QUALIFICATION OF ANALYTICAL INSTRUMENTS

CONTENTS

INDROCTION

- Analytical Instrument Qualification (AIQ) is documented evidence that an instrument performs suitably for its in-tended purpose and that it is properly maintained and calibrated. Use of a qualified instrument in analyses contributes to confidence in the veracity of generated data.
- The regulations also require the companies to establish procedures assuring that the instruments that generate data supporting regulated product testing are fit for use.
- The regulations, however, do not pro-vide clear and authoritative guidance for validation/qualification of analytical instruments.

QUALIFICATION

Action of proving and documenting that equipment or ancillary systems are properly installed, work correctly, and actually lead to the expected results.

The qualification consists of four parts:

- Design qualification
- Installation qualification
- Operational qualification
- Performance qualification

QUALIFICATION TIME LINE

Design Qualification	Installation Qualification	Operational Qualification	Performance Qualification
Before purchasing new instrument	At documented installation of new or exciting instrument	After installation major changes like Repairs, updates at regular interval. risk based	Whenever the instrument is used e.g. daily

DESIGN QUALIFICATION

- The AIQ process timeline begins with the DQ phase at the vendor site , in which the instrument is developed ,designed, and produced in a validated environment according to good laboratory practice(GLP), and current good manufacturing practices(CGMP), and ISO standards.
- It describe the user requirements and defines the functional and operational specifications of the instrument. DQ should ensure that instrument to purchased have the necessary functions and performance that will enable for suitable intended application.28

Installation Qualification(IQ)

Installation qualification is a documented collection of activities need to install an instrument in the users environment.

- system description
- Utilities/facility/environment
- Network and data storage
- Assembly and installation
- Installation verification

Operational Qualification(OQ)

After a successful IQ the instrument is reedy for OQ testing

The OQ phase may consist of these parameters.

- Fixed parameter
- Secure data storage , backup, and Archive
- Instrument functions tests

Performance Qualification(PQ)

- Once on IQ and an OQ have been performed ,PQ testing is conducted.

PQ testing should be performed under the actual running condition across the anticipated working range.

- The frequency depend on such parameter:
- Performance checks
- Preventive militance and repairs
- Standard operating procedure for operation, calibration, and maintenance
- Software validation
  - ✓ Firmware
  - ✓ Stand alone software
  - ✓ Instrument control, data acquisition, and processing software

Change Control

- Change control follows the DQ/IQ/OQ/PQ classification process .
- For DQ , evaluate the change parameters, need for the change warrants implementing it.
- If the implementation of change is needed, install the changes to system during IQ.
- OQ and PQ test need revision, deletion, or addition as the result of the installed change.

AIQ DOCUMENTATION

Two types of result from AIQ

- Static
- Dynamic

Static document:

This document obtained during the DQ,IQ and OQ phases and should be kept in a “qualification binder”.

Dynamic documents:

this document are generated during the OQ and PQ phases, when the instrument is maintained, or when it is tested for performance.

Instrument Categories

- Modern laboratories typically include a suite of tools. These vary from simple spatulas to complex automated instruments.
- The users are the most qualified to establish the level of qualification needed for an instrument. Based on the level of qualification needed, it is convenient to categorize instruments into 3 groups: A, B, and C,

  i) Group A Instruments

  ii) Group B Instruments

  iii) Group C Instruments

Roles And Responsibilities

Users

- Users are ultimately responsible for the instrument operations and data quality
- Users group includes analysts, their supervisors, and the organizational management. Users should be adequately trained in the instrument's use, and their training records should be maintained as required by the regulations.

Quality Assurance

- The quality assurance (QA) role in AIQ remains as it is in any other regulated study. QA personnel should understand the instrument qualification process, and they should learn the instrument's application by working with the users. Finally, they should review the AIQ process to determine whether it meets regulatory requirements and that the users attest to its scientific validity.

FTIR- Fourier Transform Infrared Spectroscopy

INTRODUCTION

- FTIR stands for Fourier transform infrared, the preferred method of infrared spectroscopy. In infrared spectroscopy, IR radiation is passed through a sample. Some of the infrared radiation is absorbed by the sample and some of it is passed through (transmitted).
- The resulting spectrum represents the molecular absorption and transmission, creating a molecular fingerprint of the sample. Like a fingerprint no two unique molecular structures produce the same infrared spectrum.
- This makes infrared spectroscopy useful for several types of analysis.

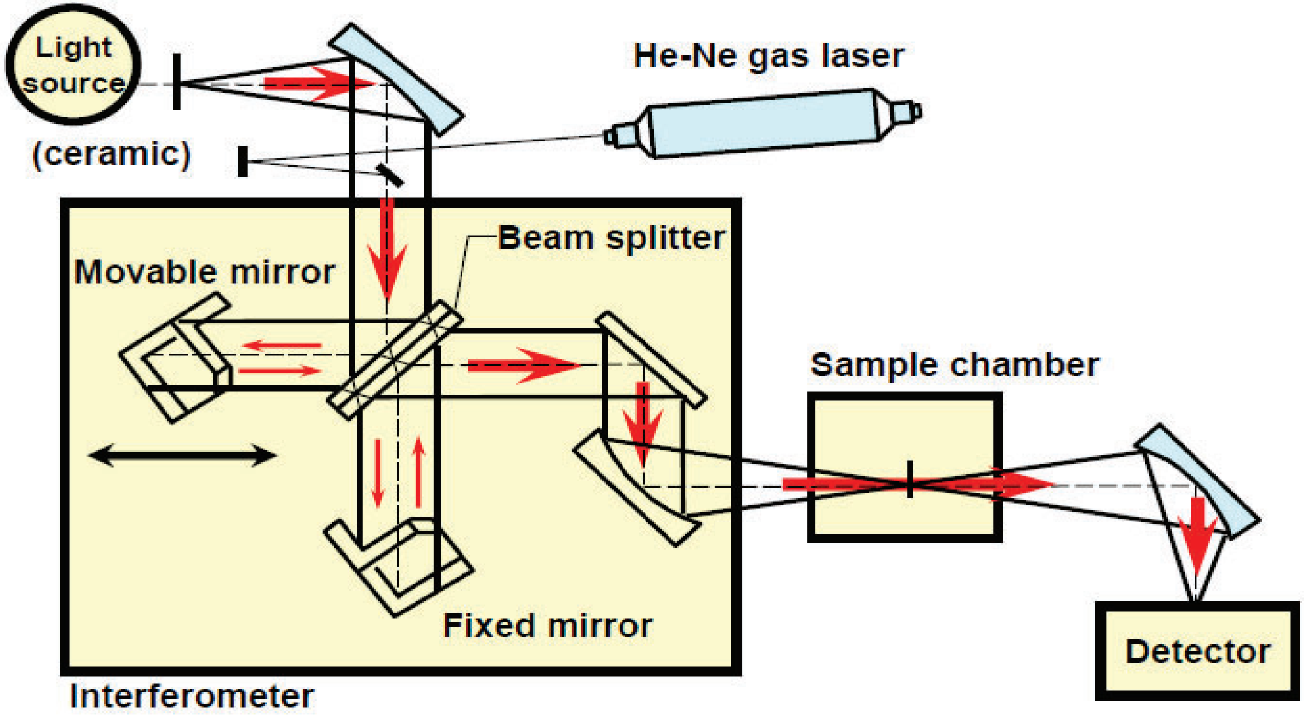
FTIR…

- A technique which is used to obtain an infrared spectrum of absorption ,emission, photoconductivity or Raman scattering of a solid ,liquid or gas.
- An FTIR spectrometer simultaneously collects spectral data in a wide spectra

