Methods for the determination of glass transition temperatures for the development of freeze drying cycles

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National Institute for Biological Standards and Control
Assuring the quality of biological medicines

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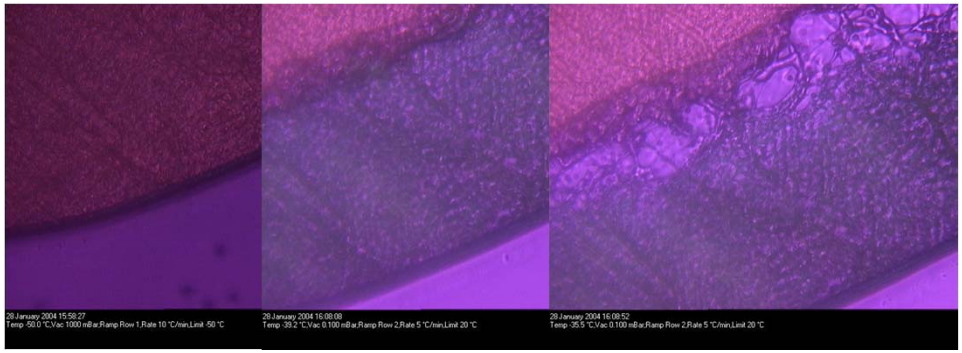
Why are NIBSC interested in lyophilization?

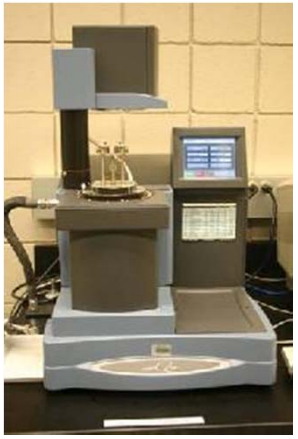
- NIBSC is a centre of the Health Protection Agency
- NIBSC remit is safeguarding the quality of biological medicines used in the UK
- NIBSC is the UK's OMCL – batch release testing of vaccines and blood products
- NIBSC is a WHO International Laboratory for Standardisation - prepares >90% of WHO International Standards – primary reference materials for biological medicines. Most of these are freeze dried for optimum stability, ease of storage and transportation

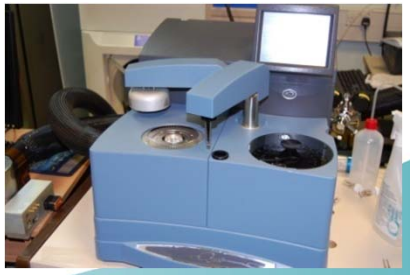
Distribute primary reference materials
Over 500 reference materials in catalogue
Over 5000 item shipments per year worldwide

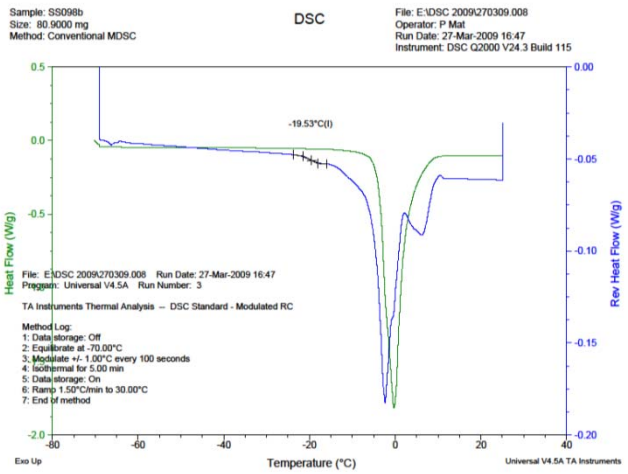
Standardisation Science

- Dedicated team for formulation/freeze drying development
- Well equipped for determination of critical parameters
- Pilot freeze drying capability (600 -900 ampoules)
- Contract & core research activities









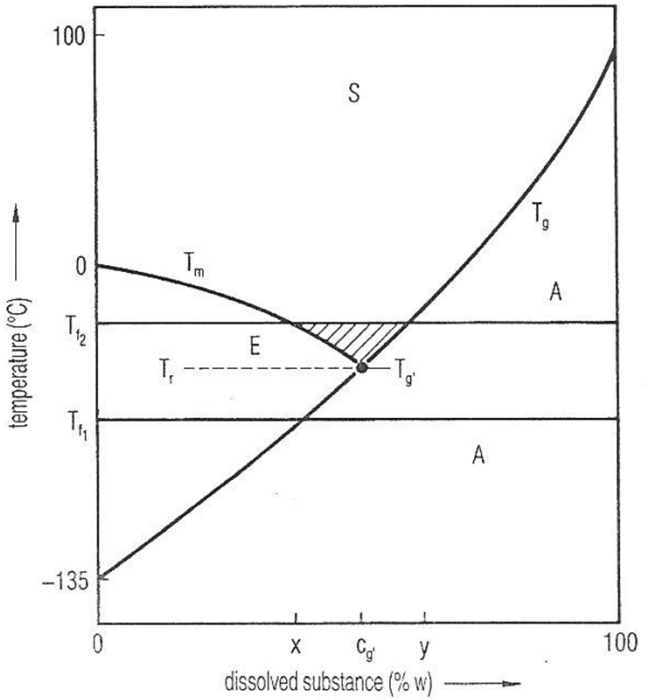
Outline of this presentation

- What is a Tg?
- Importance of Tg' in freeze drying
- Review of available thermal analysis methodologies
- Comparison esp. FDM and DSC
- Impedance - Lyotherm-2
- Use of impedance – illustration of formulation development
- DMA
- Uses of DMA
- DMA dry state

Thermal Properties

To understand the freezing behaviour of aqueous solutions there are several important types of thermal events:

- Glass Transition Temperature Tg'
- Collapse Temperature Tc
- Eutectic Temperature Teu



Idealised phase diagram for small carbohydrate showing glass transition point at which a glass is formed (adapted from Oetjen "Freeze Drying" 1999 VCH Wiley)

Critical temperature analysis methods

Current methods:

- Modulated Differential Scanning Calorimetry (mDSC)
- Differential Thermal Analysis
- Electrical Resistance
- Freeze Drying/ Cryo-Microscopy

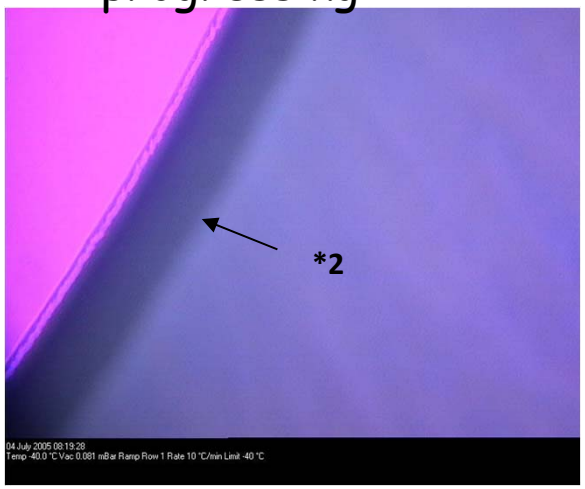
Novel methods:

- Impedance Analysis
- Dynamic Mechanical Analysis

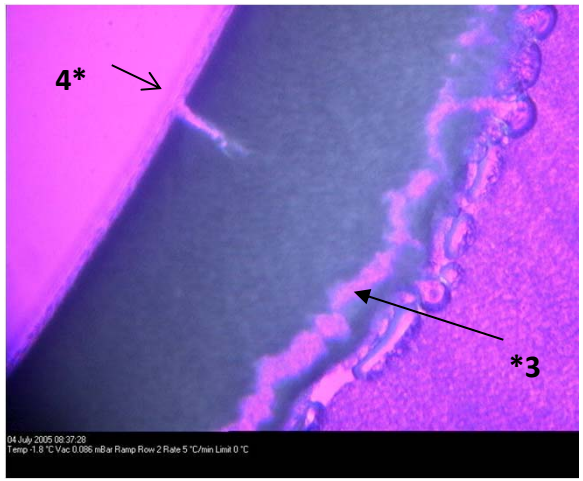
Use of analytical methods: Freeze drying microscopy

Freezing

Drying- front progressing

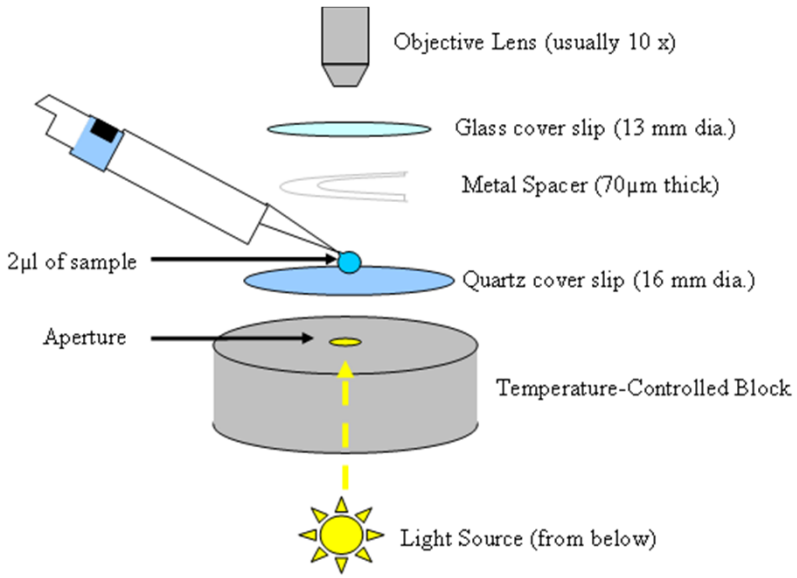


Collapse – temperature too high

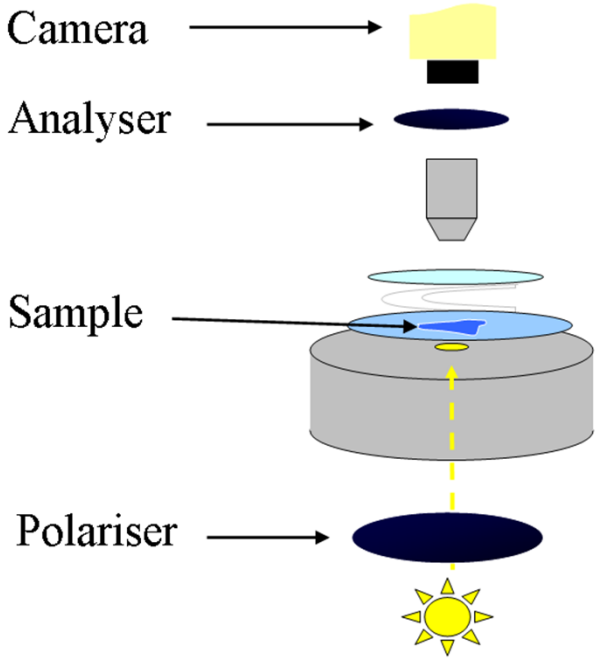


Requires <100uL sample
Vacuum control
Temperature control
Programmable ramp/hold
Video recording
Plane polarised light

Freeze drying microscopy: set up and analysis



Freeze drying microscopy sample prep

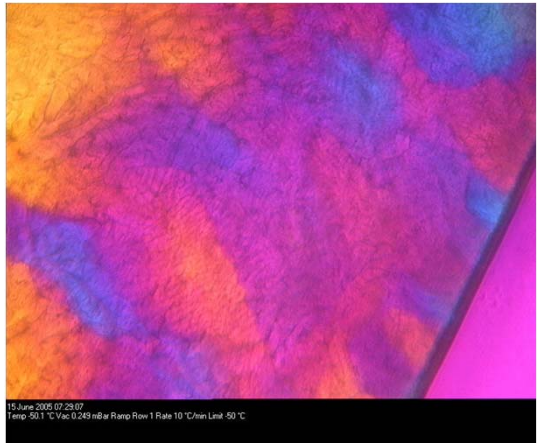


Use of plane polarised light

Figures courtesy of Dr Kevin Ward, Biopharma Technology Ltd, Winchester, UK

Freeze drying microscopy of tetanus toxoid formulation

A



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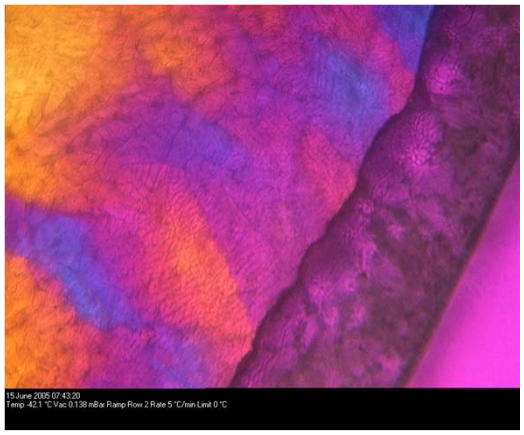

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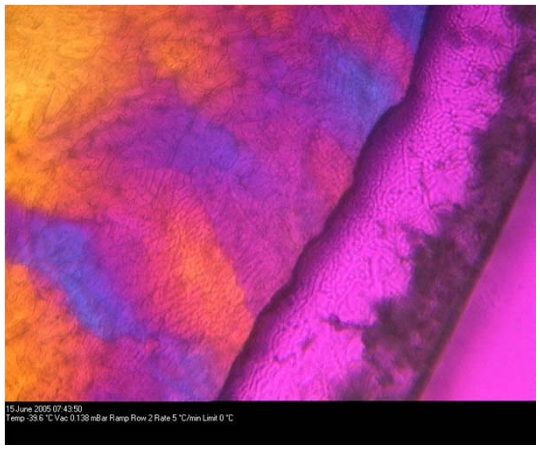


