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Introduction to Pharmaceutical Thermal Analysis: a Teaching Tool

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INTRODUCTION TO PHARMACEUTICAL THERMAL ANALYSIS: A TEACHING TOOL

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INTRODUCTION TO PHARMACEUTICAL THERMAL ANALYSIS: A TEACHING TOOL

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ABSTRACT

Significant Thermal Analysis-physical chemical data needs to be acquired by the new analyst whether an entry level chemist or a new function for the experienced pharmaceutical scientist. This teaching tool describes the introductory use of Differential Scanning Calorimetry (DSC), Thermogravimetric Analysis (TGA) and Thermomechanical Analysis (TMA) for characterizing pharmaceuticals. Optimum Experimental conditions for DSC, TGA and TMA will focus on collecting the best results and interpretations. Does the sample contain volatiles? Evaporation creates endothermic peaks, 2% water or solvent can lower the glass transition temperature (T_g) by up to 100^{o}C and affect the crystallization temperature on cooling. The decomposition temperature can be determined by DSC and TGA. Decomposition, not volatilization, can result in 5% weight loss and render no meaningful DSC data. The upper DSC temperature for practical use is based on the decomposition temperature. Identical materials can look totally different based on their storage temperature and time, cooling rate from a temperature above the T_g or above the melting temperature (T_m). TMA determines the dimensional change of a sample with respect to temperature. The heating rate, an essential feature of DSC, TGA and TMA can cause multiple variations in transitions. Thermal history of chemicals can affect the ultimate thermal analysis results. TGA can provide information about bound and unbound (free) water due to evaporation,

desorption and dehydration. Calibration of DSC and TGA are vital in establishing the precision and accuracy of these unique methods: You must learn and follow the standard protocol ASTM E968 for the heat of fusion and heat capacity as well as ASTM E967 for the determining the transition or phase temperatures of pharmaceuticals. DSC can determine the Tm, crystallization temperature T_c, T_g and the their heats of transition, e.g., fusion and crystallization.

A statistical optimum method was developed based on a great deal of supportive data was collected by varying the DSC, TGA, and TMA variables and fitting the results into an experimental design, a 2^3 factorial design. This was accomplished by the team at Cleveland State University as well as that reported in the thermal and pharmaceutical literature. Typical variables considered were sample size (e.g., 3 vs 15 mgs), heating rate (e.g., 5 vs 20^{o}C/min), atmosphere (e.g., nitrogen vs. air) and humidity exposure (e.g., 100%, wet vs. 0%, dry).

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ABBREVIATIONS

DEA- Dielectric Analysis

DSC- Differential Scanning Calorimetry

TGA- Thermogravimetric Analysis

TMA- Thermomechanical Analysis

SFI- Solid fat index

ΔH_f- Heat of Fusion

T_m - Melting Temperature, extrapolated endothermic onset temperature

T_p- Peak Melting endothermic Temperature

ΔH_c-Heat of exothermic Crystallization

T_c- Crystallization Temperature, extrapolated exothermic onset temperature

T_{cp}- Peak exothermic Crystallization temperature

ΔH_v - Heat of endothermic Vaporization

T_v - Vaporization temperature, extrapolated endothermic onset temperature

T_{vp} - Peak Vaporization temperature

T_g -Glass transition temperature

Mom- Milk of Magnesia

ASTM- American Standards for Testing Materials

OBJECTIVES

The main objective of this thesis was to introduce thermal analysis and its applications at an entry level in the pharmaceutical industry. In the process, instruments were successfully calibrated using pharmaceuticals. Studying the behavior of pharmaceuticals by different thermal analysis instruments, under different conditions and then compare the results was another objective. A statistical optimum method was developed based on a great deal of supportive data was collected by varying the DSC, TGA, and TMA variables and fitting the results into an experimental design, a 2^3 factorial design. This was accomplished by the team at Cleveland State University as well as that reported in the thermal and pharmaceutical literature. Typical variables considered were sample size (e.g., 3 Vs 15 mgs), heating rate (e.g., 5 Vs 20^{o}C/min), atmosphere (e.g., nitrogen vs. air) and humidity exposure (e.g., 100%, wet vs. 0%, dry).

A new protocol was developed to distinguish the water content in the brand and generic forms of commercially available Milk of Magnesia suspensions were developed with the aid of TGA.

