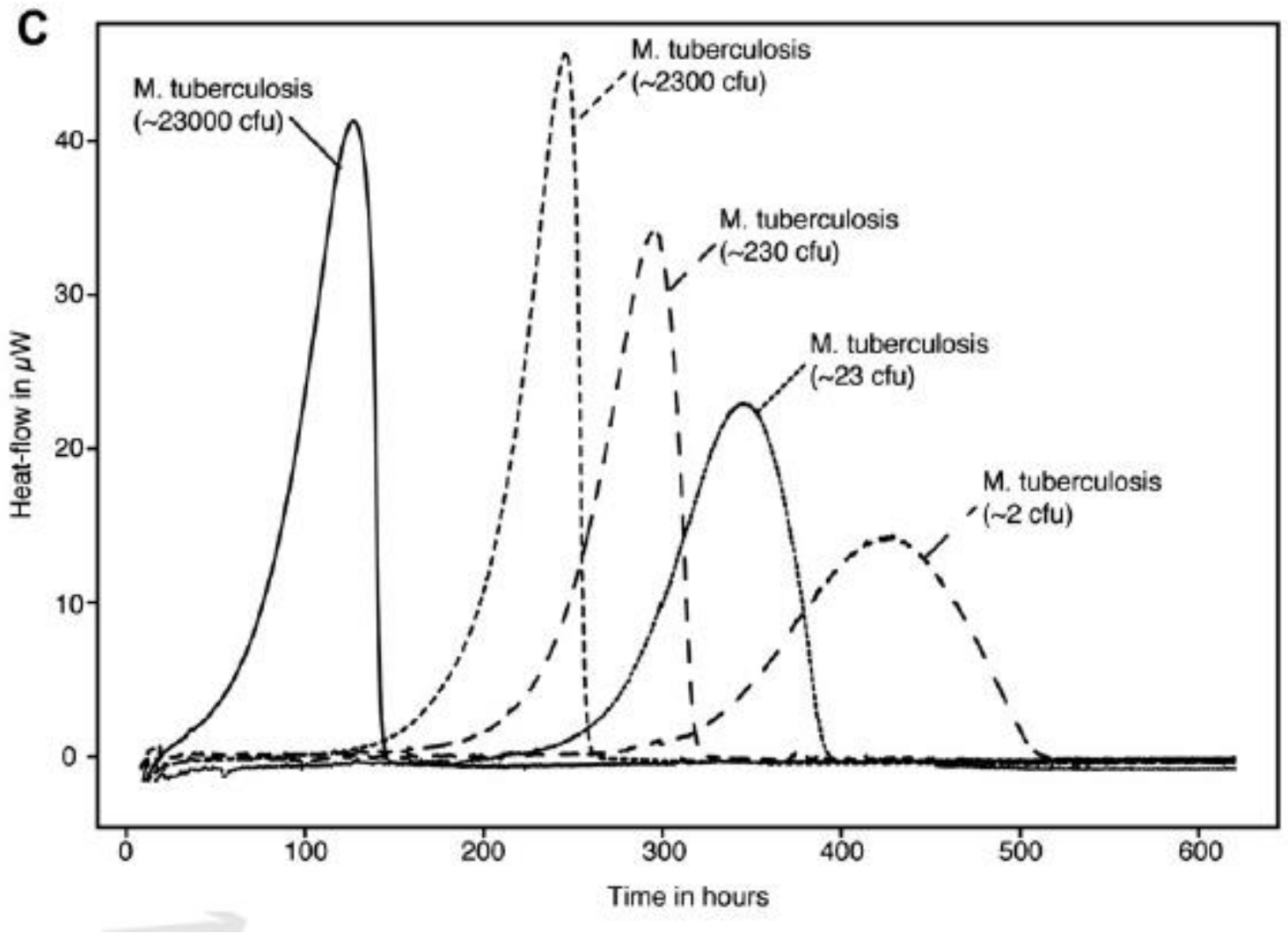
Different pathogenic mycobacteria



(MOTT – Mycobacteria other than tuberculosis)

From: O. Braissant*, D. Wirz, B. Göpfert, A.U. Daniels
Tuberculosis (2009), doi:10.1016/j.tube.2009.11.001

M. tuberculosis



From: O. Braissant*, D. Wirz, B. Göpfert, A.U. Daniels
Tuberculosis (2009), doi:10.1016/j.tube.2009.11.001

Effect of Growth Hormone on Cells

Addition of GH should give

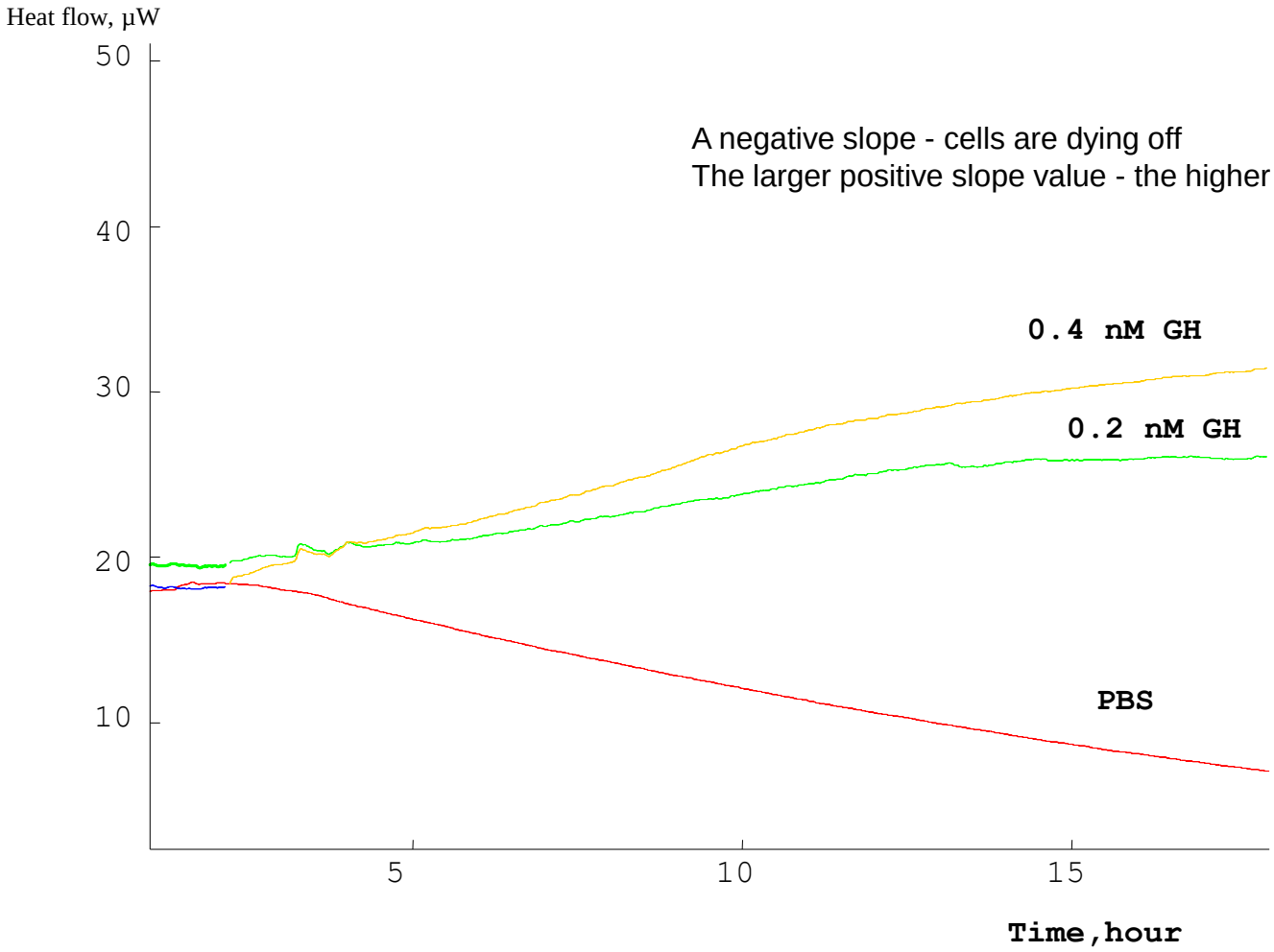
- an immediate metabolic response

followed by

- anabolism = increase in biomass

Long-term Studies of GH on Lymphoid Cells

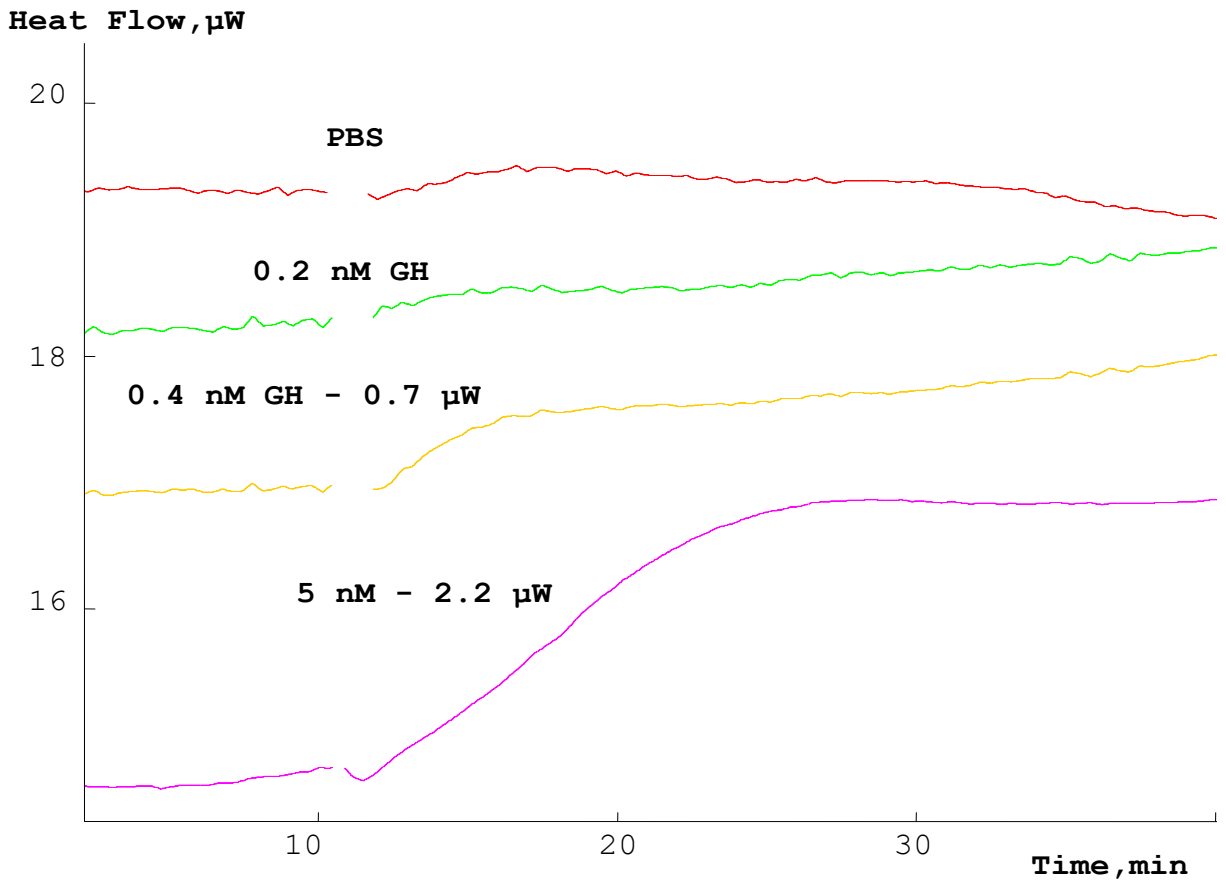
The proliferative response upon addition of human growth hormone, hGH



A negative slope - cells are dying off
The larger positive slope value - the higher proliferation rate

Schön and Walum, 1997

Initial Metabolic Response - Addition of GH



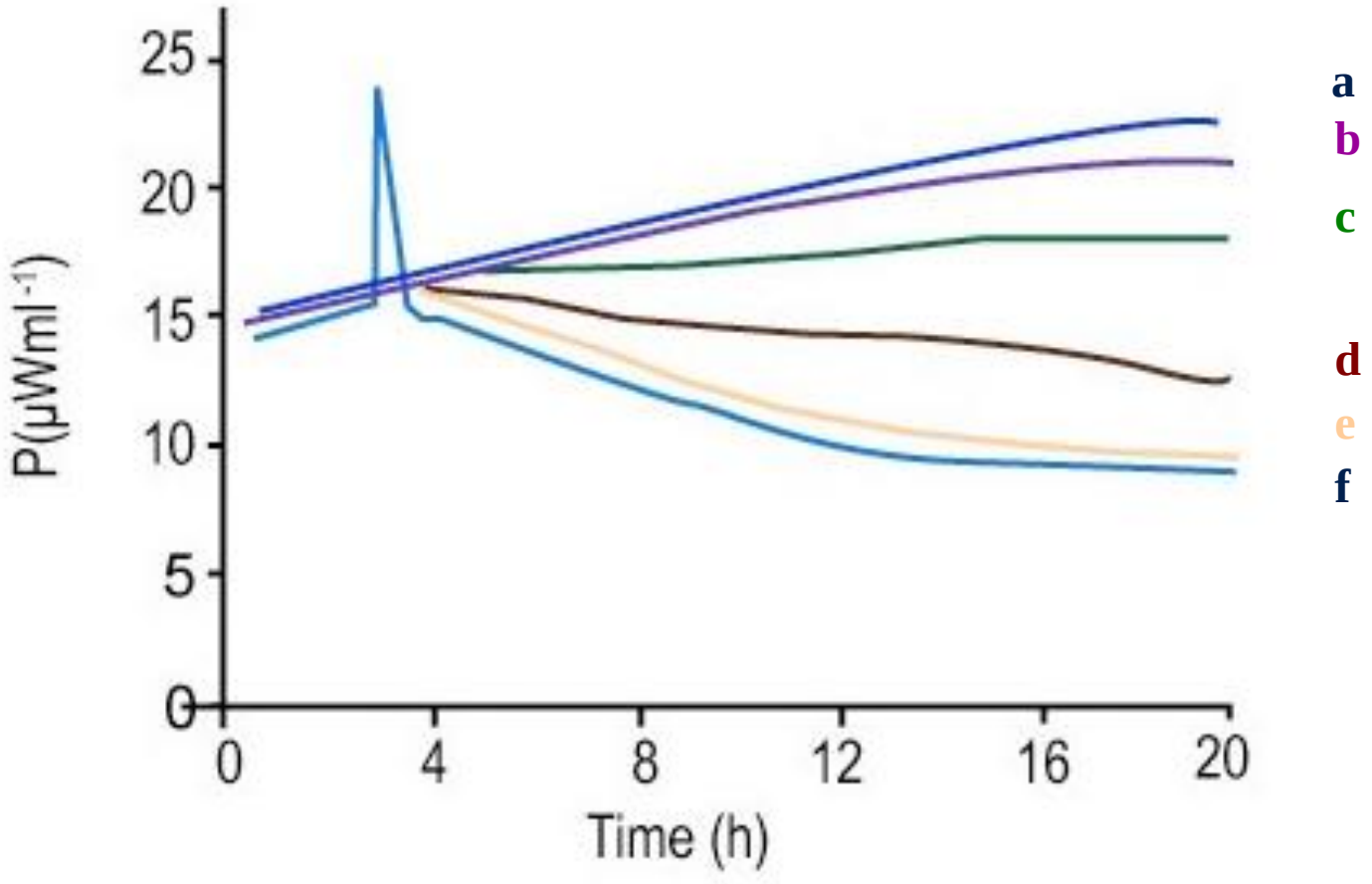
Schön and Walum, 1997

Results - GH on a Lymphoid Cell Line

- Kinetic information: 10 min for maximum metabolic GH stimulation; another 20 min before growth starts
- Dose response curves can be constructed based on the initial metabolic response
- Dose response curves can be constructed from the growth rate with high sensitivity

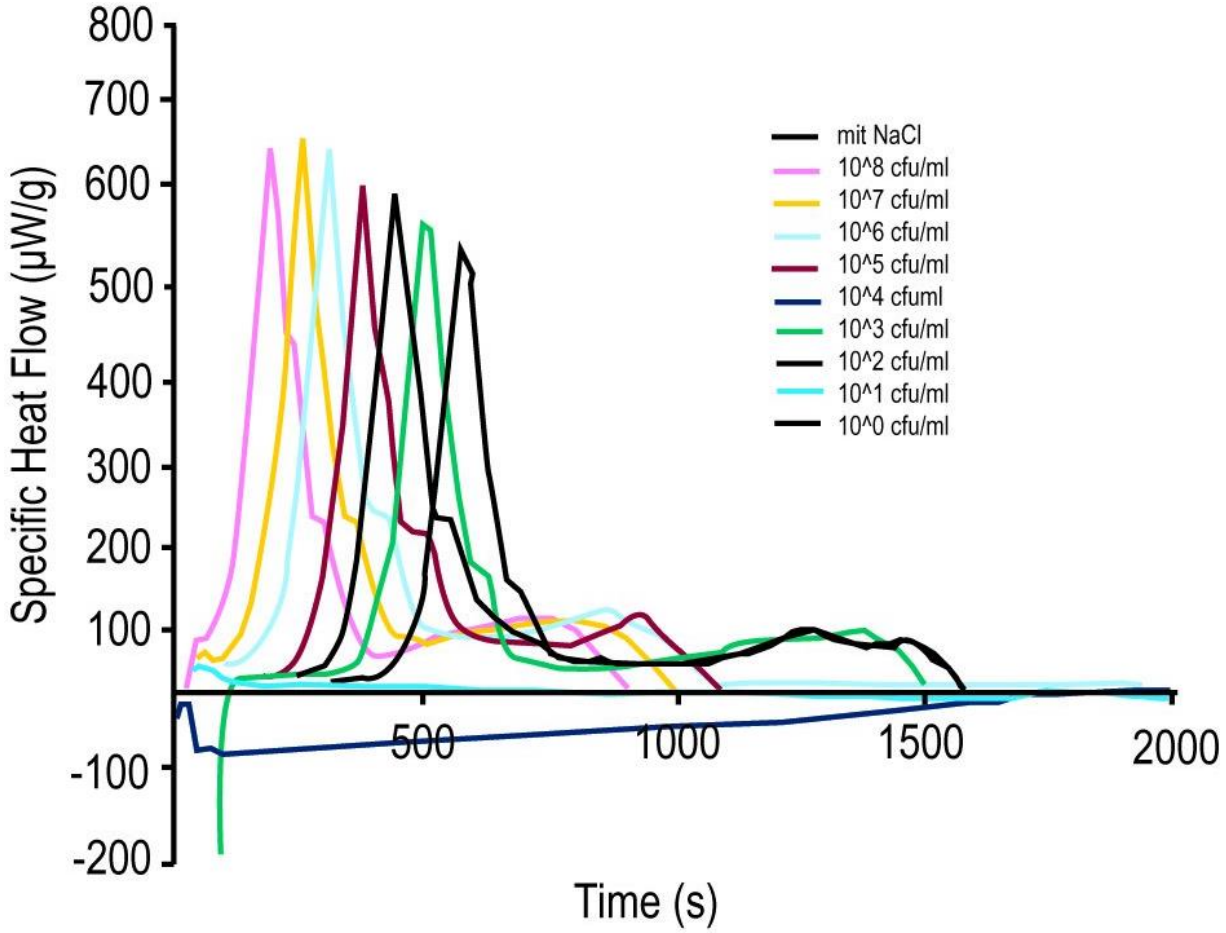
Drug Efficacy

Flow calorimetry: Leukemia (T-lymphoma) cells exposed to the anti-cancer drug methotrexate. The final drug concentrations were (a) 0, (b) 0.2, (c) 0.5, (d) 1.0, (e) 2.0, (f) 4.0µM (ref 6).



Bermudez, Backman and Schon., *Cell. Biophys*. 20, 111-123, (1992).

Microorganism Detection



Microcalorimetry - A Novel Method for Detection of Microorganisms in Platelet Concentrates and Blood Cultures. Andrej Trampuz, Simone Salzmann, Jeanne Antheaume, Reno Frei, A.U. Daniels University of Basel & University Hospital Basel, Switzerland

Background

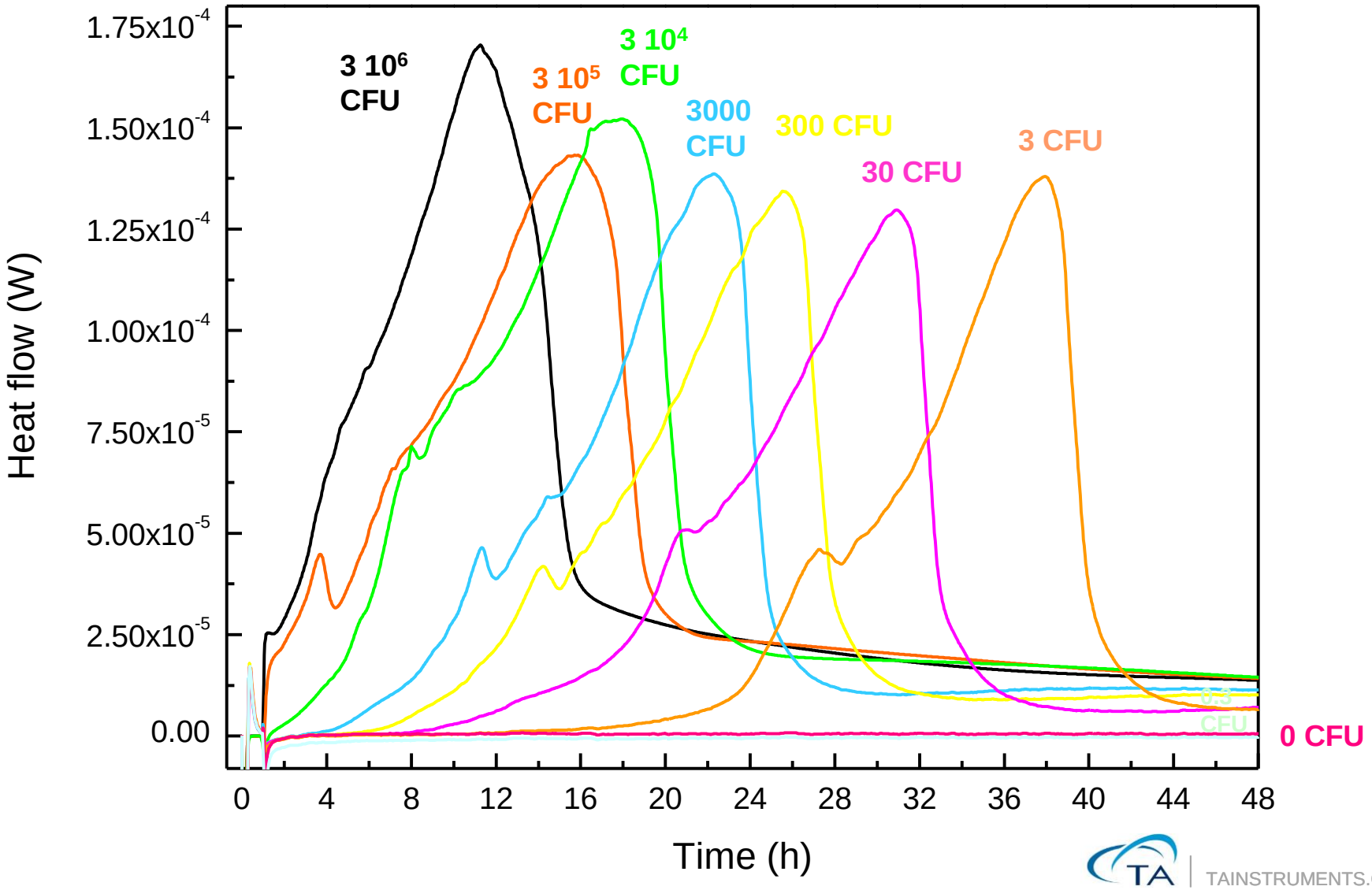
The diagnosis of implant-associated infections is difficult due to organisms attached to surfaces forming biofilms.

Diagnosis can be improved by sonicating retrieved implants to remove attached microorganisms, followed by culture and calorimetry of sonicates.

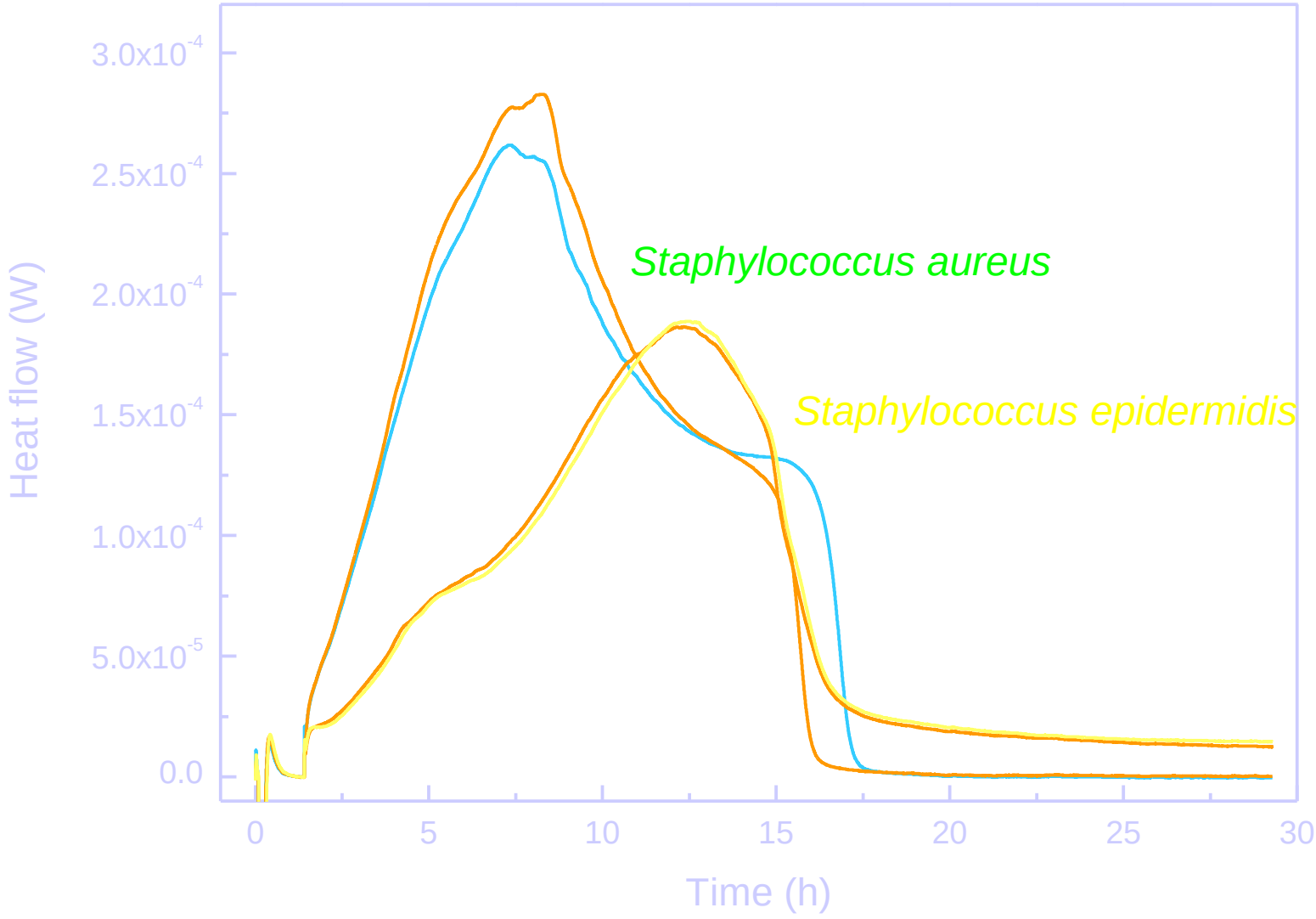
1 mL sonicate fluid was injected in ampoules containing 2 mL TSB and incubated at 37°C in calorimeter. Positivity by calorimetry defined as an increase in heat flow rate of ≥10 µW above baseline.