D

A) Liquid

B) Frozen – vacuum just applied

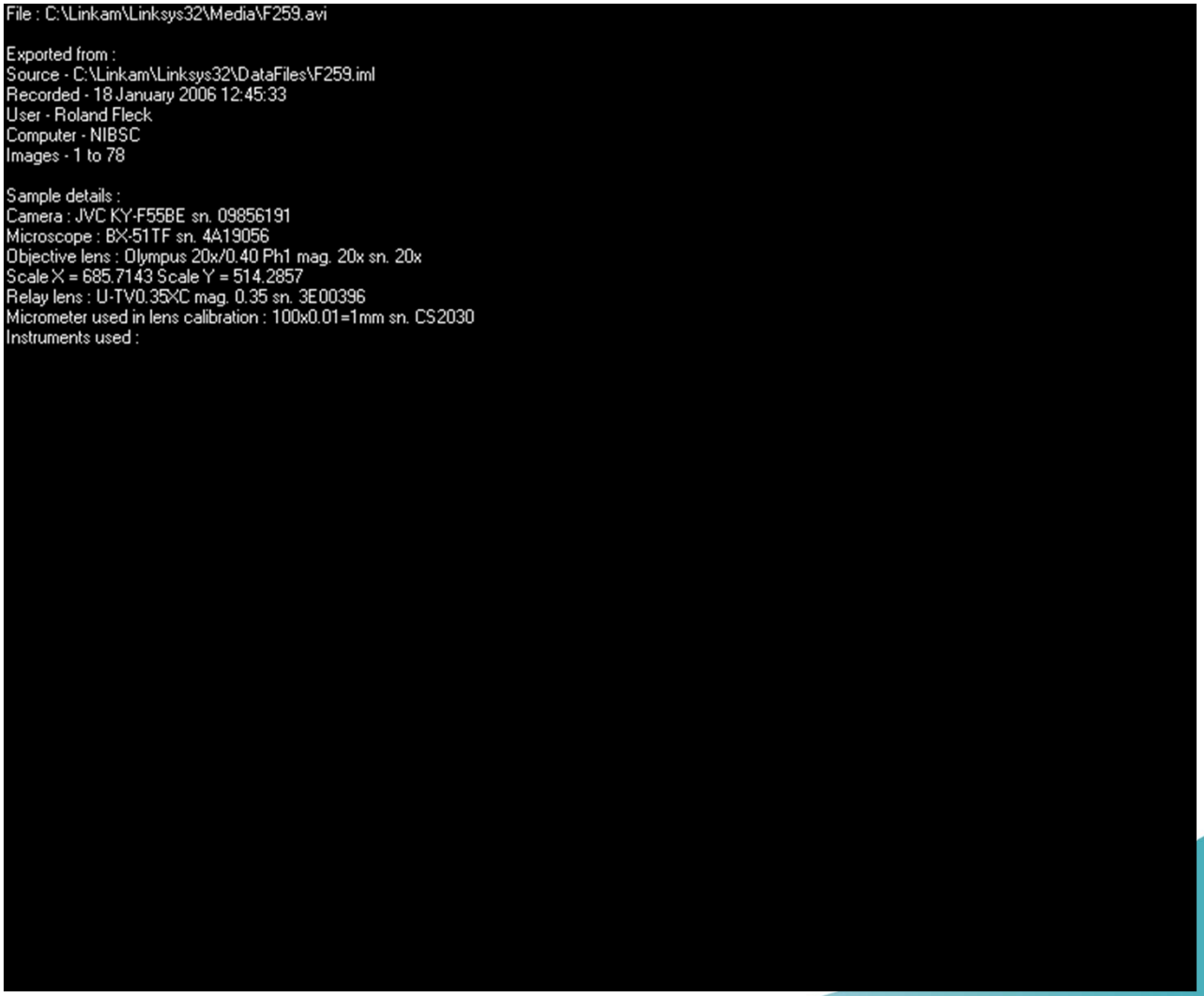
C) Drying

-42ºC,138µbar

D) Collapsed

-40 º C, 138µbar

FDM Video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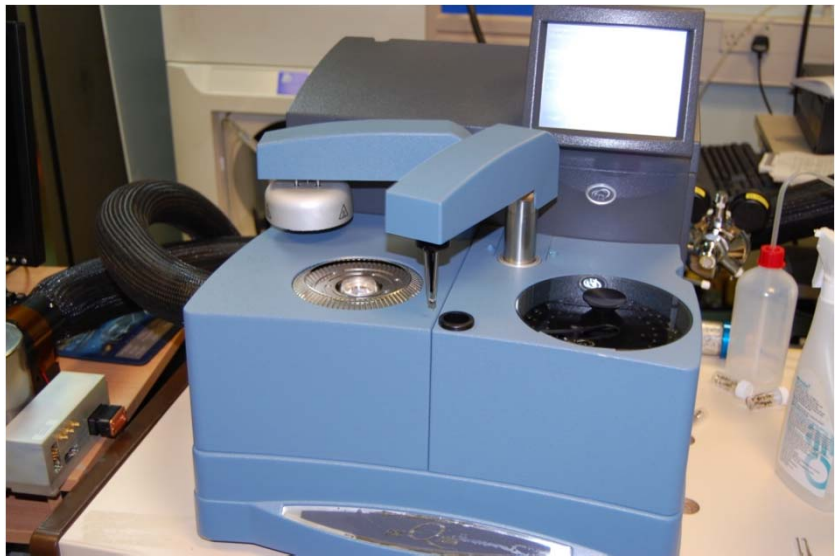
Exported from :
Source - C:\Linkam\Linksys32\DataFiles\F259.iml
Recorded - 18 January 2006 12:45:33
User - Roland Fleck
Computer - NIBSC
Images - 1 to 78
Sample details :
Camera : JVC KY-F55BE sn. 09856191
Microscope : BX-51TF sn. 4A19056
Objective lens : Olympus 20x/0.40 Ph1 mag. 20x sn. 20x
Scale X = 685.7143 Scale Y = 514.2857
Relay lens : U-TV0.35XC mag. 0.35 sn. 3E00396
Micrometer used in lens calibration : 100x0.01=1mm sn. CS2030
Instruments used :

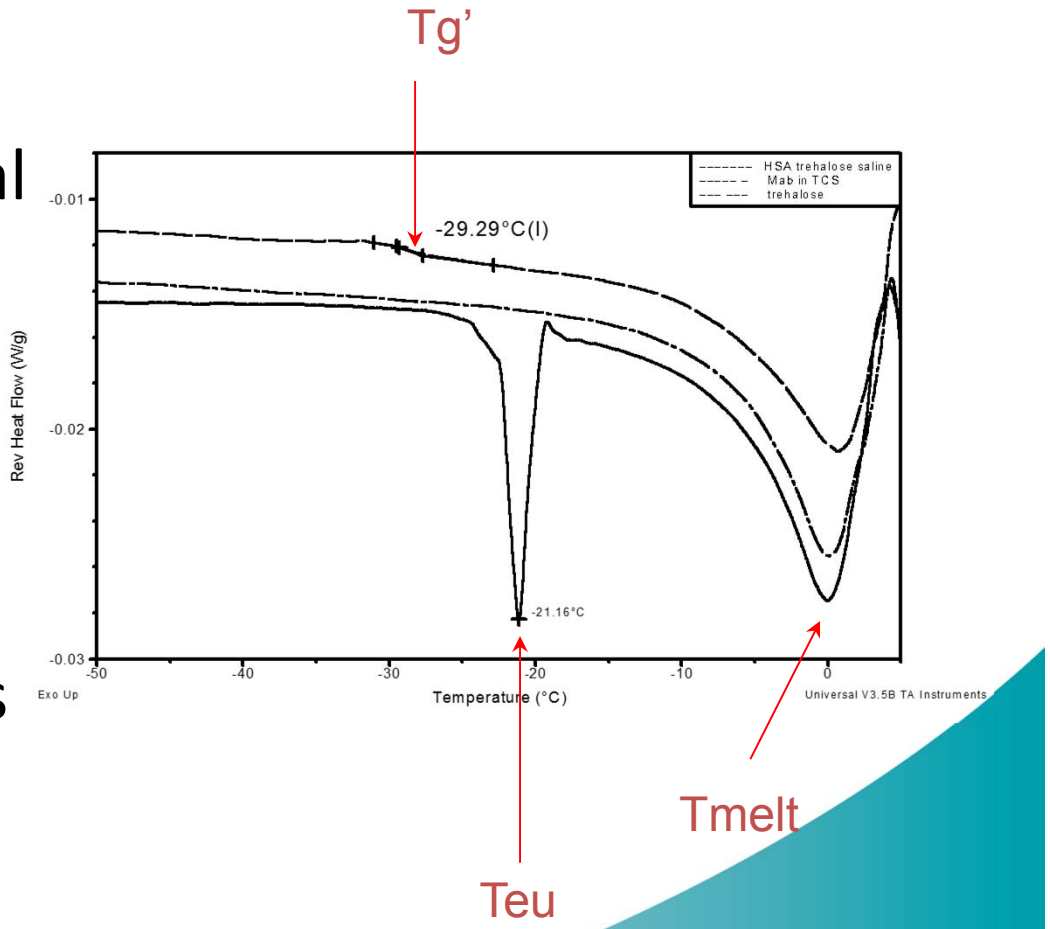
Use of DSC to determine Tg'



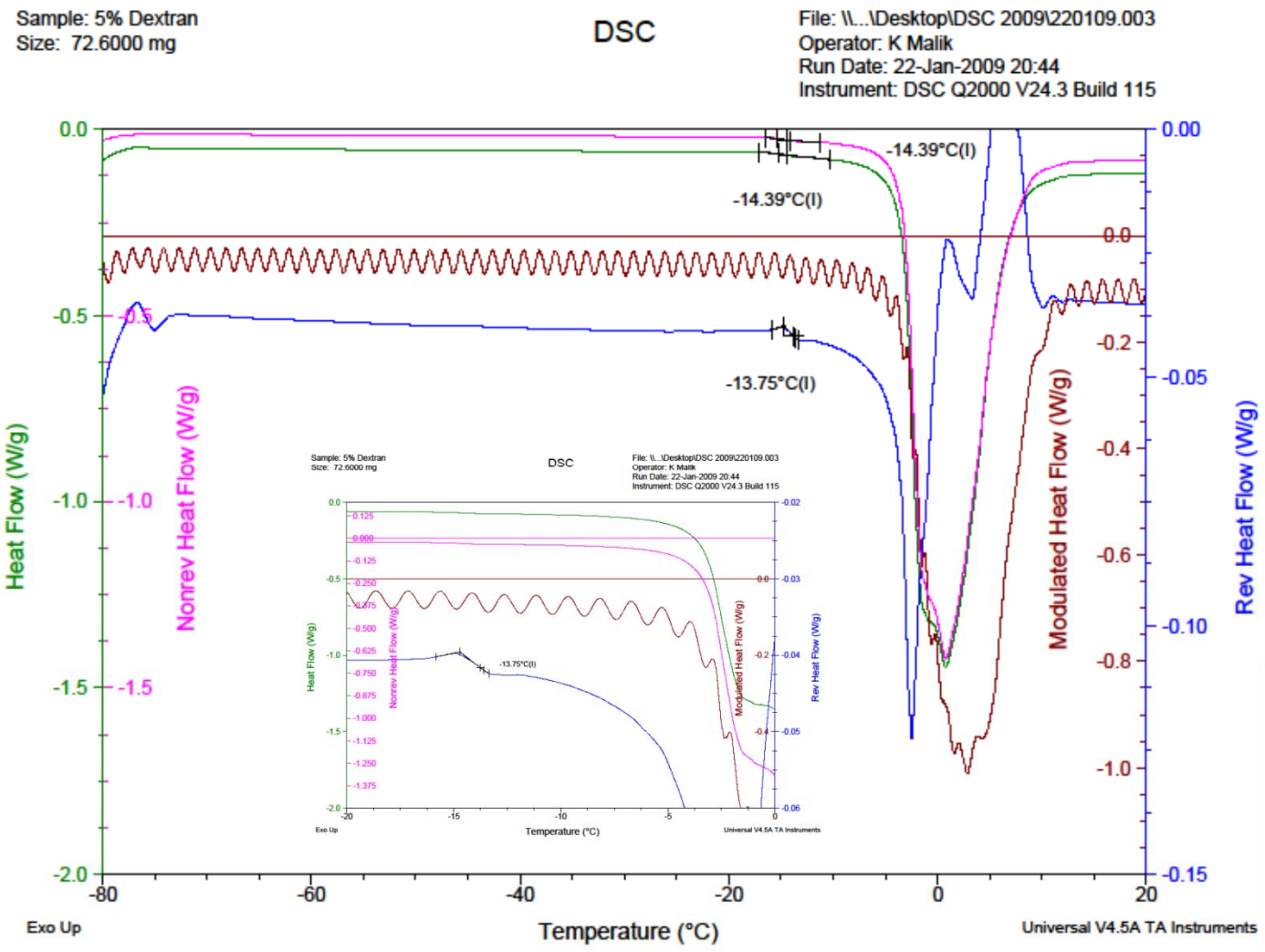
- Glass transition Tg' of frozen and Tg of dry materials
- Amorphous structure usually stable below the Tg'
- Measure Tg' by DSC in small volume pans
- Heat through Tg' and observe thermal events
- Tg' influenced by formulation
- Tg influenced by formulation and moisture content

DSC and mDSC

- Small volume encapsulated in metal pans
- Freeze rate
- Scan rates
- Empty pan reference
- Three thermal events Tm, Teu, Tg(‘)



mDSC of 5% dextran

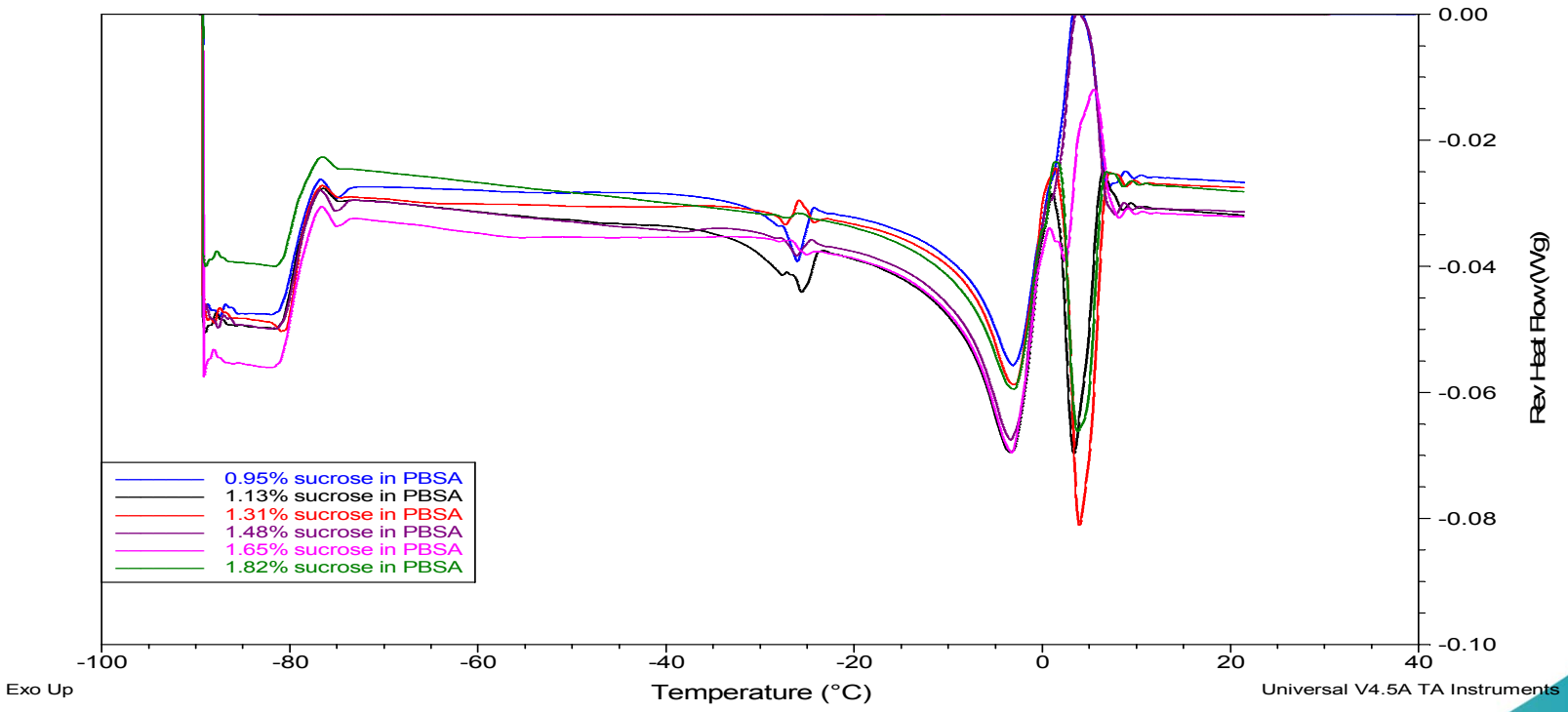


Impact of formulation on stability 4: Influenza antigen formulation

Freeze dried appearance of flu excipients (from left to right: 1%, 1.2%, 1.4%, 1.6%, 1.8%, and 2.0% sucrose in PBSA)

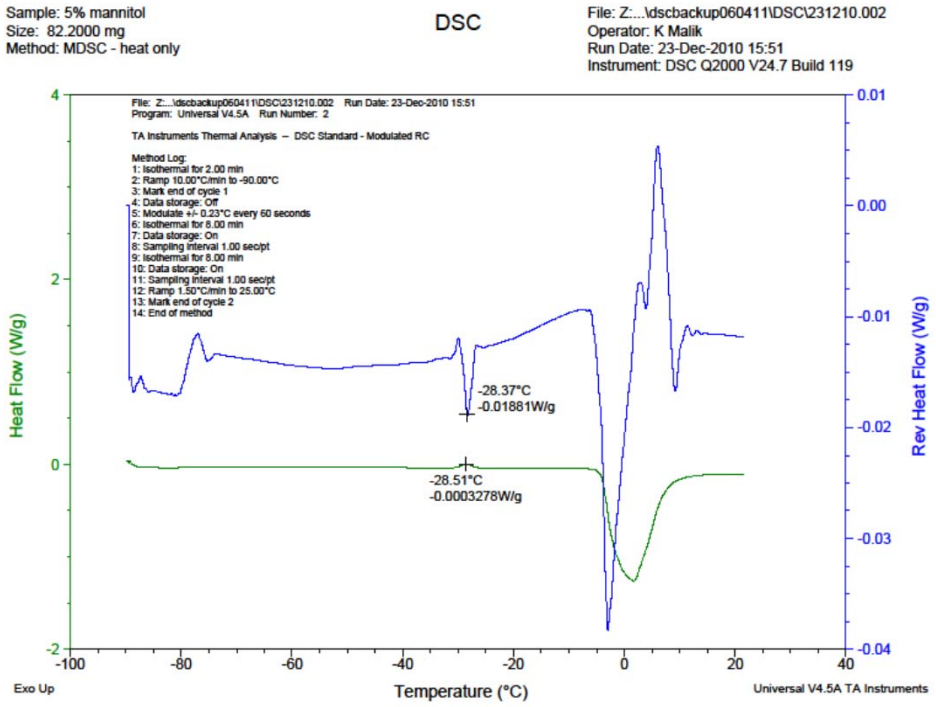
N.B. The moisture content of collapsed cakes can be as high as 4% compared to <<1% in cakes with good appearance