Components of FTIR

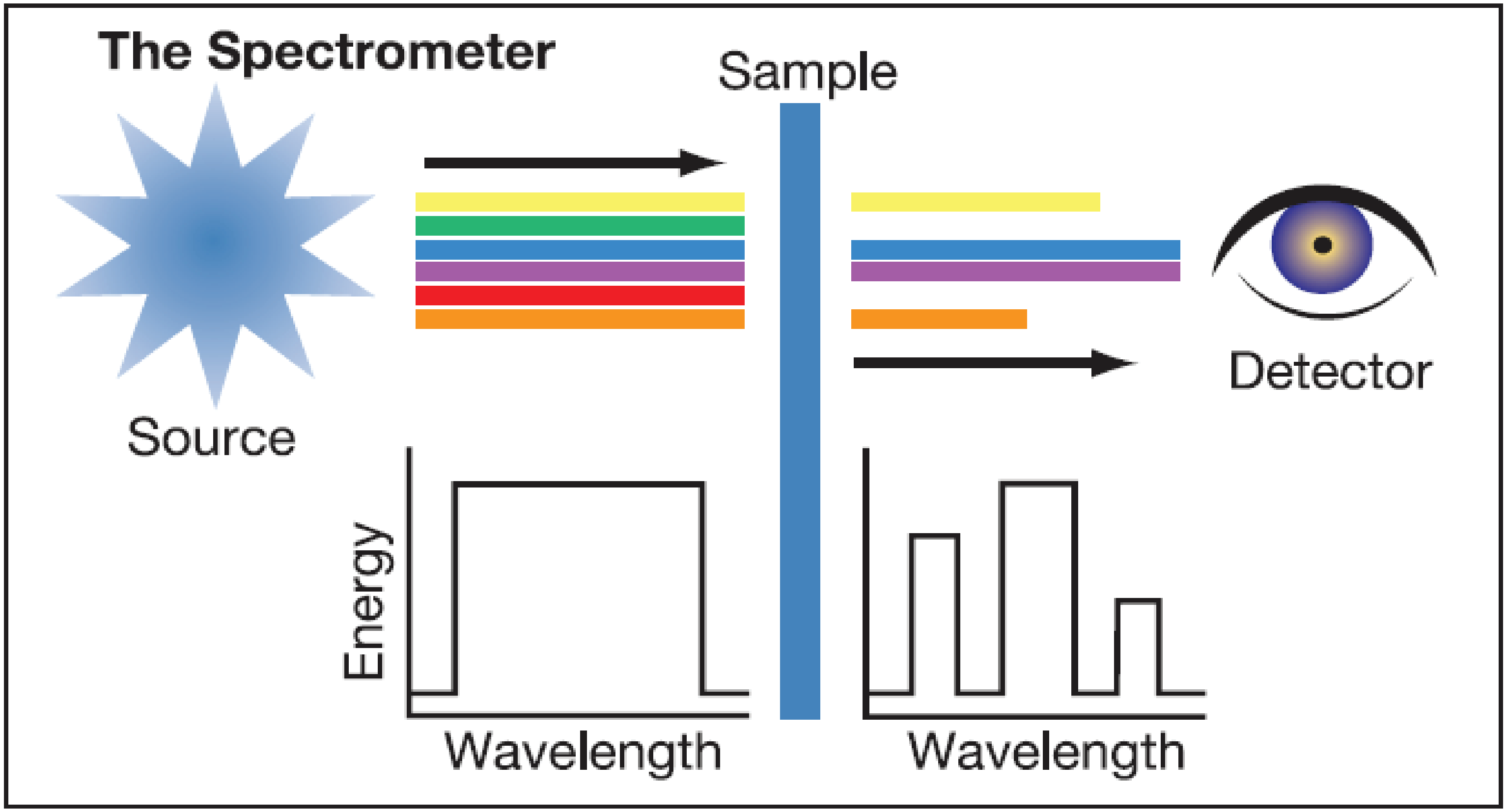
- IR Radiation source
- Beam Splitter
- Fixed mirror
- Moving mirror
- Collimating mirrors
- Sample holder
- Helium Neon laser
- Detector

FTIR - Working



Conceptual Introduction

- The goal of any absorption spectroscopy(FTIR, ultraviolet-visible ("UV-Vis") spectroscopy, etc.) is to measure how much light a sample absorbs at each wavelength. The most straightforward way to do this, the "dispersive spectroscopy" technique, is to shine a monochromatic light beam at a sample, measure how much of the light is absorbed, a Fourier-transform spectroscopy is a less intuitive way to obtain the same information.
- Rather than shining a monochromatic beam of light (a beam composed of only a single wavelength) at the sample, this technique shines a beam containing many frequencies of light at once and measures how much of that beam is absorbed by the sample. Next, the beam is modified to contain a different combination of frequencies, giving a second data point. This process is rapidly repeated many times over a short timespan. Afterwards, a computer takes all this data and works backward to infer what the absorption is at each wavelength


The Spectrometer
Sample
Source
Detector
Energy
Wavelength
Wavelength

Qualification of IR spectrophotometer

INTRODUCTION

- The present document explains about “Qualification of Equipment (IR Spectrophotometer)”,

It should be used in combination with it when planning, performing and documenting the IR spectrophotometer qualification process.

- The document contains the Introduction and general forms for Level I and II of qualification, which are common to all type of instruments. For FTIR spectrometers, an example has been added to give instrument-specific proposals that may be used in combination with the general requirements presented in the core document "Qualification of Equipment”, when drawing up a Level I checklist.

Calibration

Calibration is the process by which ensure that an instrument readings are accurate the reference to establish standard. Calibration is performed by using primary standard. It is done to check the zero error deflection by using standard reference.

Calibration Management

- Parts of a calibration management system
- Procedure(s)
- Documentation
- Calibration standards
- Calibration management software
- Calibration interval adjustment
- OOC/OOT evaluation
- What can go wrong and how to avoid it

Wave Number Precision

This is performed for substances with well known peak wave number (s) position such as

- Carbon di oxide
- Water vapour
- Polystyrene
- Ammonia
- Test is performed to know whether the exact peak wave numbers are at that time of validation.
- Result between the peak wavenumbers position for a substance with a well known peak wave numbers and values indicated by the system.

Wave Number Precision…

O % Transmittance:

- A sample which do not allow the transmission of light is measured in order to investigate the o percentage transmittance.
- This test thus can be used to find out error caused by stay light and secondary emission spectra .

100 % transmittance:

- This is investigated by performing analysis with out a sample .
- By performing analysis with out sample 100 % transmittance can be investigated.

Linearity of curve

- A calibration curve for the % transmittance and the concentration is created and the linearity of the inspected.
- Reproducibility:
- A stable sample is measured twice with in a short period and confirmed whether the variation in the measurement values such as wave numbers and transmittance are obtained.

Validation of FTIR

- To perform FTIR validation and to confirm that it is operating properly, diverse IR inspection was performed by measuring the spectra of polystyrene film.

Installation of validation programme :

Software validates the Nicolet iS50 FTIR

Design qualification

- Supplier must be provided documented evidence that the product has been designed, developed, manufactured in a quality environment e.g. iso 9001:2000 certification.
- Supplier must be provide phone and on site support in case of defects.
- Following information should be necessary in design qualification of FT-IR.

Level I. Selection Of Instruments & Suppliers

Level I. Selection Of Instruments & Suppliers

- Example of check-list (Non-Exhaustive)
- Manufacturer:_____________
- Provider/ Distributor:____________
- Name Of Instrument and Type: ___________

Selection of Instruments & Suppliers…

Attribute (This list may be adapted if necessary)	Specifications	Benefits (Instrument/ supplier)	Assessment (Pass/Fail)
SPECTROPHOTOMETER			
Detector range	The optical bench shall include a DTGS detector with a frequency range of 7400 to 350 cm-1		
It shall include a Compressed air interferometer			
Spectral resolution			
The instrument shall Come with an air-cooled Standard infrared source.			

Selection of Instruments & Suppliers…

Attribute (This list may be adapted if necessary)	Specifications	Benefits (Instrument/supplier)	Assessment (Pass/Fail)
Wave number accuracy	The instrument shall have a spectral resolution not exceeding 1.0 cm-1		
The interferometer shall have at least four basic velocity levels: software shall permit the selection of a greater number of velocities between the basic levels.	Wave number accuracy shall be better than +- 0.01 cm-1		
The mirror's greatest velocity shall allow a speed of at least five sweeps per second			
The laser and infrared beams must be coaxial to enable rapid, easy alignment of the system, depending on the samples or accessories			

Selection of Instruments & Suppliers…

Attribute (This list may be adapted if necessary)	Specifications	Benefits (Instrument/ supplier)	Assessment (Pass/Fail)
The optical bench shall have main experimentation module with a device to allow purging with nitrogen			
At purchase, the main experimentation module shall be designed so it can receive 13 and 5 mm potassium bromide pellets			

Level II of Equipment Qualification or installation qualification

Installation and Release for use

- It is recommended to check all requirements set during the selection of the instrument, and calibration should be performed before putting into service by an accredited external service supplier,

or

- Internally by appropriately qualified personnel, using certified reference buffers according to an approved procedure.
- Correct software installation is verified
- The supplier instruction for installation is read