CHAPTER I

INTRODUCTION TO THERMAL ANALYSIS

Thermal analysis is defined as "series of techniques for measuring the temperature dependency of a physical property of a certain substance while varying the temperature of the substance according to a specific program." The substance referred to here includes reaction products (1).

Physical properties include mass, temperature, enthalpy, dimension, dynamic characteristics, and others, and depending on the physical properties to be measured, the techniques of thermal analysis are classified as shown in Table 1.

Physical property	Defined technique	Physical property	Defined technique
Mass	Thermogravimetry (TG)	**Dimensions**	Thermal Expansion measurement
	Isotonic mass change method		Thermomechanical analysis (TMA)
	Evolved gas detection (EGD)	**Dynamic characteristics**	Dynamic thermomechanical measurement
	Evolved gas analysis (EGA)	**Acoustic characteristics**	Thermoacoustic emission measurement
	Emanation thermal analysis		Thermoacoustic measurement
	Thermal particle analysis	**Optical characteristics**	Thermooptical measurement
Temperature	Heating curve method	**Electric Characteristics**	Thermoelectric measurement
	Differential thermal analysis (DTA)	**Magnetic characteristics**	Thermomagnetic measurement
Enthalpy	Differential scanning calorimetry (DSC)		

Table 1 Classification of techniques of thermal analysis (ICTA) [(1)]

(1) The Society of Calorimetry and Thermal Analysis, Japan(ed): Foundation and Application of Thermal Analysis, P.1(1985)

Conventionally thermal analysis has been mainly employed in measurements for research and development, but in recent times it is used in many practical applications, as the testing standards on the basis of thermal analysis have been established, for example, in quality control in the production field, process control, and material acceptance inspection.

It is also applied in wide fields, including polymer, glass, ceramics, metal, explosives, semiconductors, medicines, and foods.

The main aim of this study is to introduce thermal analysis at an entry level chemist or a new function for the experienced pharmaceutical scientist. This teaching tool describes the introductory use of Differential Scanning Calorimetry (DSC),

Thermo-Mechanical Analysis (TMA) and to some extent Thermo-gravimetric Analysis (TGA) for characterizing pharmaceuticals.

A statistical optimum method was developed based on a great deal of supportive data that was collected by varying the DSC, TGA, and TMA variables and fitting the results into an experimental design, e.g. a 2^3 factorial design. This was accomplished by our research group at Cleveland State University as well as that reported in the thermal and pharmaceutical literature. Typical variables considered were sample size (e.g., 3 vs 15 mgs), heating rate (e.g., 5 vs 20 ^{o}C/min), atmosphere (e.g., nitrogen vs. air) and humidity exposure (e.g., 100%, wet vs. 0%, dry).

CHAPTER II

THERMOGRAVIMETRIC ANALYSIS

In order to obtain meaningful data from the DSC a sample is analyzed in a TGA from where the optimum conditions are obtained for testing with a DSC.

Principle: TGA measures the amount and the rate of weight change of a material with respect to temperature or time in controlled environments. The TGA shown in the figure 1 is a TA 2950. A TGA consists of three major parts a furnace, 1. A microgram balance, 2. An auto sampler and 3. A thermocouple. The furnace can raise the temperature as high as 1000^{o}C which is made of quartz. The auto sampler helps to load the samples on to the microbalance. The thermocouple sits right above the sample. Care should be taken at all times that the thermocouple is not in touch with the sample which is in a platinum pan.

Figure 1 Thermogravimetric Analyzer

Sample Preparation

Sample preparation has a significant effect in obtaining good data. It is suggested that maximizing the surface area of the sample in a TGA pan improves resolution and reproducibility of weight loss temperatures. The sample weight affects the accuracy of weight loss measurements. Typically 10-20mg of sample is preferred in most applications. Whereas, if the sample has volatiles 50-100mg of sample is considered adequate. It is to be noted that most TGA instruments have baseline drift of ±0.025mg which is ±0.25% of a 10mg sample.

Experimental Conditions

Heating Rate

Samples are heated at a rate of 10 or 20°C/min in most cases. Lowering the heating rates is known to improve the resolution of overlapping weight losses. Advances in the technology have made it possible for variable heating rates (High Resolution TGA) to improve resolution by automatically reducing the heating rate during periods of weight loss.