Use of DSC to predict successful FD outcome



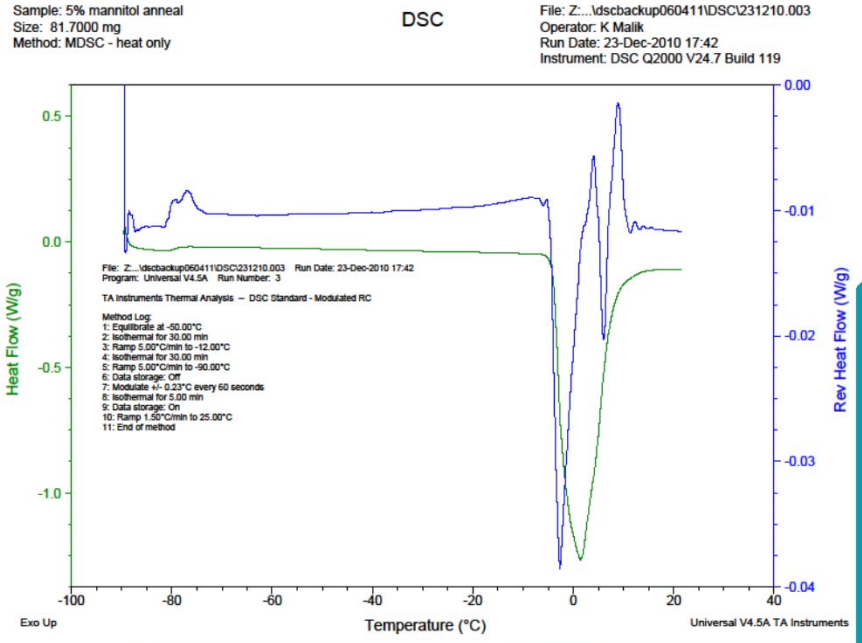
- Impact of sucrose content on NaCl eutectic

DSC for monitoring annealing

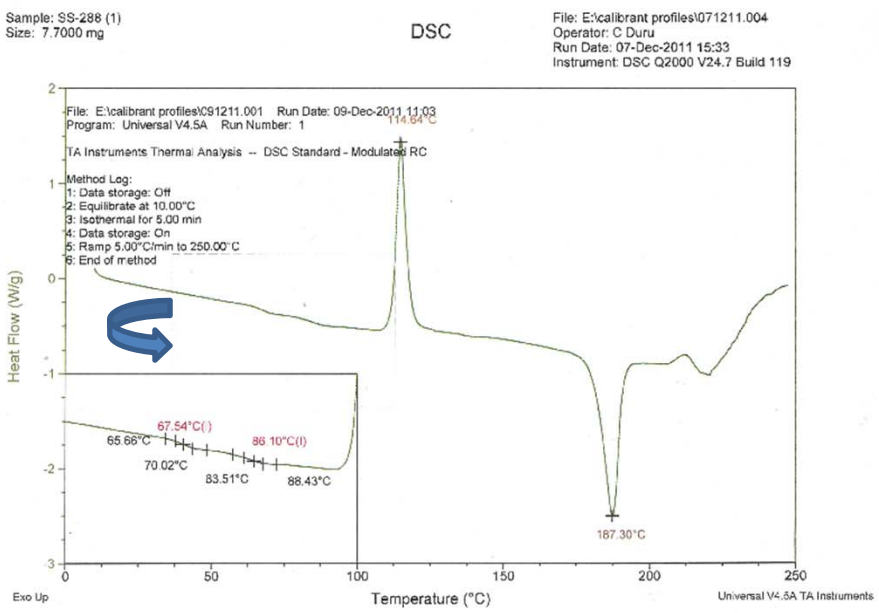


Mannitol solution frozen and warmed without annealing step

Mannitol solution, frozen & then annealed at -12 C, re-cooled and warmed

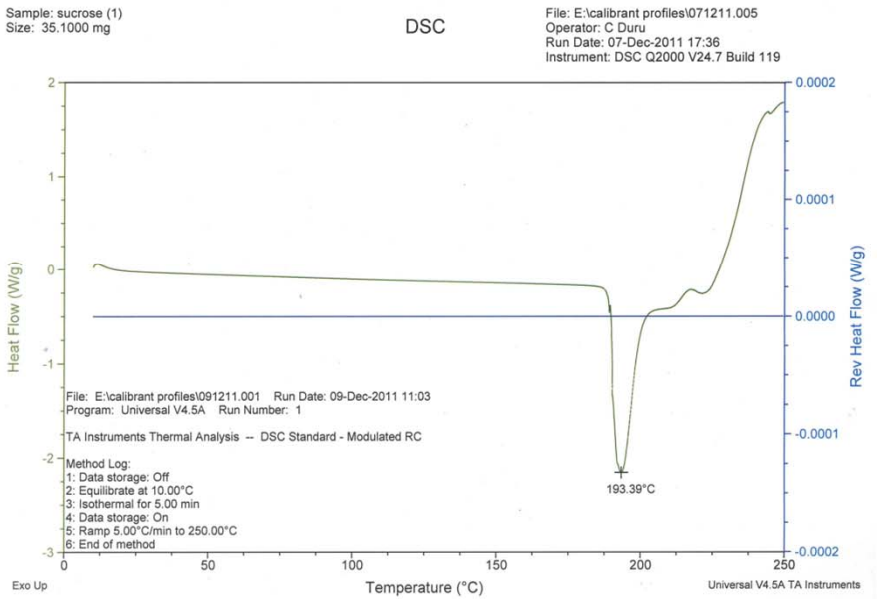


Tg of dry state sucrose



Amorphous (freeze dried) sucrose

Crystalline sucrose



Comparison of thermal analysis methods on some biologicals

Description	Freeze-Drying Microscope		DSC		Conductivity		DTA
	Freeze Temp	Collapse Temp	Tg'	Teu	SLTT Start	SLTT End	SLTT
IL-17 1ug/ml in 0.9% NaCl/ 0.1% Trehalose/ 0.2%HSA/ 0.01% Tween 20	-15	-20	-25	-21	-25	-23	-27
Factor XIII Concentrate (in 40mM HEPES,0.1MNaCl, 0.5% trehalose, 1% HAS)	-16	-28	-50	-24	-27	-25	-23
Meningococcal Serogroup W P/S	-18	-11	-2		-4	-0.5	-2

Novel Techniques – Impedance

- Pioneered by the late Prof Louis Rey
- Comparatively simple technology
- Comparison of changes in temperature between sample and reference material (water or other solvent e.g methanol)during cooling and warming processes
- Typically requires 2-4ml of sample but depends on equipment design

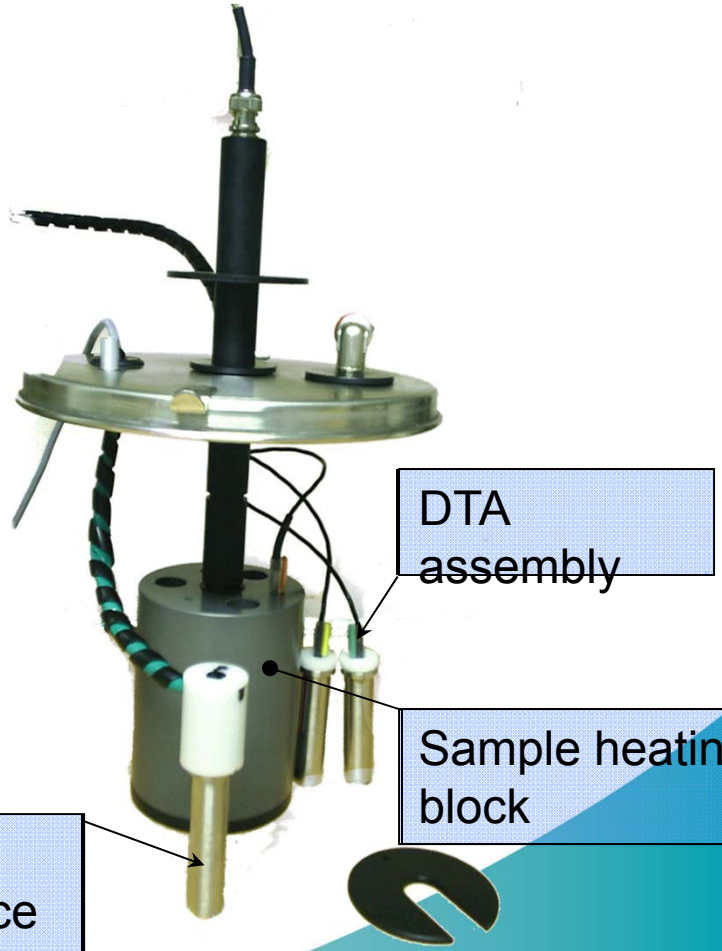
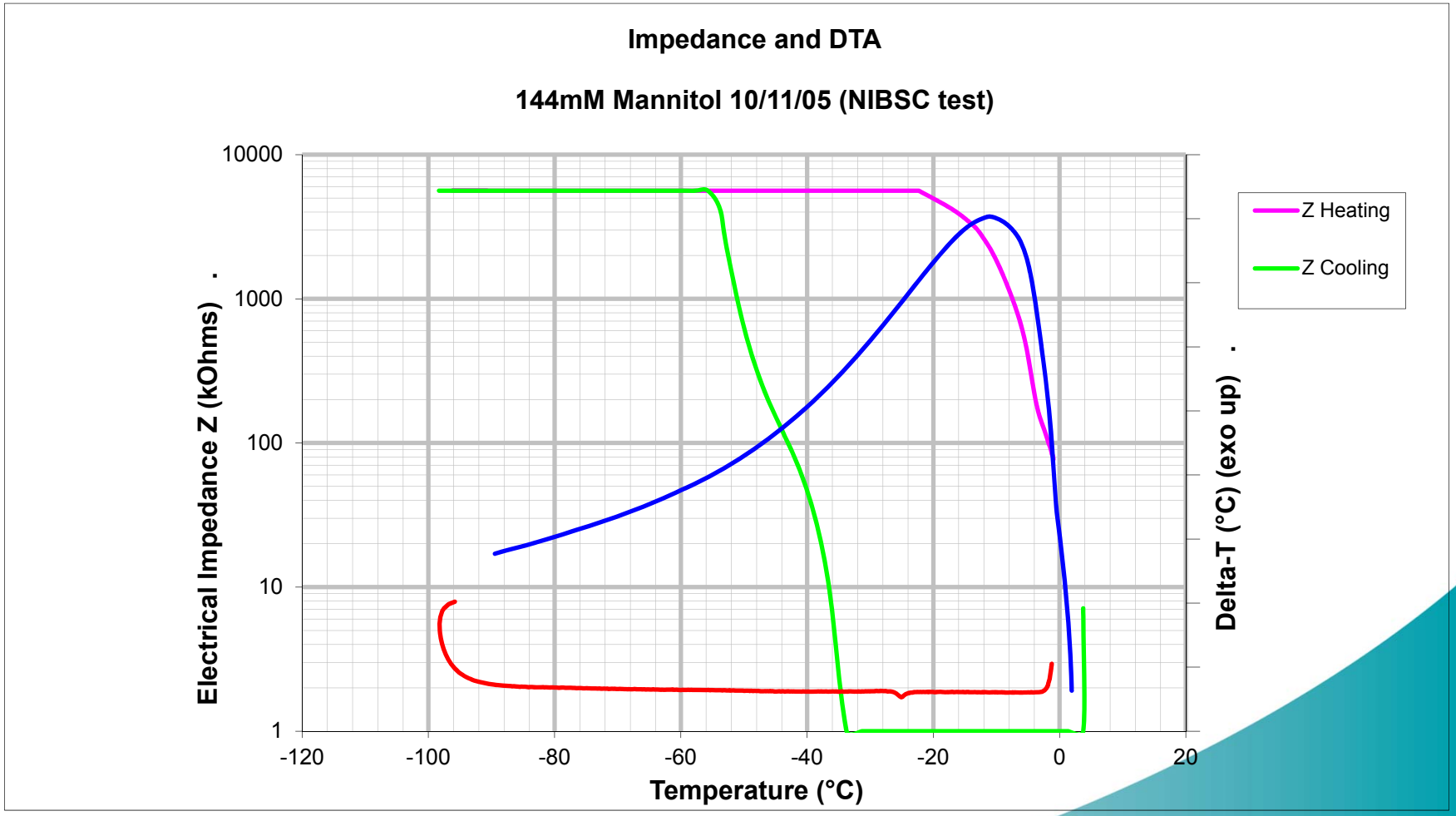
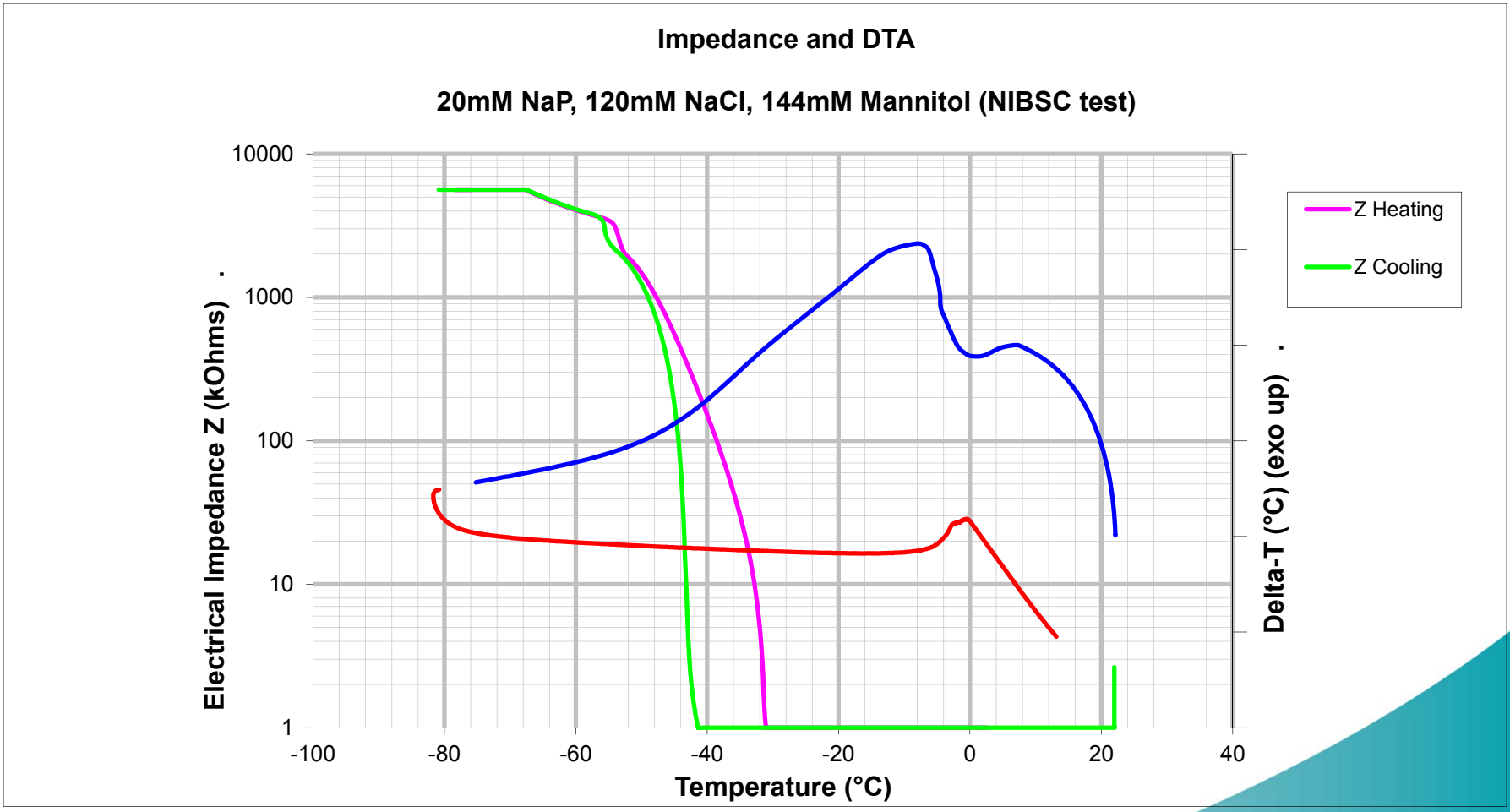
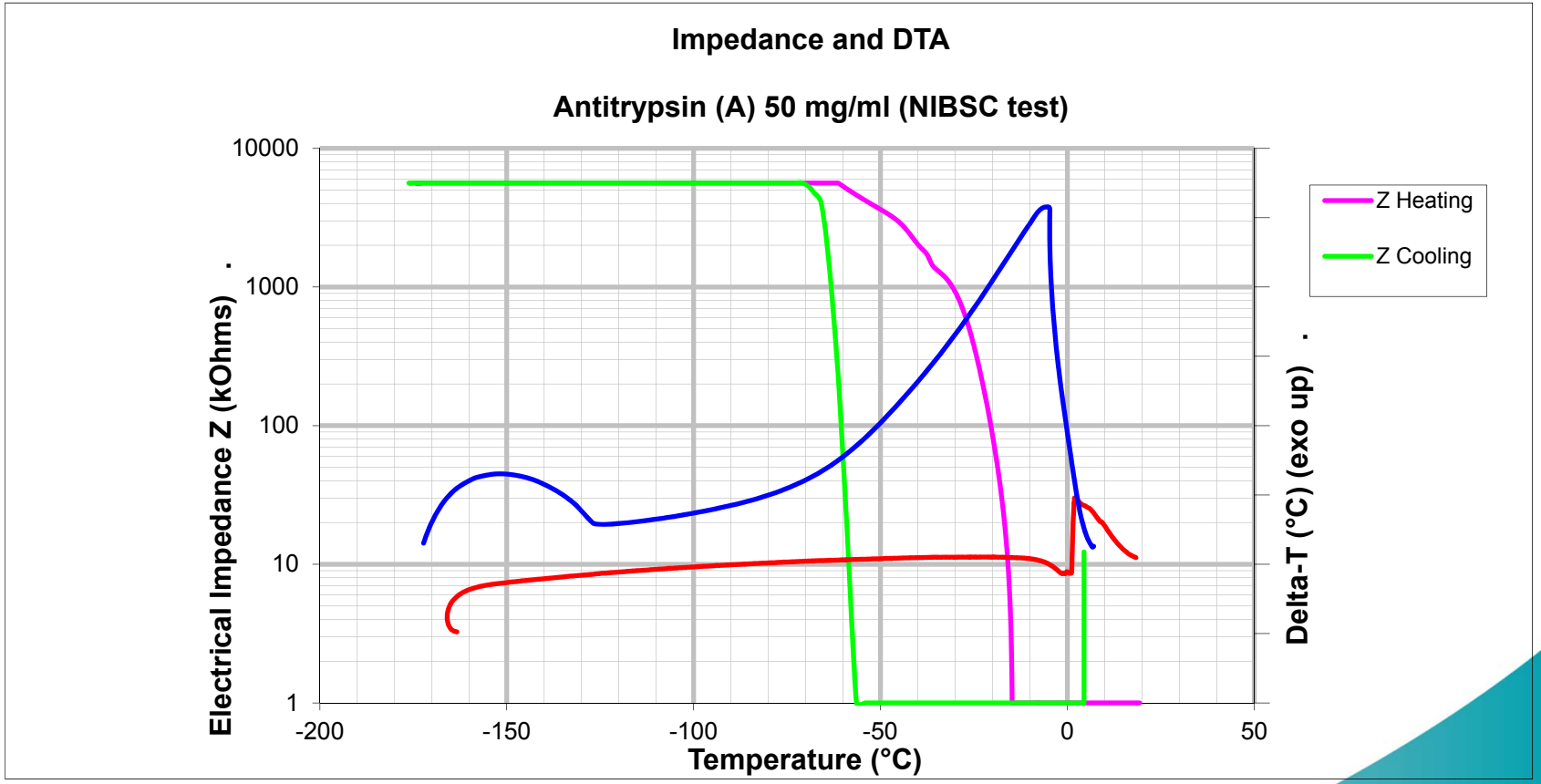
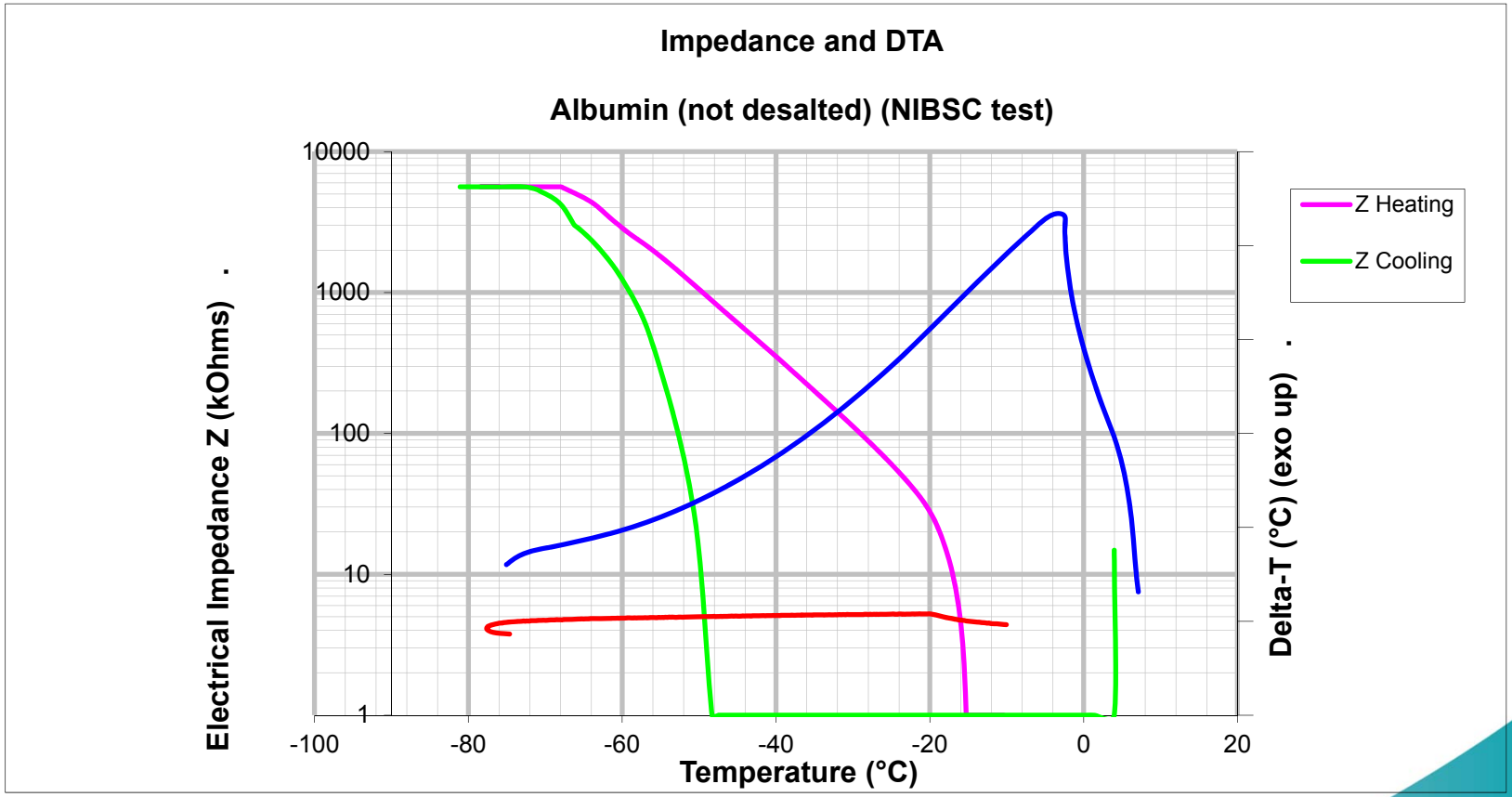