Level III. Periodic & Motivated Instrument Checks or operational qualification

Examples of requirements for IR spectrophotometers

Parameters to be checked

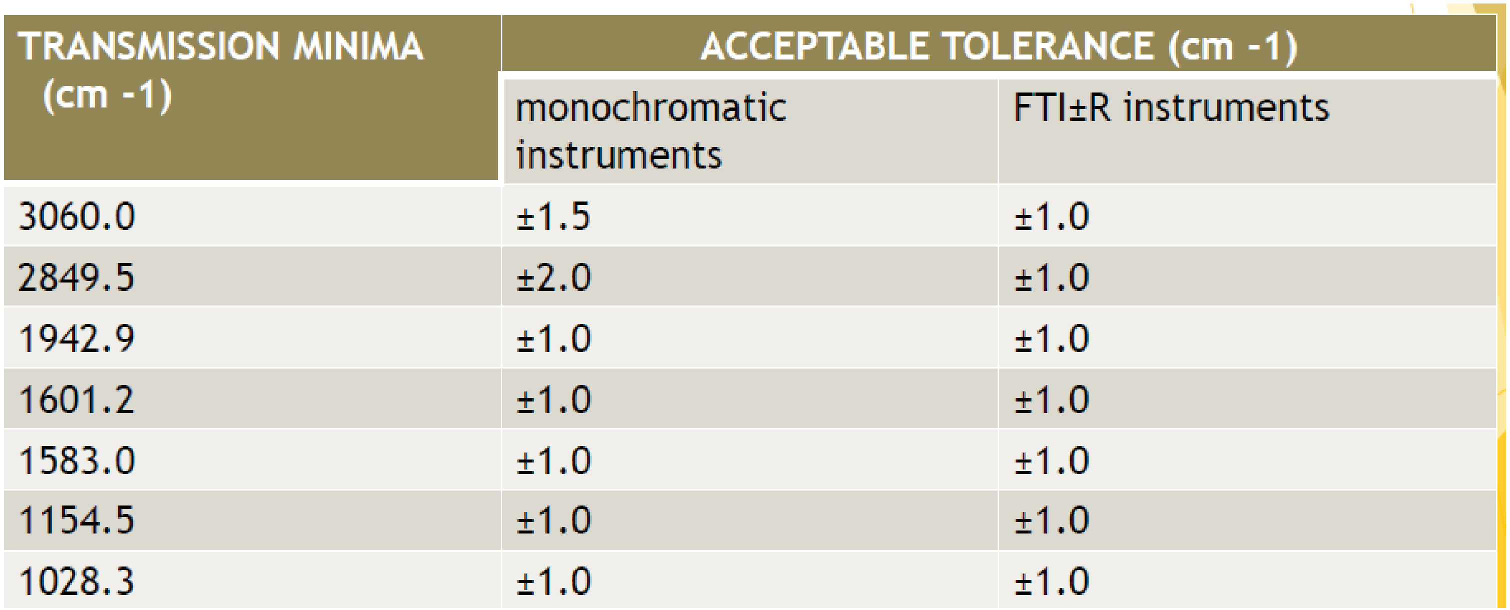
1. Wave-number scale
2. Detector energy ratio
3. Signal/Noise ratio
4. Resolution
5. Zero test
6. Contamination check (only for ATR instruments)
7. Throughput check (only for ATR instruments)

1.WAVE-NUMBER SCALE

Method and Limits:

The wave-number scale may be verified by recording the spectrum of a polystyrene film, which has transmission minima (absorption maxima) at the wave numbers

(in cm-1) shown in the table below



TRANSMISSION MINIMA (cm -1)	ACCEPTABLE TOLERANCE (cm -1)	
	monochromatic instruments	FTI±R instruments
3060.0	±1.5	±1.0
2849.5	±2.0	±1.0
1942.9	±1.0	±1.0
1601.2	±1.0	±1.0
1583.0	±1.0	±1.0
1154.5	±1.0	±1.0
1028.3	±1.0	±1.0

2. DETECTOR ENERGY RATIO

Method:

- Record the minimum energy ratio value for at least one of the following measurement points and compare it to the vendor’s specifications: -
- Energy at 3990 cm-1 / energy at 2000 cm-1
- Energy at 4000 cm-1 / energy at 2000 cm-1
- Energy at 3400 cm-1 / energy at 1300 cm-1
- Energy at 2000 cm-1 / energy at 1000 cm-1

Limits:

- Energy ratio test specifications vary for each spectrometer configuration. The optical bench shall include a DTGS detector with a frequency range of 7400 to 350 cm-1

3.SIGNAL/NOISE RATIO

Method:

- Record the maximum noise level for each of the following regions:
- Peak-to-peak noise between:
- 4050 cm-1 and 3950 cm-1
- 2050 cm-1 and 1950 cm-1
- 050 cm-1 and 950 cm-1
- 550 cm-1 and 450 cm-1

(systems with DTGS detector only) RMS (root mean square) noise between:

- 4050 cm-1 and 3950 cm-1
- 2050 cm-1 and 1950 cm-1
- 1050 cm-1 and 950 cm-1

◦ 550 cm-1 and 450 cm-1

(systems with DTGS detector only)

Limits (% T):

- Noise level test specifications vary for each spectrometer configuration.

4. RESOLUTION

Materials:

- Certified polystyrene film of approximately 35 µm in thickness.

Method:

- For instruments having a monochromator, record the spectrum of the polystyrene film.

- For Fourier-transform instruments, use suitable instrument resolution with the appropriate apodisation prescribed by the manufacturer. There solution is checked by suitable means, for example by recording the spectrum of a polystyrene film approximately 35 µm in thickness.

Limits:

-Difference between the absorbance's at the absorption minimum at 2870 cm-1 and the absorption maximum at 2849.5 cm-1 > 0.33.

- Difference between the absorbance's at the absorption minimum at 1589 cm-1 and the absorption maximum at 1583 cm-1 > 0.08.

5. ZERO TEST

Method:

- When using a polystyrene film of approximately 35 µm in thickness as standard at the wavelength of 2925 cm-1 and 700 cm-1 , almost complete absorption of the irradiated energy can be observed.
- With this test, the remaining transmission is measured. As the maximum absorption can be observed at 700 cm-1 negative values may be observed.
- The objective of the test is to evaluate if, despite the fact that there is almost complete absorption, energy is still detectable.
- Non-valid results are an indication of a non-linear behaviour of thedetector and the electronic system.

6. CONTAMINATION TEST

- (Only for Attenuated Total Reflection (ATR) instruments)

Note: If an automated system is available, this test can be run more frequently or it can be transferred to Level IV, to be run before each analysis.

Level IV. In-use instrument checks examples of requirements for IR spectrophotometers

Parameter to be checked	Typical tolerance limits
System suitability check	According to PH,EUR or MAH dossier or validated in –house method

Performance Qualification

POWER SPECTRUM

- Power spectrum gives the plot of portion of signals power (energy per unit time) falling with in the given frequency bins .
- This test eliminates the intensity of power spectrum at a specified wave numbers.
- When the measured intensity is equal to or larger than the criterion value, the test is passed.

Acceptance Criteria

WAVE NUMBER(CM`	STANDARD VALUE FTIR
4600	10% or min of max
4000	25% or min of max
3000	50% or min of max
Power max value	50.0
700	10% or min of max
500	2% or min of max
403	0.5% or min of max
351	0.01% or min of max

Resolution

- The resolution is checked by recording the spectrum of polystyrene film of approximately 35 micro meter in thickness.
- The difference between %transmittance at absorption maximum A at 2870 and absorption minima B at 2849.5 must be greater than 18.
- The difference between percentage transmittance at absorption maxima c at 1589 and absorption minima 1583 must be greater than 12.

Wave Number Accuracy

The wave number scale is usually calibrated by the use of several characteristic wave number of a polystyrene film.

3060.0(+/-1.5)cm^-1
2849.5(+/-1.5)cm^-1
1942.9(+/-1.5)cm^-1
1601.2(+/-1.5)cm^-1
1583.0(+/-1.5)cm^-1
1154.5(+/-1.5)cm^-1
1028.3(+/-1.5)cm^-1

Wave number reproducibility

- This program specifies three to measure the peak wave numbers.
- Then it obtains the actual peak wave numbers at each point by measuring the polystyrene film twice. It should satisfy 5 cm^-1 around 3000 cm^-1 of polystyrene absorption wave number, 1 cm^-1 around 1000 cm^-1.
- The software determines whether the differences between each of two measurements are within the allowable range and it labels the result PASS if they are with in the range.
- Ep 4.0 doesn’t include this inspection.