Purge gas

Nitrogen is the most common gas used to purge samples in TGA due to its inert nature. Whereas, helium provides the best baseline. Air is known to improve resolution because of a difference in the oxidative stability of components in the sample. Vacuum may be used where the sample contains volatile components, which helps improve separation from the onset of decomposition since the volatiles come off at lower temperatures in vacuum, e.g. oil in a rubber tire product..

Miscellaneous

There are generally two limitations of TGA for analyzing materials:

1. In a multiple component system, sample can decompose in a narrow temperature range. This can be overcome by
   - varying the type of purge gas and/or
   - using a high resolution technique
2. TGA is quantitative but cannot identify the decomposition products. Hence TGA coupled with Mass Spectrometer or FTIR can be used also for quantitative use to some extent.

Calibration

Blank test

Without sample, air is passed at 20 ml/mm, and the temperature is raised up to 1000 °C at heating rate of 10°Cmin^{-1}. By this blank test, the general condition of the apparatus can be known. The TGA curve can drift slightly as the temperature is increased. This is owing to the changes in the buoyancy and convection. When noise appears in the TG curve, the possible cause may include contact between sample dish and thermocouple, contact between quartz suspension wire and purge gas feed pipe, and contact between weight pan and arid glass cap. Vibration and shock may also cause noise. When the sample pan or suspension wire is contaminated with deposit of decomposition product or the like, the TGA curve shows a slight decreasing curve.

Calibration of mass changes

Since the TGA is usually measured by the rate of the weight change to the sample

weight, calibration of absolute value of weight is hardly necessary, but it may be calibrated in the following manner: A weight of 20 mg is read to a precision of 10 microgms by a precision balance, and the mean (So) is determined. The furnace is put on, and when the TGA signal is stabilized, the instrument balance control is adjusted to set the automatic zero. Then the furnace is put into place and the furnace is set again, and the TGA signal value is read. This value is S_1. Repeating the same operation several times, the mean of S_1 is obtained as *S.* In this operation it is known that a signal corresponding toS_1 mg is delivered with the weight of So mg is placed on the balance. The measuring precision of TGA is within ±1 % of the range. When calibrating the apparatus, the calibration function is utilized.

Calibration of temperature

The temperature of the TGA may be calibrated in two manners: the method of making use of the melting point of a pure metal, and the method of utilizing the Curie point temperature. In the former method, one of the metals listed in Table 2 is processed in a ribbon shape, and it is suspended on the TGA suspension wire, and a weight of about 100mg is attached at its tip. When the pure metal is fused by heating, the weight drops, and a weight drop appears on the TGA curve.

In the latter method, the standard substance in Table 3 verified by International Congress on Thermal Analysis, ICTA, is measured. The standard substances in Table 3 are Ferro magnets, and have different Curie temperatures. It is intended to calibrate by measuring the apparent weight change appearing in steps at Curie temperatures by making use of a permanent magnet.

Name of pure substance	Melting point /^{o}C	Heat of fusion J/g
Indium	156.6	28.59
Tin	231.9	60.62
Lead	*327.5*	23.22
Zinc	419.6	111.4
Aluminum	660.3	397.0

Table 2 Melting Point and Heat of Fusion of Pure Substances

(1)The Society of Calorimetry and Thermal Analysis, Japan (ed.): Foundation and Application of Thermal Analysis, p. 179(1985)

	Substance	**Temperature/^{o}C**
GM761 (for TGA only)	**Permanorm 3**	**259**
	Nickel	**353**
	Mumetal	**381**
	Permanorm 5	**454**
	Trafoperrn	**750**

Table 3 Standard Substances for Temperature Calibration Verified by ICTAC[(1)]

Weight calibration is not necessary if the TGA analysis is to be performed in percent weight loss only.

Based on the TGA data, thermal stability of materials and their compositions can be predicted depending on the weight changes caused by evaporation, dehydration, oxidation and decomposition, up to temperatures as high as 1000^{o}C. A typical example is the TGA of calcium oxalate hydrate, heated to 1000^{o}C which shows three steps in its decomposition curve. The weight loss data is recorded every half second throughout the run time.

A typical TGA curve is shown in Figure 2.