Image of Lyotherm -2 courtesy of Biopharma Technology Ltd, UK

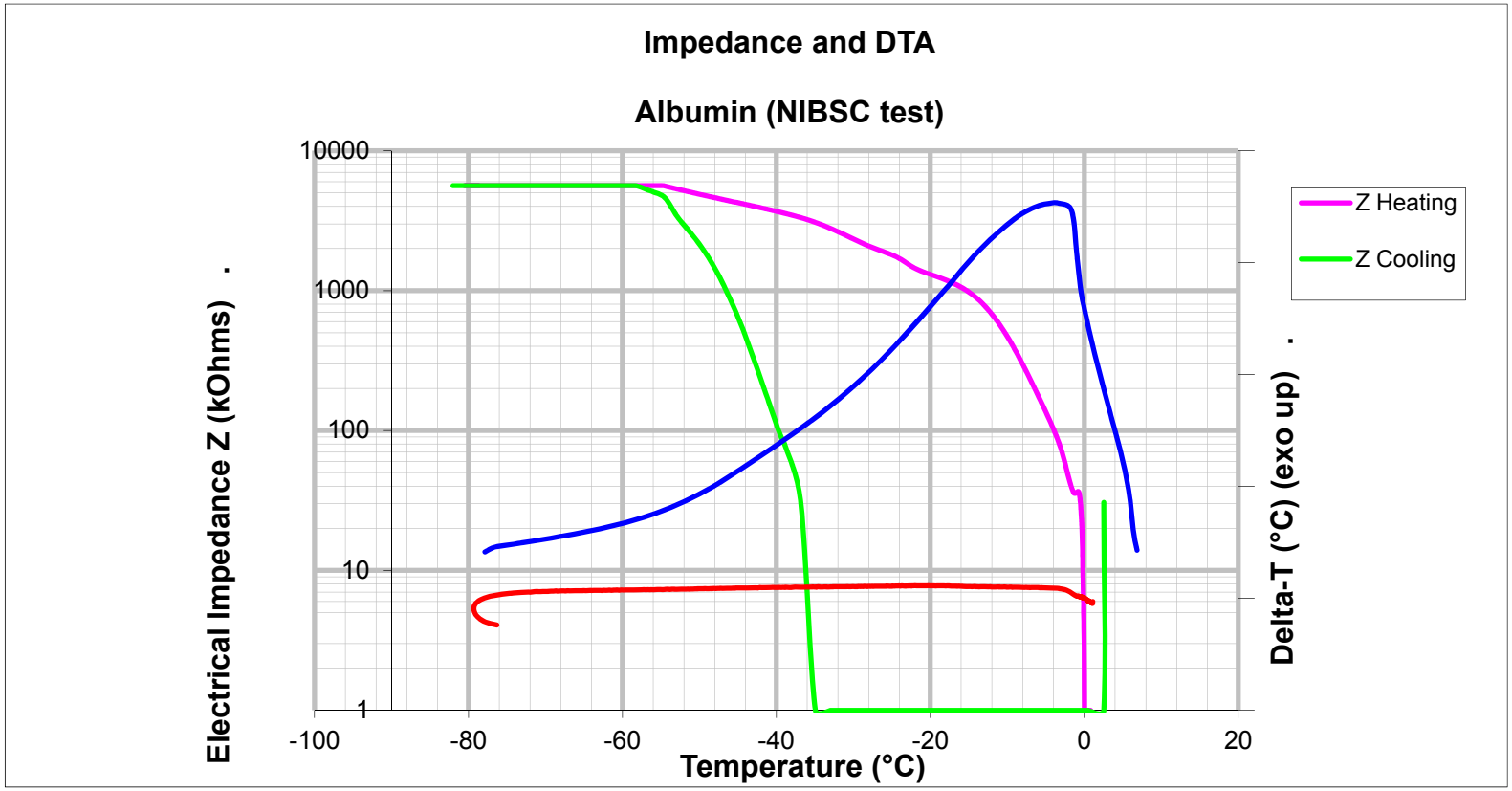

Impedance and DTA
144mM Mannitol 10/11/05 (NIBSC test)
Electrical Impedance Z (kOhms)
10000
1000
100
10
1
-120
-100
-80
-60
-40
-20
0
20
Temperature (°C)
Delta-T (°C) (exo up)
Z Heating
Z Cooling


Impedance and DTA
20mM NaP, 120mM NaCl, 144mM Mannitol (NIBSC test)
Electrical Impedance Z (kOhms)
10000
1000
100
10
1
-100
-80
-60
-40
-20
0
20
40
Temperature (°C)
Delta-T (°C) (exo up)
Z Heating
Z Cooling

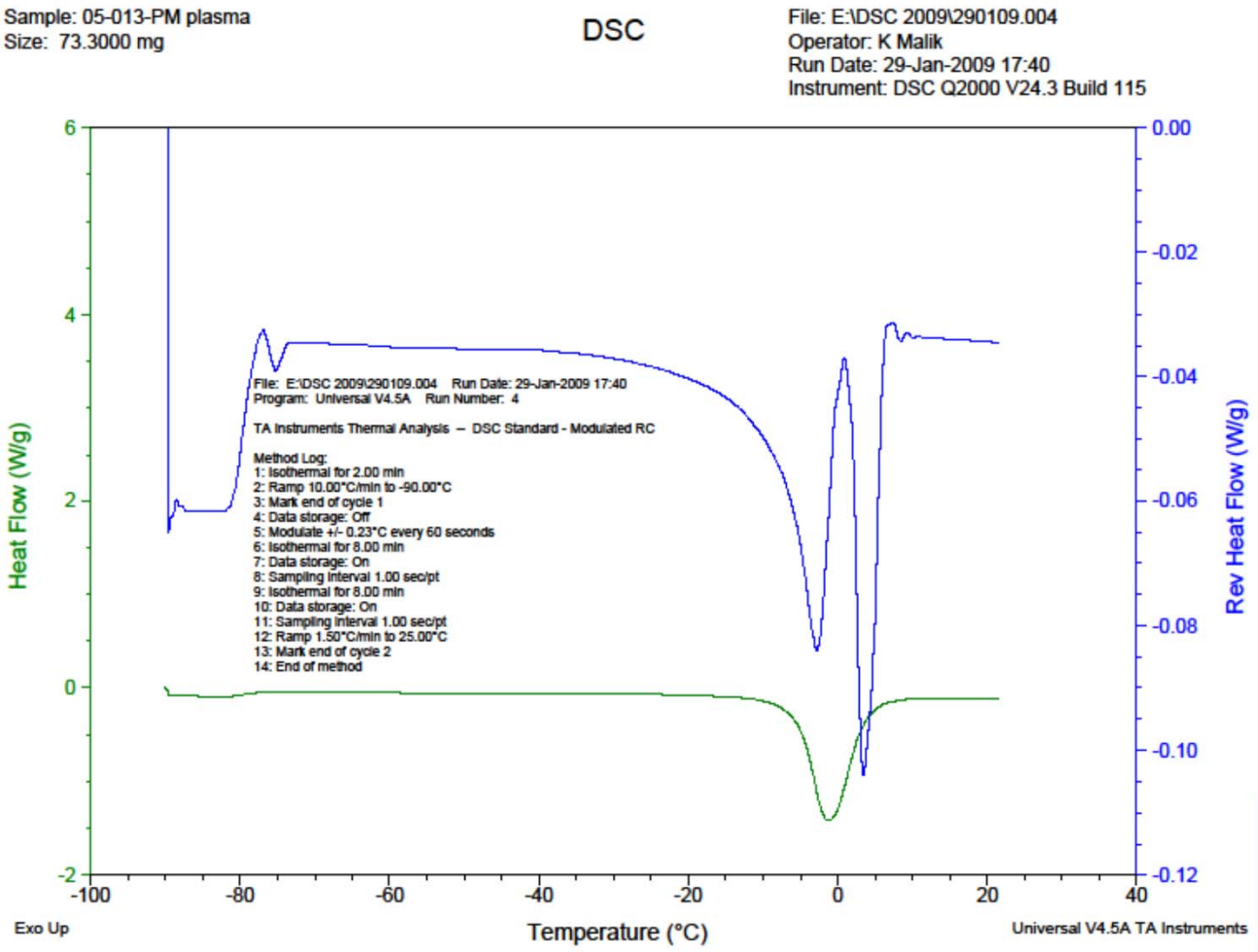

Impedance and DTA
Antitrypsin (A) 50 mg/ml (NIBSC test)
10000
1000
100
10
1
Electrical Impedance Z (kOhms)
-200
-150
-100
-50
0
50
Temperature (°C)
Delta-T (°C) (exo up)
Z Heating
Z Cooling


Impedance and DTA
Albumin (not desalted) (NIBSC test)
Electrical Impedance Z (kOhms)
10000
1000
100
10
1
-100
-80
-60
-40
-20
0
20
Temperature (°C)
Delta-T (°C) (exo up)
Z Heating
Z Cooling