Transmittance reproducibility

- This program specifies peak wave number at three points and the transmittance at each point is measured it twice.
- The transmittance reproducibility should satisfy 0.5%T when the several point of polystyrene absorption from 3000 cm^-1 to 1000 cm^-1 are measured twice.
- Then it determined whether the differences between the two data are within the allowable range and it labels the result PASS if they in the range.
- All of above furnished data should be represented in.

As per ASTM E1421-94 level Zero

- This software complies describes with in the description in the ASTM(American society for testing and materials).
- The FTIR abnormalities or large changes over short term and long term is assessed by these tests.
- The three parameters checked by this program are:

ENERGY SPECTRAM TEST

ONE HANDRED PERCENT LINE TEST

POLYSTYRENE TEST

ENERGY SPECTRUM TEST

- Power spectra obtain in the inspection are compared with reference data and the spectra are checked for the changes over long periods.

ONE HUNDRED PERCENT LINE TEST

- 100% T line spectra are calculated for power spectra and are measured continuously in inspection and the spectra are checked for the changes over short periods.

POLYSTYRENE TEST

- Polystyrene test data should be represented in validation report.
- Evaluation is performed using difference between spectra obtained for polystyrene film and in inspection and the stored reference data.
- All of the above furnished

ADVANTAGES

- Better sensitivity and brightness
- High wavenumber accuracy
- Enhanced frequency Resolution
- Wavenumber range flexibility
- Less time consuming
- Data's can be stored & reanalysed

DISADVANTAGES

- More expensive
- Require precision for mirror movement
- Detection of compound is influenced by
- Water vapour, path length & chemical interference

Applications

- Opaque or cloudy samples
- High resolution experiments (as high as 0.001 cm-1 resolution)
- Trace analysis of raw materials or finished products
- Depth profiling and microscopic mapping of samples
- Kinetics reactions on the microsecond time-scale
- Analysis of chromatographic and thermo gravimetric sample fractions

DIFFERENTIAL SCANNING CALORIMETER

- The differential scanning calorimeter (DSC) is a fundamental tool in thermal analysis. It can be used in many industries – from pharmaceuticals and polymers, to nanomaterial's and food products.
- A number of physical and chemical effects can be produced by temperature changes, and methods for characterizing these alterations upon heating or cooling a sample material are referred to as thermal analysis.
- The physical and chemical changes a sample undergoes when heated, are characteristic of the material being examined. By measuring the temperature at which such reactions occur and the heat involved in the reaction, the compounds present in the material can be characterized. The majority of known inorganic compounds have been so characterized.
- The physical and chemical changes that take place when unknown sample is heated provide the information that enables the identification of the material. These changes also indicate the temperature at which the material in question ceases to be stable under normal conditions.
- Common methods of thermal analysis are DSC, DTA, TGA, and TMA.

Differential Scanning Calorimetry History

- This technique is developed by E.S.Watson and M.J.O’Neill in 1962.
- Introduced commercially at the Pittsburgh Conference on analytical Chemistry and Applied Spectroscopy.
- First Adiabatic differential scanning calorimeter that could be used in Biochemistry was developed by P.L.Privalov in 1964.

Principle

- In DSC the heat flow is measured and plotted against temperature of furnace or time to get a thermo gram. This is the basis of Differential Scanning Calorimetry (DSC).
- The deviation observed above the base (zero) line is called exothermic transition and below is called endothermic transition.
- The area under the peak is directly proportional to the heat evolved or absorbed by the reaction, and the height of the curve is directly proportional to the rate of reaction.
- Calorimetry - The study of heat transfer during physical and chemical process.
- Calorimeter - A device for measuring the heat transferred.

Principle…

- Differential scanning calorimetry (DSC) is a technique for measuring the energy necessary to establish a nearly zero temperature difference between a substance and an inert reference material, as the two specimens are subjected to identical temperature regimes in an environment heated or cooled at a controlled rate.
- It is the most widely used method of thermal analysis in pharmaceutical field.
- Thus, when an endothermic transition occurs, the energy absorbed by the sample is compensated by an increased energy input into the sample in order to maintain a zero temperature difference.
- Because this energy input is precisely equivalent magnitude of energy absorbed in transition, direct calorimetric measurement of transition is obtained from this balancing energy.
- On the DSC chart recording, the abscissa indicates the transition temperature and the peak measures the total energy transfer to or from the sample.

What Does DSC Measure

- DSC measures the amount of energy (heat) absorbed or released by a sample as it is heated, cooled or held at constant temperature. DSC also performs precise temperature measurements.

Used

- Melting point
- Crystallization
- Glass Transition

O.I.T. (Oxidative Induction Time)

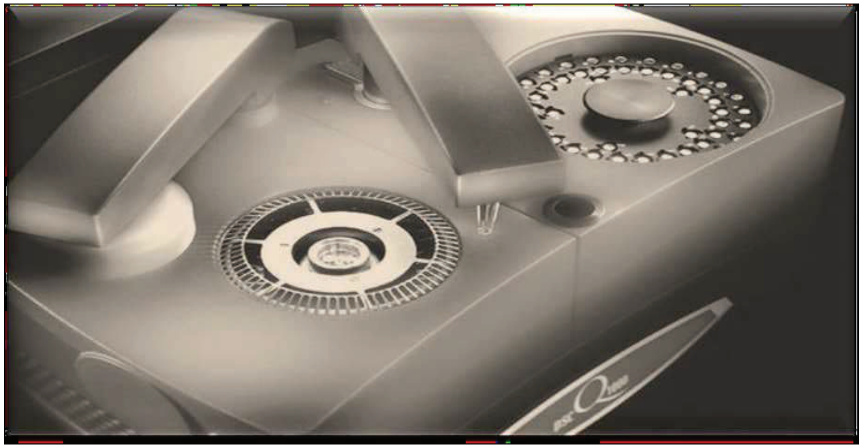
It is a standardized test performed in DSC that measures the level of stabilization of the material tested. The time between melting and onset of decomposition in isothermal conditions is measured.

- Polymorphism
- Purity
- Specific Heat
- Kinetic Studies

Curing Reactions - The process in which an adhesive undergoes a chemical reaction and becomes a solid by forming a bonded joint. The reaction may be initiated by heat, light, UV radiation, water etc.

Denaturation - A process pertaining to change in the structure of a protein from regular to irregular arrangement of polypeptide chains.

Conventional DSC



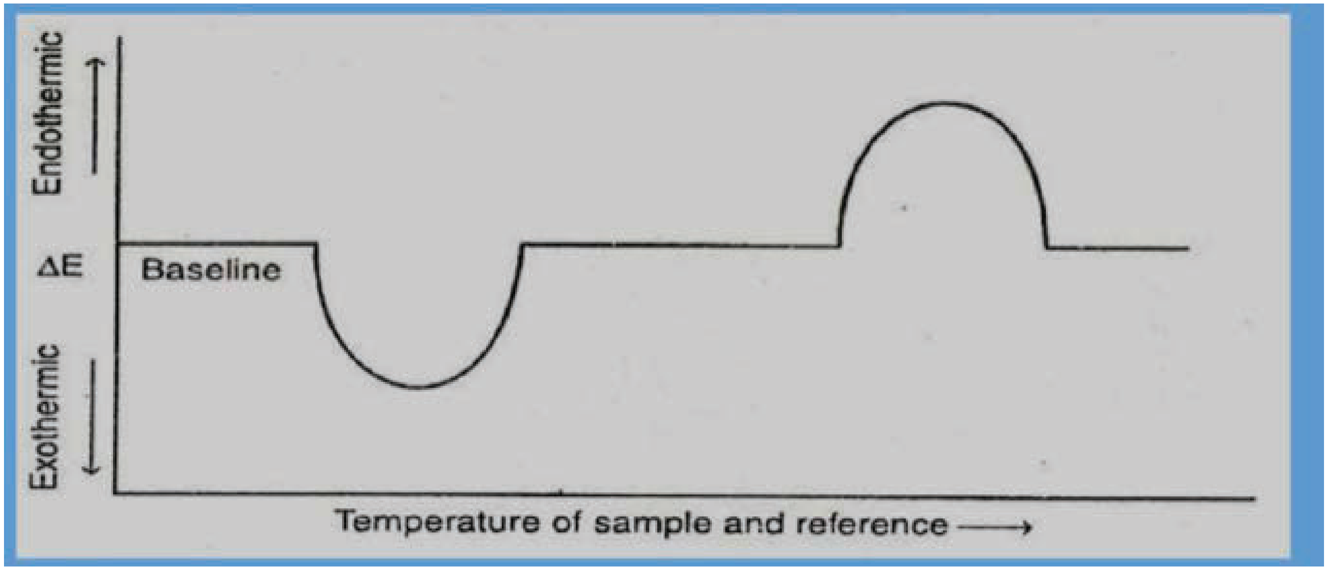
- **In general an endothermic reaction on a DSC arises from**
- Desolvations
- Melting
- Glass transitions and
- Decompositions.