Trampuz, A. et al. JCM (2006), **44**, 628

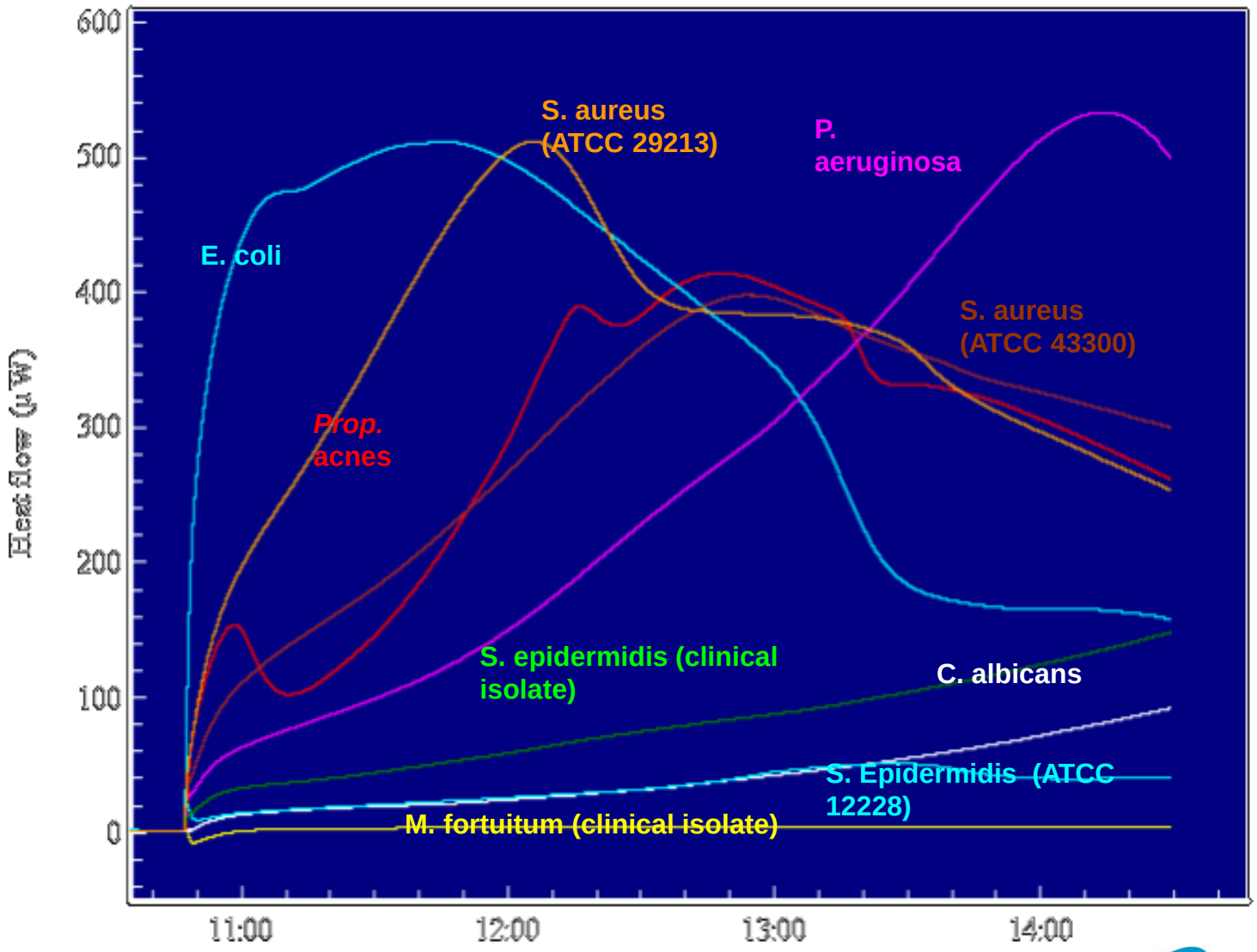
S. epidermidis in 3 mL TSB



S. aureus & *S. epidermidis* in TSB



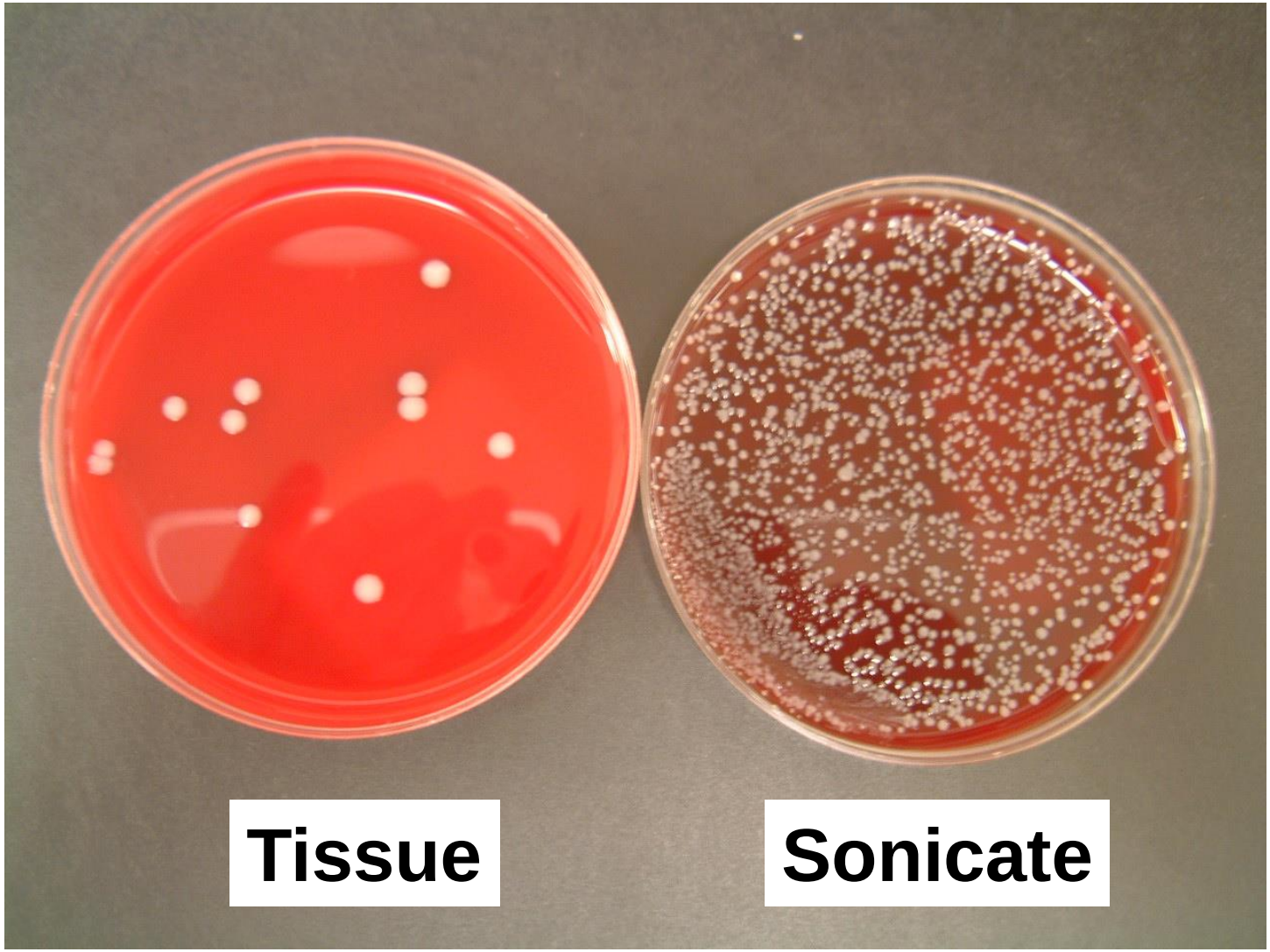
Suspension of Microorganisms in TSB



Results

Characteristic (n = 646)	Aseptic cases (n = 475)	Implant infection (n = 171)
Type of prosthesis		
Joint prosthesis (n = 167)	**31%**	**44%**
Internal osteosynthesis (n = 479)	**69%**	**56%**
Diagnosis		
Tissue culture (next slide)	**9%**	**77%**
Sonicate culture (≥5 cfu/plate)	**5%**	**89%**
Sonicate Gram stain	**0%**	**52%**
Calorimetry (> 30 µW) (n = 409)	**13/306 (4%)**	**98/103 (95%)**

Results

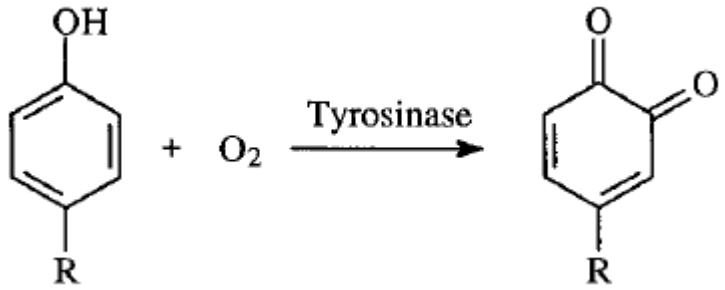


Advantages of Calorimetry

- Rapid detection of microbial growth (within hours)
- High sensitivity and specificity
- Potential rapid antimicrobial susceptibility testing (using selective growth media)
- Potential rapid identification

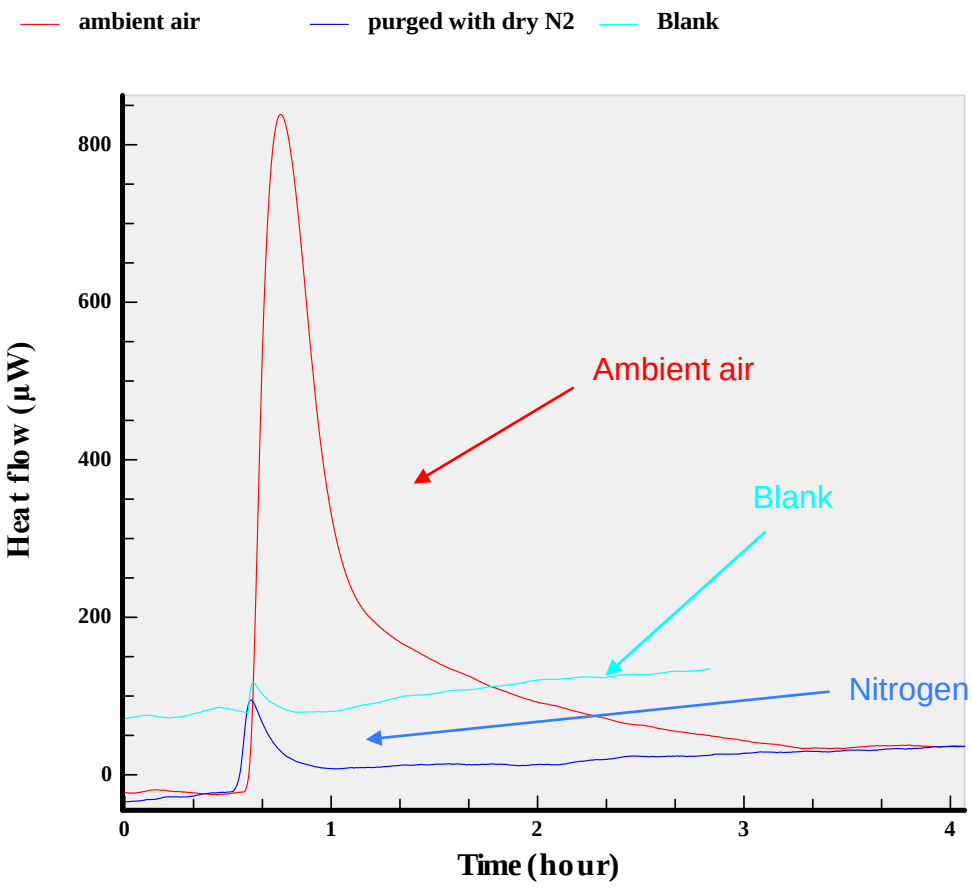
Enzymatic Reaction of Tyrosine

- L-tyrosine (or 4-hydroxyphenylalanine) is a para isomer and is the most common isomer form found in nature. There are two additional isomers, namely meta- and ortho-tyrosine (3- and 2- hydroxyphenylalanine, respectively), which rarely occur in nature.
- Tyrosinases are enzymes that oxidize a broad range of phenols into ortho-quinones.
- When tyrosine is exposed to a tyrosinase in the presence of oxygen the benzene ring is oxidized at the hydroxyl group and converted to an ortho-quinone molecule, which changes the color of the solution.



Tianhong Chen, Heather D. Embree, Li-Qun Wu, Gregory F. Payne "*In Vitro* Protein-Polysaccharide Conjugation. Tyrosinase-Catalyzed Conjugation of Gelatin and Chitosan" Biopolymers, **64**, 292-302 (2002)

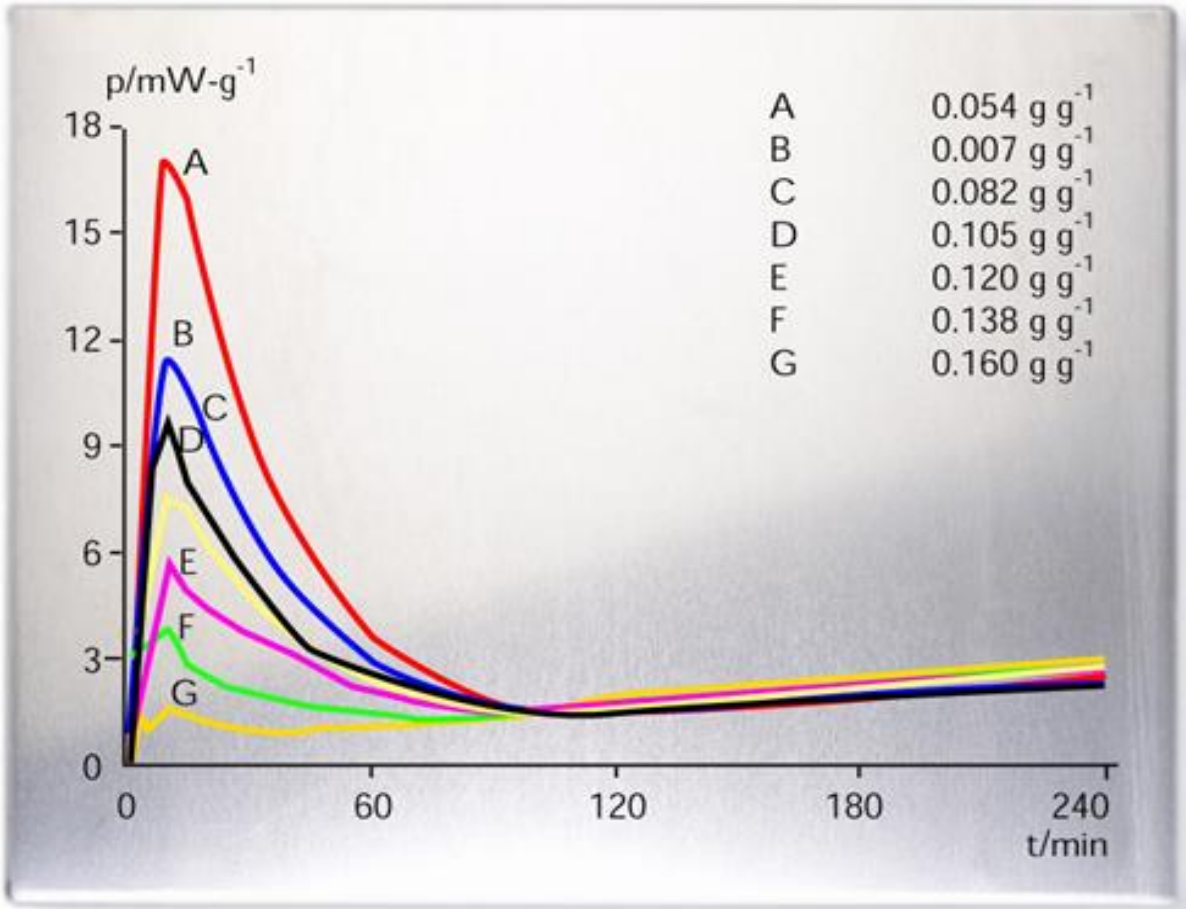
Oxidation of Tyrosine by Tyrosinase



Experiment performed in a TAM Air with Admix ampoule.

- The exothermic oxidation of the tyrosine is detected by the calorimeter. The magnitude of the reaction (and heat flow) is dictated by the oxygen content inside the ampoule.
- Data shifted on Y-axis for comparison.
- Picture below shows the comparison of the solution color change with oxygen content. Blank - left, Nitrogen head space – center, and ambient air – right.

Germination of Quinoa Seeds

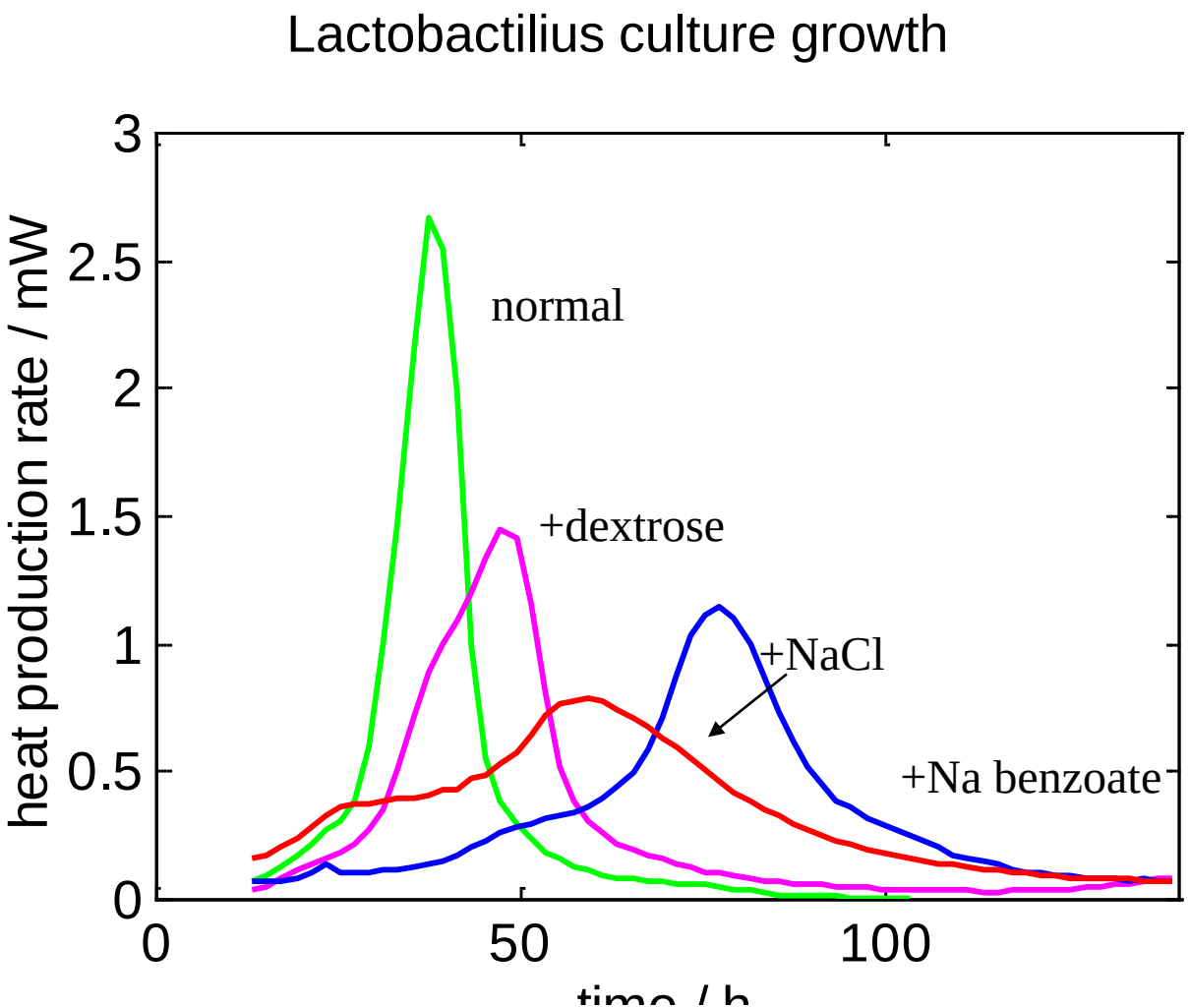


Sigstad and Garcia, *Thermochim. Acta* 366, 149-155, (2001).

Milk Fermentation

- milk + 1% starting culture
- normal, +dextrose, +NaCl, +Na-benzoate
- Closed 20 mL glass ampoules at 19°C

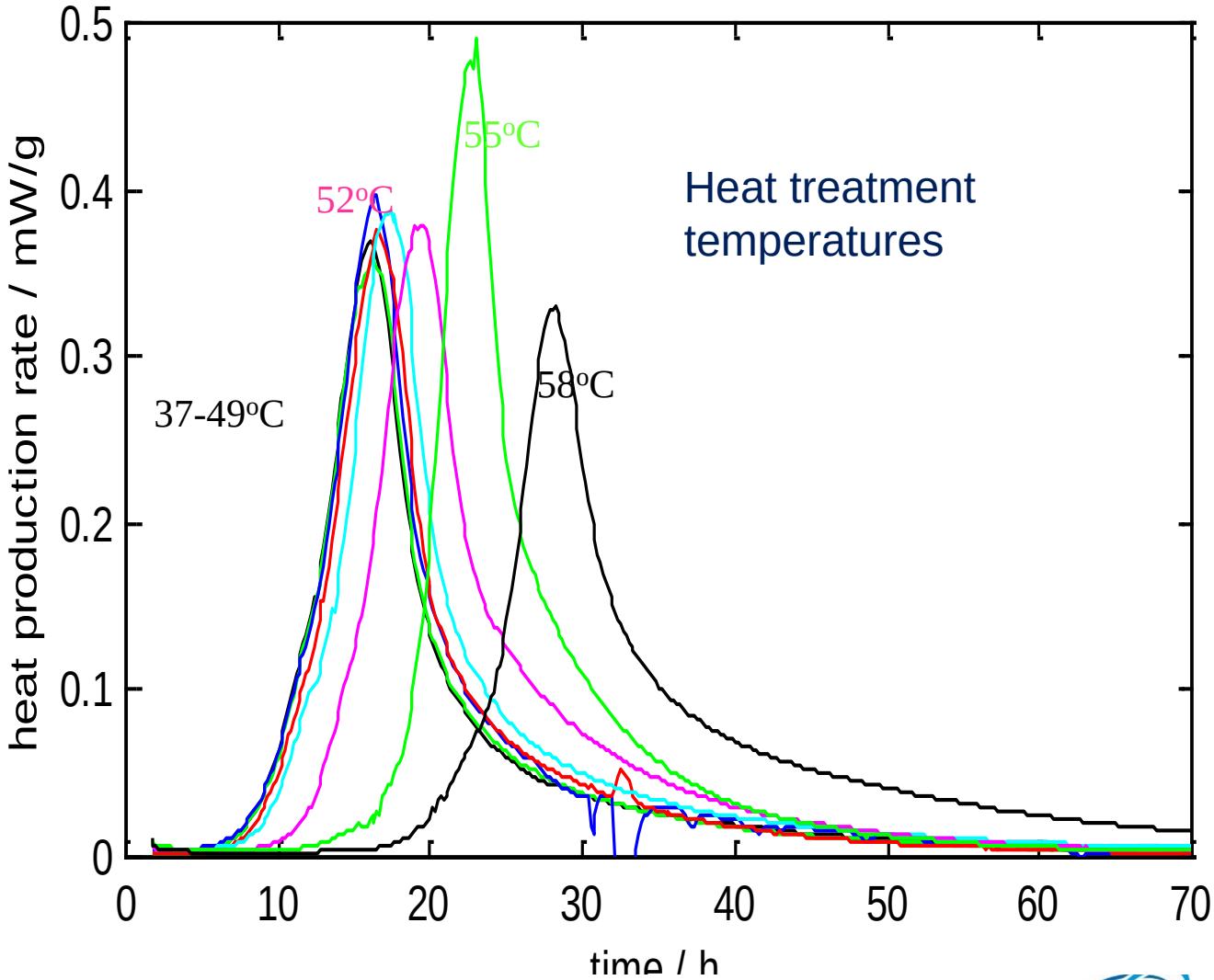
Milk Fermentation



Carrot Juice Spoilage

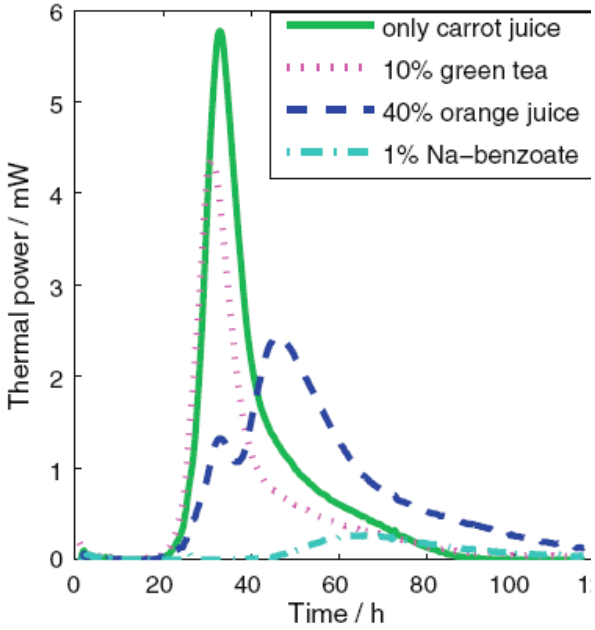
- 8 samples heat treated at 37-58ºC
- Closed 20 mL glass ampoules at 25ºC (accelerated)

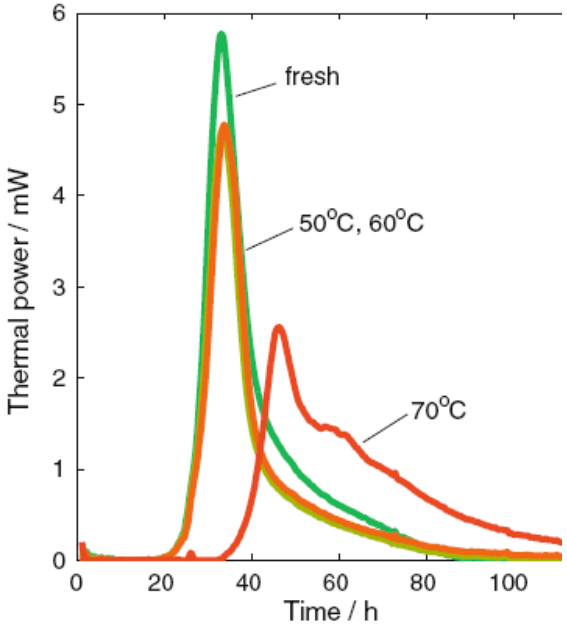
Carrot Juice Spoilage Measured at 25°C

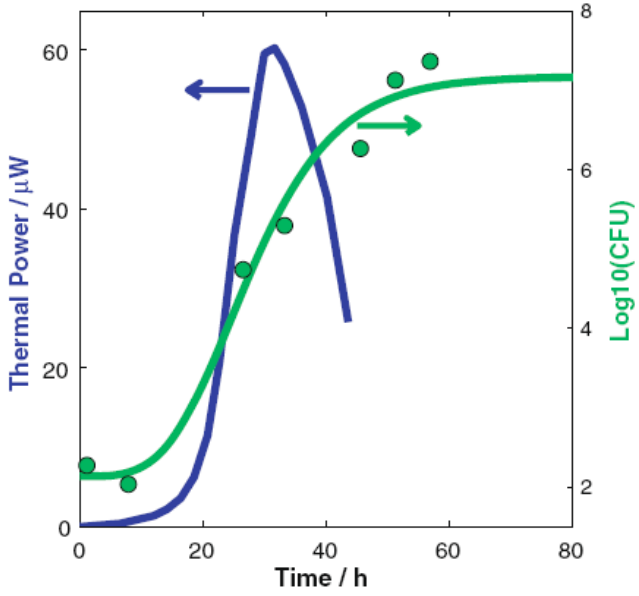


Microbiological spoilage prevention

- The goal of preventing microbial spoilage can be reached by many methods, e.g., natural and synthetic chemical preservatives, and thermal treatments.
- Fresh carrot juice is an extremely perishable food-stuff as it has a neutral pH, high sugar content and also contains many soil microorganisms.