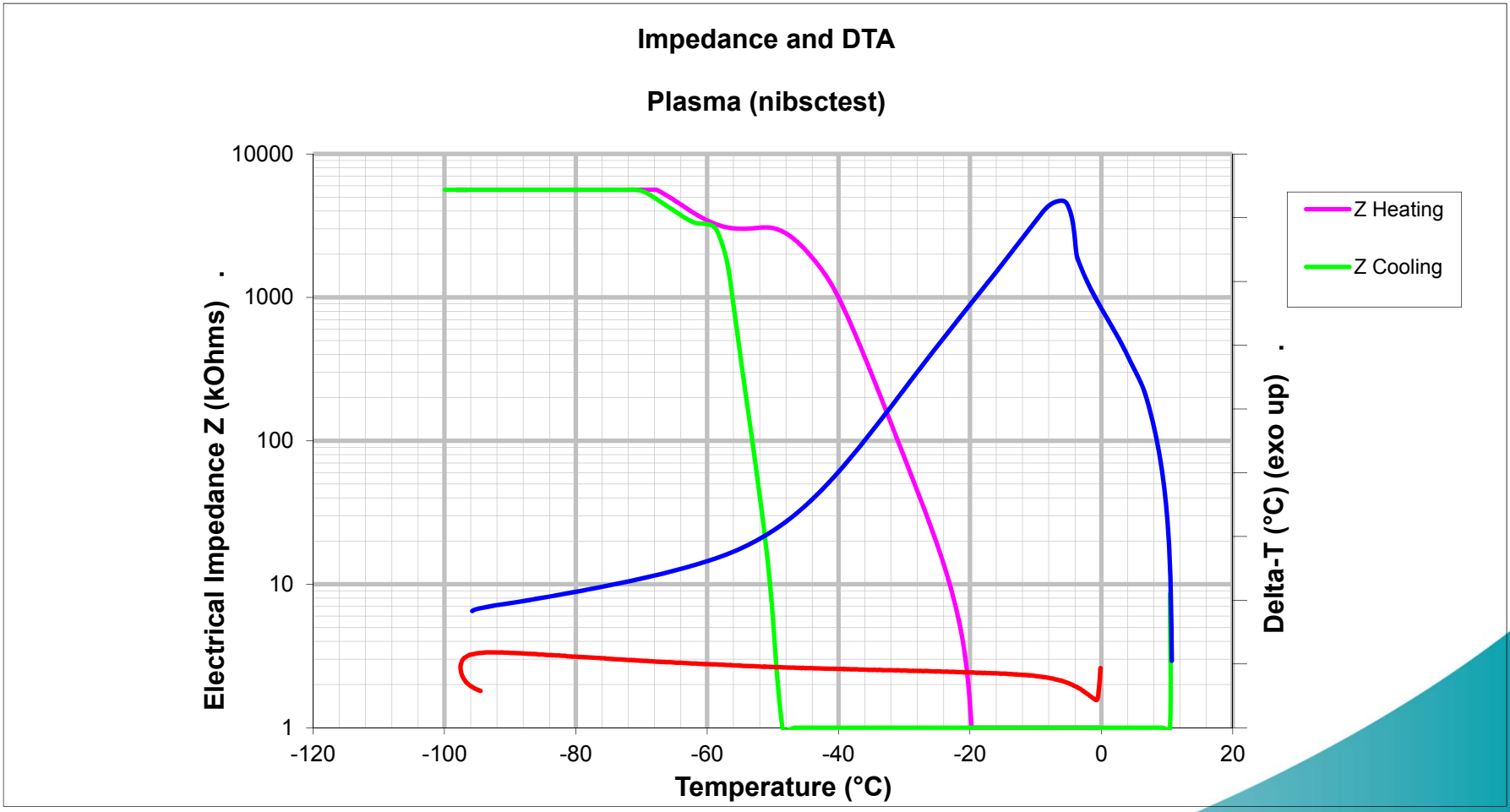
Albumin desalted



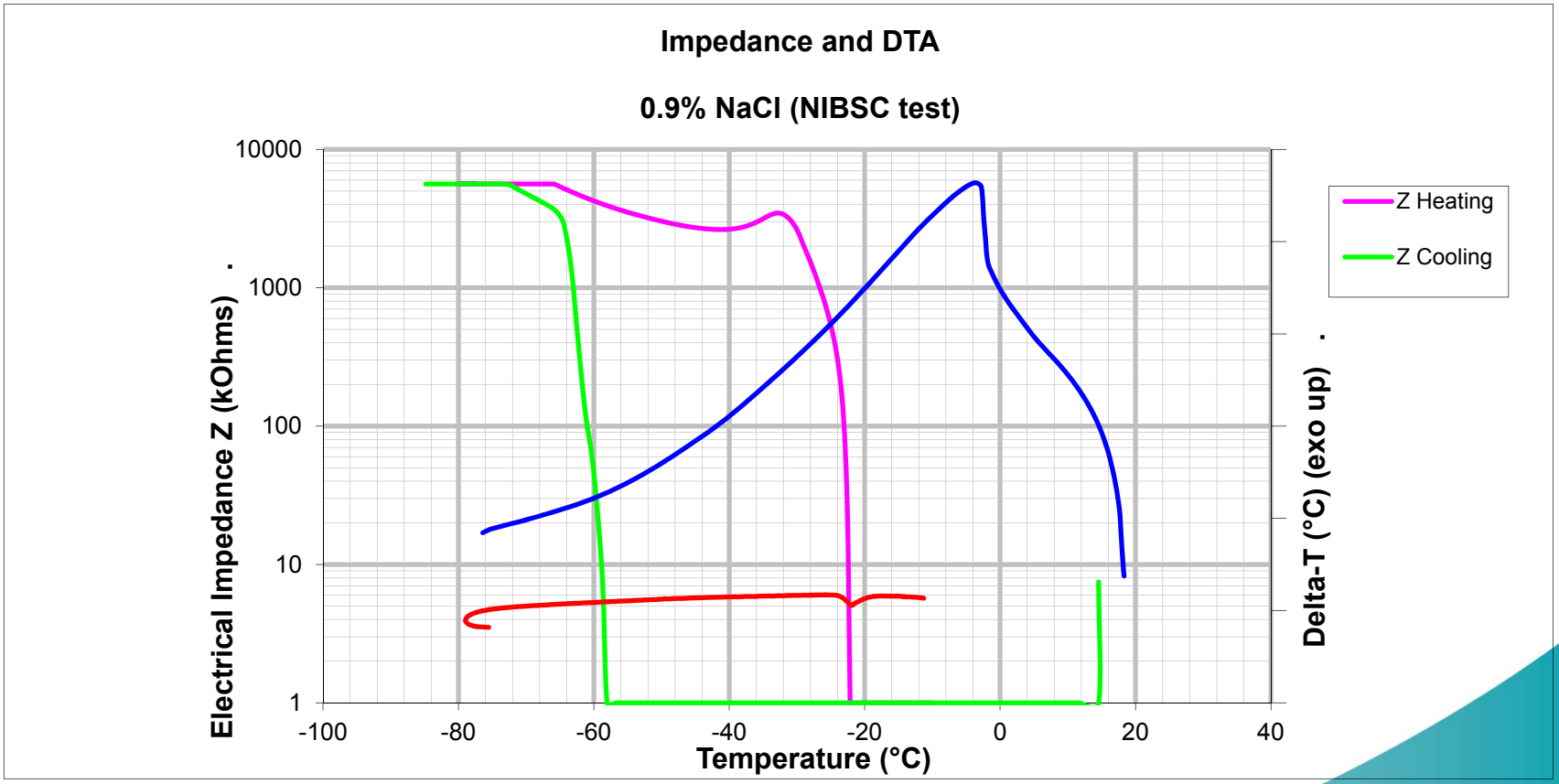
Modulated DSC of plasma



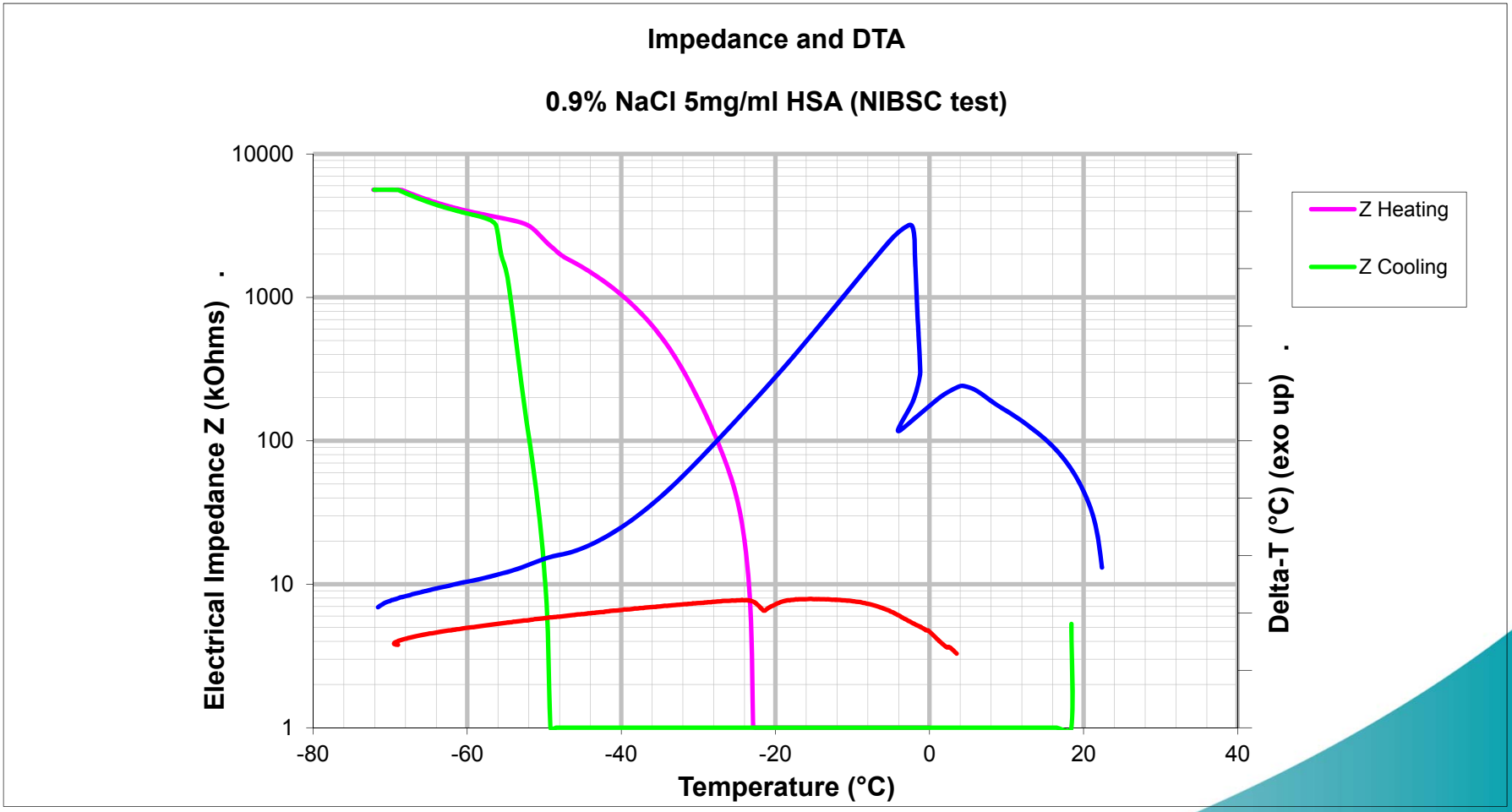
Impedance analysis of plasma



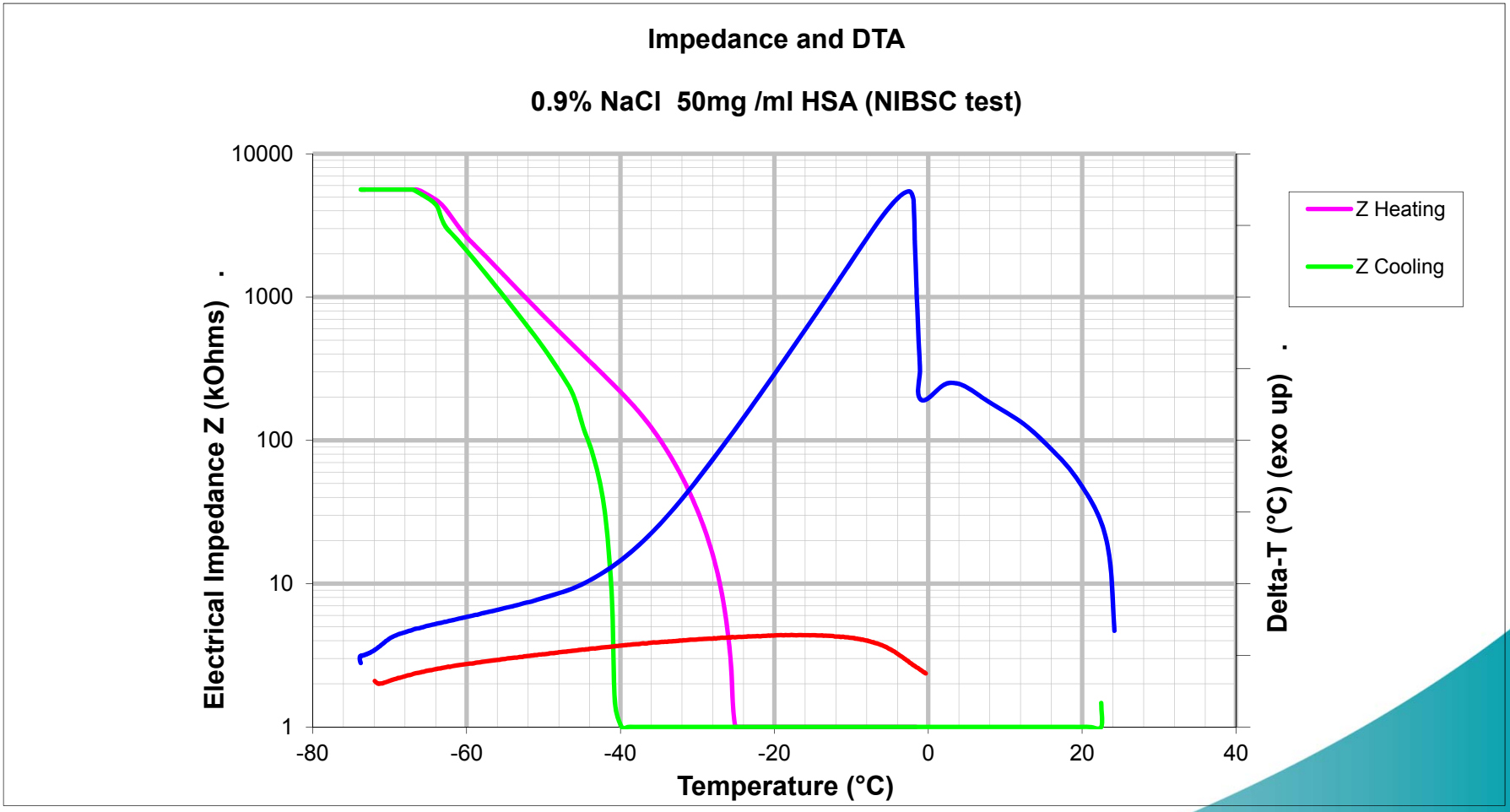
Impedance and DTA profiles of 0.9%w/v NaCl



Impedance and DTA profiles of 0.9%w/v NaCl + 5mg/ml HSA



Impedance and DTA profiles of 0.9%w/v NaCl + 50mg/ml HSA

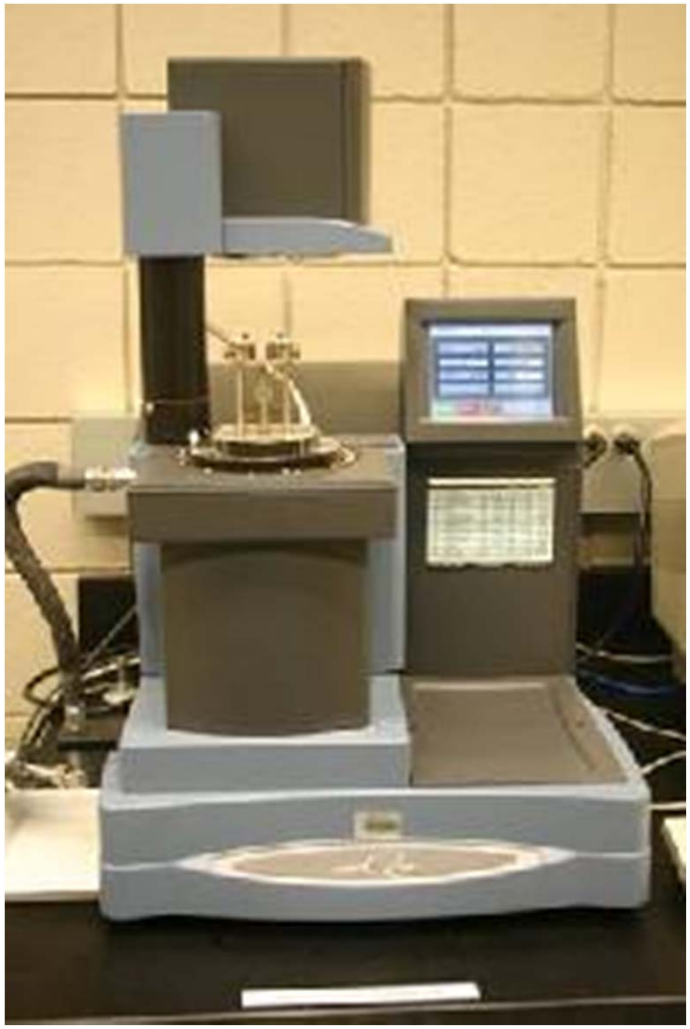


Comparison of Saline albumin & saline trehalose formulations freeze dried

Freeze drying outcomes left to right A-G

- A. 0.9% NaCl (Blue ring): **Crumbled to powder**
- B. 0.9% NaCl and 5mg/ml human serum albumin (Green ring): **Robust cakes, lossen when agigtated**
- C. 0.9% NaCl and 10mg/ml human serum albumin (Red ring): **Robust loose white cakes**
- D. 0.9% NaCl and 50mg/ml human serum albumin (Black ring): **Robust off white cakes, top surfaces cracked**
- E. 0.9% NaCl and 10mg/ml trehalose (Blue stripe): **Robust loose cakes**
- F. 0.9% NaCl and 20mg/ml trehalose (Green stripe): **Collapsed**
- G. 0.9% NaCl and 50mg/ml trehalose (Red stripe): **Cakes formed but very poor appearance, honey combed top surface**

Dynamic Mechanical Analysis DMA



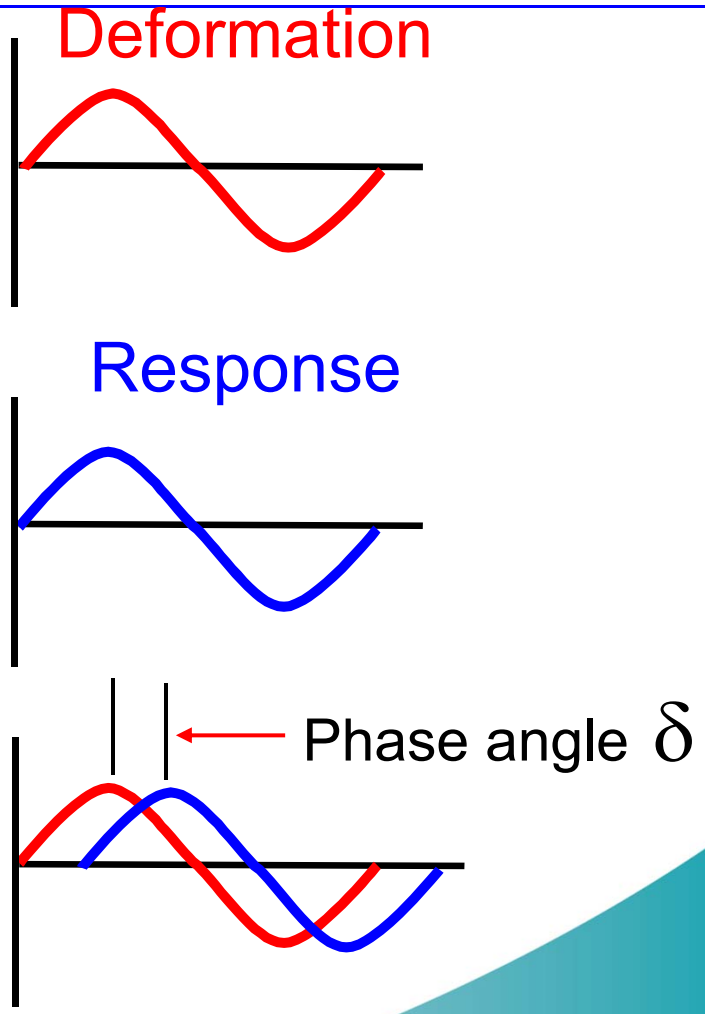
DMA involves applying a periodic stress (frequency/frequencies) to a sample and then measuring the resultant strain.