Conventional DSC…

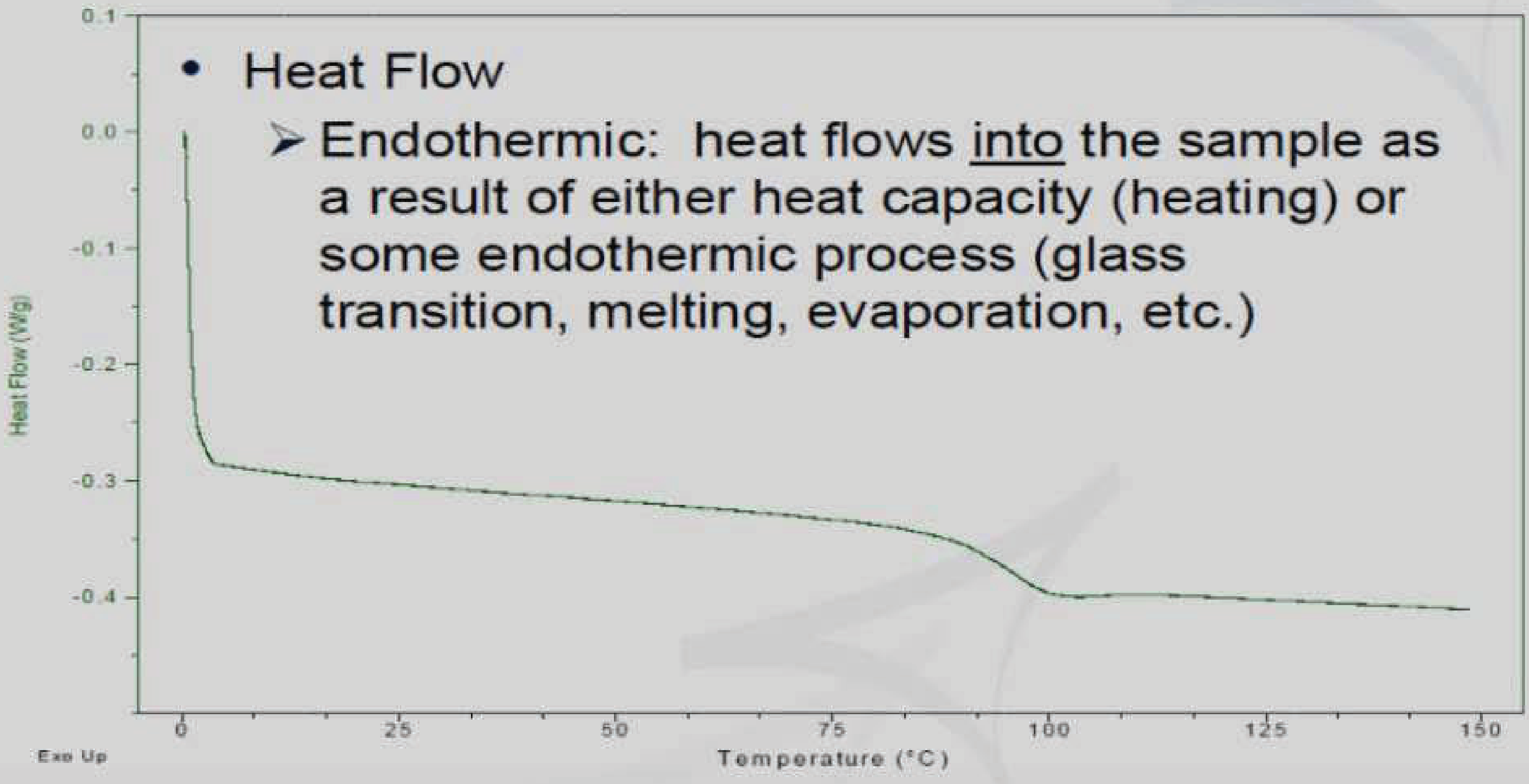
An exothermic reaction measured by DSC is usually indicative of molecular reorganizations such as

1) Crystallization
2) Curing
3) Oxidation.

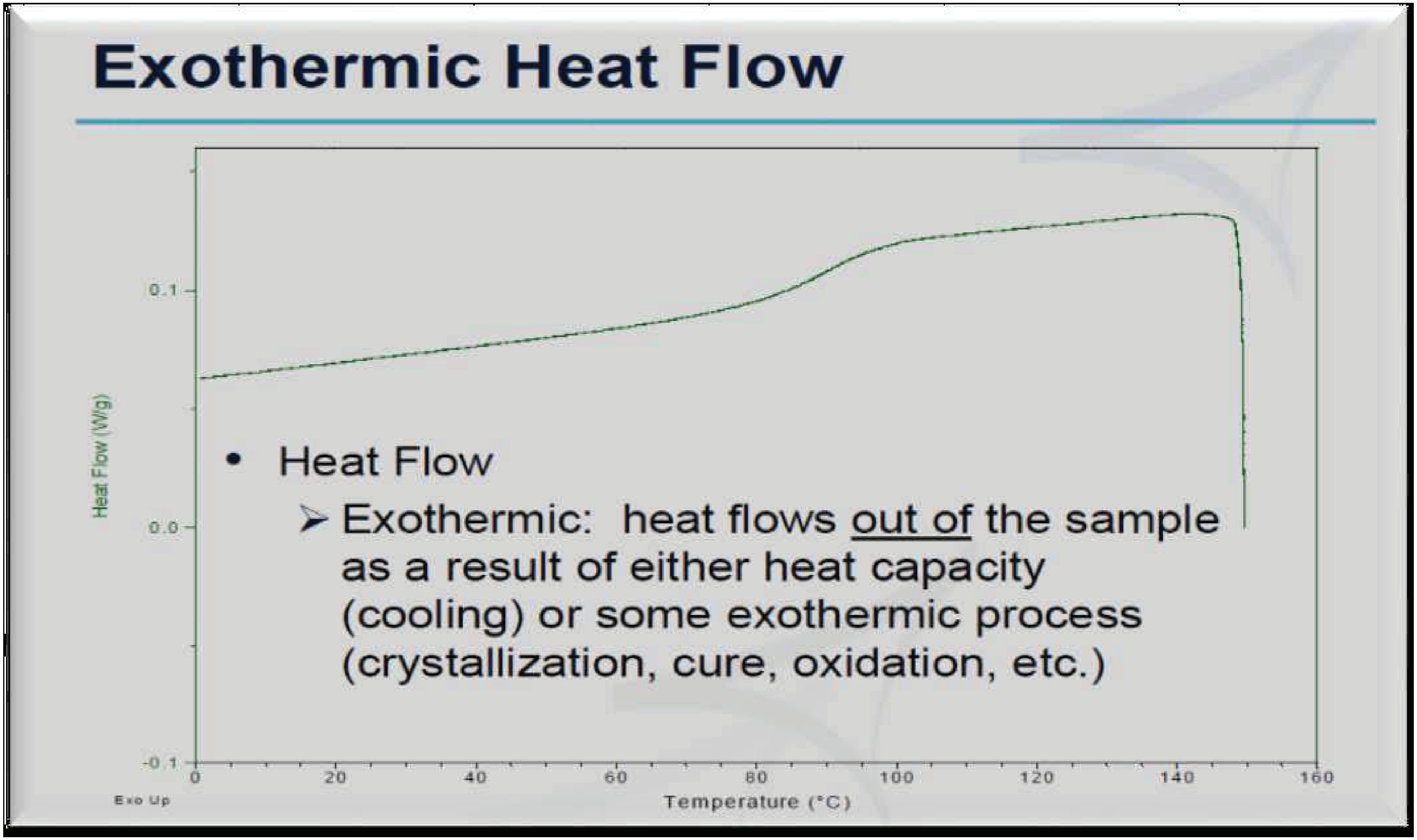
The differential heat input is recorded with a sensitivity of +/- 0.1 mill calories per second and the temperature range over with the instrument operates is -175^0 C to 725 ^0 C.