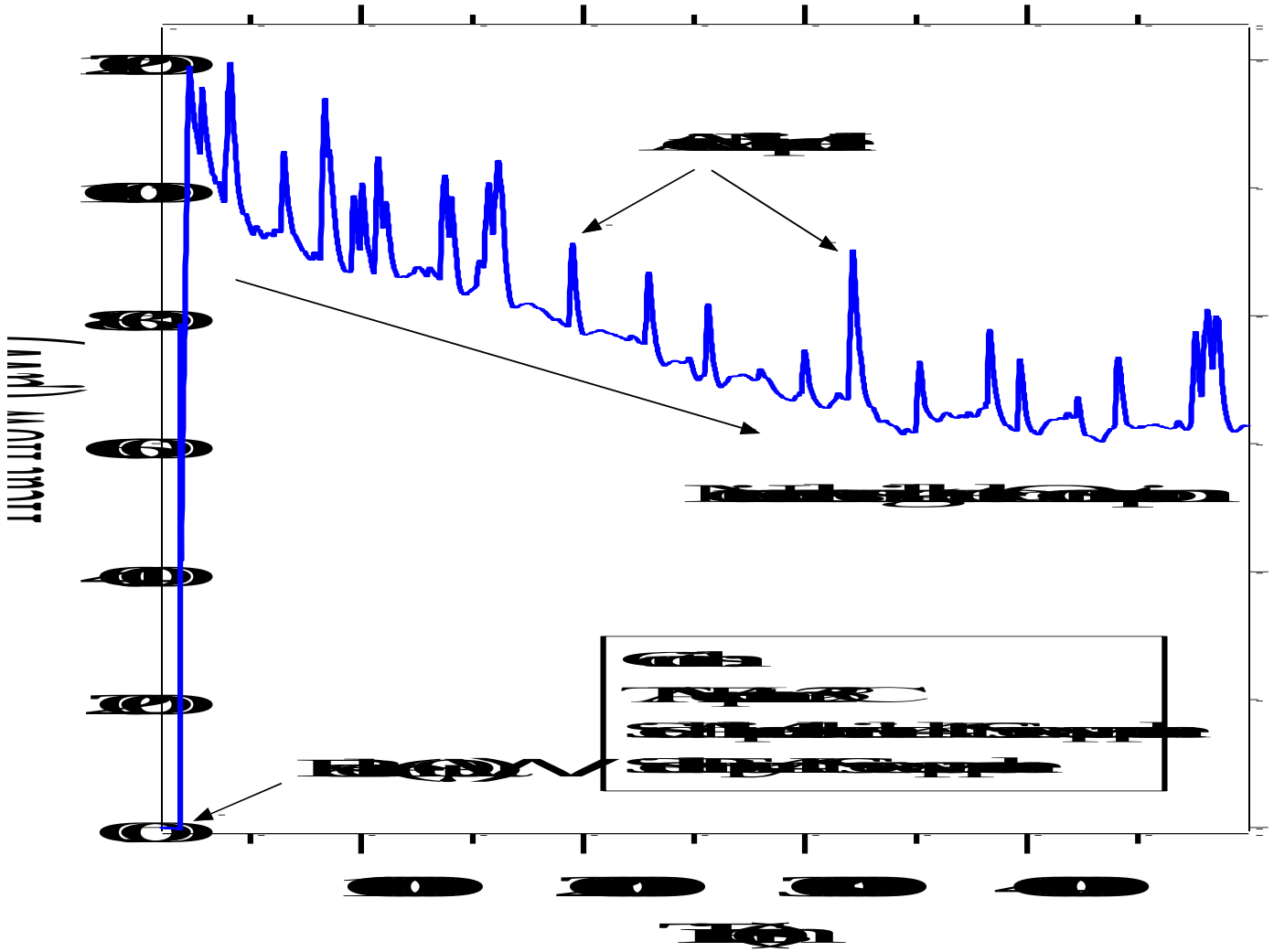






L. Wadsö, F. Gómez Galindo / Food Control 20 (2009) 956–961

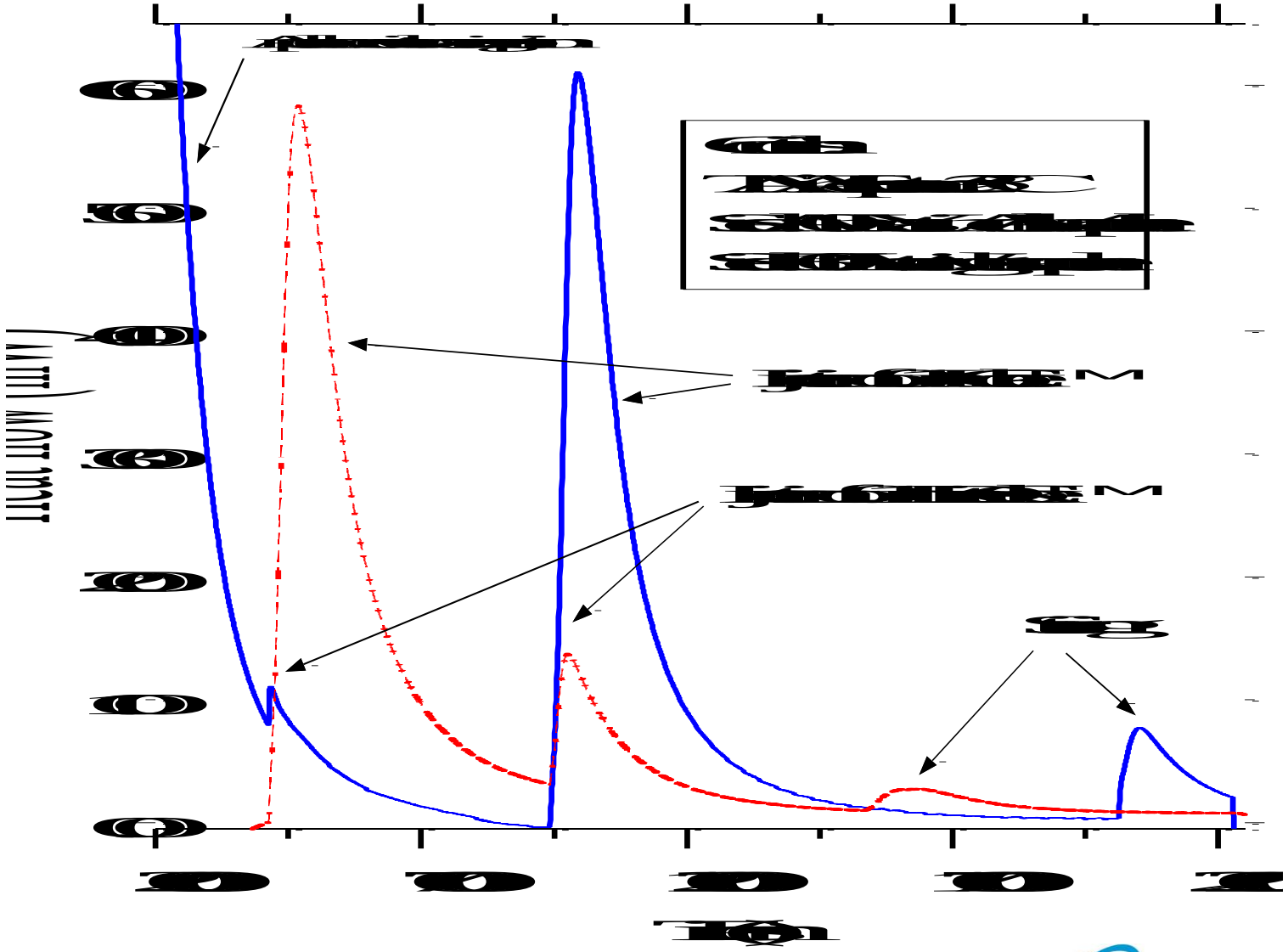
Live Insect with Single Blade of Grass



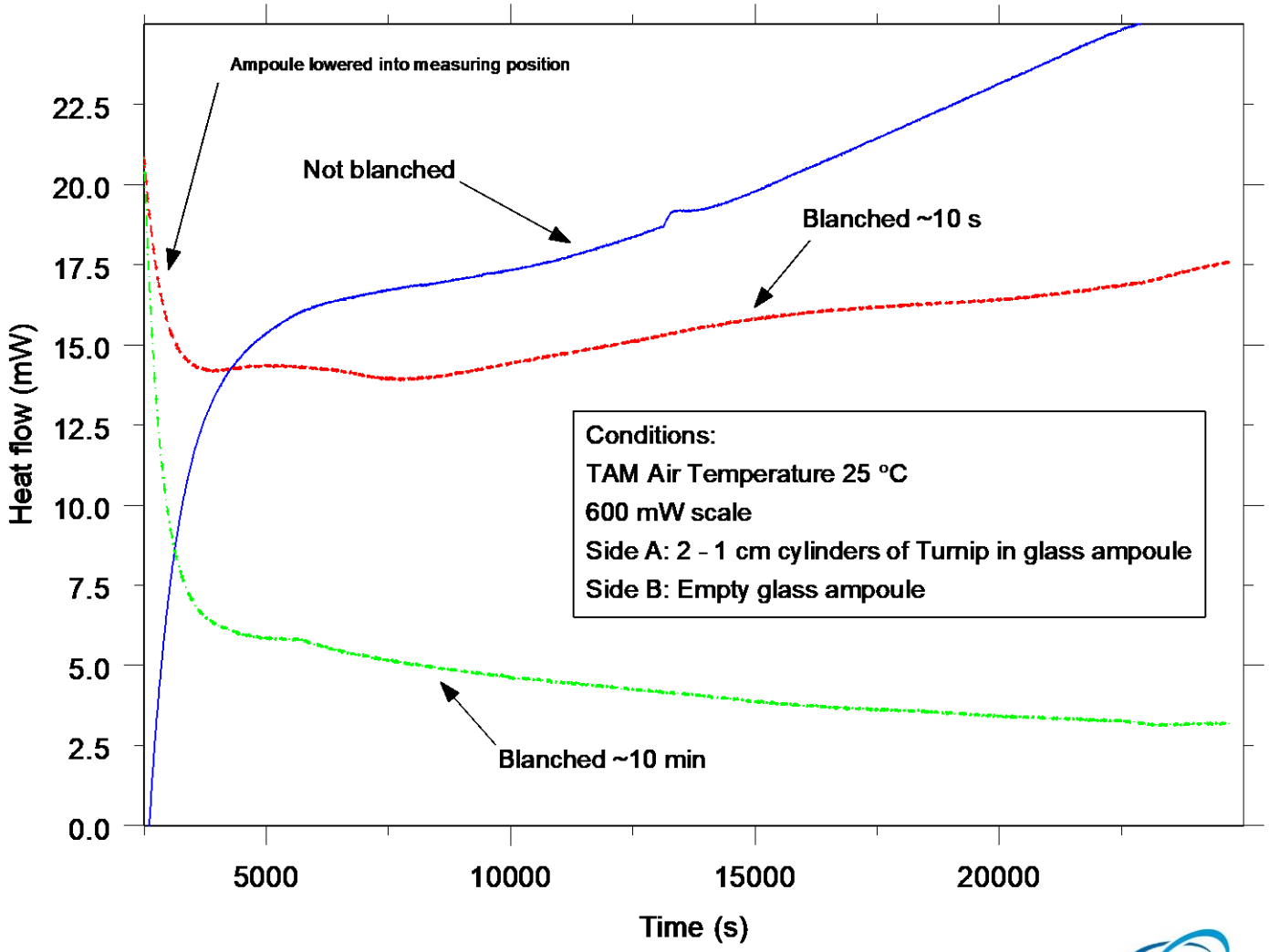
Note: Single red ant gave ~30 μW signal (not shown)*

* No insects were injured during the course of this experiment.

Effect of Sucrose on Yeast Growth – TAM Air



Effect of Blanching on Turnip



TAM Applications

Cement Hydration

Sample Ampoules

- Static 20 mL disposable glass ampoules.
- Mixing of solid/liquid outside calorimeter

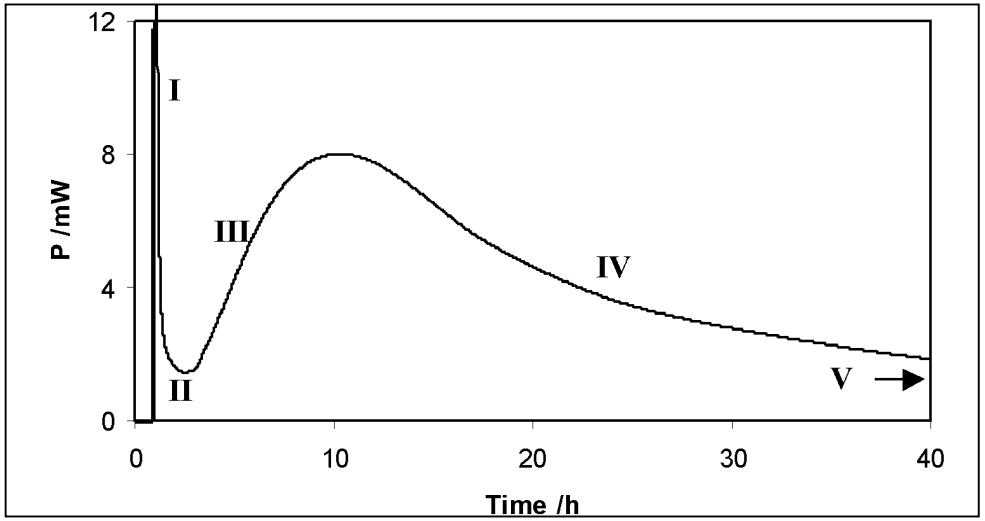
Portland Cement Basics

- Silicates hydrate to give strength giving gel, “glue”
- Aluminate and ferrite phases necessary to get a molten phase during production of cement
- Aluminates react rapidly, interact with admixtures, workability, set, early strength development
- Gypsum added during grinding to slow down aluminate hydration rate
  - Higher C_3A , lower C_4AF generally more reactive
  - Different sulfate forms have different solubility
    - *Dr. Sandberg, Grace Construction Products, US (**2002**)*
- ASTM Methods available in 2009 related to cement isothermal calorimetry (www.ASTM.org).
  - C1679-08 “Standard Practice for Measuring Hydration Kinetics of Hydraulic Cementitious Mixtures Using Isothermal Calorimetry.”
  - C1702-09 “New Test Method for Standard Test Method for Measurement of Heat of Hydration of Cement with Heat Conduction Calorimetry.”

Portland Cement Basics

The hydration process undergoes a number of phases (*Young*, 1985)

- (I) Rapid initial processes
- (II) Dormant period
- (III) Acceleration period
- (IV) Retardation period
- (V) Long term reactions



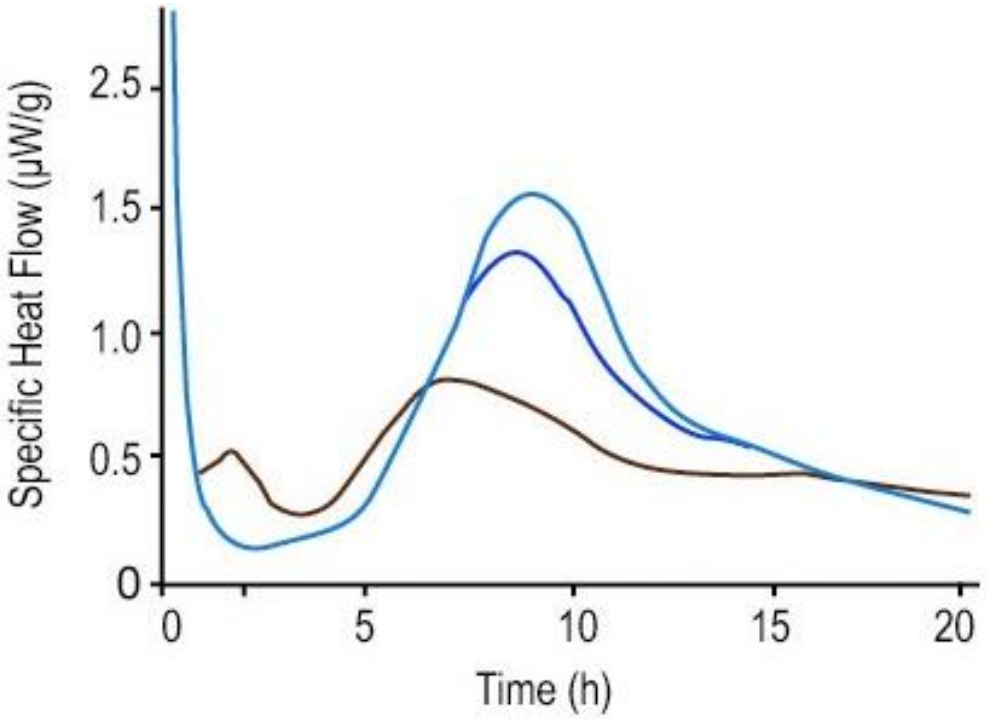
The phases has been described in more detail (*Sandberg*, 2002)

- (I) Dissolution of ions and initial hydration
- (II) Formation of ettringite
- (III) Initiation of silicate hydration
- (IV) Depletion of sulphate

Dr. Sandberg, Grace Construction Products, US (**2002**)

Setting Time of Cement

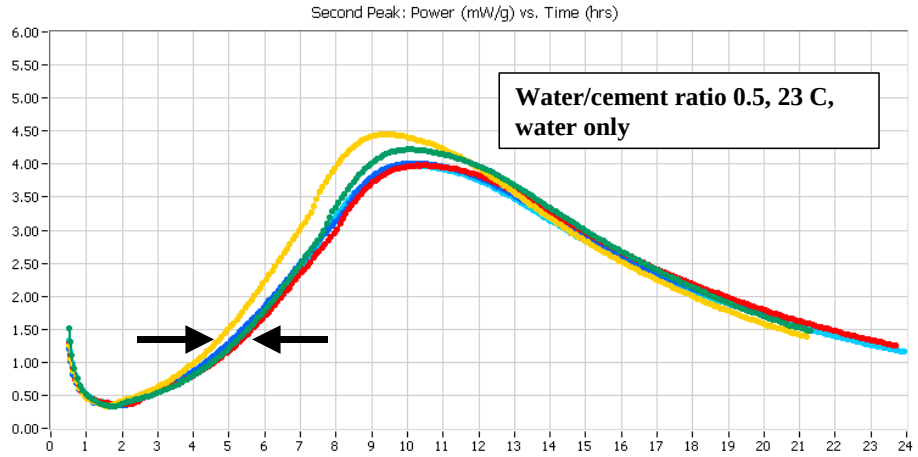
- Assessment of setting time and early stiffening (diagram)
- Influence of concrete admixtures
- Influence of glass fillers, waste products, slags etc.
- Influence of contaminants, e.g. in water
- Assessments of the efficiency of mixing

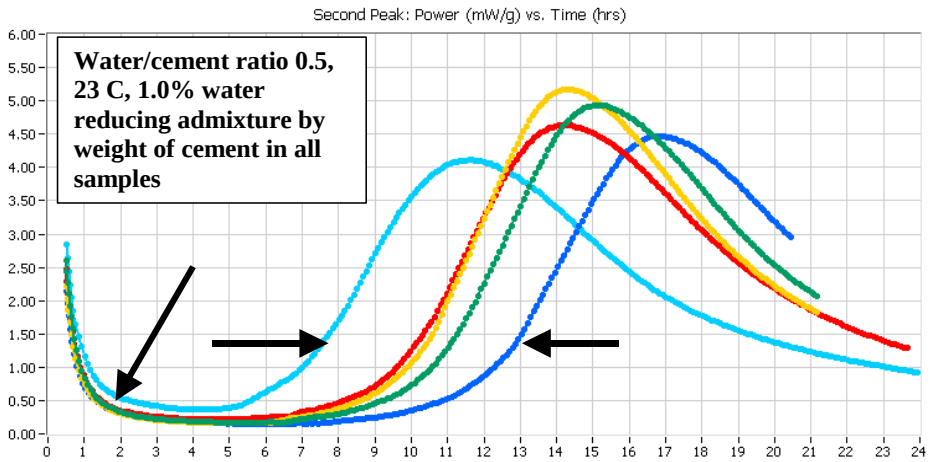


Dr. Sandberg, Grace Construction Products, US (**2002**)

Effects of Admixtures

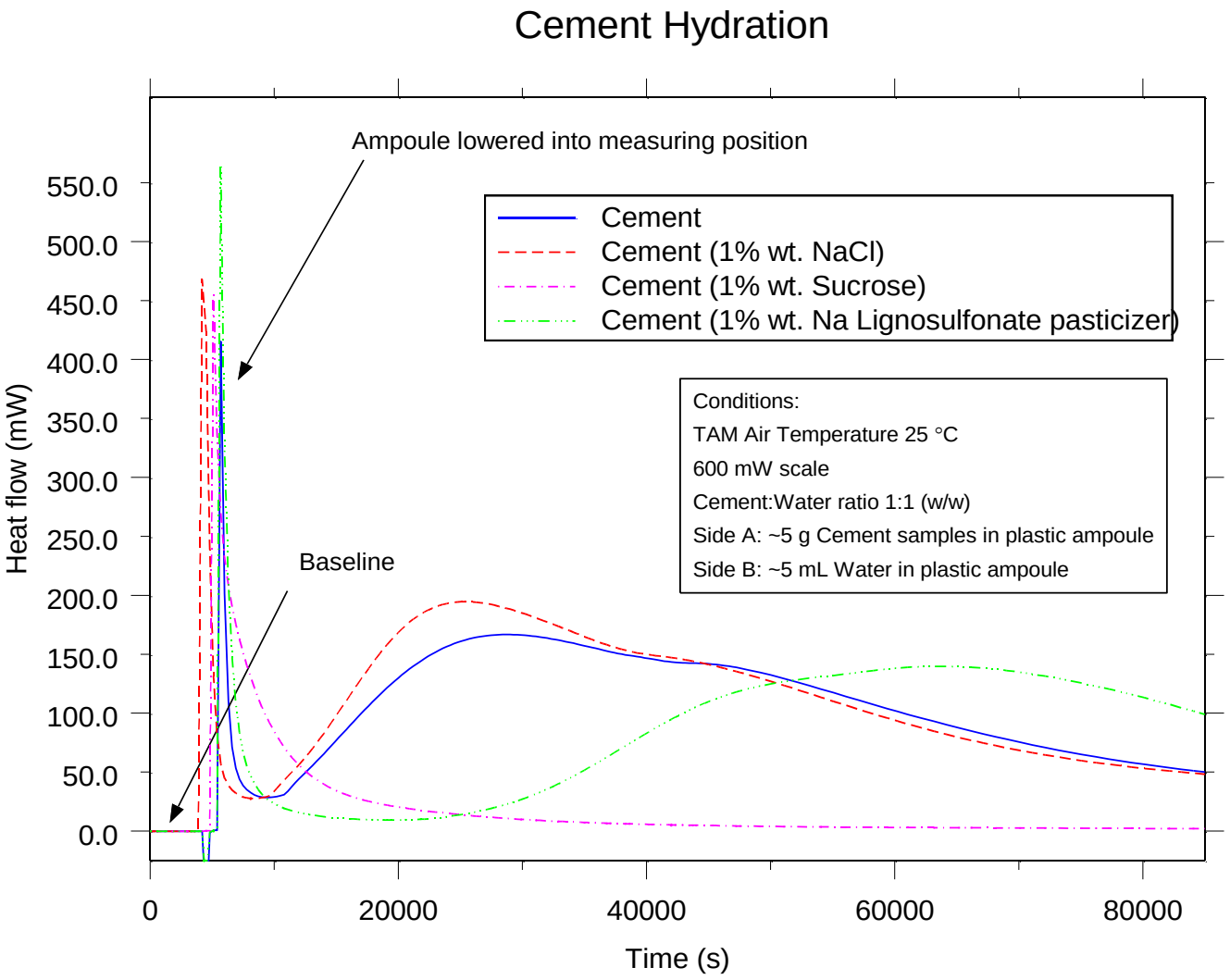
- Admixture is any material which affects the process and properties of cement
- Only small differences between cement lots when tested <u>without</u> admixture
- Very large differences between cement lots when tested <u>with</u> same admixture!!!





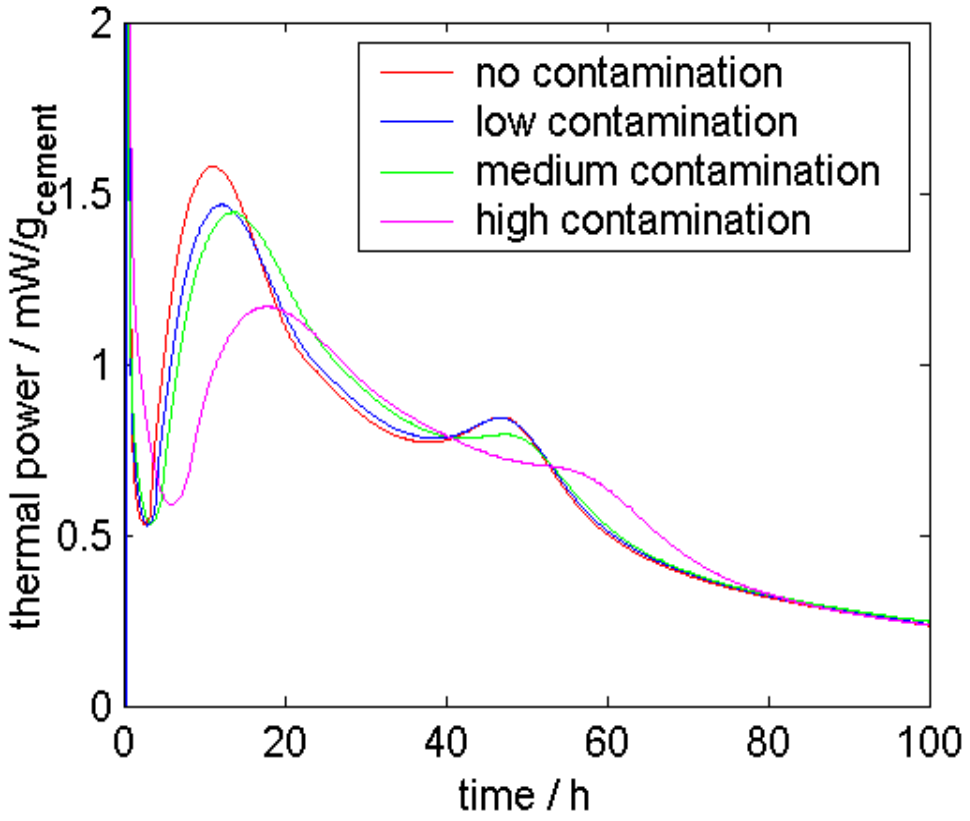
Dr. Sandberg, Grace Construction Products, US (**2002**)

Cement Hydration



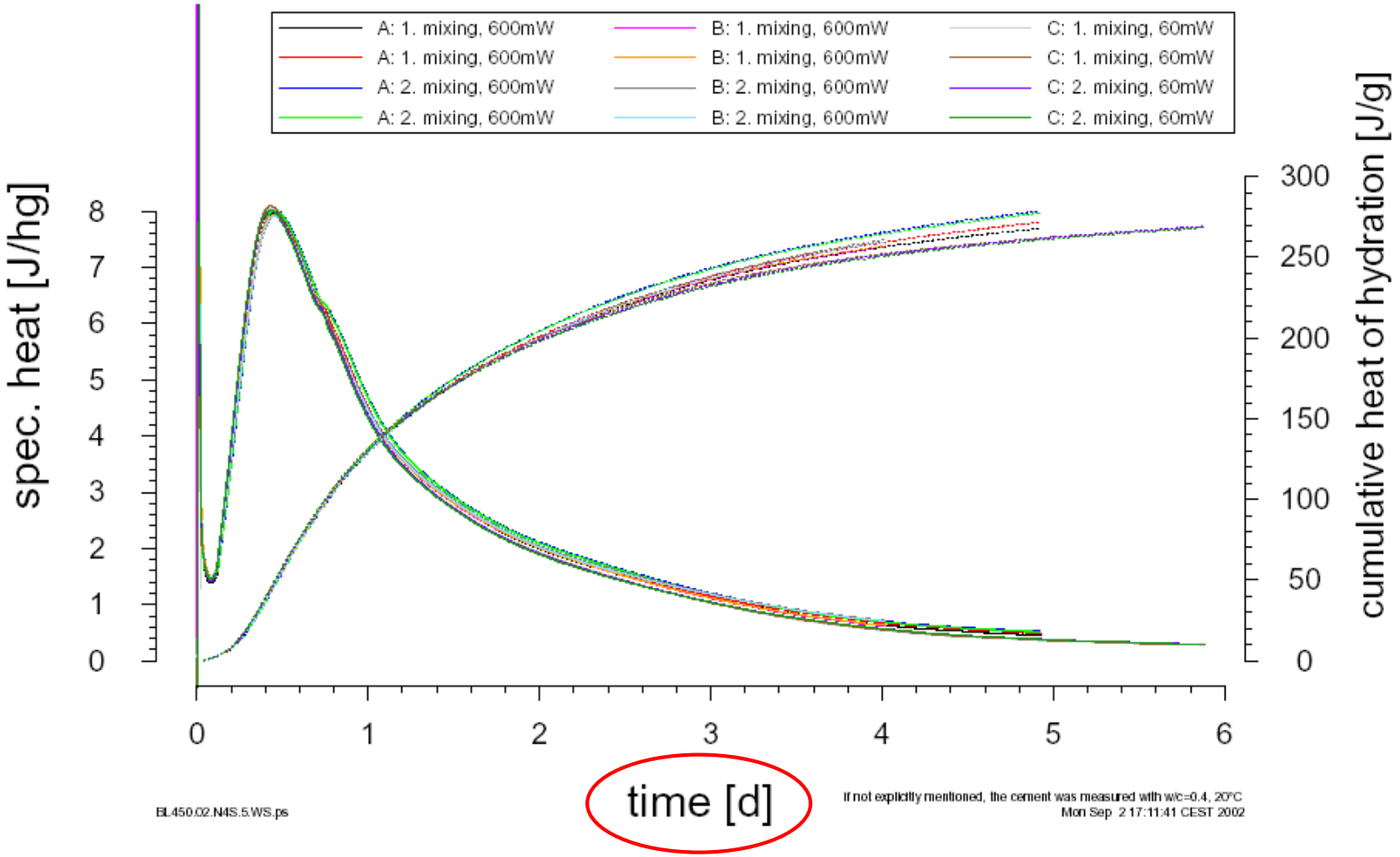
Effect of Contamination

Influence on hydration rate of a mixture of *soil and sawdust* (0; 0.9; 2.5 and 5.9% of w/c=0.6 cement mortar).