A dynamic stiffness can then be calculated and the damping (tan delta). When a sample passes through its Tg the tan delta increases dramatically.

Dynamic Mechanical Testing

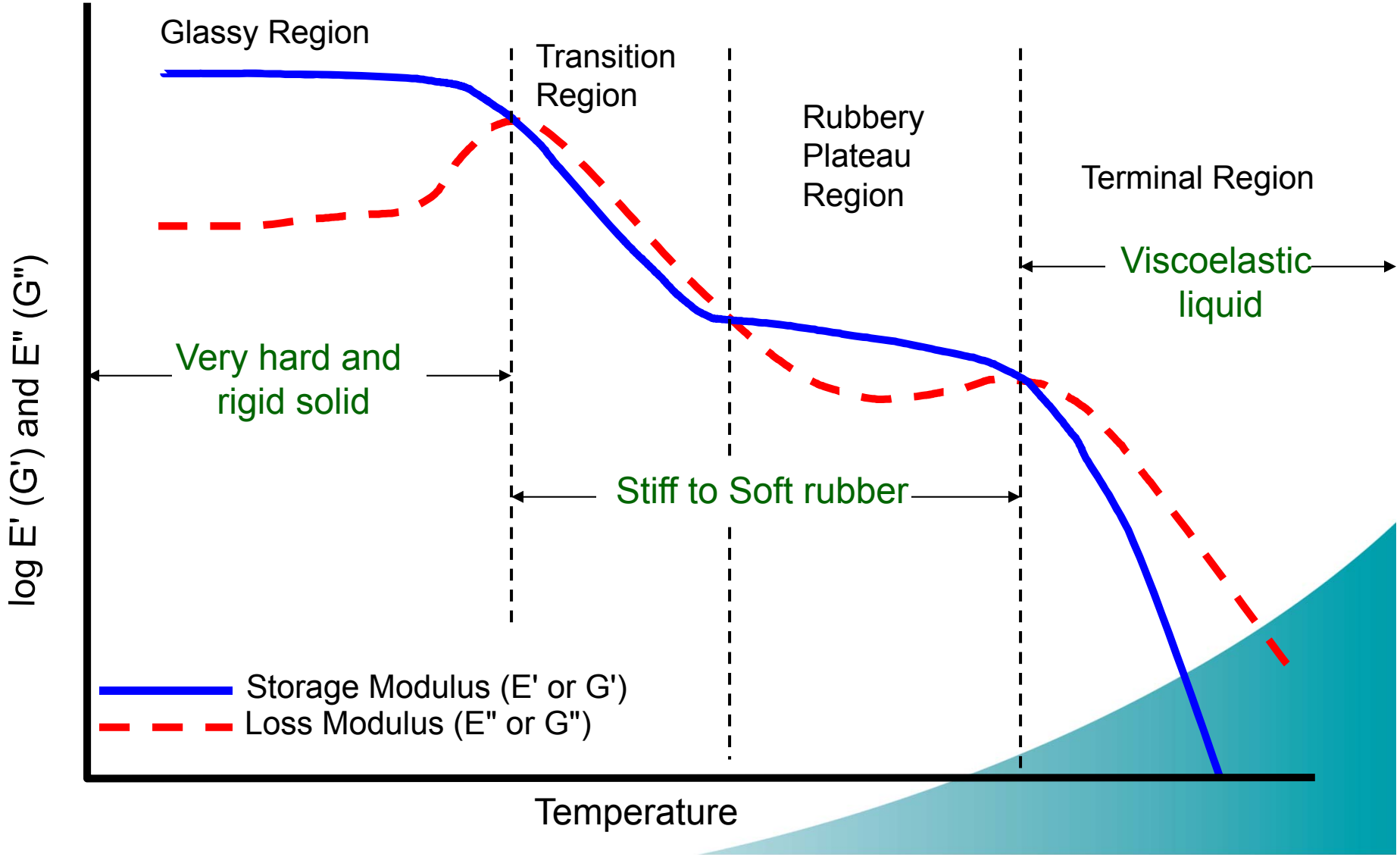
Slide courtesy of Phil Davies
TA Instruments

- An oscillatory (sinusoidal) deformation (stress or strain) is applied to a sample
- The material response (strain or stress) is measured.
- The phase angle δ, or phase shift, between the deformation and response is measured.



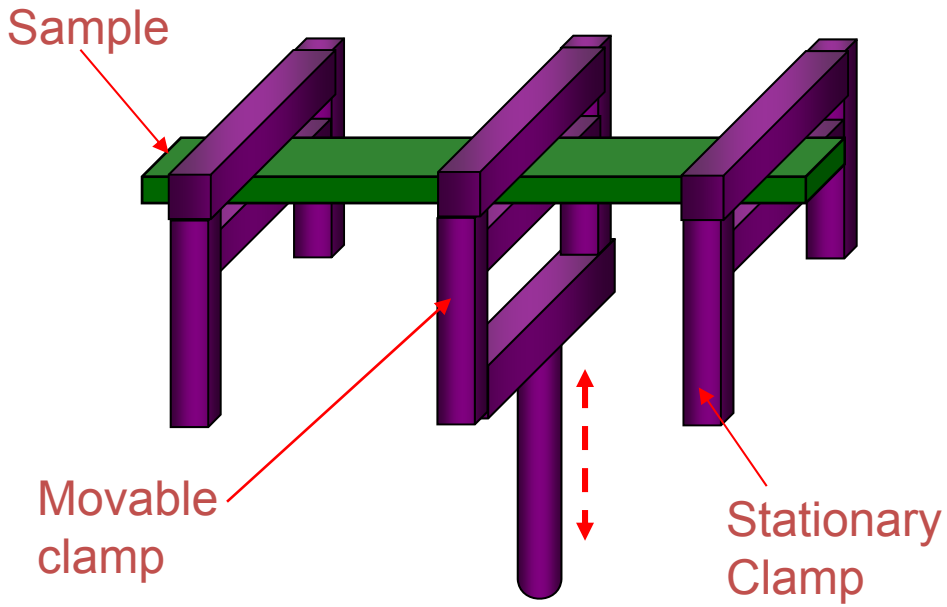
Viscoelastic Spectrum for a Typical Amorphous Polymer

Slide courtesy of Phil Davies
TA Instruments



Measure principles of Q800 DMA

Slide courtesy of Phil Davies
TA Instruments



- Good general purpose mode (thermoplastics, elastomers)
- Highly damped materials can be measured
- Best mode for evaluating the cure of supported material

Sample preparation for DMA:

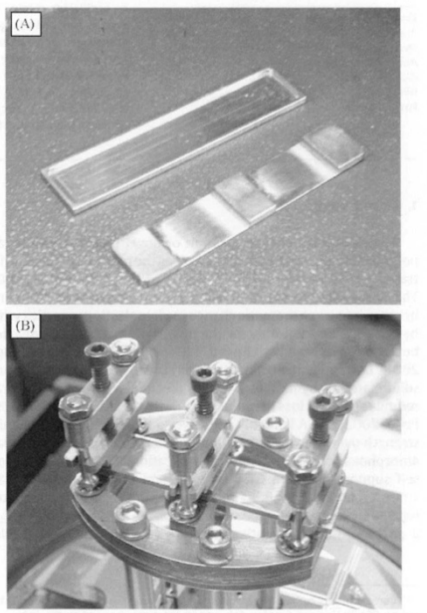


Fig. 1. (A) The new powder DMA sample holder, which consists of a rectangular stainless steel open container with inner dimensions of 60 mm × 11 mm × 1 mm into which the powder mix was placed. (B) Holder clamped into the DMA with the powdered sample sandwiched between the bottom of the holder and the detachable lid.

From Mahlin et al (2009) *Int J Pharm* 371;120-5

Can use wick or add liquid directly

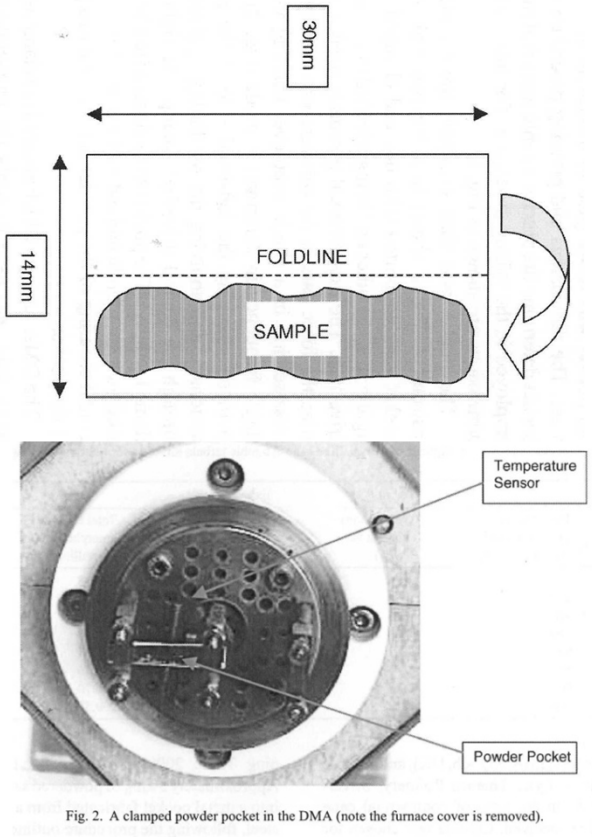


Fig. 2. A clamped powder pocket in the DMA (note the furnace cover is removed).

From Royall et al (2005) *Int J Pharm* 301;181-91

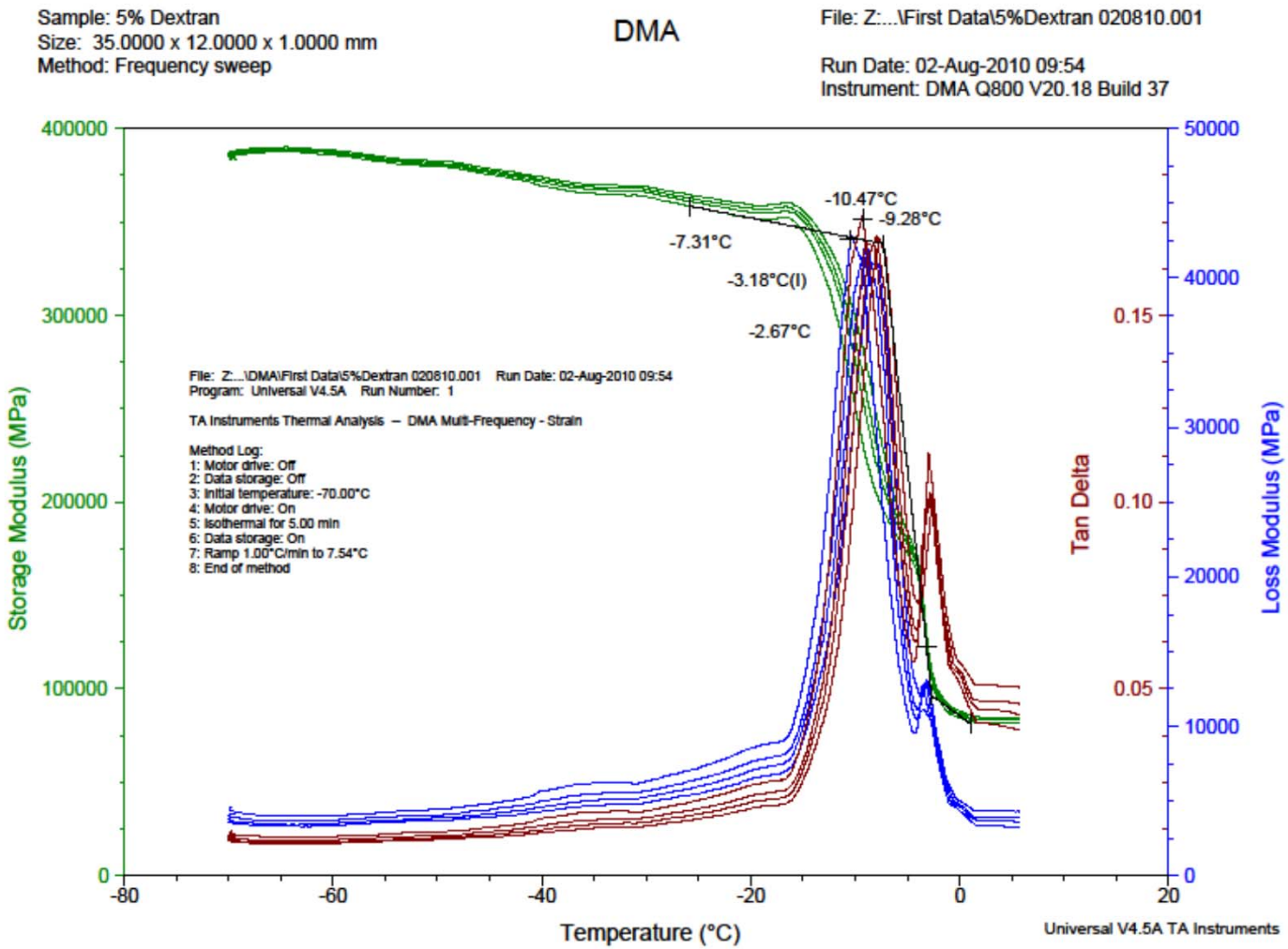
Novel technique - DMA

- The Modulus: Measure of material's overall resistance to deformation. E = Stress/Strain
- The Elastic (Storage) Modulus: Measure of elasticity of material. The ability of the material to store energy.

  E' = (stress/strain)cos δ
- The Viscous (Loss) Modulus:The ability of the material to dissipate energy. Energy lost as heat.