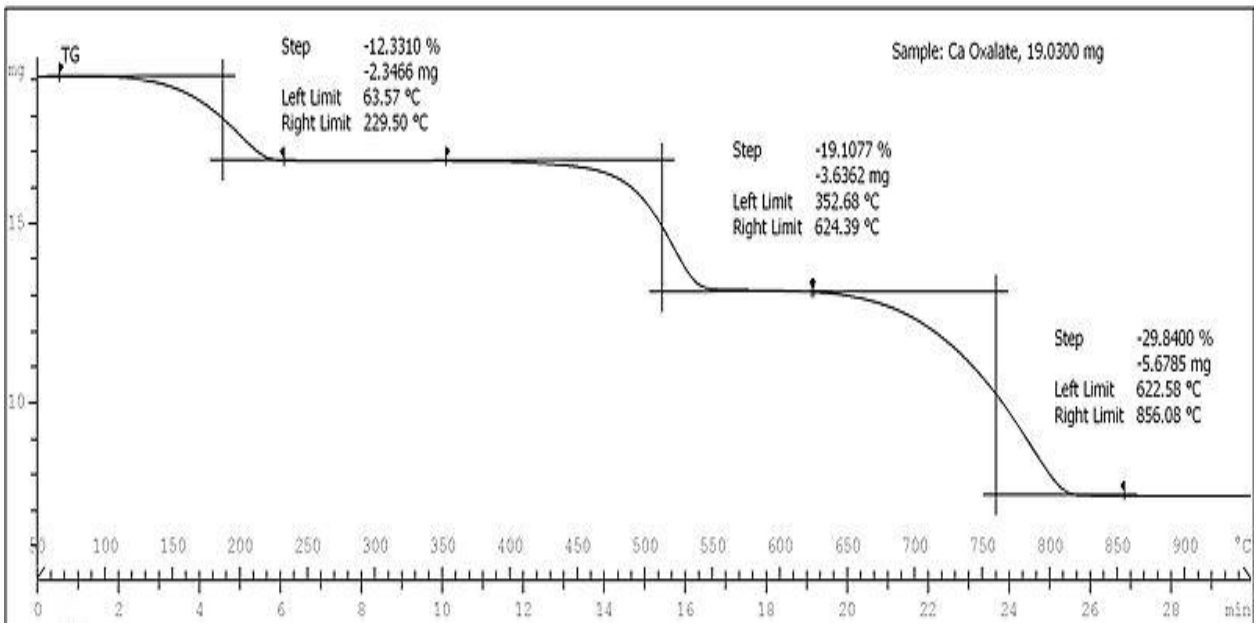

Figure 2 TGA Curve of Calcium Oxalate

Each step is explained by the following chemical reaction (TA Instruments "Weight Loss Determined from Mass Spectrometry Trend Data in a Thermo-gravimetric/Mass Spectrometer System" Carlton G. Slough TA Instruments, 109 Lukens Drive, New Castle DE 19720, USA)

At 200°C

$CaC_2O_4 \bullet H20 \text{ --------} \rightarrow CaC_2O_4 + H_20 \uparrow (12.33\ \%)$

At 500°C

$CaC_2O_4 \text{ ----------} \rightarrow CaCO_3 + CO \uparrow (19.10\ \%)$

At 75O°C

$CaCO_3\text{----------} \rightarrow CaO + CO_2 \uparrow (29.84\ \%)$

Calcium oxalate hydrate is a well known material for the calibration of the TGA.

Applications

There is a wide range of applications of TGA, e.g,

- Composition of multi-component system
- Thermal stability of materials
- Oxidative stability of materials
- Estimated lifetime of a product
- Decomposition Kinetics of materials
- The effect of reactive or corrosive atmosphere on materials
- Moisture and volatiles contents on materials.

  Evaporation of free (unbound) water begins at room temperature due to dry gas flowing over the sample. Dehydration/Desolvation of bound water almost always begins at temperatures above room temperature and typically 125°C.

Decomposition can have multiple stages (weight losses) but the presence of multiple weight loss steps can also indicate the presence of multiple components in the sample.

One of the applications is determination of the bound and unbound water in the suspension of Milk of Magnesia, used as a laxative. A detailed study was conducted in comparison of the generic and a brand MoM and is described in Chapter III.