*Dr. L. Wadsö, University of Lund, Sweden (**2002**)*

TAM Air Reproducibility



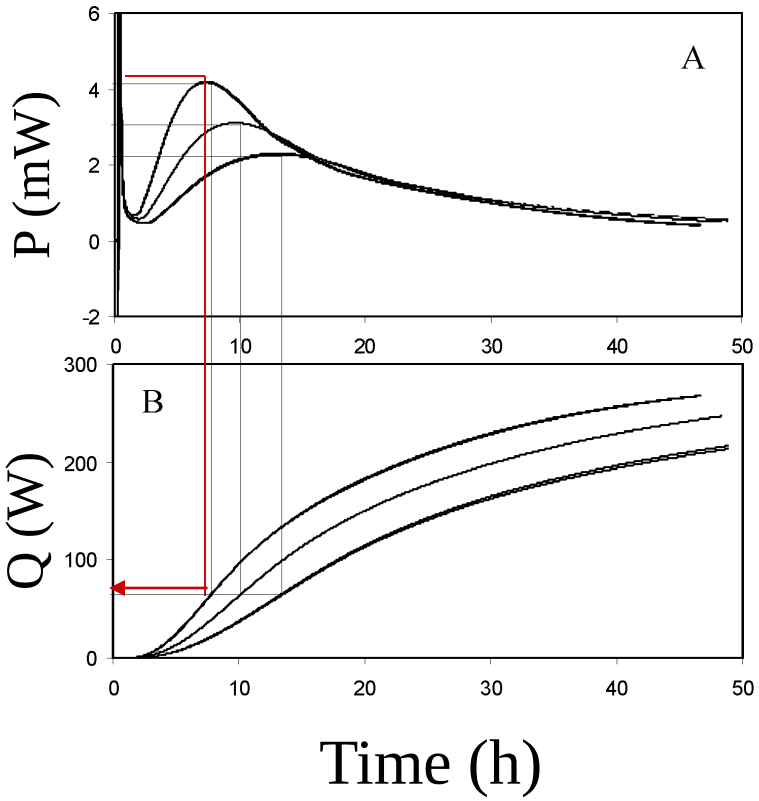
*Dr. Moro , Holcim Group Support, Switzerland (**2002**)*

Temperature Dependency of Cement Hydration

Measurements at 20, 25 and 30 °C

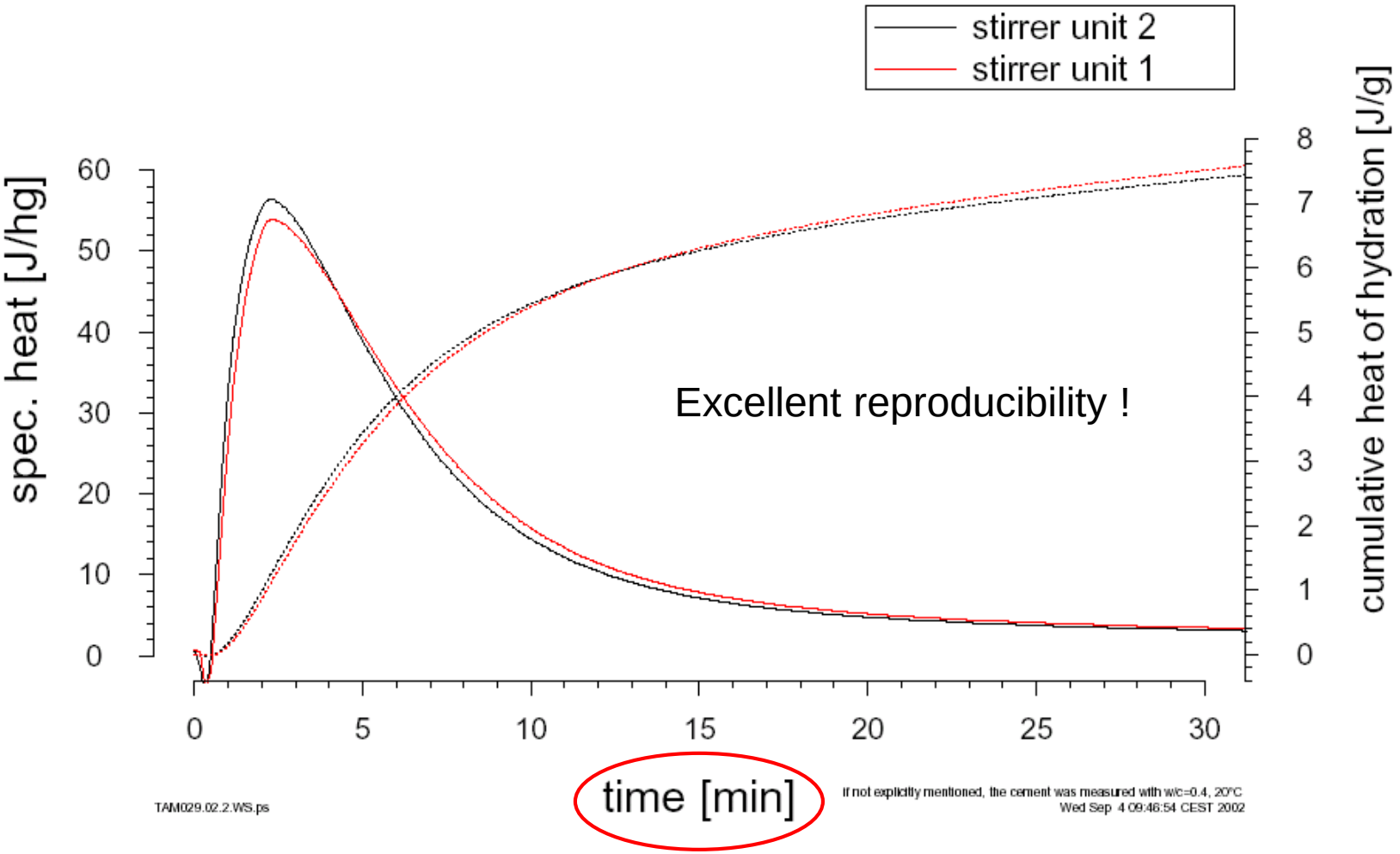
P reflects the rate of the process

Q reflects the extent of the process



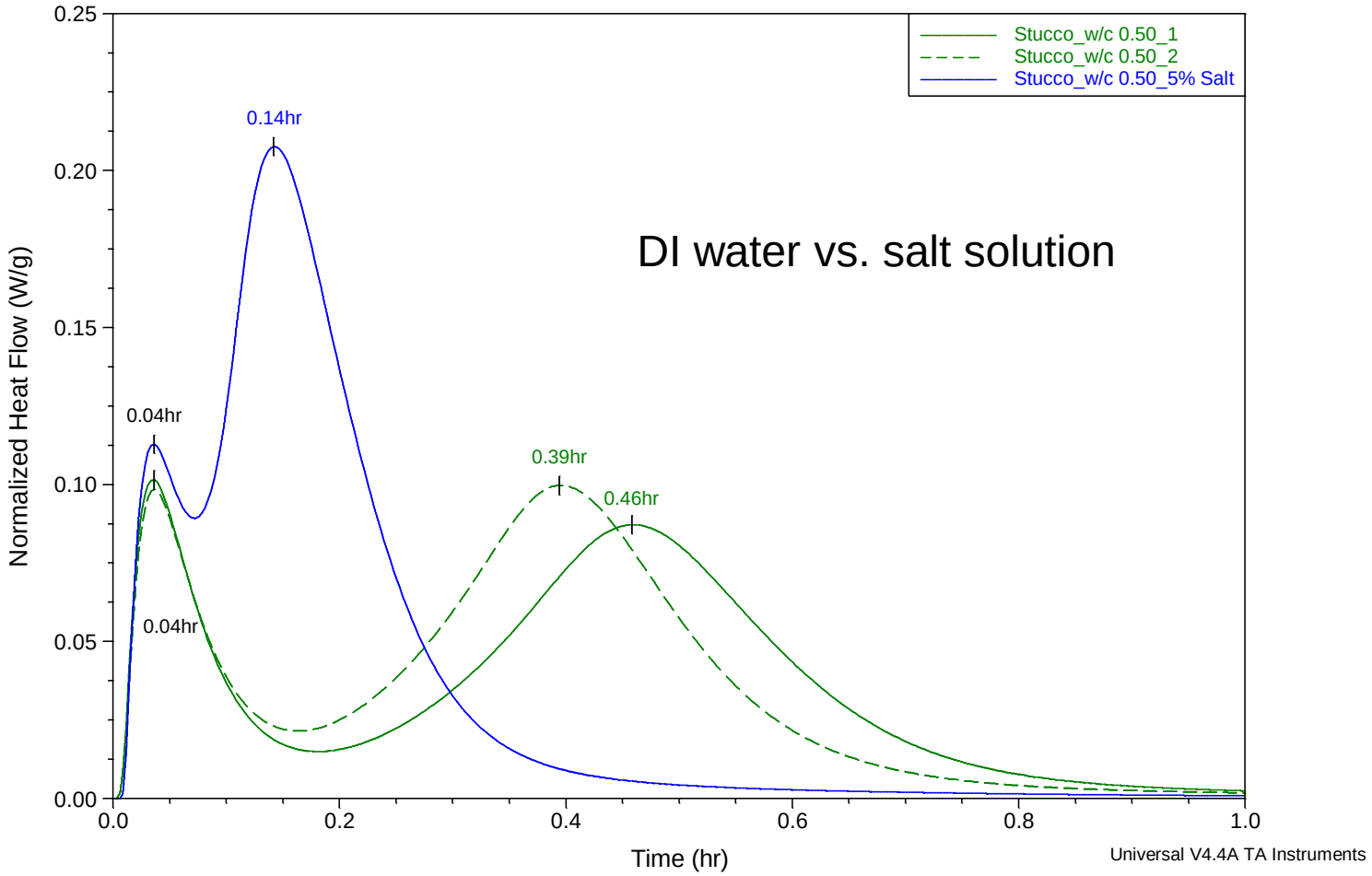
Dr. Johansson , Thermometric AB, Sweden (**2002**)

Admix Ampoule -Two Identical Ampoules

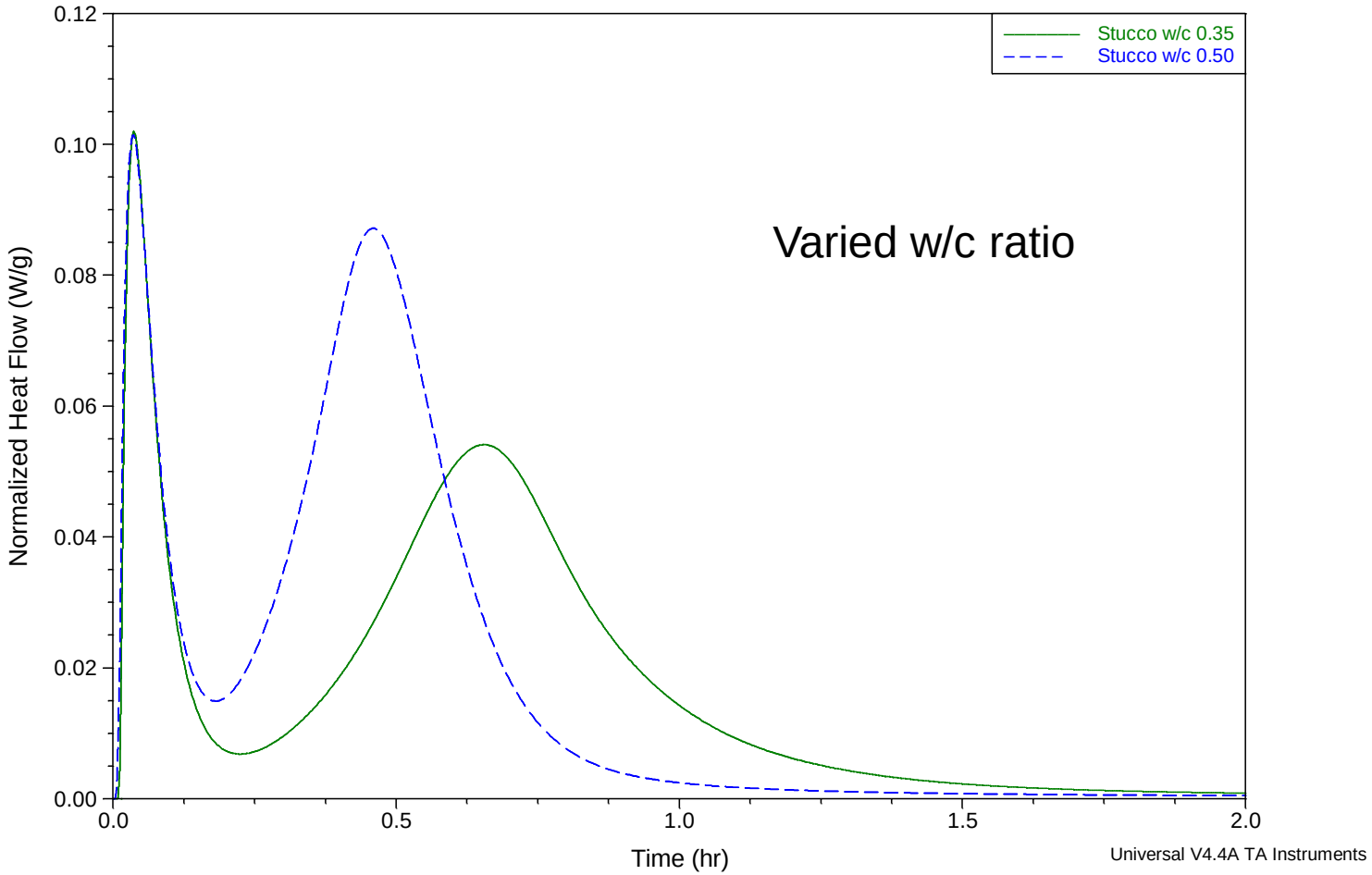


*Dr. Moro , Holcim Group Support, Switzerland (**2002**)*

Admix Ampoule Experiment



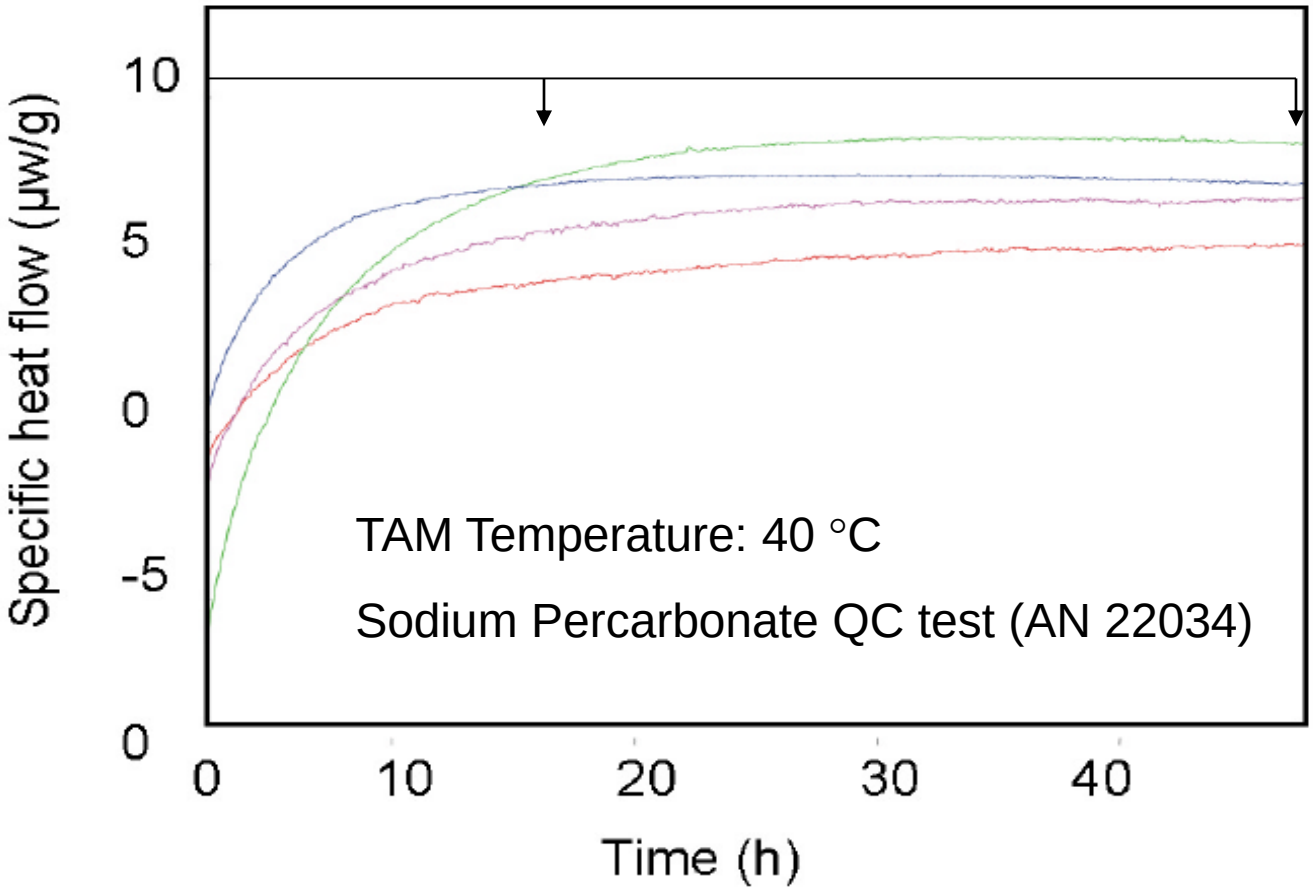
Admix Ampoule Experiment



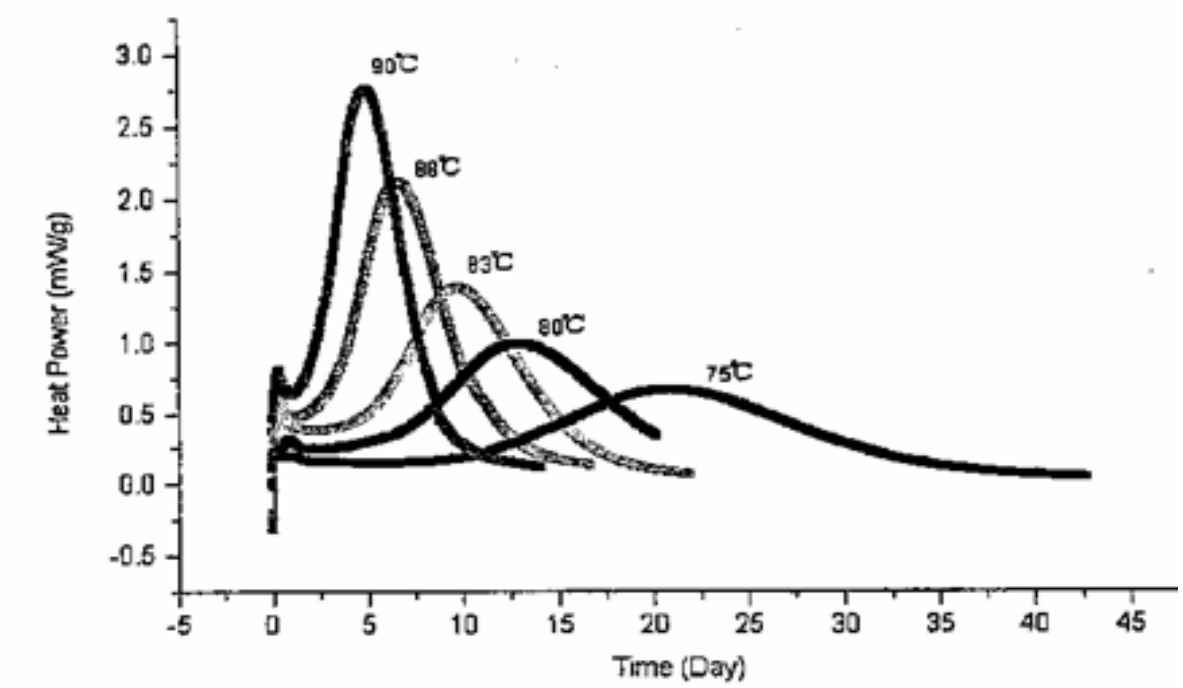
TAM Applications

Material Science and Industrial Applications

Stability with TAM



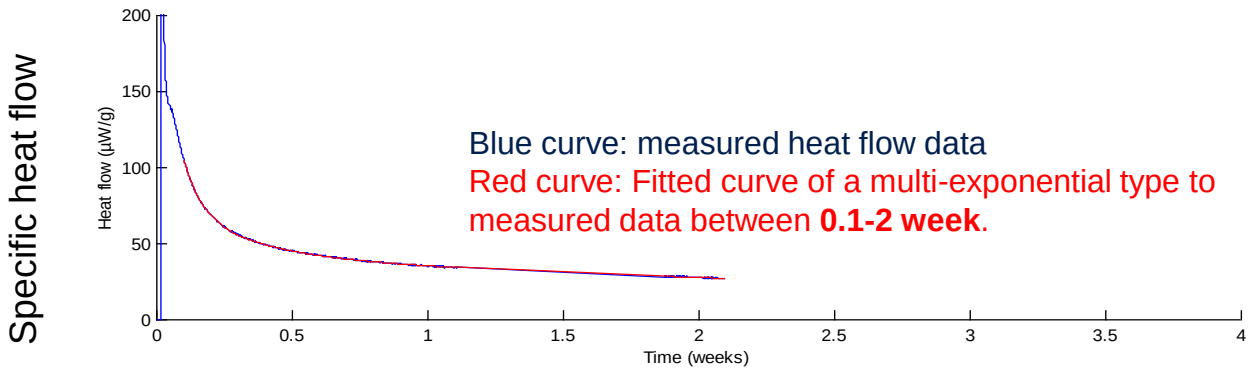
Stability of Organic Peroxides



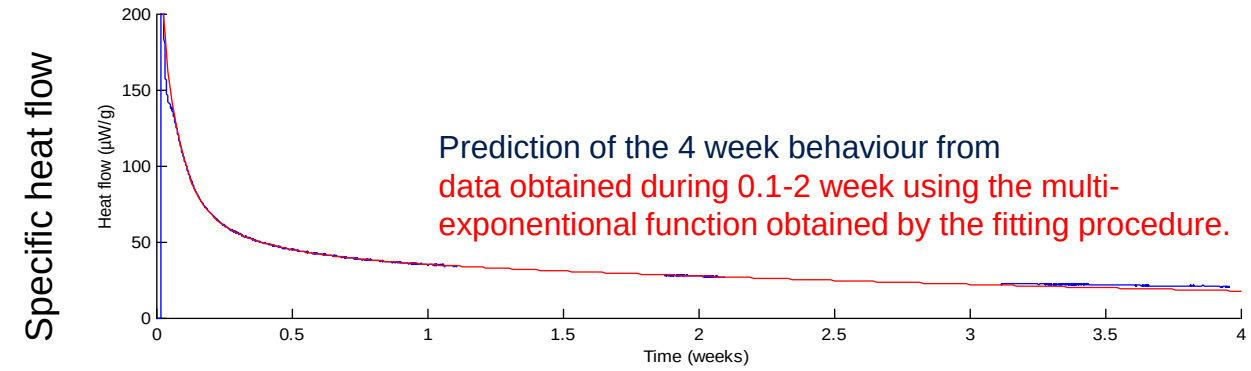
Autocatalytic behaviour of 80% wt. cumene hydroperoxide

Hou, Houng-Yi; Shu, Chi-Min; Duh, Yih-Shing; AIChE Journal, (2001), Vol 47, No 8, 1983

Long-term Behaviour from Short-term Data

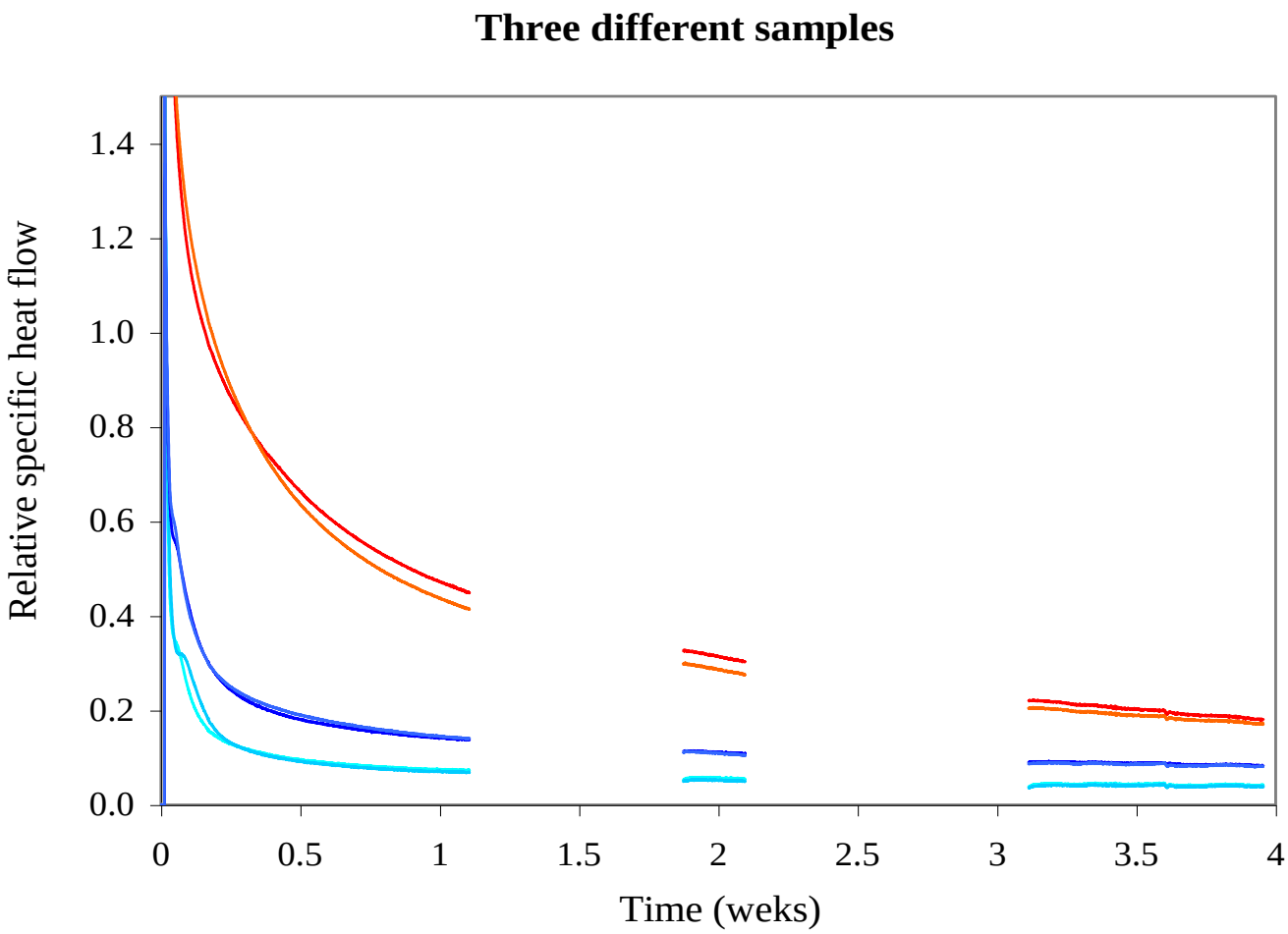


Blue curve: measured heat flow data
Red curve: Fitted curve of a multi-exponential type to measured data between **0.1-2 week**.