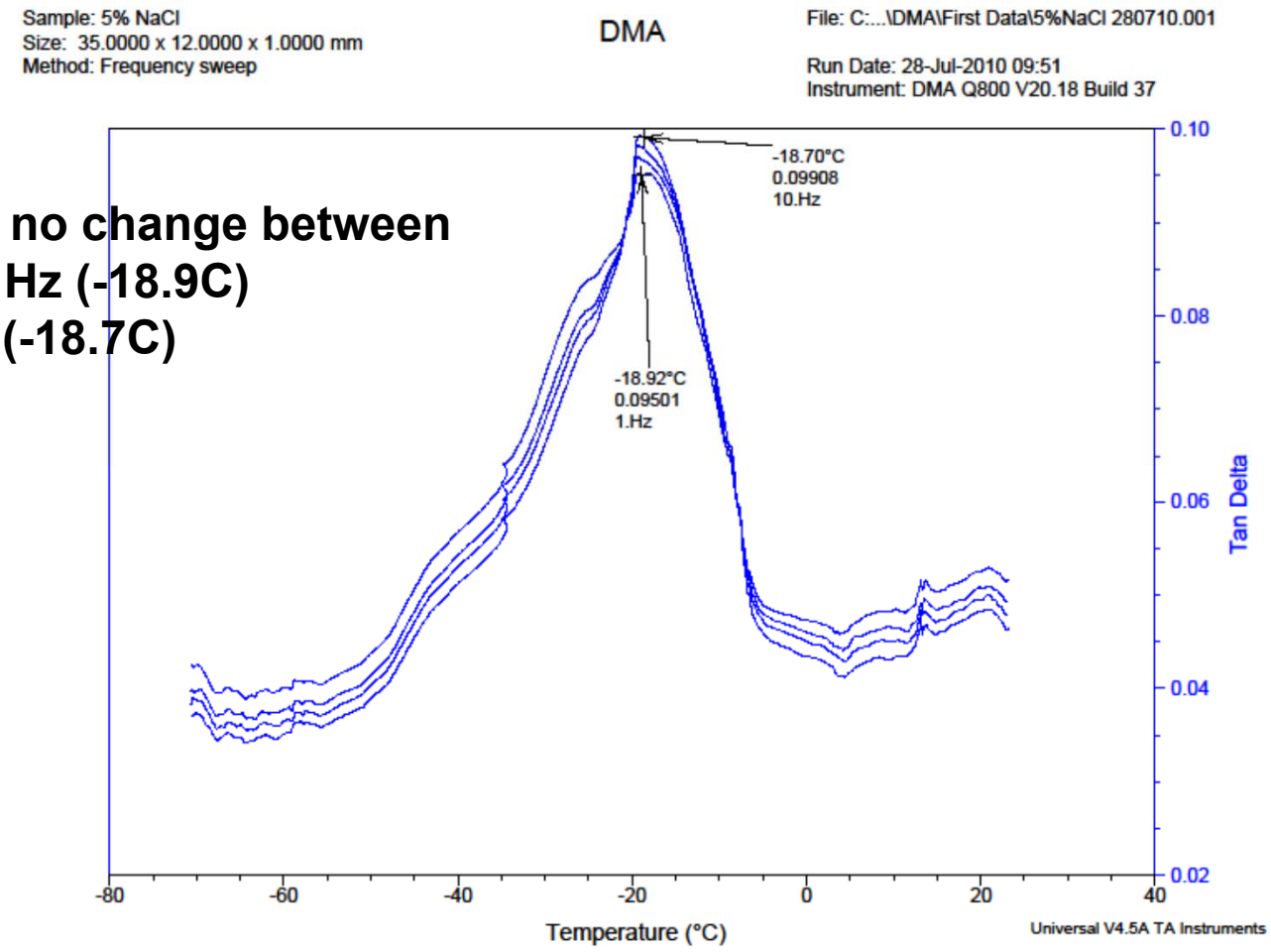
  E" =(stress/strain)sin δ
- Measure of material damping – such as vibration or sound damping. Tan δ = E"/E'

DMA of Tg' for dextran (5%)



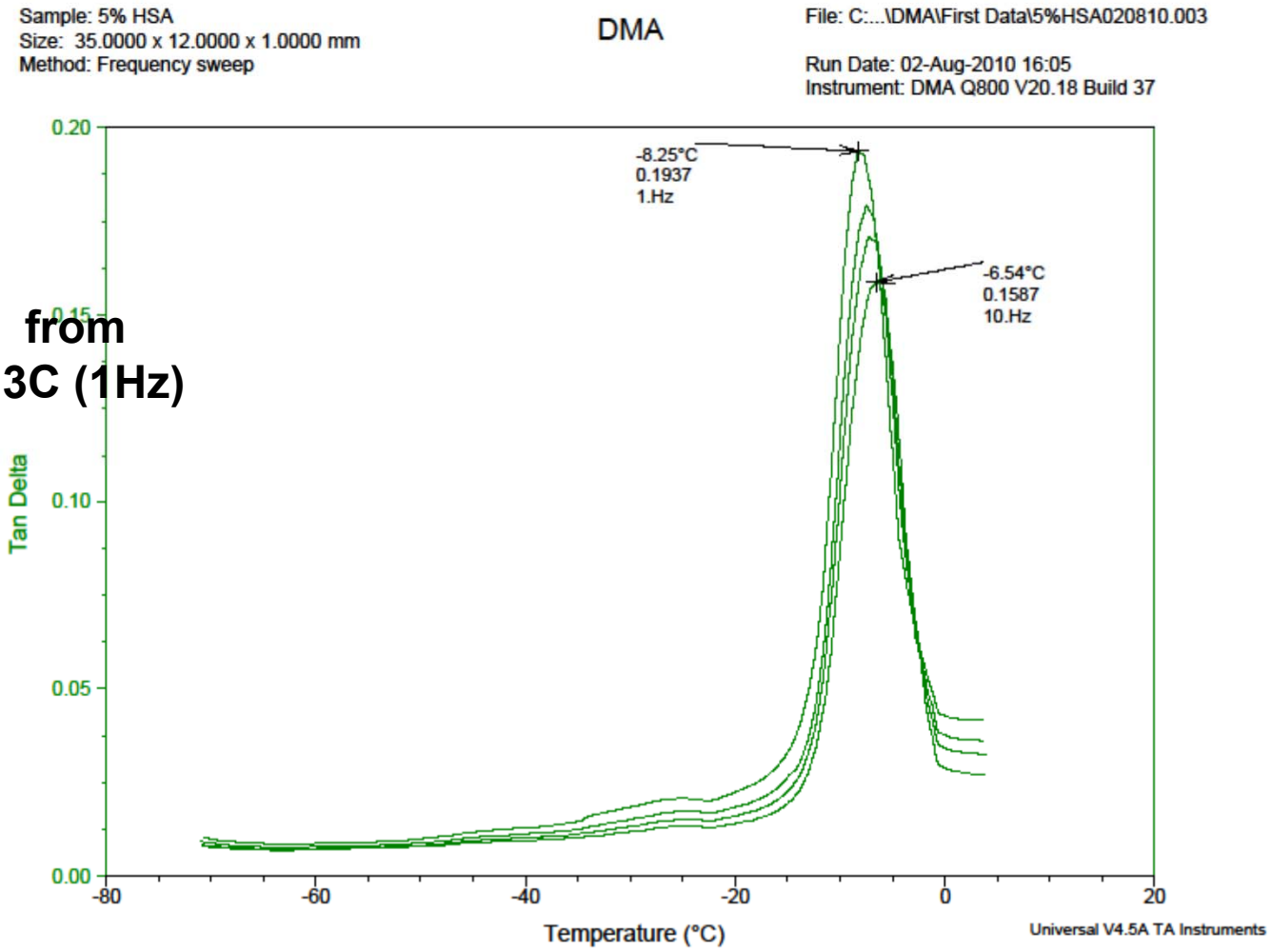
DMA of crystalline material

Effectively no change between
Tan delta 1Hz (-18.9C)
And 10 Hz (-18.7C)



DMA of amorphous material

Shift in Tan delta from -6.5C (10Hz) to 8.3C (1Hz)



E' Onset, E" Peak, and tan δ Peak

Slide courtesy of Phil Davies
TA Instruments

- E' Onset: Occurs at lowest temperature - Relates to mechanical Failure
- E" Peak: Occurs at middle temperature - more closely related to the physical property changes attributed to the glass transition in plastics. It reflects molecular processes - agrees with the idea of T_g as the temperature at the onset of segmental motion.
- tan δ Peak: Occurs at highest temperature - used historically in literature - a good measure of the "leatherlike" midpoint between the glassy and rubbery states - height and shape change systematically with amorphous content.

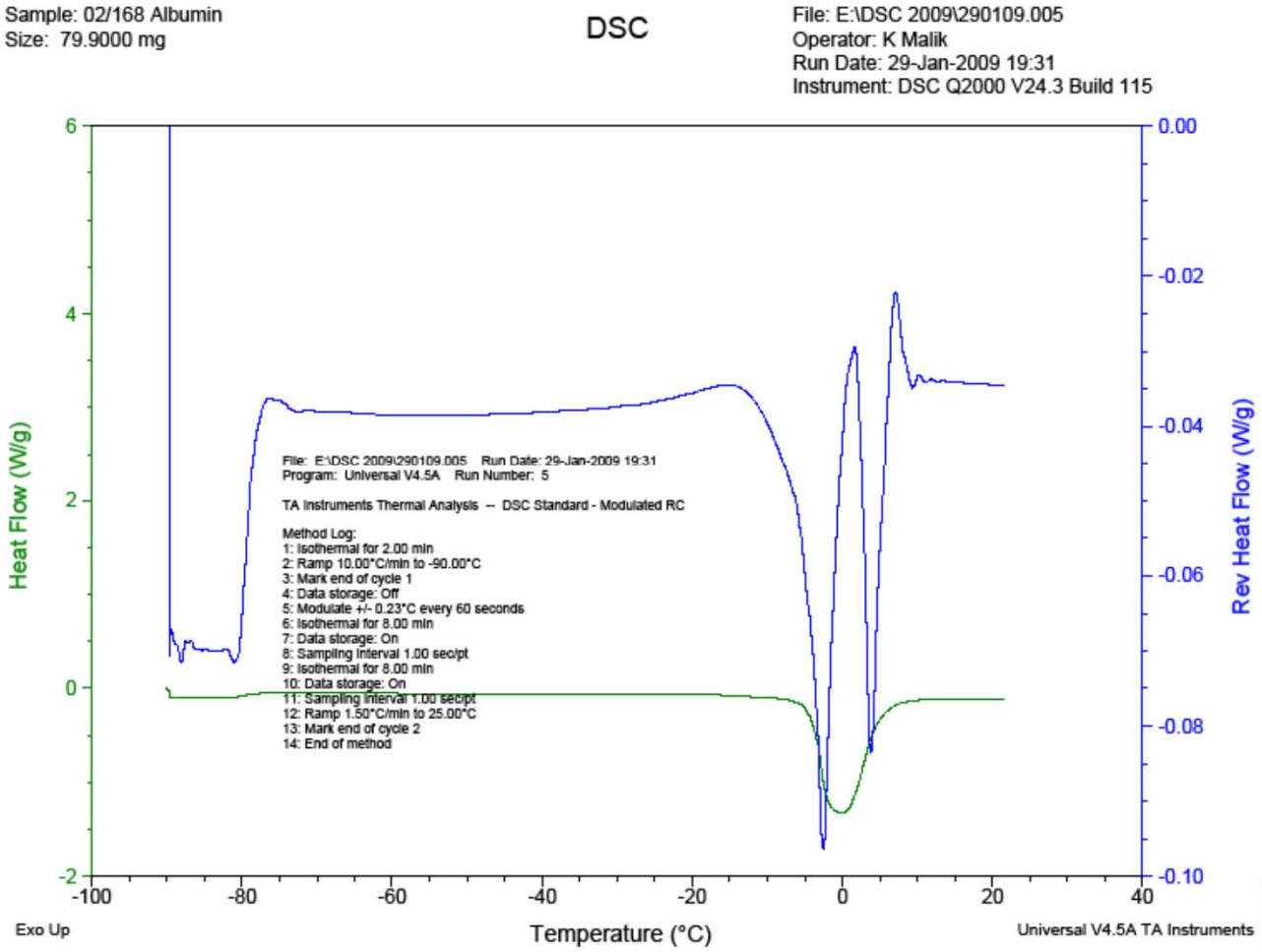
Reference: Turi, Edith, A, Thermal Characterization of Polymeric Materials, Second Edition, Volume I., Academic Press, Brooklyn, New York, P. 980.

Impact of analysis method on Tg' determination by DMA

Product	Tanδ max °C	Fall in Modulus (midpoint) °C	Onset of fall in Modulus °C	Typical DSC result °C
Trehalose (n=4)	-23.5	-23.4	-30.4	-29
Lactose (n=2)	-21.5	-27.5	-36.5	-32
Albumin (n=3)	-7.2	-13.4	-25.3	-9(Tc)

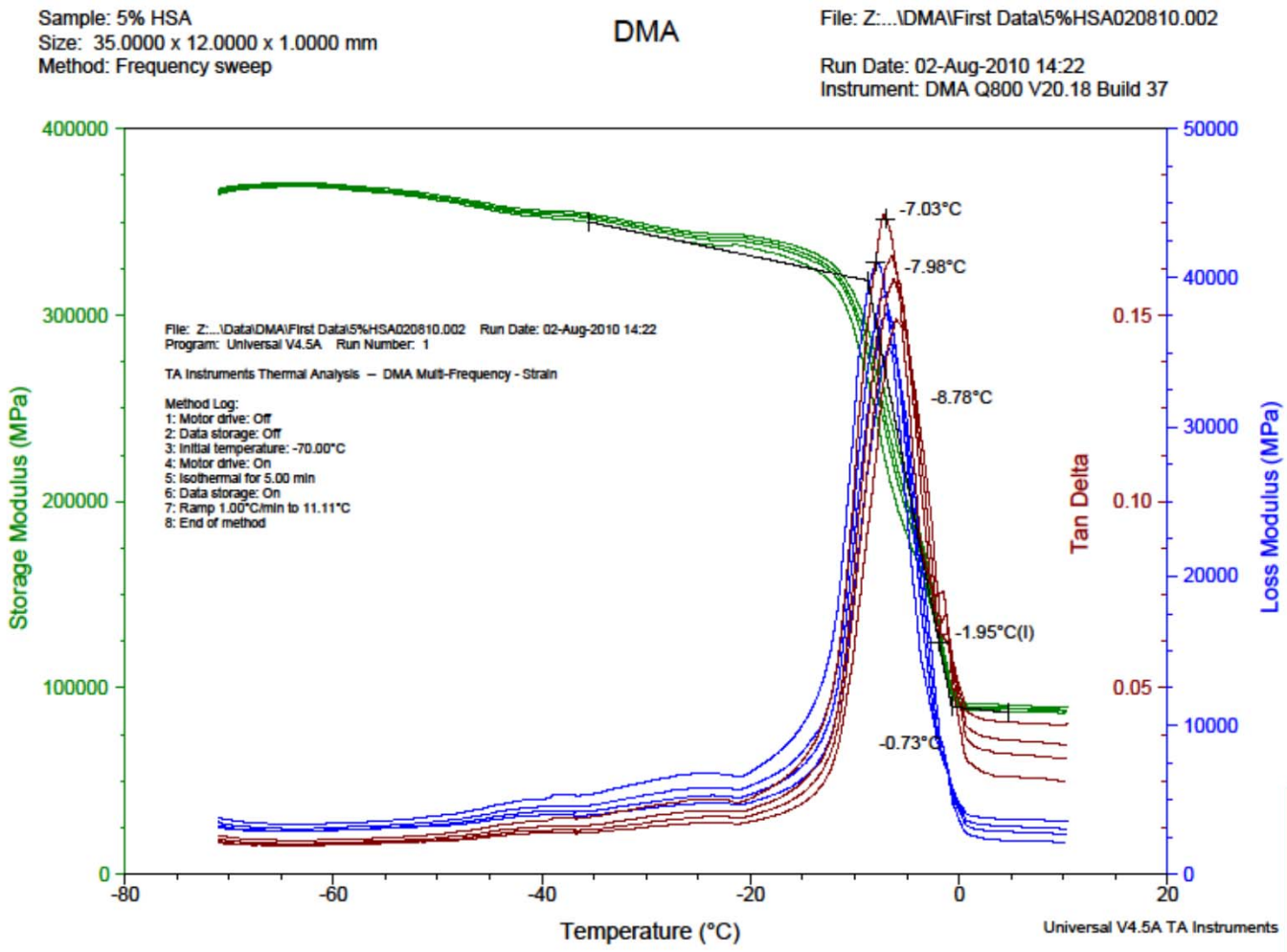
Comparison of Tg' values assigned by Tanδ max, midpoint of loss modulus and onset of loss modulus. (Gearing et al (2010) *Cryobiology* 61; 27-32)