Endothermic Heat Flow



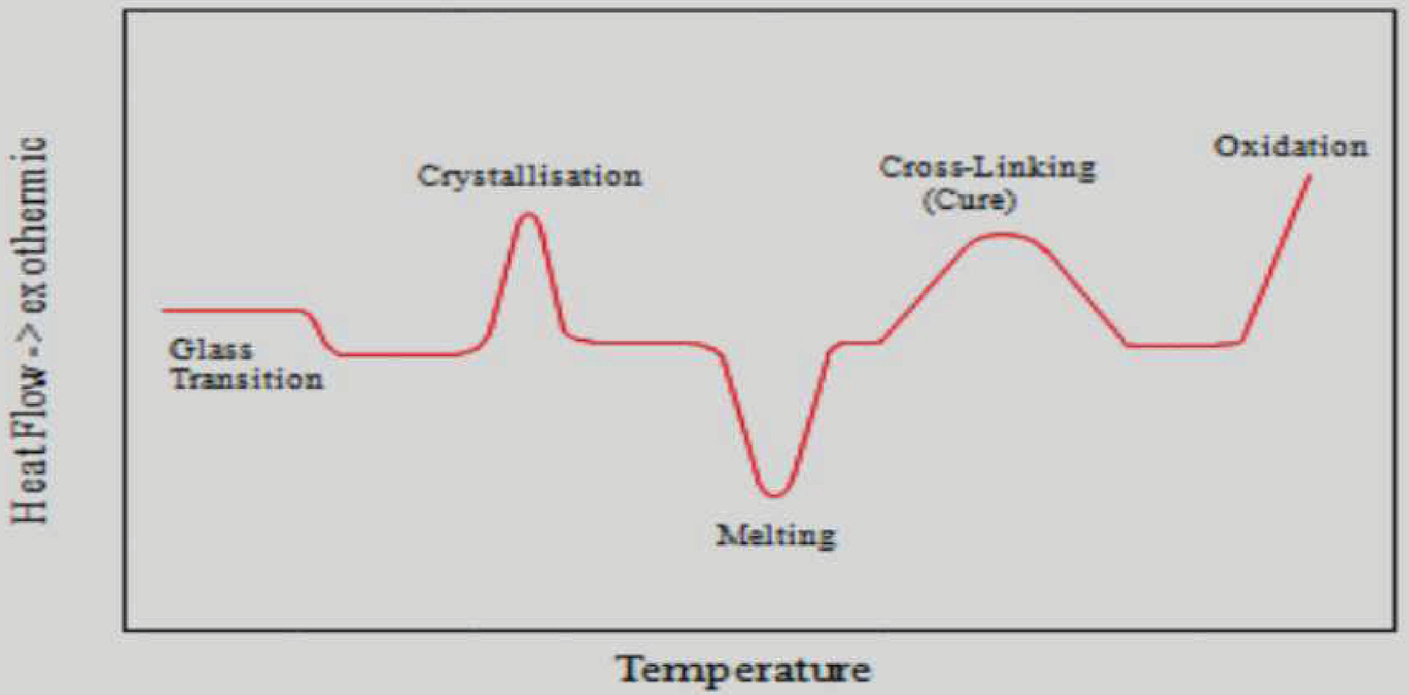
- Heat Flow
  - ➢ Endothermic: heat flows <u>into</u> the sample as a result of either heat capacity (heating) or some endothermic process (glass transition, melting, evaporation, etc.)



Exothermic Heat Flow

- Heat Flow
  - Exothermic: heat flows out of the sample as a result of either heat capacity (cooling) or some exothermic process (crystallization, cure, oxidation, etc.)

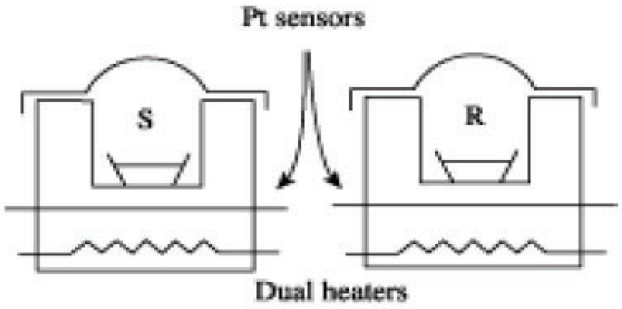
DSC Thermogram



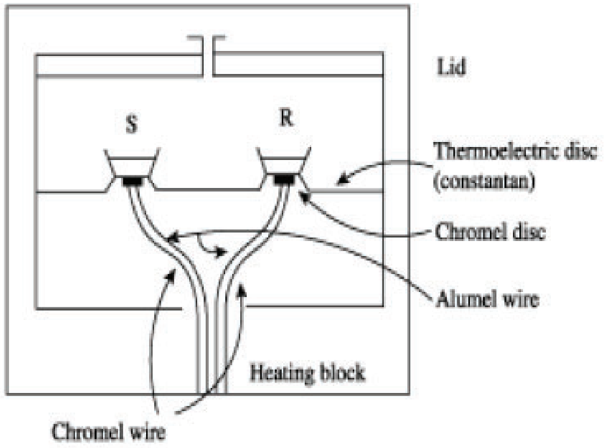
Types of DSC Technologies

Two basic types of DSC instruments:

- Power compensation DSC and
- Heat-flux DSC



Power compensation DSC



Heat flux DSC

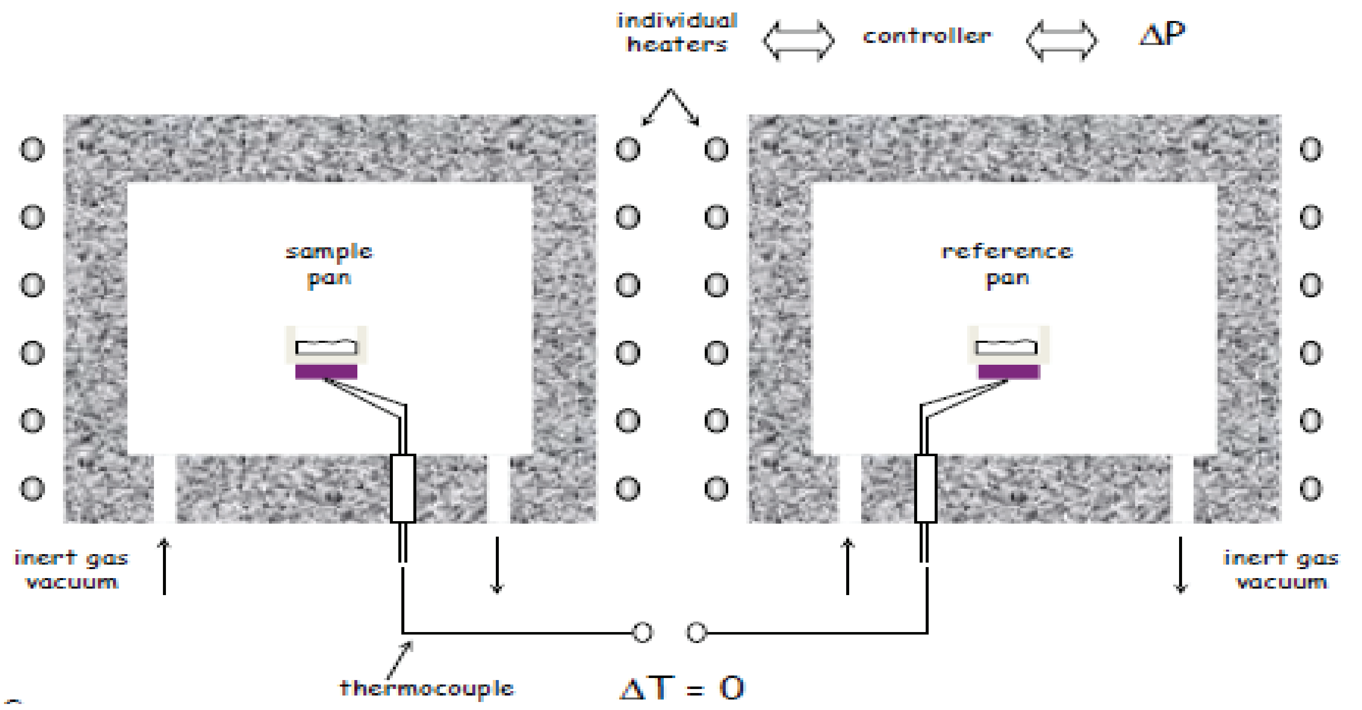
Power Compensation DSC

It is developed by Perkin Elmer, USA. It directly measures heat flow between sample side and reference side using two separate, low mass furnaces.

Principle: An exothermic or endothermic change occurs in the sample, when the sample is heated, power (energy) is applied or removed from the furnace to compensate for the energy change occurring in the sample is measured.