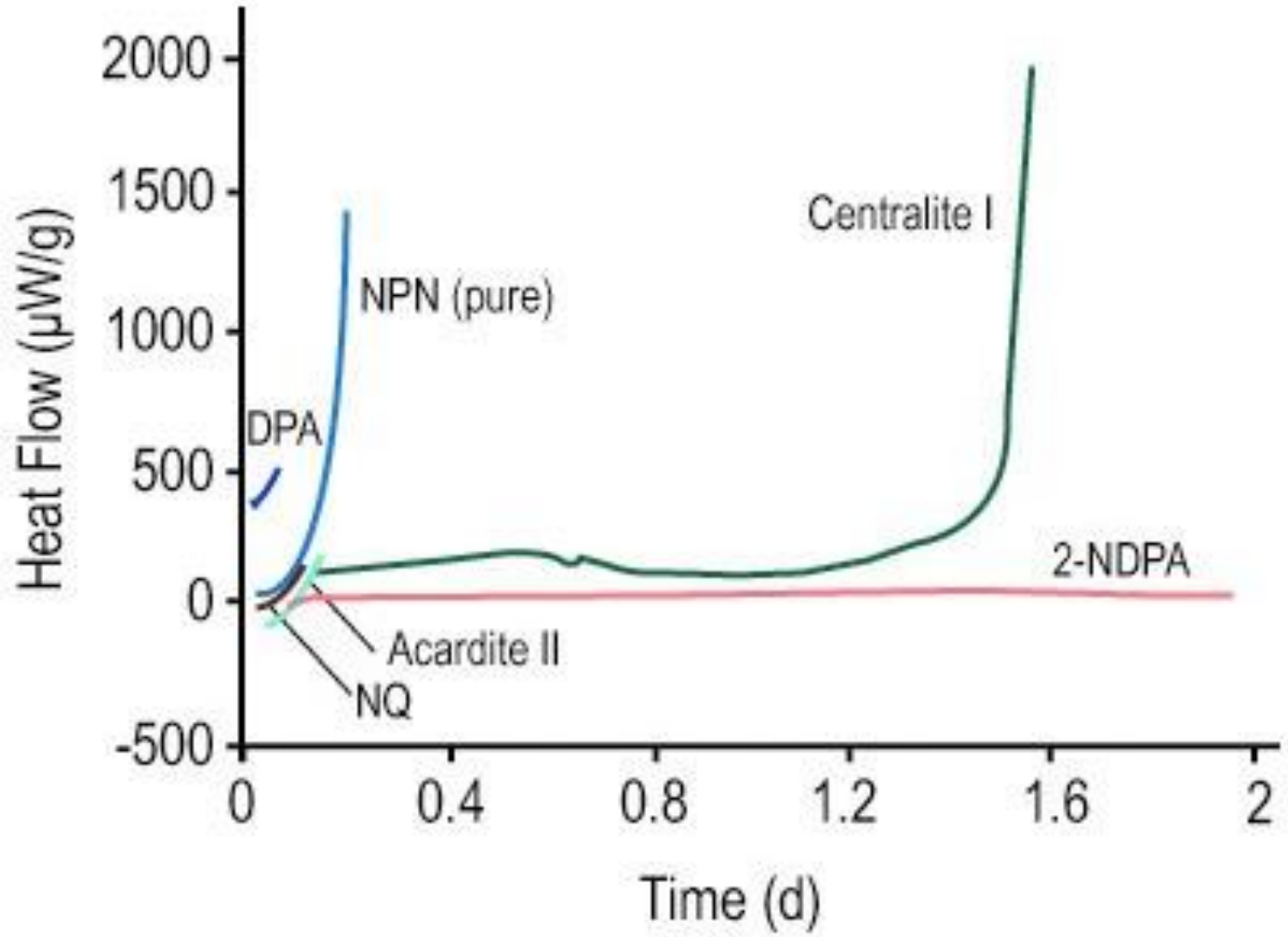


Prediction of the 4 week behaviour from data obtained during 0.1-2 week using the multi-exponentional function obtained by the fitting procedure.

Repeatability



Stabilization of an Energetic Plasticizer (NPN)



Wingborg and Eldsäter, *Propellants, Explosives and Pyrotechnics* **27**, 314 -319, (2002).

NATO Standard

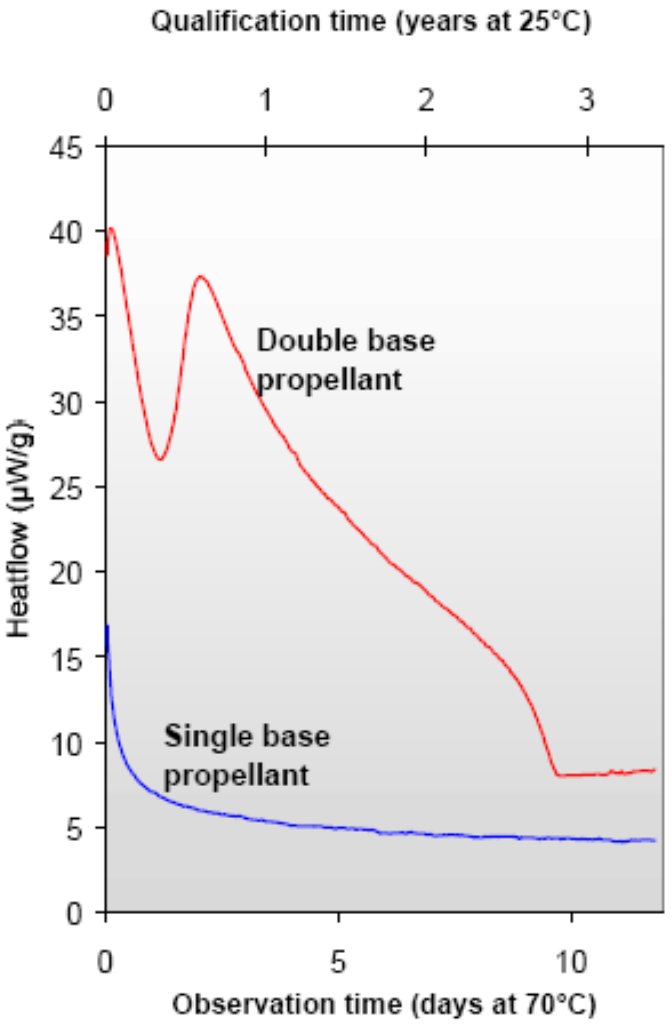
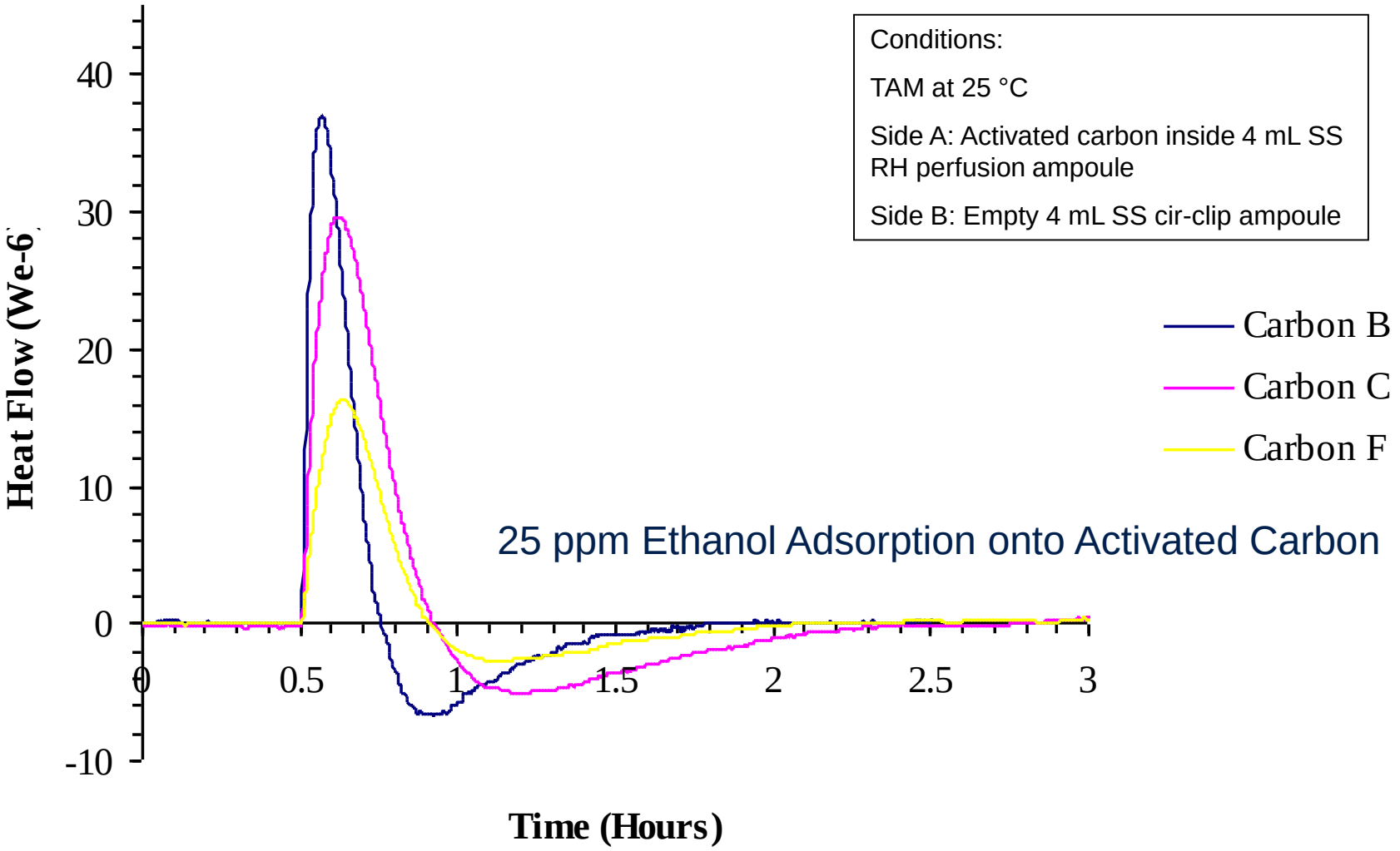


Figure from Bodycote Materials testing

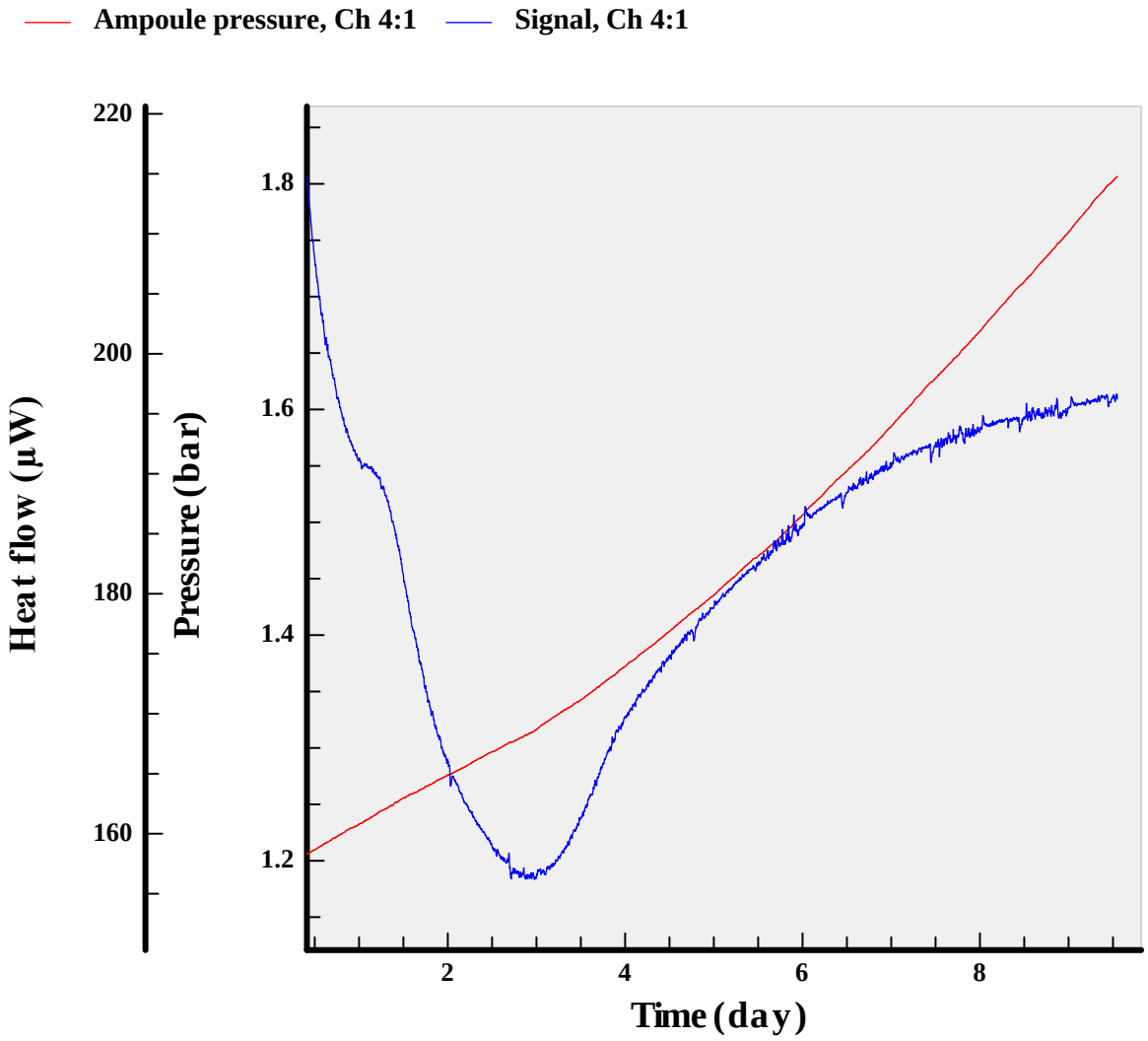
- STANAG 4582 – ratification in process
- Dedicated for stability testing of nitrate ester based propellants by use of heat flow calorimetry (HFC)
- A method to establish chemical stability of SB, DB and TB propellants for a minimum of 10 years when stored at 25 °C

RH Perfusion – Two Gas Sources

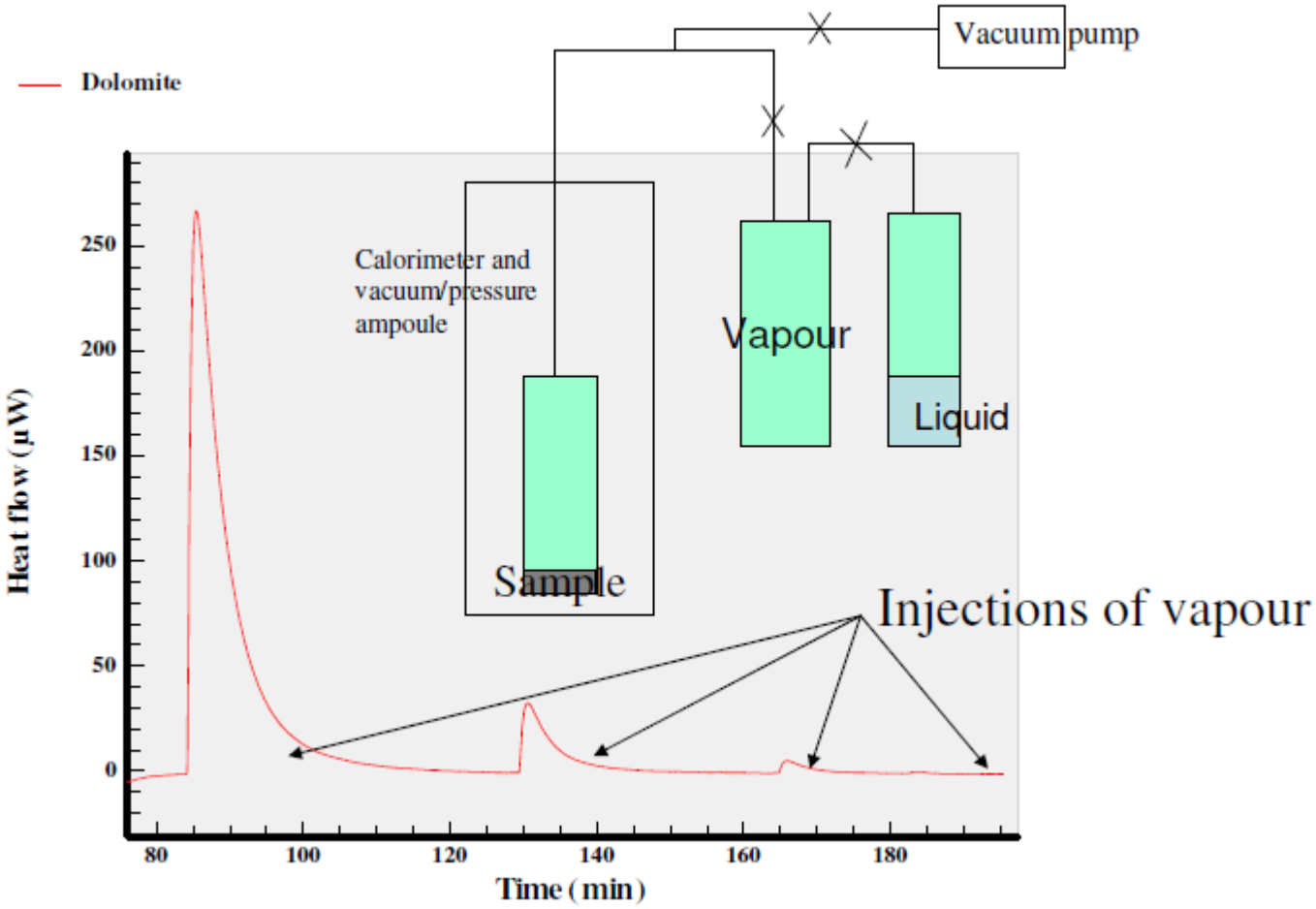


Note: Each sample weighed approximately 15 mg. Heat flow signal was not normalized.

Percarbonate – Vacuum/Pressure Ampoule



Moisture Sorption - Vacuum/Pressure Ampoule



Dolomite = Calcium magnesium carbonate $CaMg(CO_3)_2$

Oxidation of Polymers

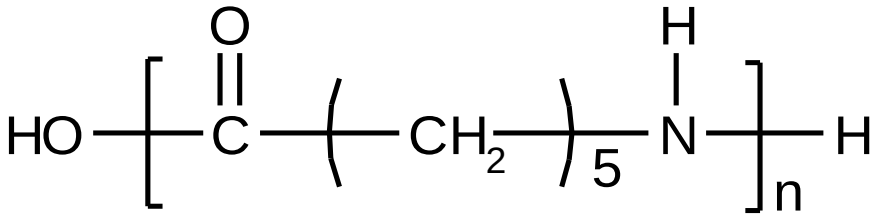
- Radical chain reaction
  - Initiation, Propagation, Termination, and Chain branching
- Important intermediates
  - Hydroperoxides and radicals
- Solid state oxidation may be heterogeneous in nature
  - Localization and spreading
- Stabilizers are usually added to prevent oxidation
- Effects of prolonged oxidation
  - Chain scission and embrittlement

Characterization of Oxidation

- The effects of oxidation is typically studied by analyzing the formation of volatile, non-volatile and polymer bound oxidation products as a function of time.

- Alternative: Use rate-sensitive techniques to monitor the in-situ oxidation.

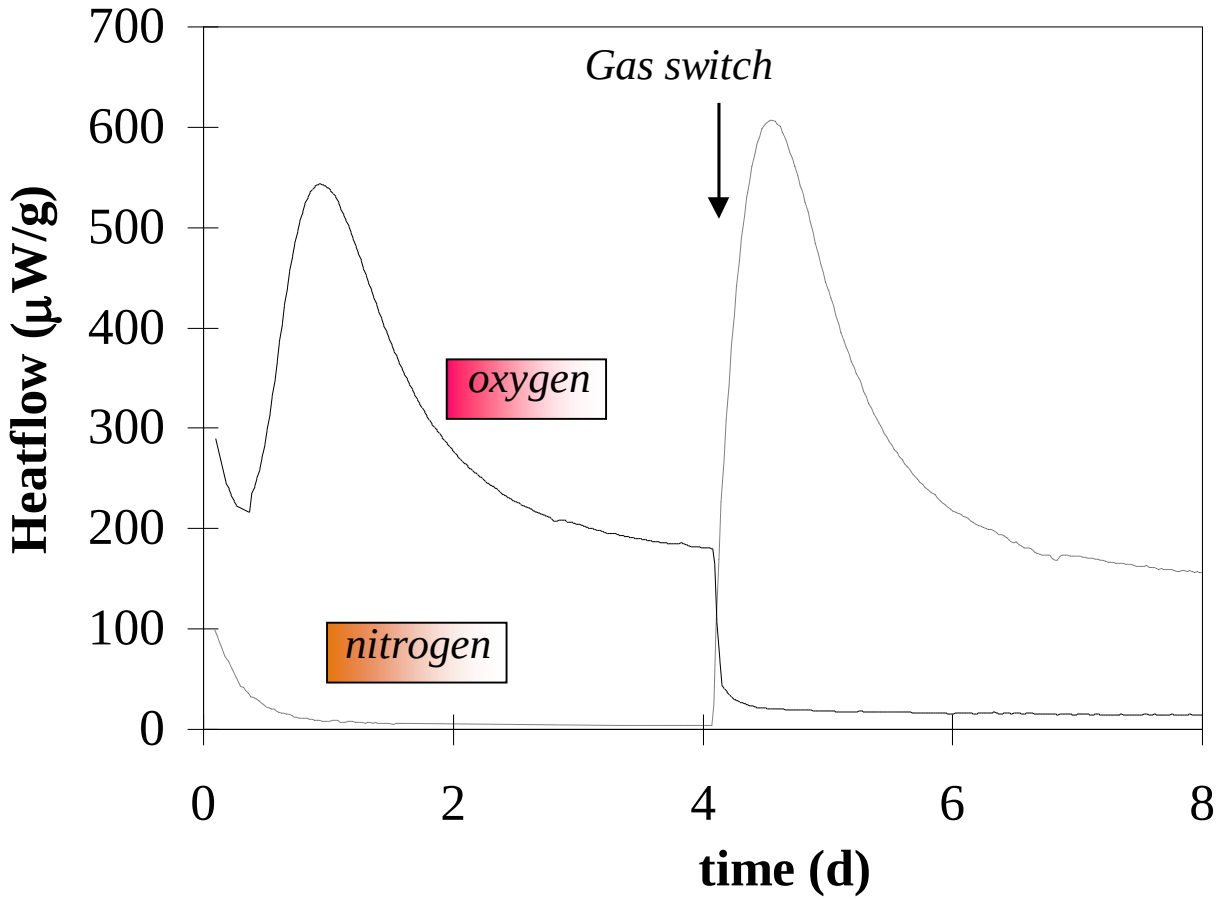
Polyamide 6



- Polyamide 6 film from Nyltech, Italy
- Thickness: 40 µm
- Crystallinity: 40 %
- Melting point: 210°C
- Glass transition temp: 70°C (dry), -10°C (100 % r.h.)
- Stabilizer: Irganox 1098 (hindered phenol)
- Sensitive to oxidation!

Response to Nitrogen / Oxygen

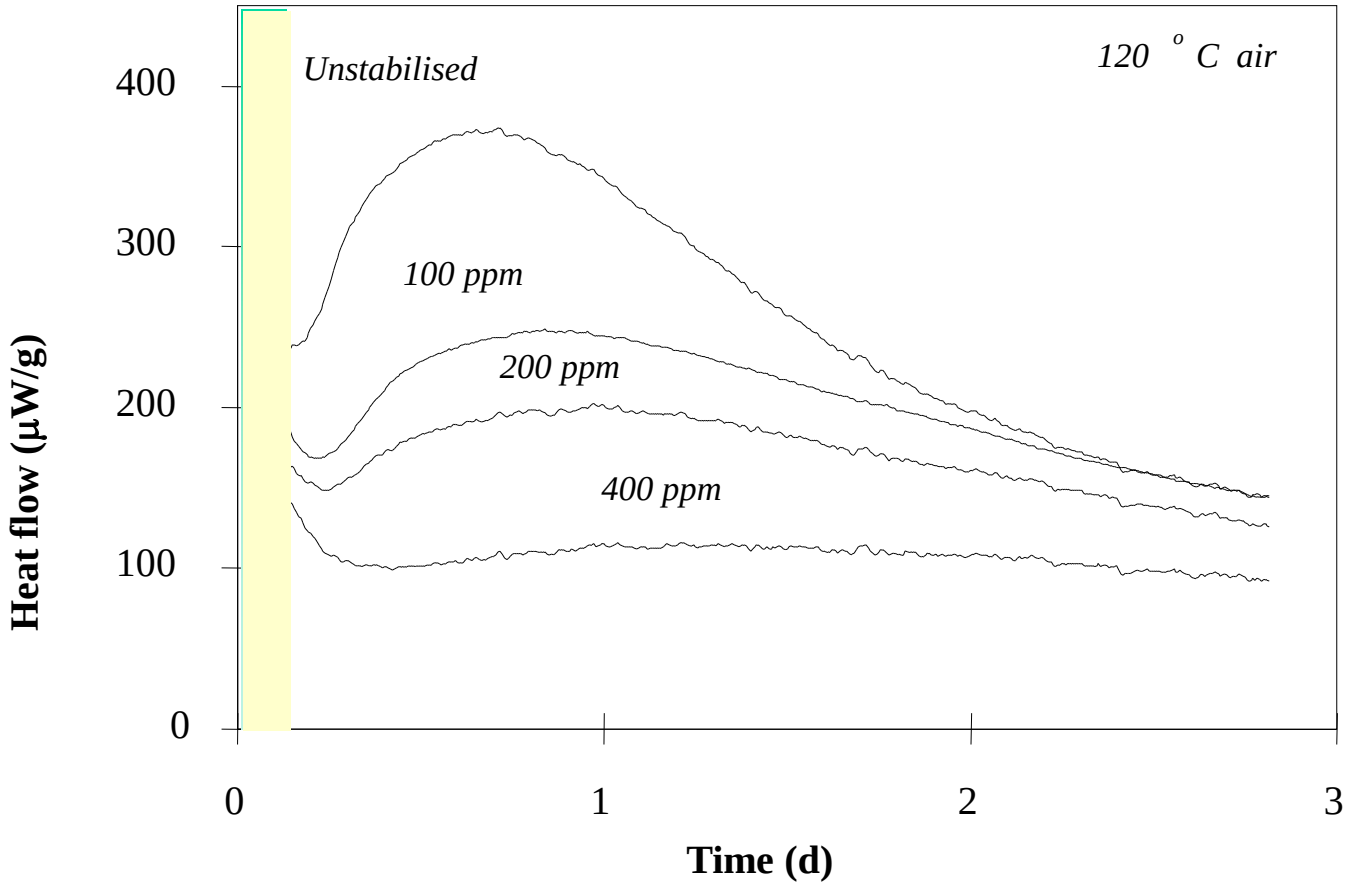
Heat flow curves for unstabilized polyamide 6 film exposed to nitrogen and oxygen at 110°C. After four days, the gases were switched .



Forsström, D., Thesis,"Novel techniques for characterisation of the oxidative stability of polyamides", KTH, Dept. Polymer Technology (1999)

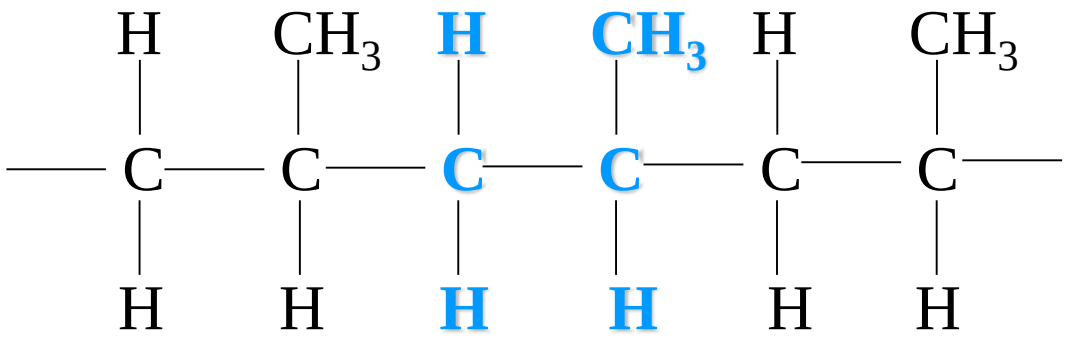
Efficiency of Stabilizers

Influence of Irganox 1098 on the Heat Flow time curve for PA6. The presence of the stabiliser suppresses the heat flow signal indicating the preventing action.