Comparison DSC and DMA

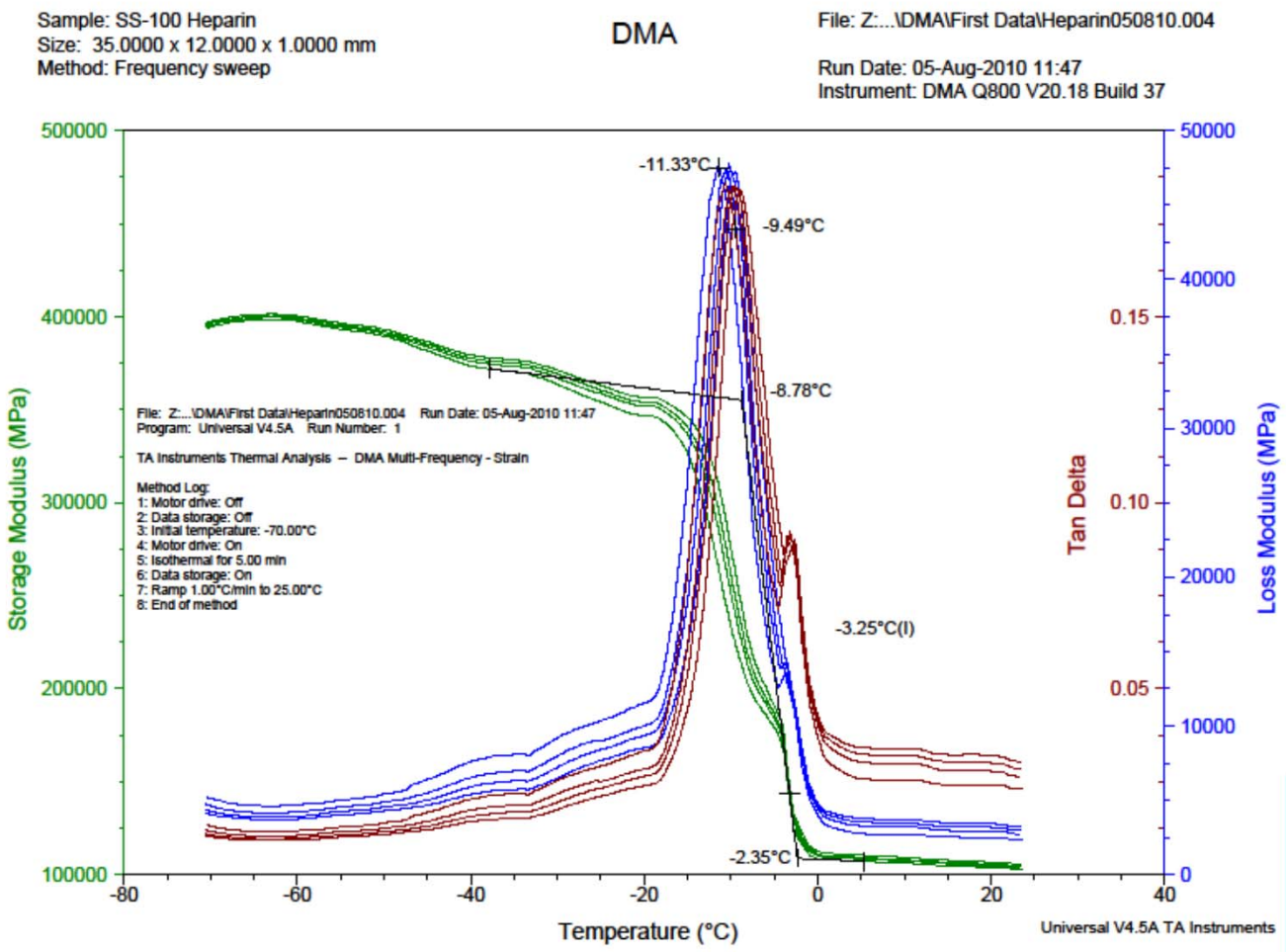


mDSC

DMA of HSA (5%)


Sample: 5% HSA
Size: 35.0000 x 12.0000 x 1.0000 mm
Method: Frequency sweep
DMA
File: Z:...\DMA\First Data\5%HSA020810.002
Run Date: 02-Aug-2010 14:22
Instrument: DMA Q800 V20.18 Build 37
File: Z:...\Data\DMA\First Data\5%HSA020810.002 Run Date: 02-Aug-2010 14:22
Program: Universal V4.5A Run Number: 1
TA Instruments Thermal Analysis -- DMA Multi-Frequency - Strain
Method Log:
1: Motor drive: Off
2: Data storage: Off
3: Initial temperature: -70.00°C
4: Motor drive: On
5: Isothermal for 5.00 min
6: Data storage: On
7: Ramp 1.00°C/min to 11.11°C
8: End of method
-7.03°C
-7.98°C
-8.78°C
-1.95°C(I)
-0.73°C
Storage Modulus (MPa)
Tan Delta
Loss Modulus (MPa)
Temperature (°C)
Universal V4.5A TA Instruments

DMA - Frequency scan Heparin



Reproducibility over 3 runs

Product	# 1 °C	#2 °C	#3 °C	Mean °C	CV %
Human plasma albumin	-7.22	-7.03	-8.2	-7.5	8.4
Heparin	-11.33	-11.06	-10.06	-10.8	6.1
Dextran	-10.5	-10.9	-10.3	-10.6	2.7

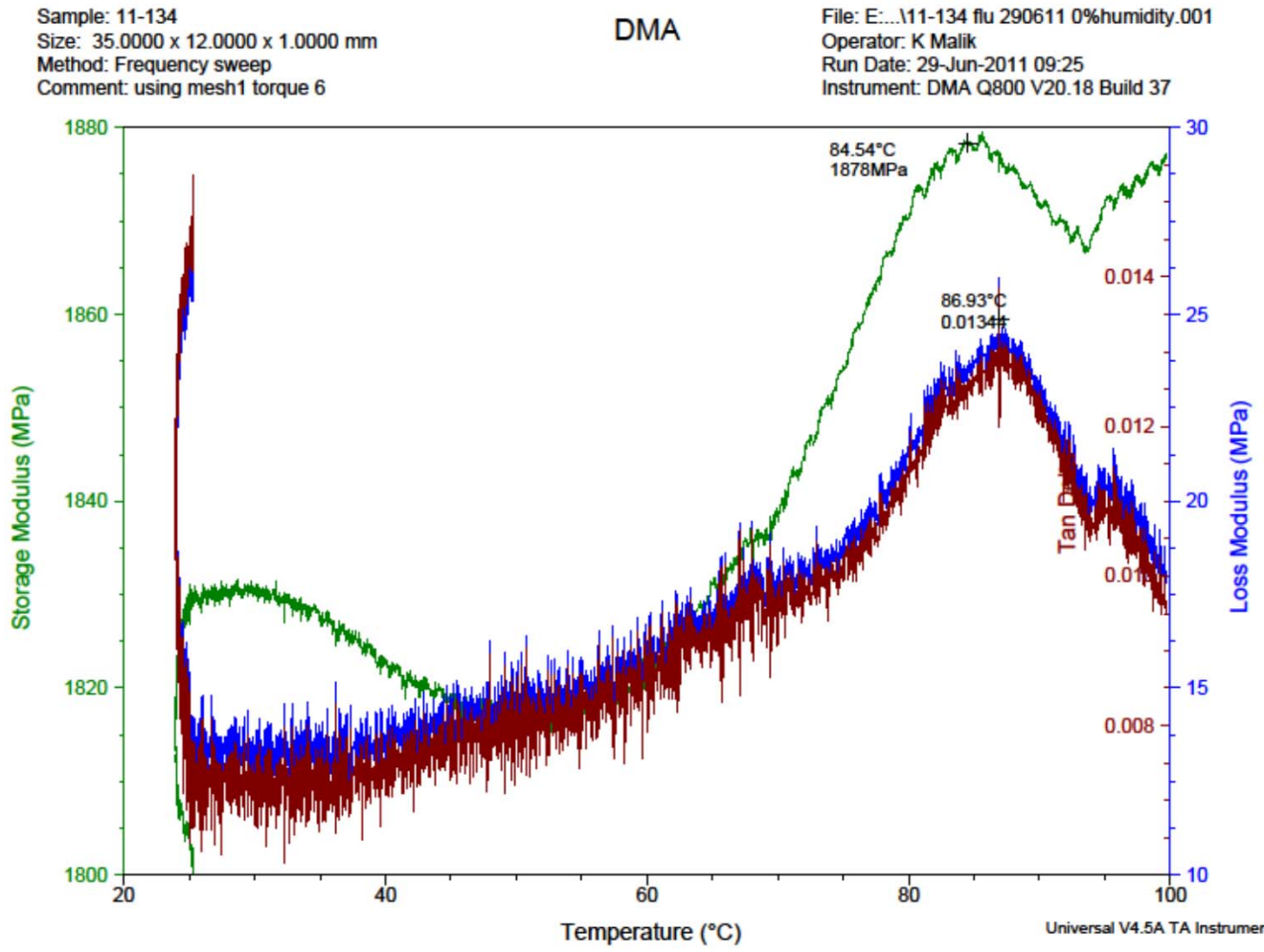
Where can DMA data fit into predicting freeze drying conditions ?

- Tg' are kinetic events
- DMA often used in food industry
- Recent literature indicates in high concentrations of protein higher Tc values than Tg' by DSC
- DMA values are higher than traditional Tg'
- Where no Tg' deliverable by other techniques DMA values may be useful
- Need to build data on DMA Tg' when used in freeze drying

Using DMA to study the impact of RH

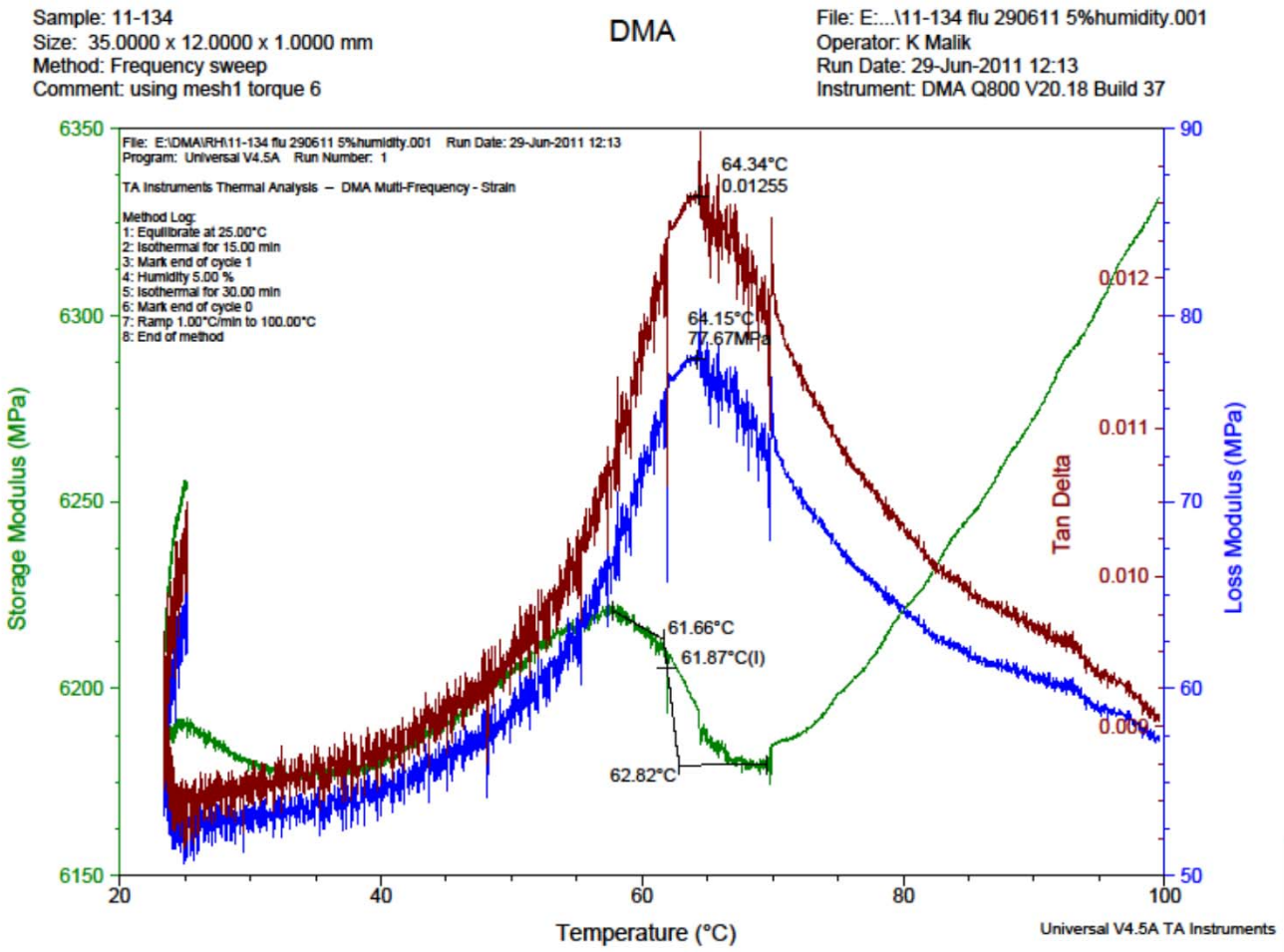
- The DMA system can deliver controlled relative humidity to the sample & by using a porous sample holder (in informal research collaboration with TA Instruments Ltd) we should be able to study the change in Tg with RH as well as temperature
- Studies begun with simple excipients in Jan 2011
- Intend to apply this technology to IS materials in order to better understand the impact of formulation and freeze drying conditions on the stability of IS materials on long term storage –especially where storage is in vials and not our familiar ampoule format

Flu antigen: amorphous material: 0% RH



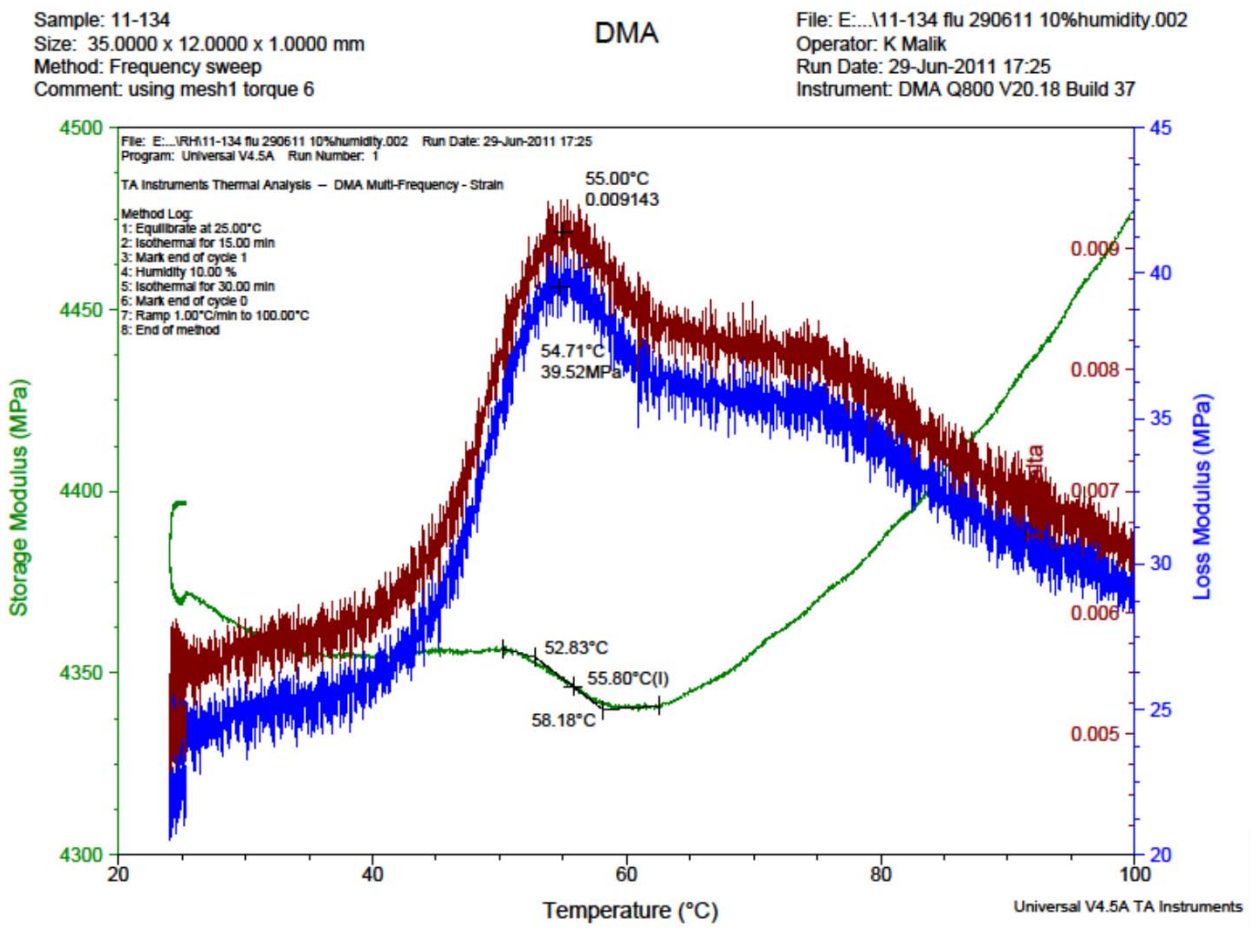
87ºC

Flu antigen: 5% RH



64ºC

Flu antigen: 10%RH



55ºC

Comparison on TA methods

Method	mDSC	Resistivity	DTA	FDM	Impedance	DMA
Pro's	• Sensitive, • Versatile –can measure Tg & Tg'	• Simple • Inexpensive	• Simple • Inexpensive	• parallel technique • Temp & vacuum • Small volume	• Simple • May detect weak events	• Sensitive
Con's	• Expensive, • Sample container, • Overlap of thermal events	• Broad transition • May miss weak events • dominated by eutectics?	• Broad transition • Multiple transitions	• small volume • interpretation	• familiarity • interpretation	• familiarity • Expensive • Broad responses

Conclusions

- Thermal analysis is a vital tool in predicting the impact of formulation changes on the freeze drying conditions required
- Can use TA to optimise freeze drying processes
- Different TA methods can be applied - pro's and con's
- Other techniques (e.g. FDM, tomography)may add visualisation
- Impedance provides an alternative indicating softening may occur below Tg'
- DMA is a technique which can offer versatile alternative for both Tg' and Tg determinations
- Dry state Tg determinations are a useful guide for studying impact of moisture on glass stability though may not be the whole story

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