- The system is maintained in “Thermal Null” state all the times.
- The amount of power required to maintain the system in equilibrium is directly proportional to the energy changes.
- Sample holder - it is made up of aluminium, platinum or stainless steel.
- Sensors - platinum resistant sensors are generally used. Separate sensors are used for are used for sample and reference cells.
- Furnace - separate blocks of furnace are used for sample and reference cells.
- Temperature controller - differential thermal power is supplied to heaters to maintain the temperature of the sample and reference at the programmed value.

Power Compensation DSC



Heat Flux DSC

- It is proposed by Boersma.
- The sample and reference cells are heated at a constant rate and thermocouples are used to detect the temperature
- differential between sample side and reference side using single, large mass furnace.

Principle

- The introduction of a controlled heat leak between the sample and reference holders enabled a quantitative measurement of energy changes to be made. Heat flux can be measured directly if a sample is surrounded by a thermopile.
- The peak area is related to the enthalpy change by a calibration factor which is partially temperature dependent

Heat Flux DSC…

Sample holder - sample and reference holders are connected by a low resistance heat flow path. The material with which the sample holder is made may be aluminium, stainless steel, platinum.

- **Sensors** - temperature sensors are thermocouples.
- **Furnace -** same block is used for sample and reference.
- **Temperature controller -** temperature difference between sample and reference is measured.
- A metallic disc made of constantan alloy is the primary means of heat transfer. Sample and reference sit on raised constantan discs.
- Differential heat flow to sample and reference is measured by thermocouples which are connected in series, located at the junction of constantan disc and chromel wafers.
- With this, it is possible to achieve heating or cooling rates of

1000c /min to 00c /min(isothermal).

It needs mathematical equations to get the heat flow. **Dh/Dt = Cp Dt/Dt + F(t,t)**

DSC Heat Flow Equation

- **Dh/Dt** - DSC Heat Flow Signal, **Cp** - Sample Heat Capacity = Sample Specific Heat X Sample Weight, **Dt/Dt** - Heating Rate
- **F(t,t)** - Heat Flow That Is A Function Of Time At An Absolute Temperature (Kinetic)

Instrumentation

This instrument works on the temperature control of two similar specimen holders.

It consists of two circuits

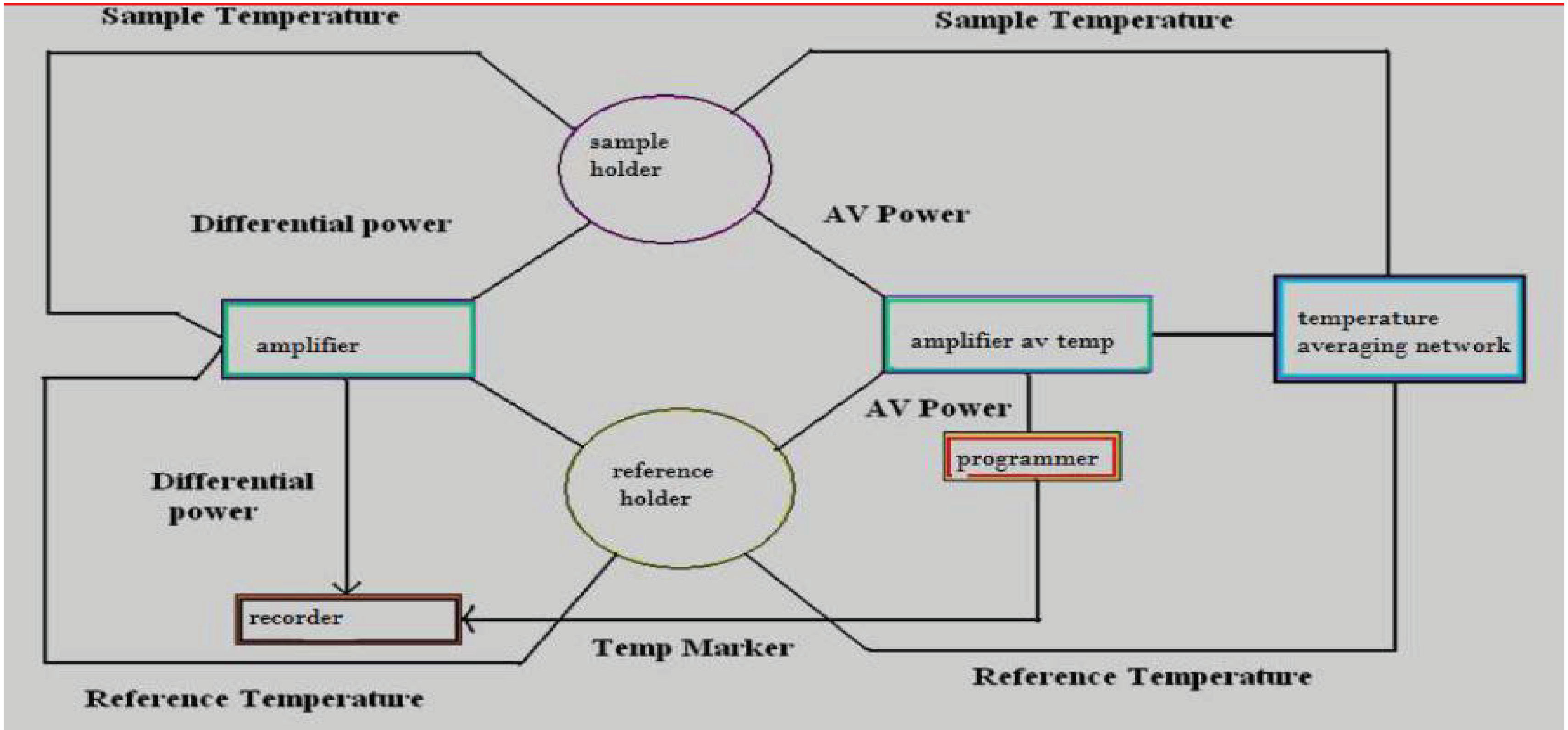
1. Left half - differential temperature control circuit
2. Right half - average temperature control circuit

- In the average temperature control circuit an electrical signal which is proportional to the dialled temperature of the sample and reference holders, is generated through the programmer.
- In the differential temperature control circuit, signals representing the temperature of sample and reference are compared. If no reaction taking place in the sample, the differential power input to the sample and reference heater is almost zero. If a reaction is taking place (ΔH is not zero) a differential power is fed to heaters. A signal proportional to this differential power along with the sign is transmitted to the recorder pen. The integral of the peak so obtained gives the internal energy change of the sample.

Cleaning The Sample Cell

- If the cell gets dirty – Clean it with brush
- Brush gently both sensors and cell if necessary
- Be careful with T zeroTM thermocouple
- Blow out any particles are remaining.

Block Diagram For DSC Instrument



Block Diagram for DSC Instrument

DSC Instrument…

- **Reference Materials** - An inert material like α-alumina is generally used. Empty pan can also be used, if the sample weight is small. With higher sample weights it is necessary to use a reference material, because the total weight of the sample and its container should be approximately the same as the total weight of the reference and its container.
- The reference material should be selected so that it possesses similar thermal characteristics to the sample. The most widely used reference material is α-alumina, which must be of analytical reagent quality. Before use, α-alumina should be recalcined and stored over magnesium perchlorate in a desiccator.
- Kieselguhr is another reference material normally used when the sample has a fibrous nature. If there is an appreciable difference between the thermal characteristics of the sample and reference materials, or if values of ΔT are large, then dilution of the sample with the reference substance is sensible practice. Dilution may be accomplished by thoroughly mixing suitable proportions of sample and reference material.

DSC Instrument…

- **Purge Gases -** Sample may react with air and may oxidize or burn. The problem is overcome by using inert gases. Inert gases are used to control moisture in the surrounding atmosphere. Commonly used inert gases are nitrogen, helium, argon etc. Inert gases should ensure even heating and helps to sweep away the off gases that might be released during sublimation or decomposition.
- **Nitrogen -** It is the most commonly used inert gas. It increases the sensitivity of the experiment. Typical flow rate is 50 ml/min.
- **Helium -** It has high thermal conductivity. It increases the resolution of the peaks. The upper temperature limit for this gas is up to 3500c. Flow rate is 25 ml/min
- **Air or oxygen -** Sometimes it is deliberately used to view oxidative effects of the sample. Flow rate is 50 ml/min
- **Heating Rate** - Faster heating rate will increase the sensitivity but will decrease the resolution. Slow heating rate will decrease the sensitivity but will increase the resolution. Good starting point is 100 24-12-2015 c/min.