Forsström, D., Thesis,"Novel techniques for characterisation of the oxidative stability of polyamides", KTH, Dept. Polymer Technology (1999)

Polypropylene (PP)



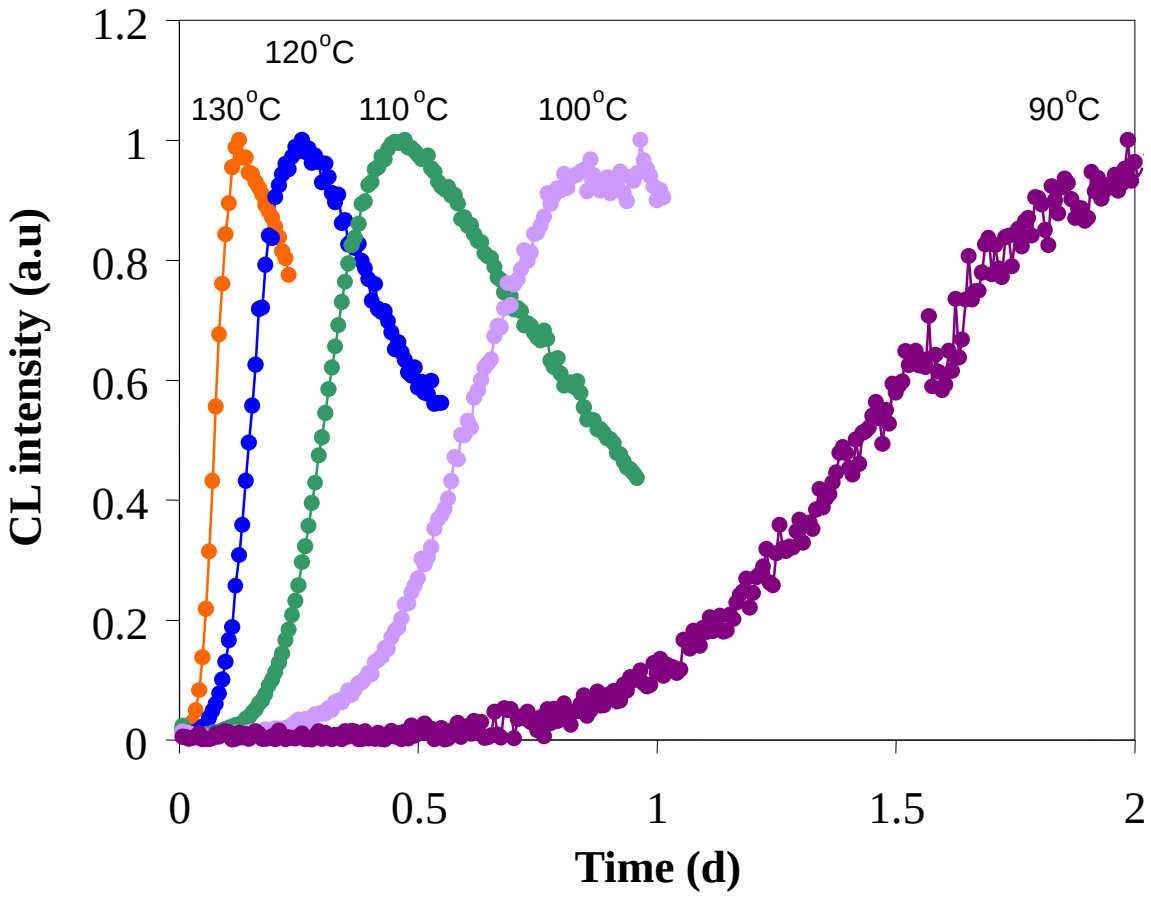
- Isotactic PP from DSM Research
- Powder: Individual particle mass: 50 – 100 µg
- Crystallinity: 53 %
- Melting point: 165°C
- Glass transition temp: -10°C
- Sensitive to oxidation at ambient conditions!

Rate-Sensitive Techniques

- Thermal Activity Monitoring (TAM)
  - Measure the heat flow by the use of thermopiles (J/s)
  - TAM is a non specific technique, i.e. sensitive to all heat producing processes (physical and chemical)
  - TAM is a quantitative technique

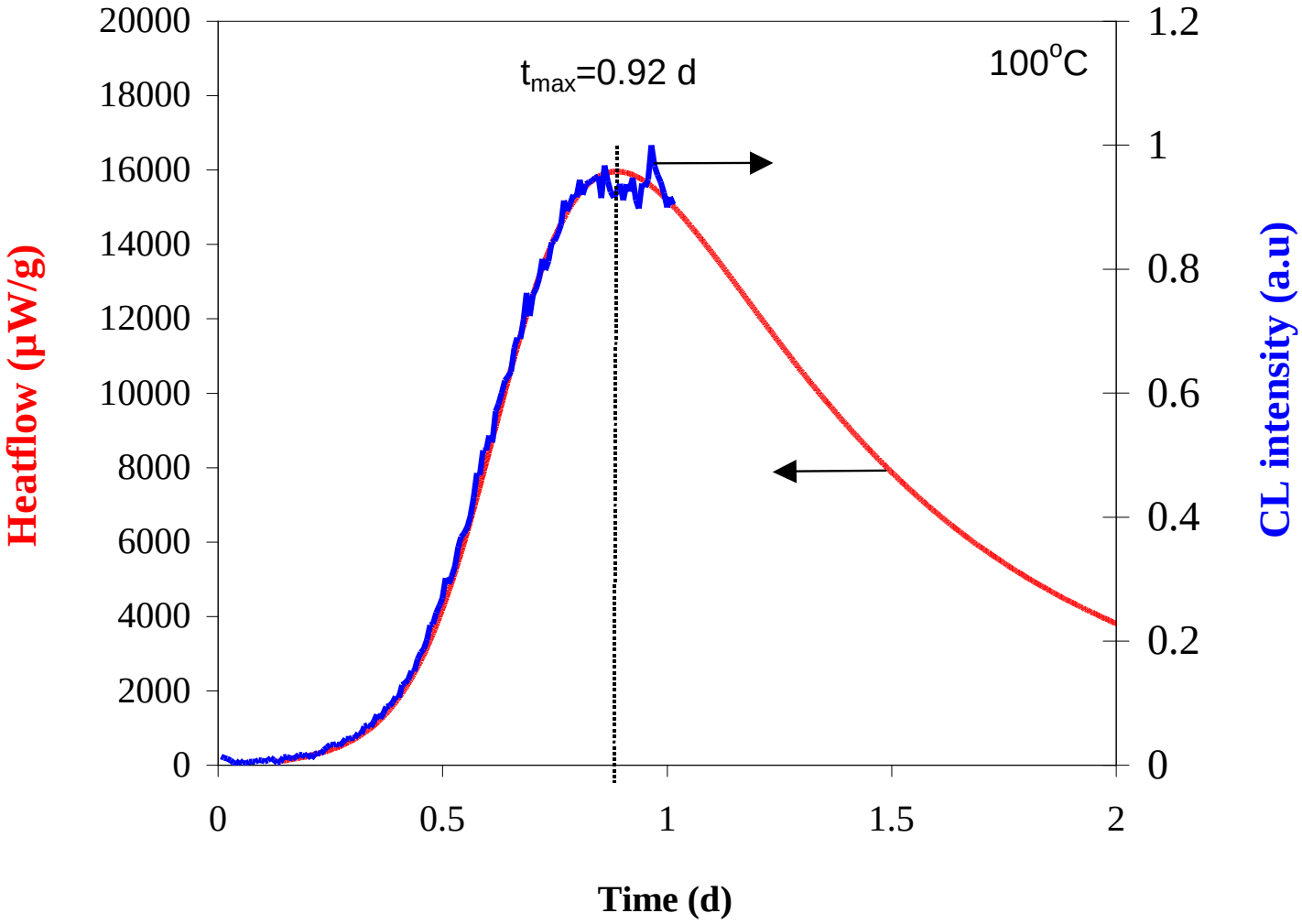
- Chemiluminescence Techniques – CL / ICL
  - Measure the light intensity by the use of a photon multiplier (counts/s)
  - CL is sensitive to all light-producing reactions
  - The most referred mechanism to explain the CL emission is the Russel mechanism *i.e*. a bimolecular termination reaction of peroxy radicals.

ICL - Oxidation of Unstabilized PP Powder in Air

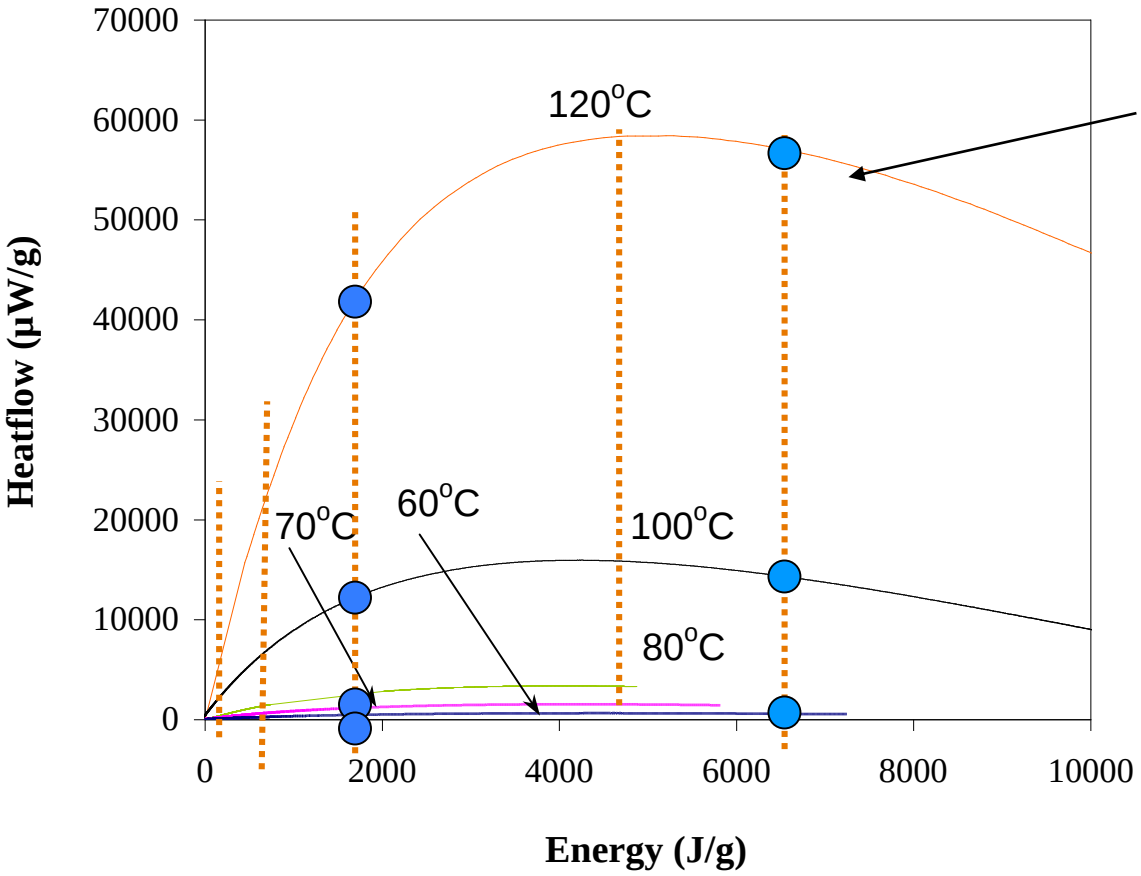


- The "oxidation rate" is not constant but vary in a characteristic way.
- The service life of the PP sample roughly corresponds to the onset of the accelerating part of the curve.

TAM ⇔ ICL 100°C


20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
Heatflow (µW/g)
t_{max}=0.92 d
100°C
1.2
1
0.8
0.6
0.4
0.2
0
CL intensity (a.u)
0
0.5
1
1.5
2
Time (d)

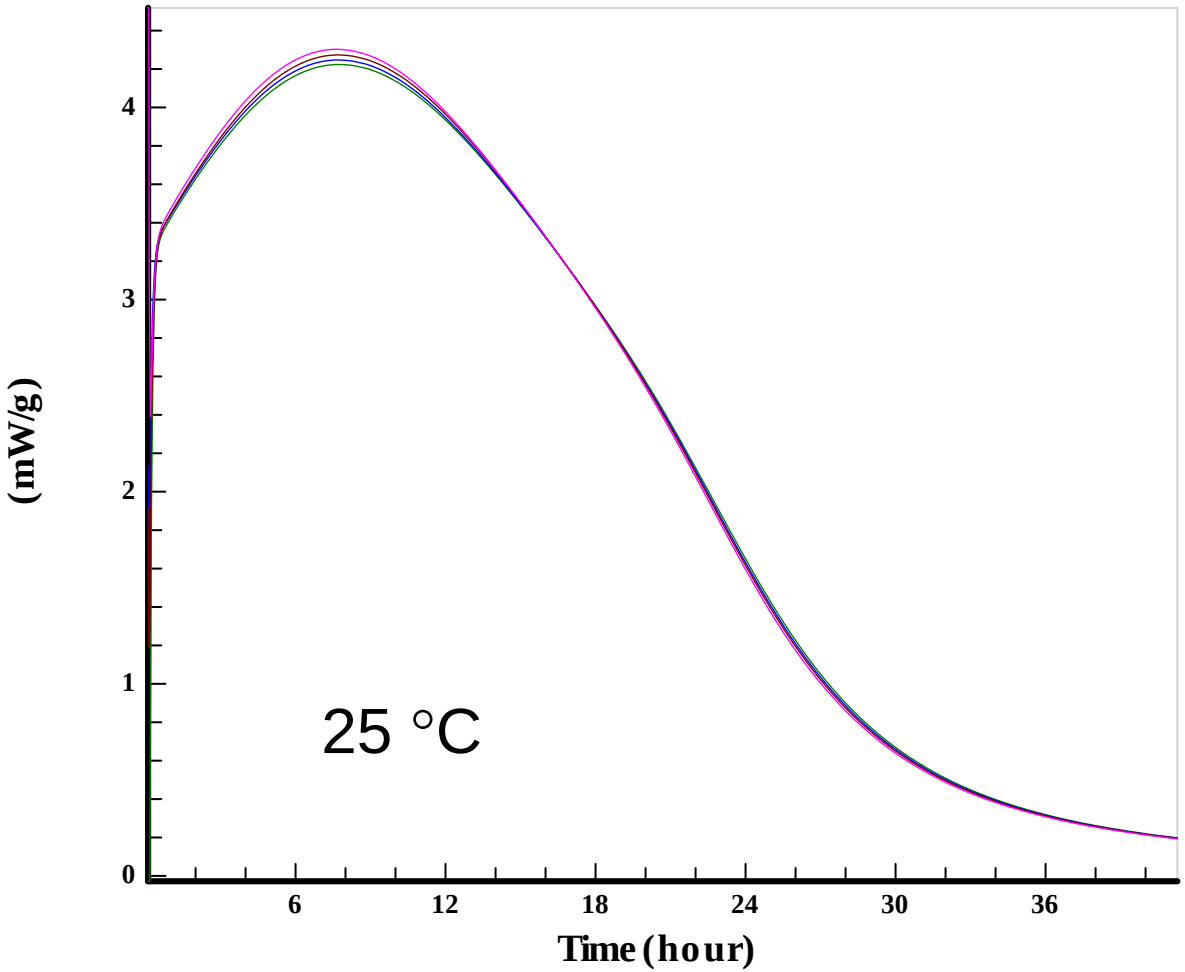
TAM: Heat Flow versus Energy

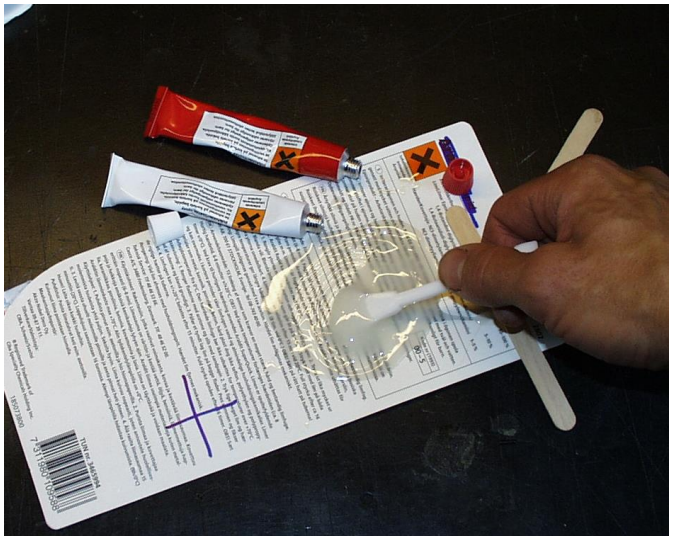


- Heat flow values are known at different temperatures for different Energies.

- Thus, an apparent activation energy (E_a) can be calculated as a function of the 'extent of oxidation'

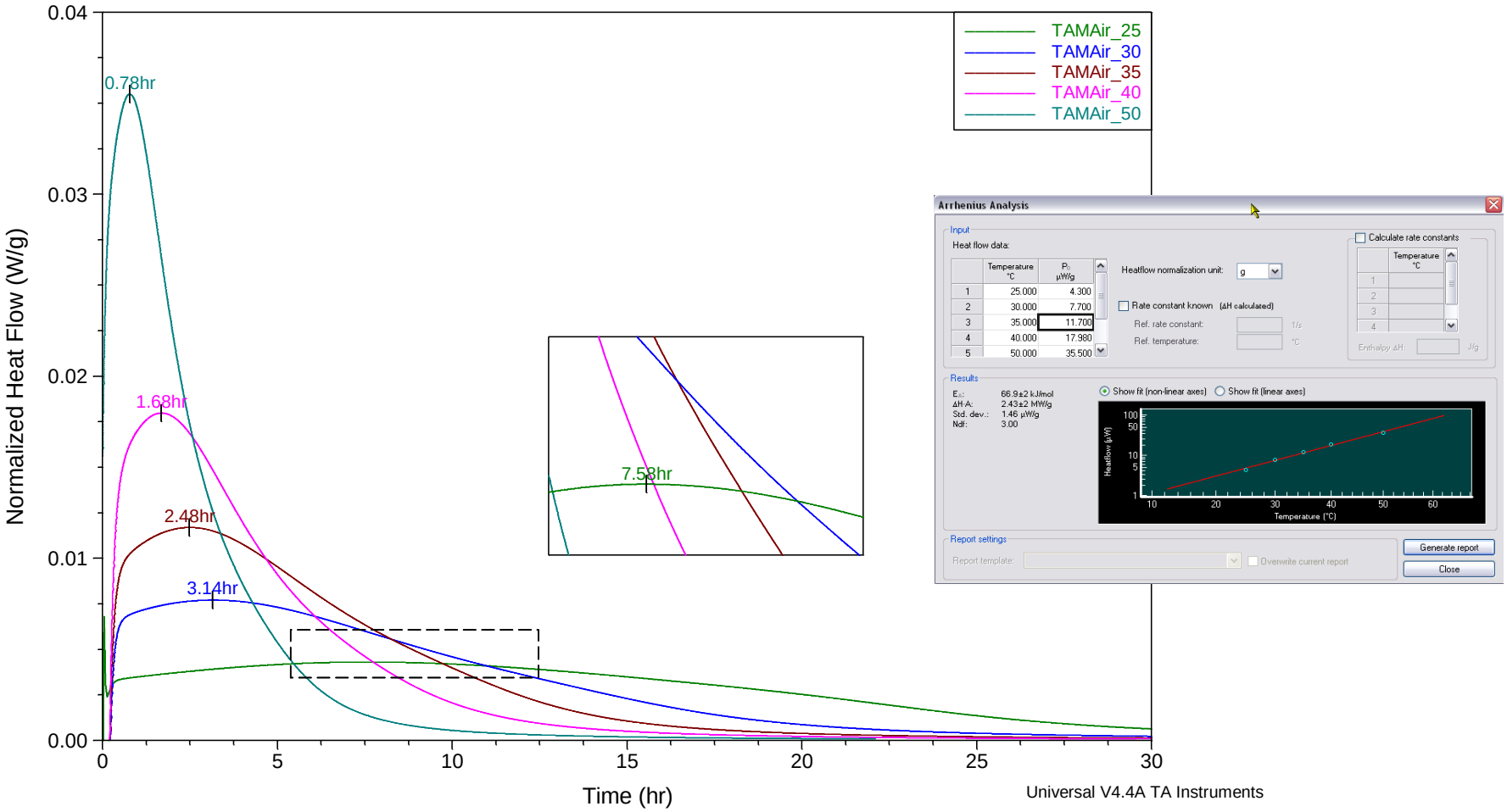
Curing of Epoxy in TAM Air





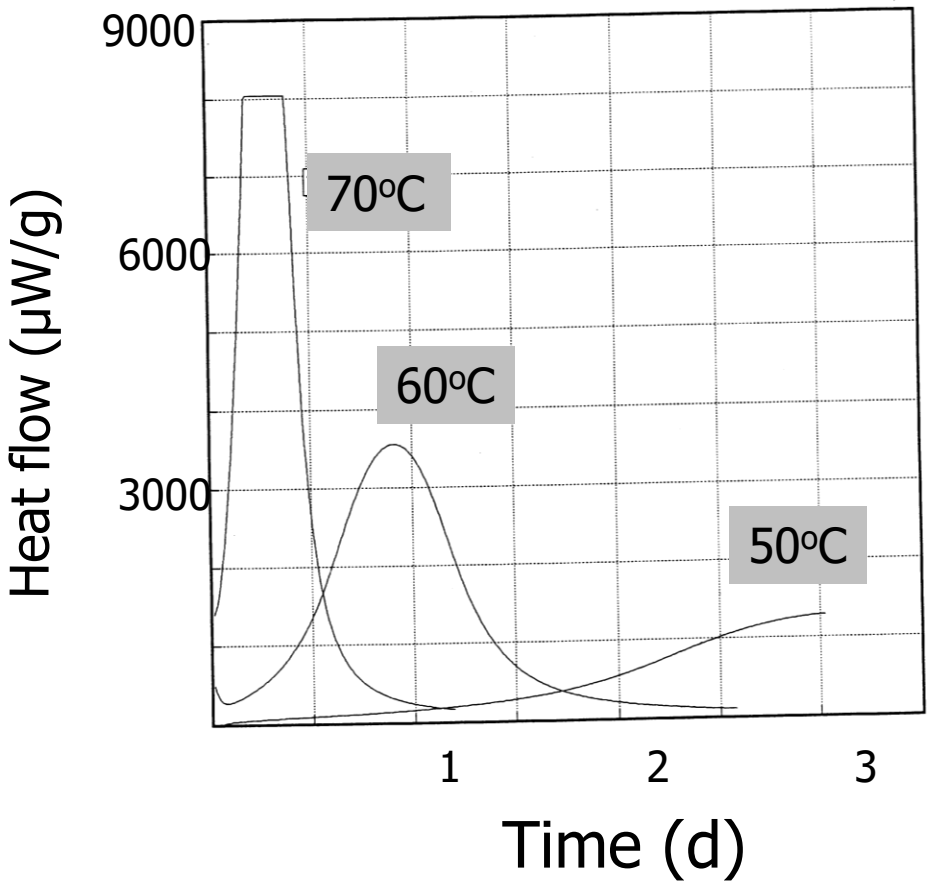
- D.E.R. 331 epoxy resin from Dow Chemical
- Jeffamine D-230 hardener from Huntsman

Curing of Epoxy in TAM Air



- D.E.R. 331 epoxy resin from Dow Chemical and Jeffamine D-230 hardener from Huntsman

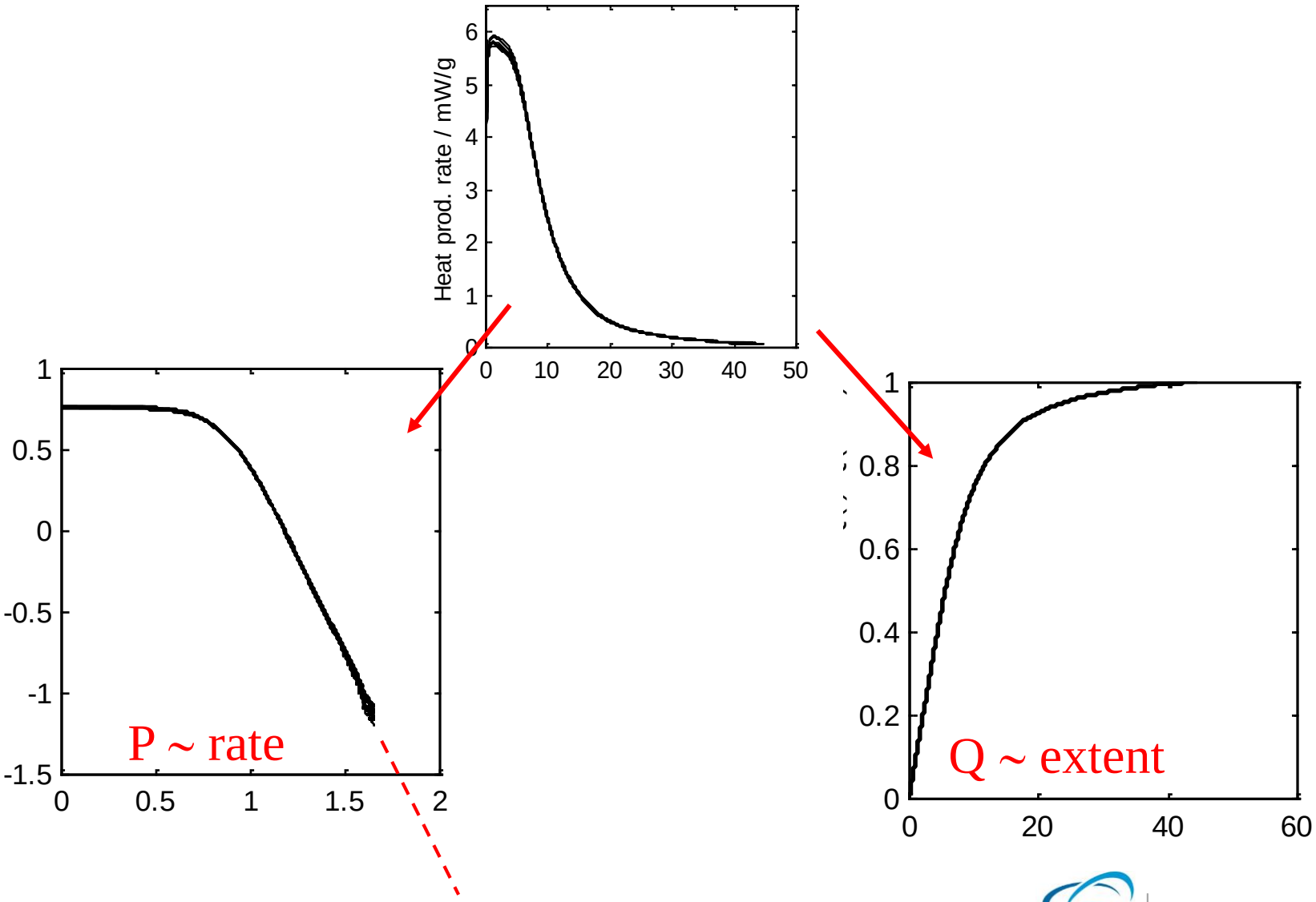
Adhesive: ESP 110



- Heat flow values are in the range of mW/g at 50°C and increases with temperature.
- Possible exothermic reactions: curing, oxidation.
- ESP 110 – usually cured at 150°C
- Closed 3 ml glass ampoules