Factors Affecting Thermogram

Sample shape:

The shape of the sample has little effect on the quantitative aspect of DSC but more effect on the qualitative aspects. However, samples in the form of a disc film or powder spread on the pan are preferred. In the case of polymeric sheets, a disc cut with a cork-borer gives good results.

Sample size:

- About 0.5 to 10mg is usually sufficient. Smaller samples enable faster scanning, give better shaped peaks with good resolution and provide better contact with the gaseous environment. With larger samples, smaller heats of transitions may be measured with greater precision.

1) Heating rates

2) Atmosphere and geometry of sample holders

- There are a number of variables that affect DSC results includes the type of pan, heating rate, the nature and mass of the compound, the particle size distribution, packaging and porosity, pre-treatment and dilution of the sample. It is used for purity analysis2o4-f12a-2b0o15ve 98% pure compounds.

DSC Calibration

Baseline

- Evaluation of the thermal resistance of the sample and reference sensors
- Measurements over the temperature range of interest

2-Step process

- The temperature difference of two empty crucibles is measured
- The thermal response is then acquired for a standard material, usually sapphire, on both the sample and reference platforms
- Amplified DSC signal is automatically varied with the temperature to maintain a constant calorimetric sensitivity with temperature

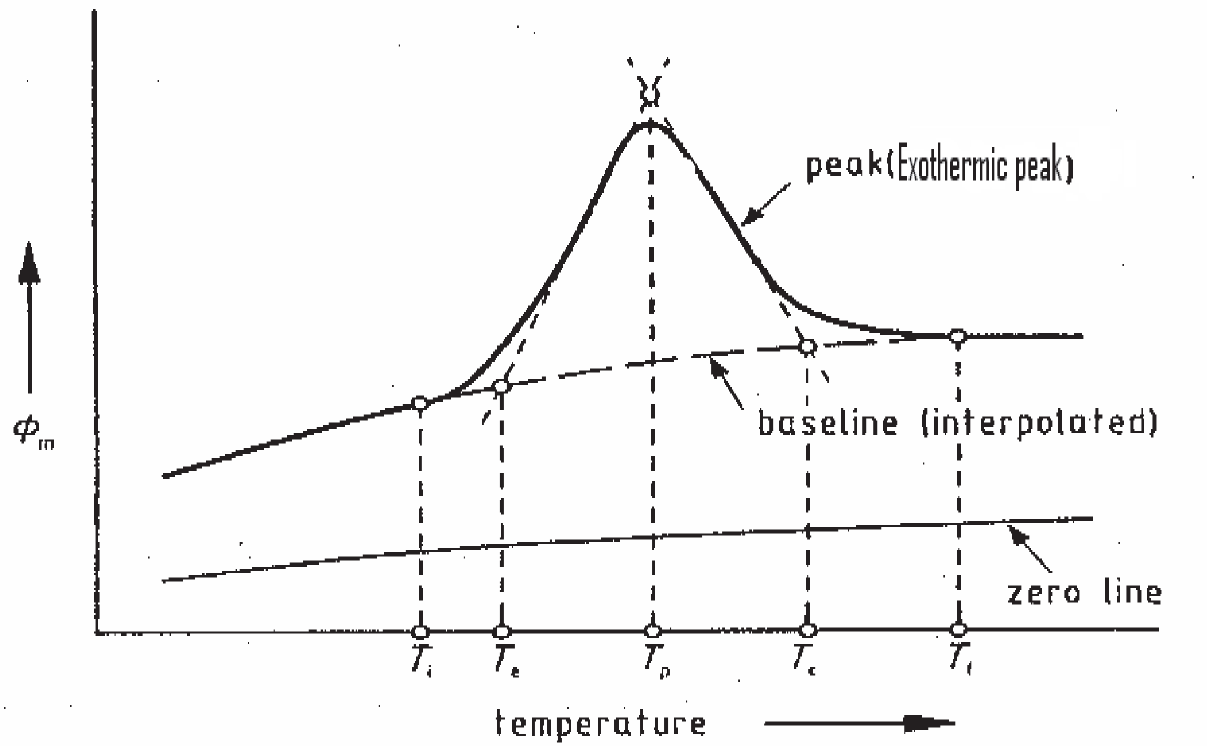
DSC Calibration…

Temperature

Goal is to match the melting onset temperatures indicated by the furnace Thermocouple readouts to the known melting points of standards analyzed by DSC Should be calibrated as close as possible to the desired temperature range.

Heat flow

Use of calibration standards of known heat capacity, such as sapphire, slow accurate heating rates (0.5–2.0°C/min), and similar sample and reference pan weights



DSC Calibration…

Calibrants

- High purity
- Accurately known enthalpies
- Thermally stable
- Light stable (hυ)
- Non-hygroscopic
- Un-reactive (pan, atmosphere)

Metals

- Indium
- Stannous
- Aluminium

Inorganics

- KNO3
- KClO4

Organics

- Polystyrene
- Benzoic acid
- Anthracene

DSC Curve

- The result of a DSC experiment is a curve of heat flux versus temperature or versus time. There are two different conventions: exothermic reactions in the sample shown with a positive or negative peak, depending on the kind of technology used in the experiment.
- This curve can be used to calculate enthalpies of transitions, which is done by integrating the peak corresponding to a given transition. The enthalpy of transition can be expressed using equation:
- $\Delta H = KA$

DSC Curve…

- Where ΔH is the enthalpy of transition,
- K is the calorimetric constant,
- A is the area under the peak.
- The calorimetric constant varies from instrument to instrument, and can be determined by analysing a well-characterized material of known enthalpies of transition.
- Area under the peak is directly proportional to heat absorbed or evolved by the reaction,
- height of the peak is directly proportional to rate of the reaction

Factors affecting DSC curve

Two types of factors effect the DSC curve

Instrumental factors

a- Furnace heating rate

b- Recording or chart speed

c- Furnace atmosphere

d- Geometry of sample holder/location of sensors

e- Sensitivity of the recoding system

f-Composition of sample containerises of factors effect the DSC curve

Factors affecting DSC curve…

Sample characteristics

- a- Amount of sample
- b- Nature of sample
- c- Sample packing
- d- Solubility of evolved gases in the sample
- e- Particle size
- f- Heat of reaction
- g- Thermal conductivity

Factors affecting DSC curve…

Application

- Protein Stability and Folding
- Liquid Biopharmaceutical Formulations
- Process Development
- Protein Engineering
- Rank order Binding
- Antibody Domain Studies
- Characterisation of Membranes, lipids, nucleiec acids & micellar systems
- Assessment of the effects of structural change on a molecules stability
- Measurement of Ultra-light molecular interactions
- Assessment of bio comparability during manufacturing.

Calibration of DSC

- Design specification:

Principle	Heat flux type
Heat flow range	+or – 40 micro W
Hold time	0-999 min,hour
Noise level	1 microwW
Size(mm)	300wx 490Dx 290H
Temperature range	-150to 600 degree celcious
Programme rate	0-99 degree K /min,K/hour
Cooling time	About 6 mins from 600 degree c to 40 c
Atmosphere	Inert gas or air
Power supply	100/120 VAC800AV

Installation Qualification

- The DSC should be
- In a temperature controlled area (15 degree C to 30 degree C)
- A clean environment
- An area with ample working and ventilation space.
- On
- A stable , heat resistant , and fire resistant work surface

Performance Qualification

- The most common procedure is to run an indium standard under the normal test condition and measure the heat of fusion value and melting onset temp.
- For many industries limits of:
- +- 0.5 degree celcious for temperature or 1% for heat of fusion may be accepted, though tighter limits +-0.3 degree celcious and 0.1%may also be adopted.

Baseline slope calibration

- This calibration involves heating an empty cell through the entire temp range expected in subsequent exp.
- Empty standard DSC cell run from 25 to 400 degree celcious.
- Heat flow signal should be 0.
- No sample cell and it should have minimum slope.

Temperature Calibration

- Establishment of relationship between temperature measured T meas and true temperature T tr.
- T true=Tmeas +ΔT corr (T meas)
- Eenthalpy calibration:
- Eestablishment of the relationship between enthalpy change measured Δtrs meas and the true enthalpy change Δtrs H absorbed or released by sample as result of a transition at the transition temperature Ttrs.
- $\Delta trs\ H = kH(T\ tr)$ΔH meas
- Heat flow calibration

CONCLUSION

- The purpose of the analytical instrument is to generate reliable data.
- The qualification of analytical instrument has become a subjective and often fruitless document generating exercise.
- Ability to use of instrument to deliver reliable and consistent data.

REFERENCES

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