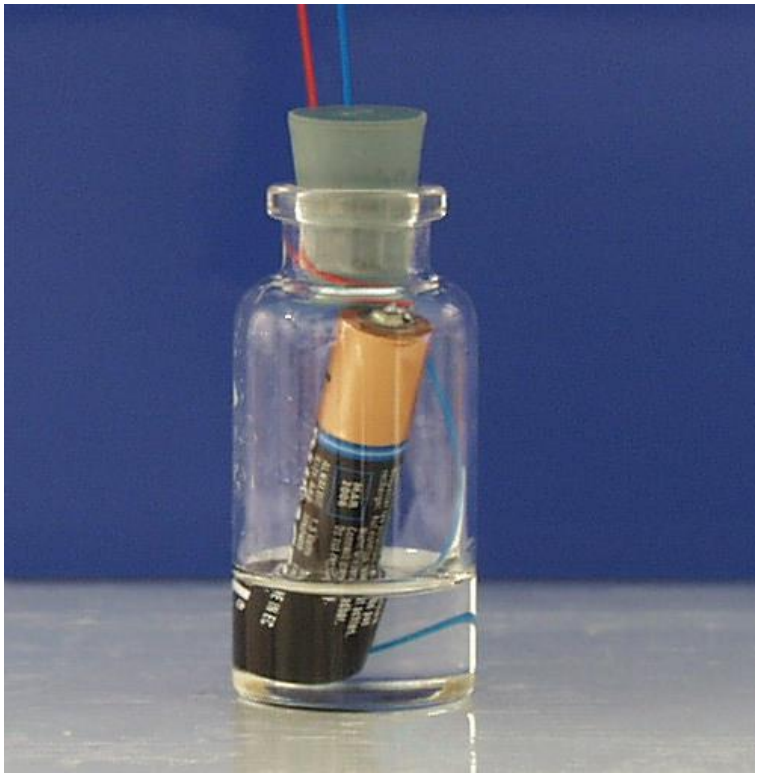
Data by Lars-Gunnar Svensson, Celsius Materials CMK, Karlskoga, Sweden

Epoxy Curing

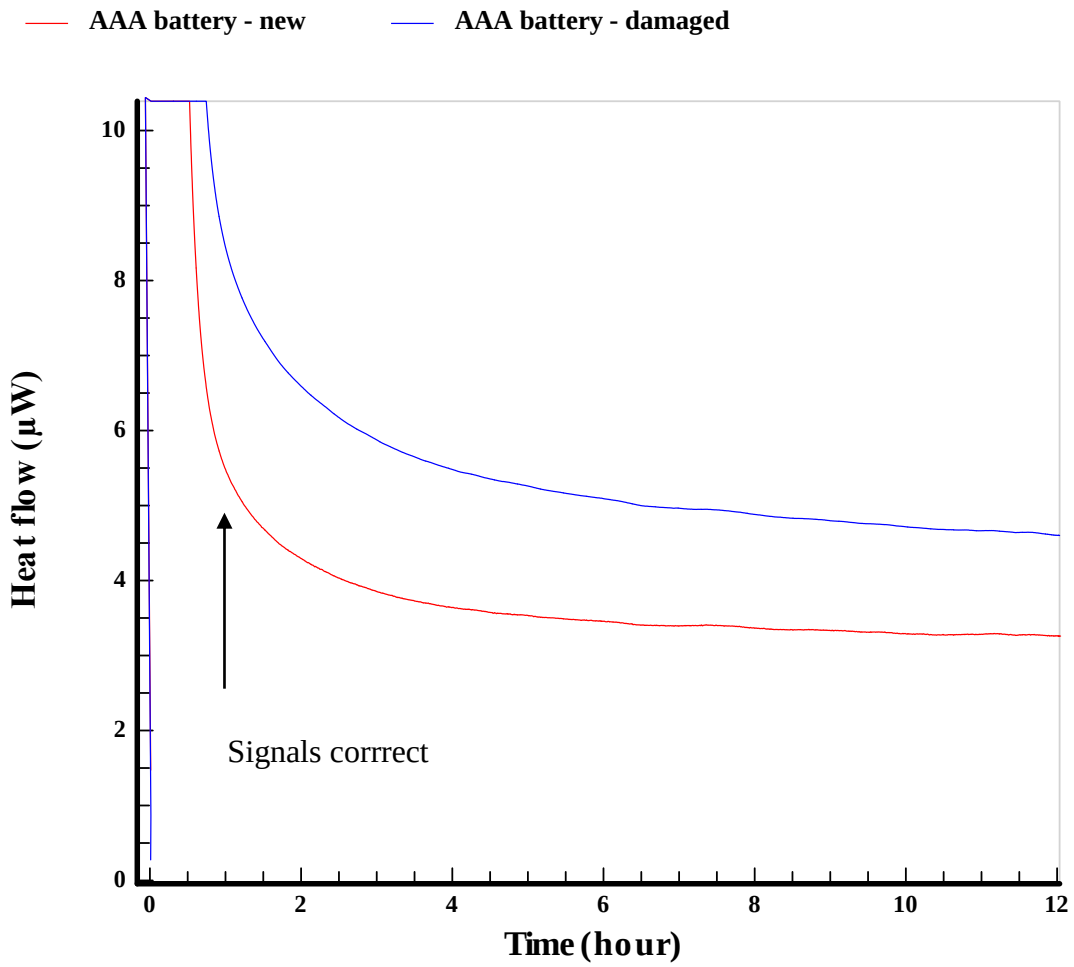


Battery Discharge

- Standard AAA battery in calorimeter discharged through a load or a resistance.



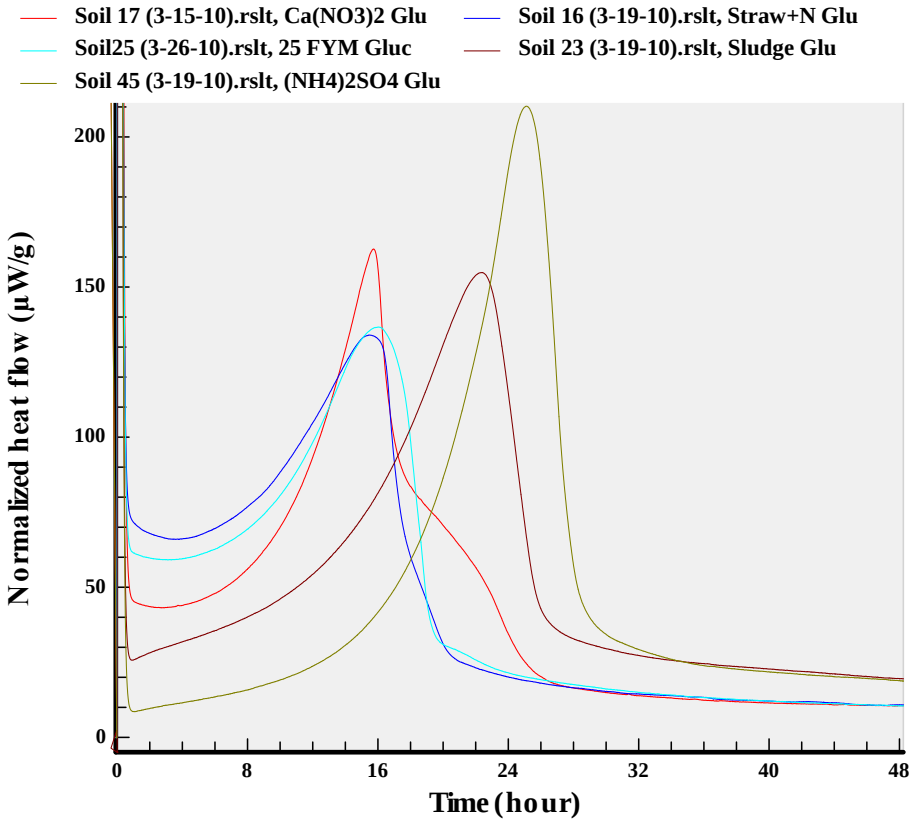
Self-Discharge (No Load) of Alkaline Battery



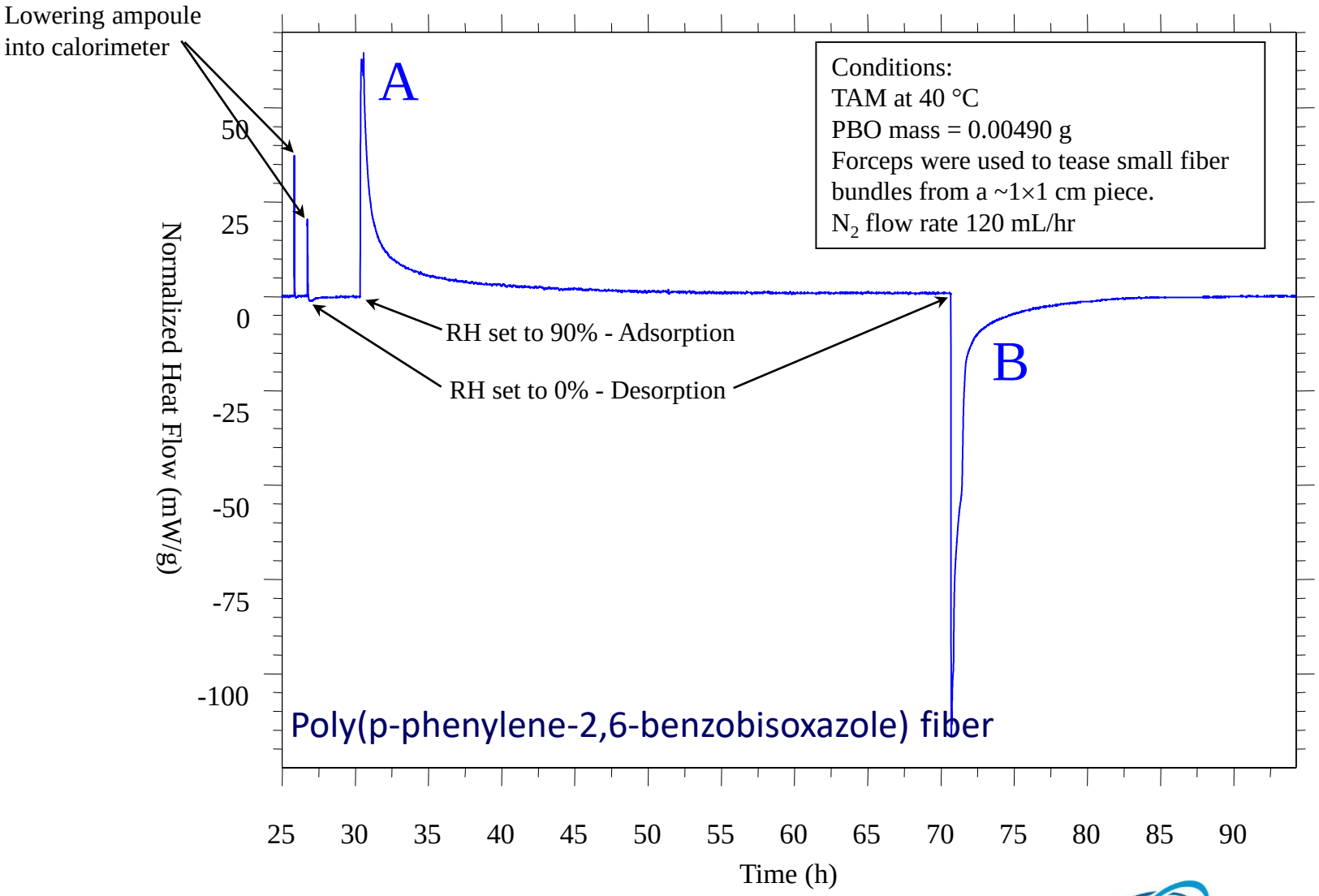
- TAM III at 25 °C
- 4 mL SSt screw cap ampoules

Larger Sample Applications

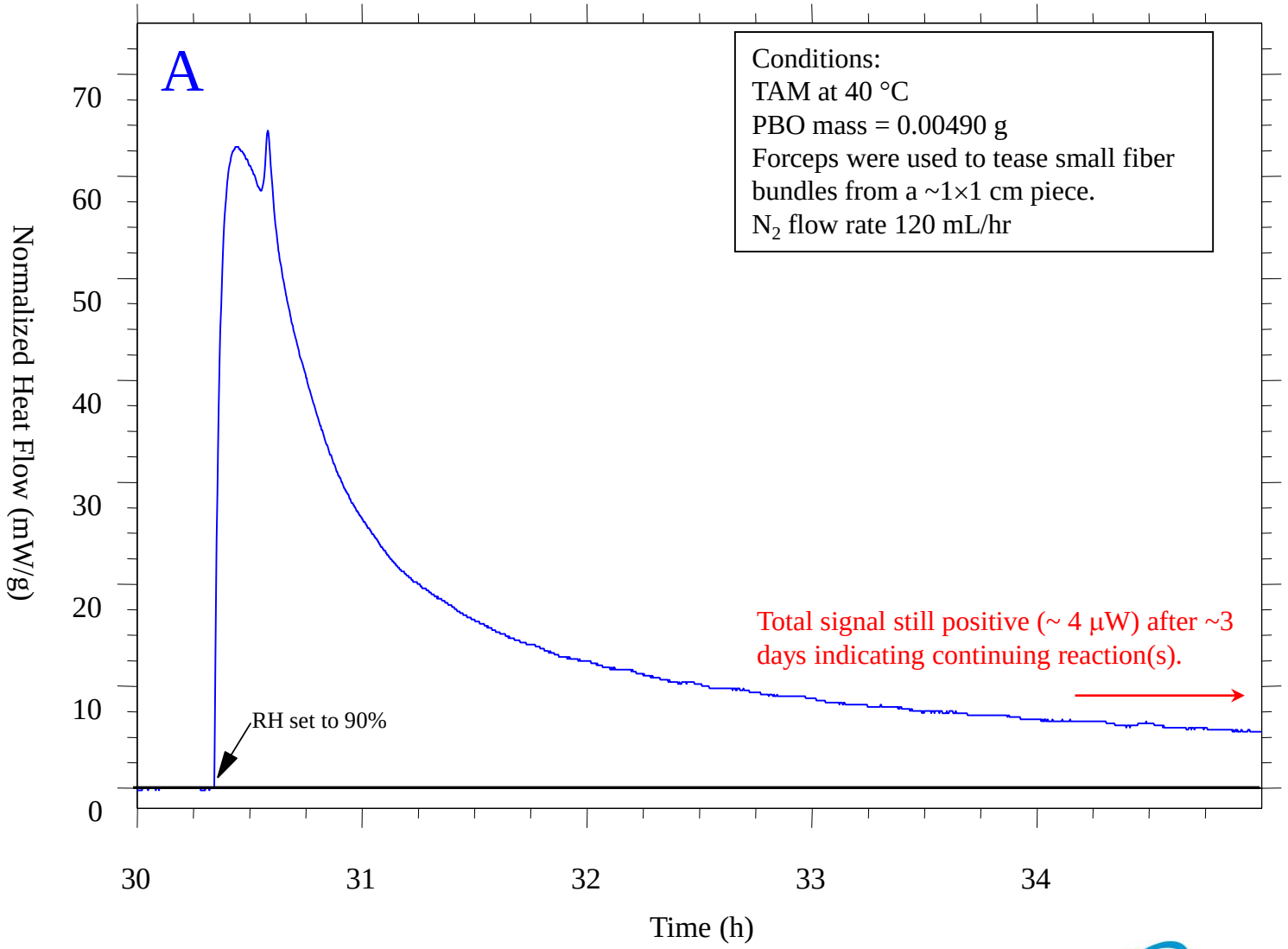
- Environmental science
- Food applications
- Large samples



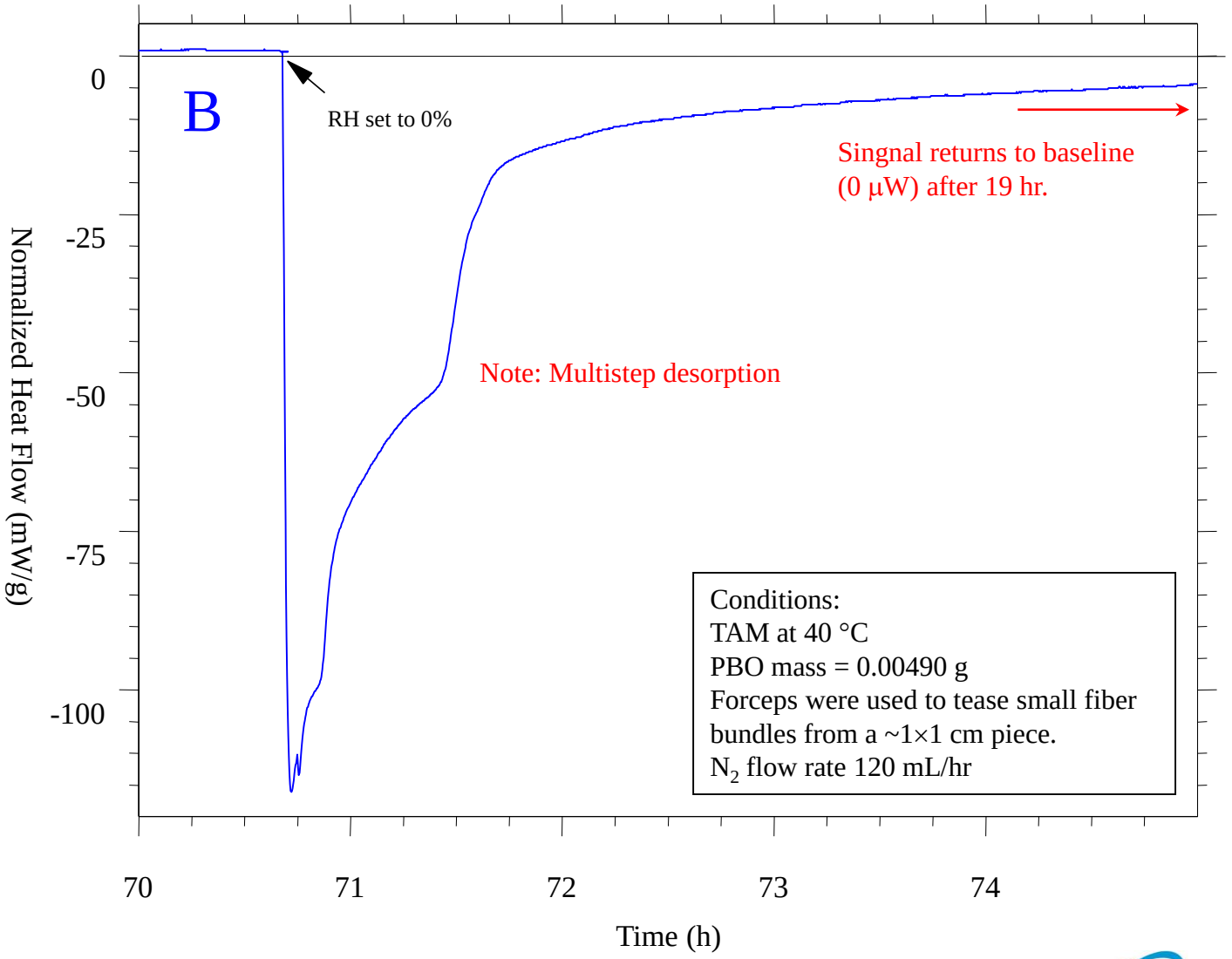
RH Perfusion – Step RH



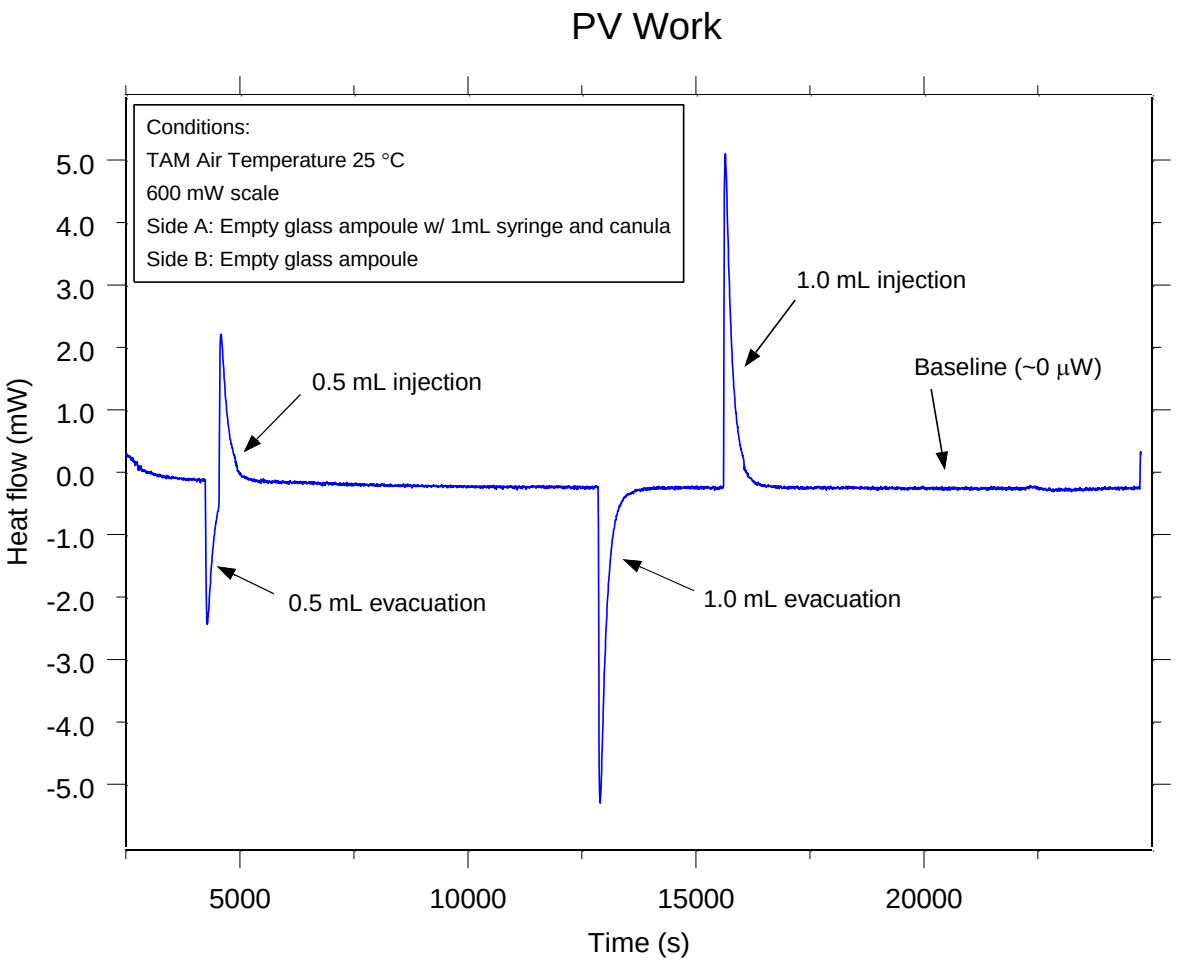
RH Perfusion – Step RH



RH Perfusion – Step RH



Pop Quiz!!!



- Lowering ampoules will create frictional heat and work (or pressure) on the calorimeter due to small air gap between ampoule and calorimeter wall.
- This example shows that heat flow increases as pressure in the ampoule increases.

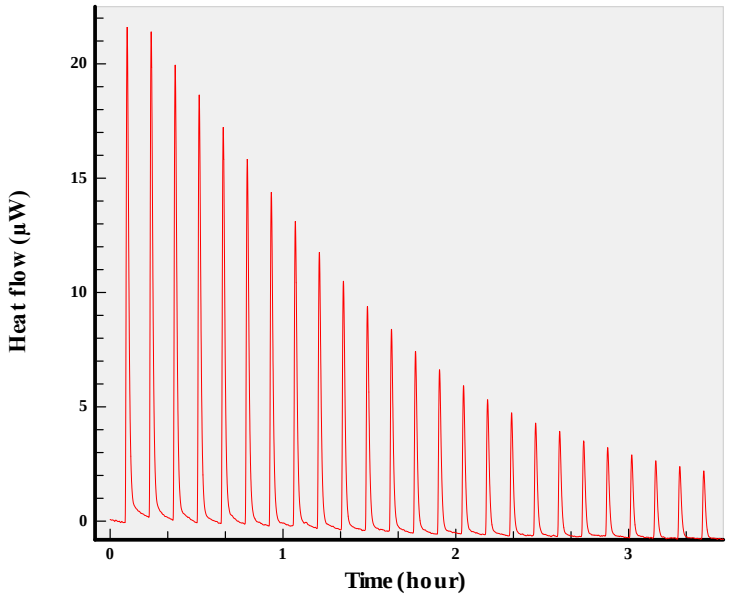
TAM Applications

Isothermal Titration Calorimetry

ITC in Biochemistry/Biophysics

An important technique for the characterization of molecular interactions and for the understanding of binding thermodynamics.

Most commonly utilized for biological molecules and biological systems.



ITC in Biochemistry/Biophysics

- A premier tool for the characterization of biological macromolecules
  - Antigen – Antibody
  - Peptide – Protein
  - Lipid – Protein
  - Nucleic Acid – Protein
  - Carbohydrate –Protein
  - Small Molecule/Drug – Protein
  - Protein – Protein
  - Protein – Receptor (soluble and membrane-bound)

Strength of TAM-ITC Relative to Other Methods

- High accuracy and precision
- General applicability
- No chemical modification necessary
- No immobilization necessary, although possible
- Equilibrium conditions
- Not limited by turbid or particulate suspensions
- Removable cell (important for toxic or radiological samples)

Binding Characterization

- Binding Affinity, K
  - Tightest binding measurable approaches nanomolar
    Weakest binding for biological macromolecules millimolar
- Binding Stoichiometry, n
- Binding Thermodynamics, ΔH, ΔG, ΔS and ΔC_p

  ΔH measure of the heat released or absorbed
  ΔC_p measure of the temperature dependence of ΔH ($d\Delta H/dT$)
  ΔG measure of the equilibrium constant ($\Delta G = -RT\ln K$)
  ΔS measure of ”order” in the system ($\Delta G = \Delta H - T\Delta S$)

Determination of Binding Constants

$$\Delta G = -RT\ln K_a = \Delta H - T\Delta S$$

- The more negative ΔG is, the higher the binding affinity
- Negative ΔH favors the reaction
- ΔS is positive for entropically-driven reactions
- ΔC_p determined from performing binding experiments at different temperatures, plotting T vs. ΔH. Slope is ΔC_p
- Different combinations of ΔH and ΔS can give same ΔG and thus K_a, but *not* necessarily the same specificity
- An ideal thermodynamic profile is a balance between hydrophobicity (solvent repulsion) and hydrogen bonding (target attraction)

The Titration Experiment

- The relation between ΔH and K

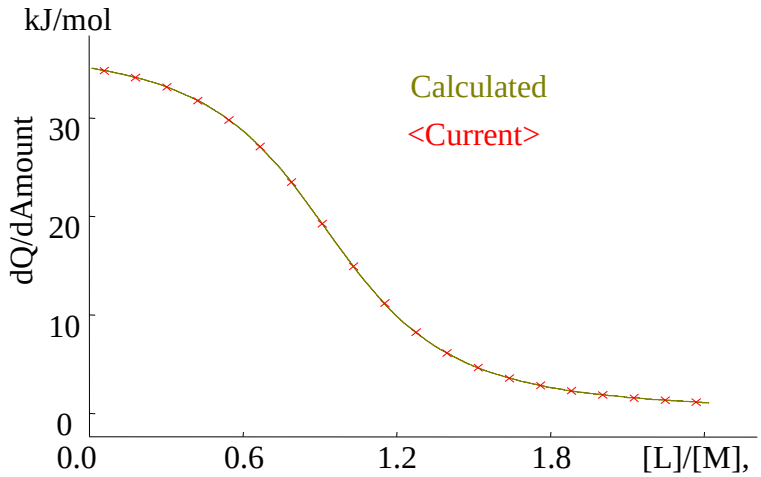
$K_a = 1/K_d$

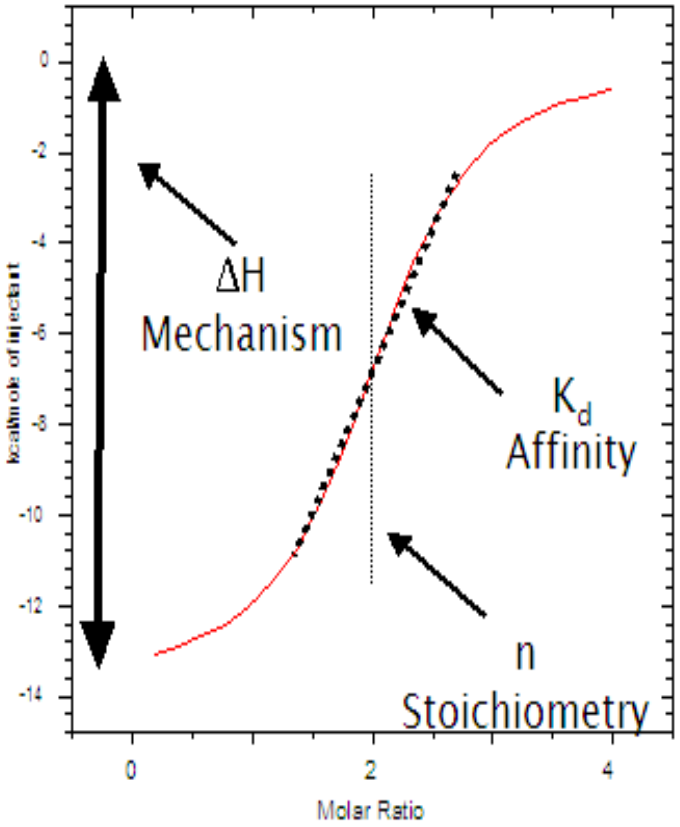
$M + L \rightleftharpoons ML$

$K = [ML] / [L][M]$

$Q = ([ML] / [M]_{total}) \cdot \Delta H$

$Q = (K \cdot [L] \cdot \Delta H) / (1 + K \cdot [L])$





Choosing a Binding Model

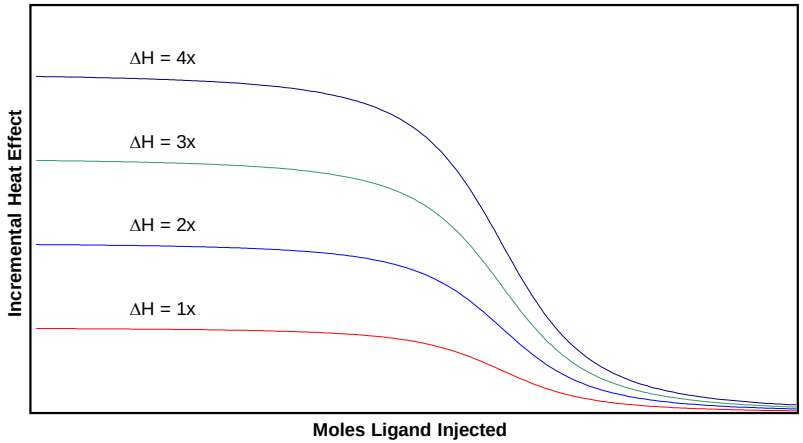
- Do you know Stoichiometry? (the number of ligands bound per macromolecule)
  - Sources: related protein/ligand complexes, X-ray or NMR structural data, CD or NMR titration data (including competitive binding), molecular modeling/docking, sequence alignments, mass spec, DSC
  - Use simplest model consistent with available information – start with Independent
- Independent? (one or several identical sites bind the same ligand with the same enthalpy and K_a, independent of each other)
- Multiple? (two or more binding sites, each capable of binding ligands, but with different enthalpies and K_a).
    - Possibly fit using several Independent models
- Cooperative? (two or more binding sites. The binding of the first ligand affects the binding of succeeding ligands)
- Other binding model?
- Try to run an experiment to determine ΔH
  - Run experiment to saturate all sites and determine enthalpy by titration curve
  - Run experiment with excess macromolecule and low ligand and calculate enthalpy per mole of ligand added.

ITC Experimental Design

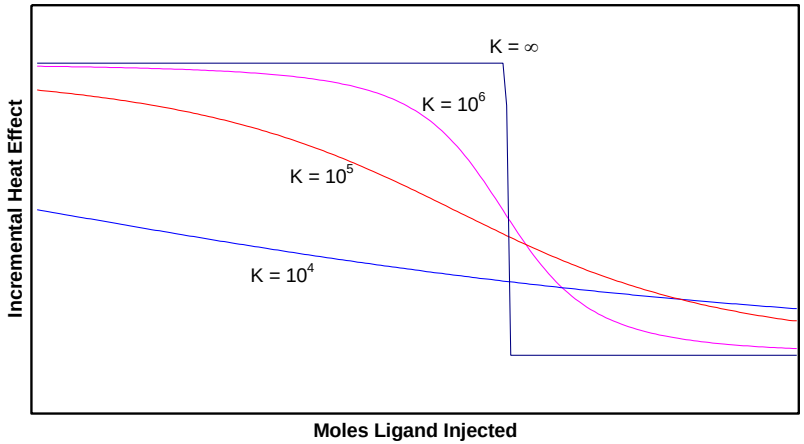
$$A + B \underset{\Delta H}{\overset{K}{\rightleftharpoons}} AB$$

$$K = \frac{[AB]}{[A][B]}$$

Constant Equilibrium Constant with Varying Enthalpy



Constant Enthalpy with Varying Equilibrium Constant



- Generally want to obtain stoichiometry (n), enthalpy (ΔH) and binding constant (K_a) from one experiment
- Enthalpy is directly measured. Receptor should be saturated with ligand at end of titration
- To obtain K_a: $10 < K_a[M]_T < 1000$
- $[M]_T$ is typically 10 – 100 mM, K_a is typically 10^3 to 10^9 M^{-1}
- Reminder: $K_D = 1/K_a$

ITC Experimental Design

- For accurate evaluation the value of C*K has to be between 1 and 1000

Note: C is the concentration of the titrand (reaction vessel)

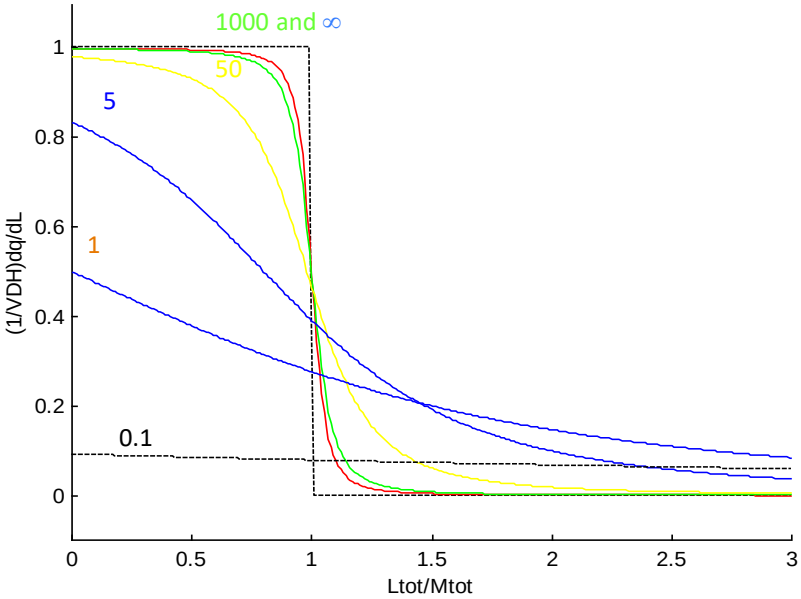
- Best precision is obtained if the titration curve has a sigmoidal shape and many data points in the transition region (*e.g*. *C***K* = 50)

- *e.g*. If *K* is estimated to 10^6 M an optimum concentration of *C* is 10^{-3} M.

ITC Experimental Design

- According to the ligand binding theory, the heat evolved for each injection depends on two variables, i.e. r and X where $r = K_a \cdot [M_t]$ and $X = [L_t]/[M_t]$.

- This left side of this equation (Independent model) can be plotted versus X for different r values, see the graph. It can be observed that the shape of the binding curve is 'nice' for an r value of 50-100. For an unknown sample it is important that the binding curves shows a good shape to obtain highest accuracy in the fitting procedure in determining the reaction enthalpy and the r value. From the r value the equilibrium constant can be found since $r = K_a \cdot [M_t]$. In practice binding curves with a good shape is obtained for r values in the range: $10 < r < 1000$.



$$\frac{1}{V \cdot \Delta H} \cdot \frac{dq}{dL_t} = \frac{1}{2}\left(1 - \frac{1 + r \cdot (X - 1)}{\sqrt{(r - X \cdot r + 1)^2 + 4X \cdot r}}\right)$$

ITC Experimental Design

- Choosing correct ligand and receptor concentrations requires an estimate of stoichiometry and K_a, often from spectroscopic measurements. ITC is then used to determine K_a accurately
  - Weak binding (low K_a) – may be limited by concentration
    - may use multiple syringes and combine results
  - Strong binding (high K_a)
    - minimize concentration or injection volume, etc…
    - try competitive binding (displacement) experiment

- Use Experiment Design module to alter K_a, binding model, stoichiometry and concentrations, and see the effect on the binding curve. Requires 'best guess' inputs of stoichiometry, K_a and binding model

- No idea of the parameters? Try 5 μM receptor (in cell), 50 μM ligand (in syringe), 15 x 15 μL injections, 25 °C

Hints for ITC Experiments

- Small heat of binding? Change temperature, pH and/or buffer
- Temperature: ΔH is temperature and system dependent, varies with ΔC_p. Conduct experiment at relevant temperature where binding has measurable ΔH.
- Different buffers have different enthaplies of ionization, affect ΔH of binding. If low enthalpies observed, use buffer with high ionization enthalpy
- Low enthalpy may indicate that a non-optimal pH is being used.
- Determine concentrations by absorbance.
  - Scanning wavelength is better than single wavelength in a situation where additives may interfere.
- Degas both ligand and macromolecule solution

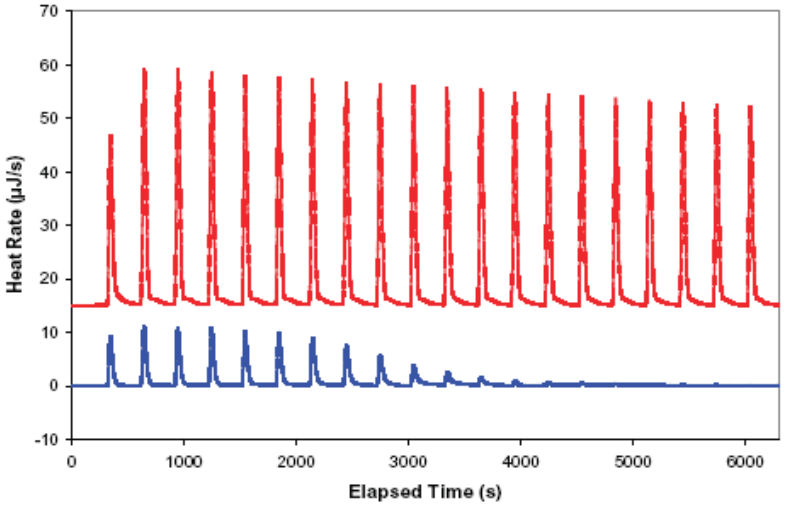
Hints for ITC Experiments

- All reactions produce heat. Ensure only the desired reaction is measured.
  - pH, ionic strength, choice of buffer and temperature
- Diluting a compound produces or absorbs heat (‘heat of dilution’).
- To minimize heat of dilution of buffer ‘contaminants’ in protein and ligand solution: 1) dialyze protein and ligand 2) use the used dialysis buffer to dissolve the ligand 3) perform ‘blank’ experiment (titrate ligand into buffer), then subtract blank from ‘real experiment’ data. Caution: analyze blank data for indications of ligand-buffer interactions.
  - Is the ligand too small to dialyze? Be sure to desalt and use dialysis buffer to prepare and dissolve ligand.

Hints for ITC Experiments

- Instrument default settings are factory-optimized for aqueous solutions. User can adjust for organic solvents. Order ITC with Kalrez (not Viton) O-rings if anticipate using organics.

- Non-aqueous solvents have different viscosities and heat capacities.
- Match [organic] in syringe and sample cell? Extremely difficult if ligand first dissolved in organic, then diluted with buffer to match [organic] in sample cell.
- Any mismatch in [organic] can result in heat of dilution masking heat of binding
- Proteins easily unfold in organics. If organics are present, is the ligand binding to the native conformation?
- Example: 5% DMSO decreases T_m of RNase A by 1 ^oC.



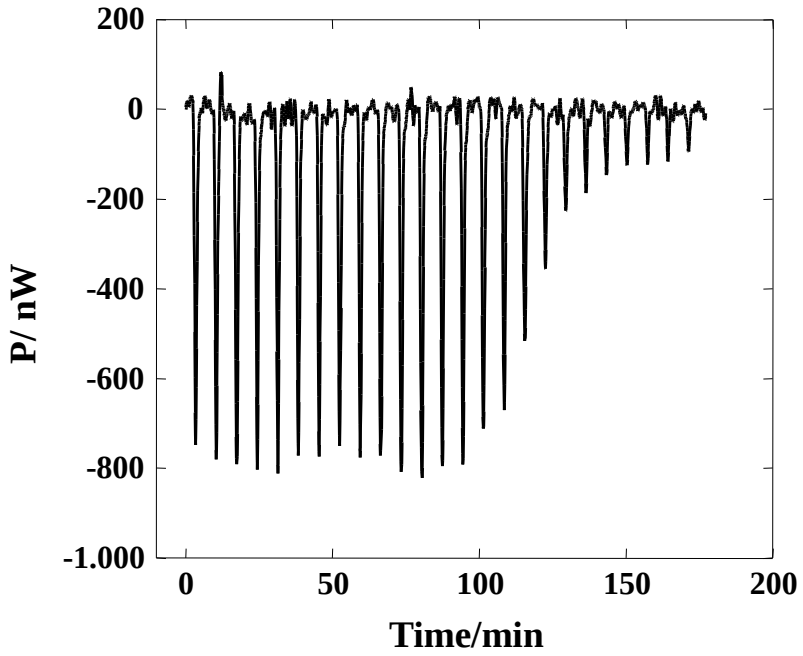
Hints for ITC Analysis

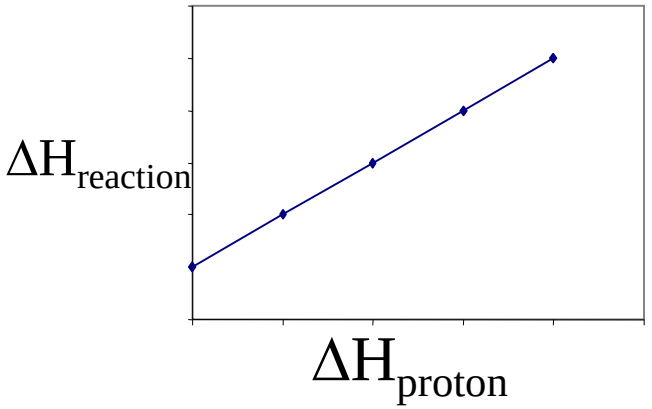
- Unexpectedly low stoichiometry could be due to:
  - [receptor] lower than anticipated
  - [ligand] higher than anticipated
  - Receptor contains contaminating proteins
  - Receptor is partially unfolded
  - Multiple binding sites
  - Wrong binding model
  - Insufficient curvature in data: change concentrations

- Solvent protonation
  - Study different buffers at the same pH
  - Plot observed enthalpy versus ionization enthalpy
    - Slope gives the number of protons released (if negative)
      - Slope equals zero than no effect of buffer selected
    - Y-intercept is the enthalpy of binding (buffer independent)
      - Different pH will result in a different plot

Proton Linkage

- $M + L + B \rightleftharpoons ML + BH^+$; $\Delta H_{reaction}$
- $M + L \rightleftharpoons ML + H^+$; $\Delta H_{binding}$
- $B + H^+ \rightleftharpoons BH^+$; ΔH_{proton}





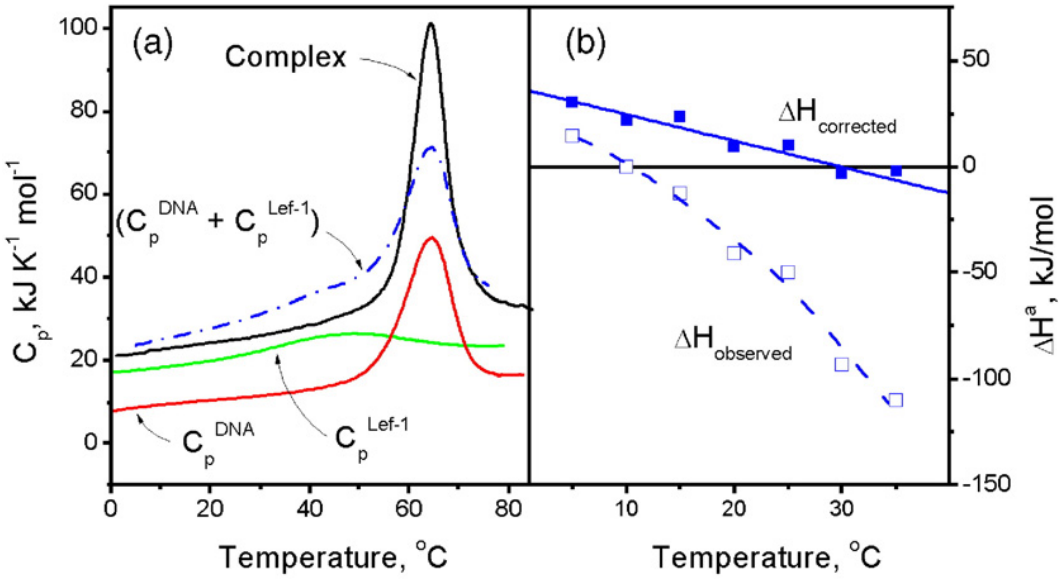
IGF-I to the soluble extracellular IGF-I receptor

DSC may be Helpful for ITC Results Correction

- Characterizing the thermodynamics of a binding reaction requires determining ΔH and ΔG at several temperatures, and obtaining ΔCp to predict the change in ΔH and ΔG with temperature
- ΔH is directly measured by ITC. ΔG is calculated from the binding constant at T:

$$\Delta G = -RT \ln K_a$$

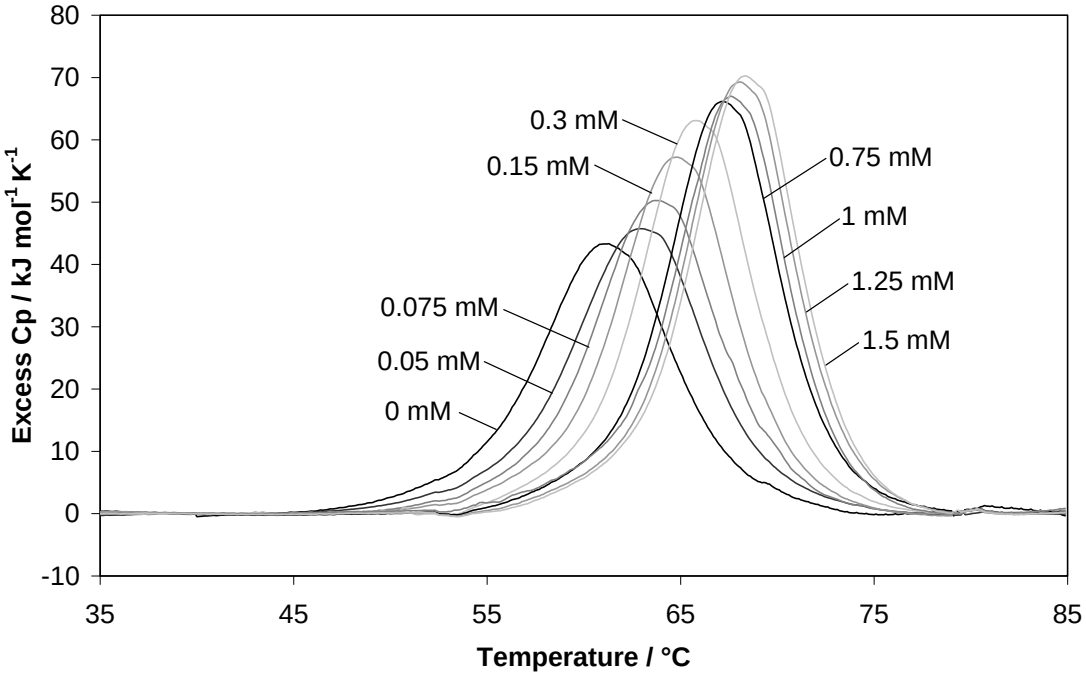
- Obtaining ΔCp by ITC requires ΔH measurements at several temperatures, then taking the slope to obtain ΔCp. Better to determine ΔCp by DSC and correct the temperature effect on ΔH .



Dragan *et al., J. Mol. Biol*. **343**, 371-393, 2004.

ITC Results by using Slow-scanning DSC

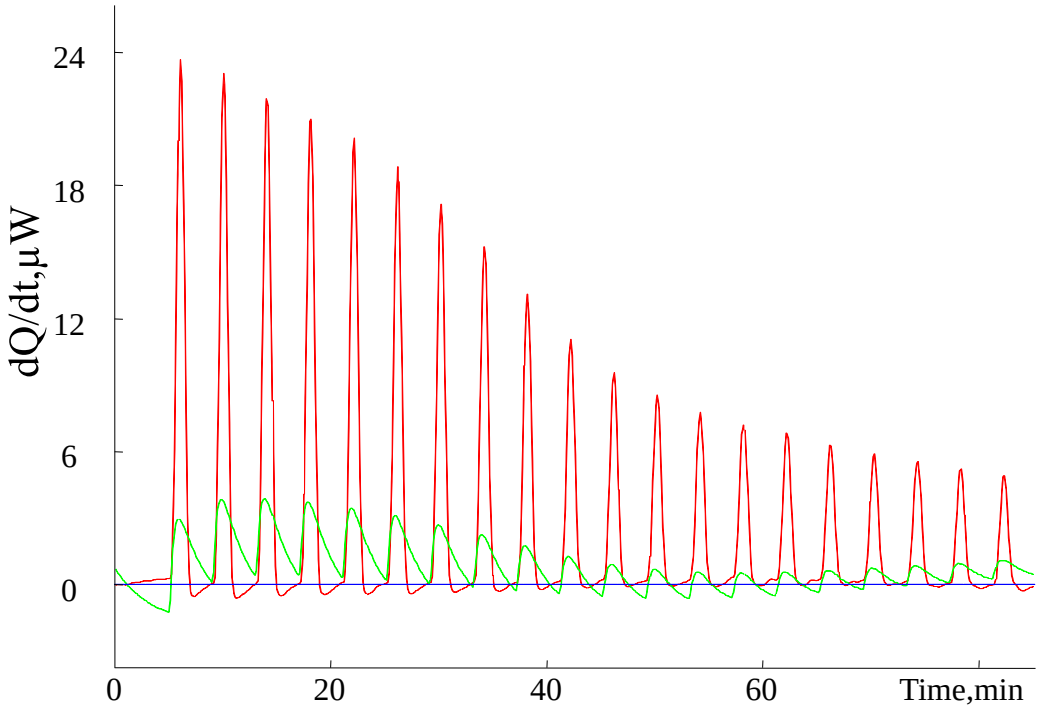
- If a ligand binds preferentially to a folded protein, the T_m of the protein will increase. Generally, the more bound ligand there is, or the tighter it binds, the more T_m increases.
- Can determine binding constant at T_m.
- But, useful if very slow or very tight binding, or organic solvents necessary.
- Valid if comparing relative binding of ligands to same protein
- DSC is a quick way to determine if two molecules interact. Also allows ΔC_p correction of ITC data



Binding of 2'-CMP to RNase A ± 5% DMSO
K_a = 5900 M^{-1} (-DMSO); 6900 M^{-1} (+DMSO) at T_m

Dynamic Calibration for ITC

dQ/dt_{Dyn} dQ/dt_{HF} Baseline



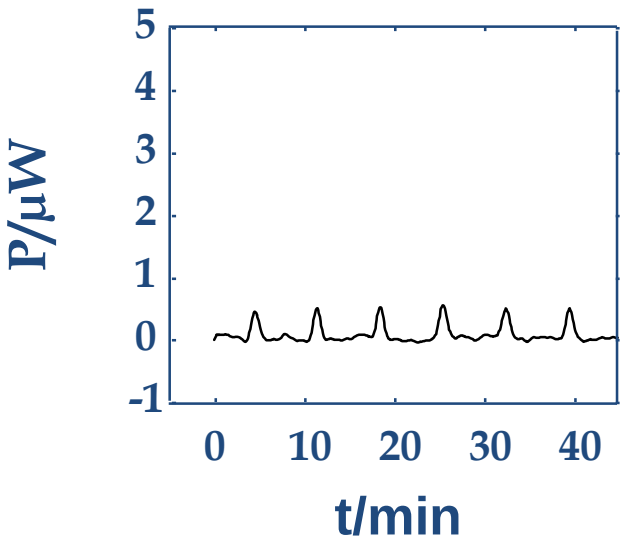
$$P_{Dyn} = P_{raw} + \tau \cdot dP_{raw}/dt$$

where $P = dQ/dt$

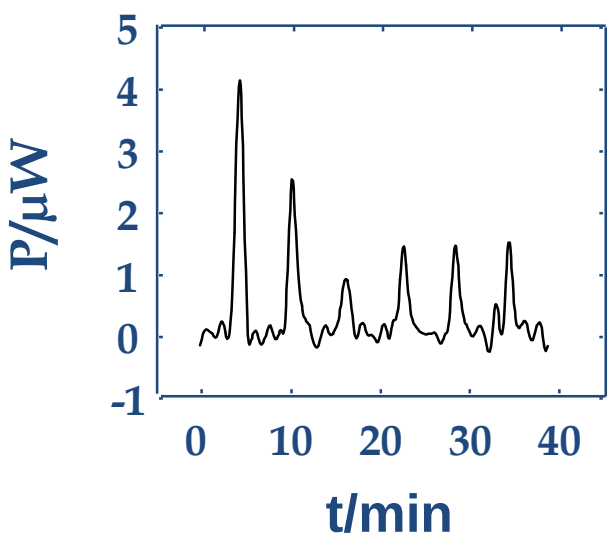
Titration Calorimetry in Rational Drug Design

- Screening of pharmaceutical hits and leads

Non-binder — Heat of dilution

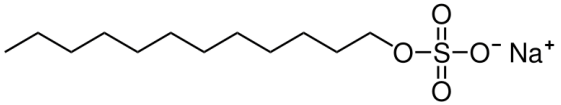


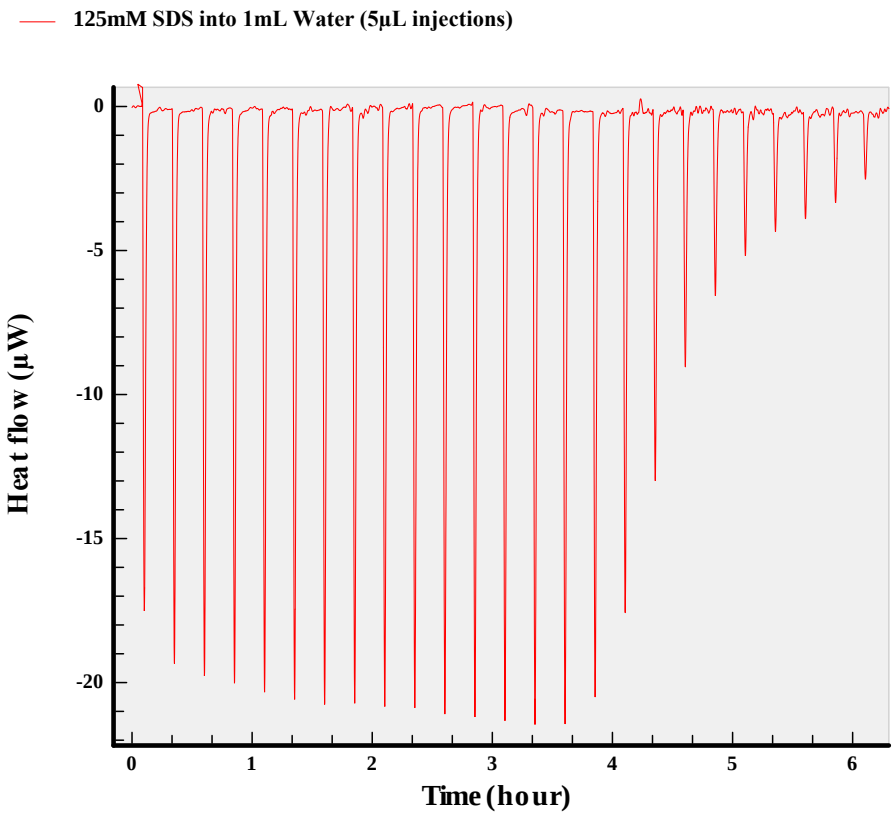
Binder

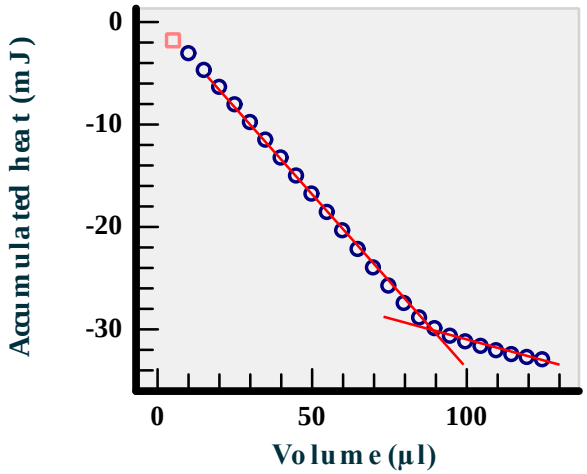


CMC of Sodium Dodecyl Sulfate (37 °C)

- 125.18mM SDS injected into 1 mL of water
- MW = 288.38 g/mol







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