In an overview of thermal analysis testing it is always preferable to do a TGA experiment on unknown samples before doing a DSC experiment (especially for pharmaceuticals). Decomposition of pharmaceuticals renders products which are insoluble and generally sticky on the inside of a DSC cell. These products will lower the life use of a DSC cell. Therefore, know the decomposition temperatures of all drugs and heat in a DSC evaluation to 50^{o}C below those temperatures.

CHAPTER III

THERMAL ANALYSIS OF WATER AND MAGNESIUM HYDROXIDE CONTENT IN COMMERCIAL PHARMACEUTICAL SUSPENSIONS OF MILK OF MAGNESIA

Introduction

In general, water can be organized by phases of matter: liquid, solid and gas. The liquid phase is the most common among all the water phases. The water molecule has a net positive charge on the hydrogen atoms and a net negative charge on the oxygen atom. The net result is that each water molecule has a dipole moment. Water is a polar liquid that can form a hydronium ion (H_3O^+) and is interactive with hydroxide ion (OH^-).

$$2\ H_2O\ (l) \rightleftharpoons H_3O^+\ (aq) + OH^-\ (aq)$$

The heat of vaporization, (ΔH_v), is the energy to change a given quantity of water into its gas phase at the standard temperature and pressure. Heat of vaporization for water is 2257 Jg^{-1}. The heat of fusion (ΔH_f) is the result of the change in the phase of water from crystalline solid to liquid which occurs at the melting temperature (T_m). Heat of fusion for water is 334 Jg^{-1}. The heat of crystallization (ΔH_c) is the result of the change in

the phase of water from liquid to a crystalline solid which occurs at the crystallization temperature ($T_{c)}$). Heat of crystallization for water is 334 Jg^{-1}[3].

The following is a summary from W.J. Sichina paper "Characterization of Water of Hydration of Pharmaceuticals Using the DSC". He developed a test to characterize the properties associated with the waters in a pharmaceutical material. The method includes automated sample pan puncturing accessory for the study of free and bound waters in pharmaceuticals. [2]

Milk of Magnesia is a suspension of magnesium hydroxide Mg $(OH)_2$ in water**.** It is widely used as an antacid to neutralize stomach acid and laxatives. Low solubility of $Mg(OH)_2$ in water makes it a weak base and considered as a strong electrolyte. The United States Pharmacopeia states that single strength Milk of Magnesia should contain not less than 90.0 % and not more than 115.0% of the labeled amount of 80 mg of $Mg(OH)_2$ mL^{-1}. It is commercially produced by the precipitation of magnesium hydroxide paste from seawater. The paste can have varying degrees of viscosity, which determines whether a suspending agent is required or not. Water, whose melting /crystallization temperature and enthalpy are not significantly different from those of normal (bulk) water, is called free water or freezing water or unbound water. Those water species exhibiting large differences in transition enthalpies and temperatures, or those for which no phase transition can be observed calorimetrically are referred to as bound water.

The purpose of these experiments is to find the best analytical method to determine bound, unbound water and water activity. **Oven-dry** and **moisture analyzer**

methods are traditional methods, which are used in this experiment as controls. They are used to determine the total water lost from the test samples. Since both traditional methods can only determine the total amount of water lost, these methods cannot be used to determine bound or unbound water.

TGA rapidly measures changes in mass as a sample is heated and is eventually vaporized. This can be used to create a water loss profile that can show the different temperature ranges in which water and other components of a sample vaporize. This method is also used to determine the amounts of bound and unbound water and from which can be used to determine the total amount of water lost in a sample upon heating to high temperatures. This will be compared to the traditional controls to determine if the novel methods can be accurately used to determine bound, unbound, and total water in a sample of milk of magnesia.

Thermogravimetry Method (TGA)

A TA Instruments Hi-Res Thermogravimetric Analyzer Model 2950 was used to measure bound and unbound water in milk of magnesia samples. The samples were prepared by placing one drop of material on to a pre-tared platinum TGA pan. The pan was placed onto the auto-loading mechanism of the TGA analyzer and an automated loading sequence was initiated. TGA data was analyzed using *Universal Analysis 2000*, by TA Instruments, version 4.4A. The data was plotted and analyzed using the first derivative of the percent (%) mass loss versus temperature in °C. From there, each peak was identified and the percent material loss was calculated. Also identified were the initial and end

points at which mass loss began and ended. All remaining material in the sample was calculated as percent residue. The results are shown in figures 3 and 4 and Table 4.

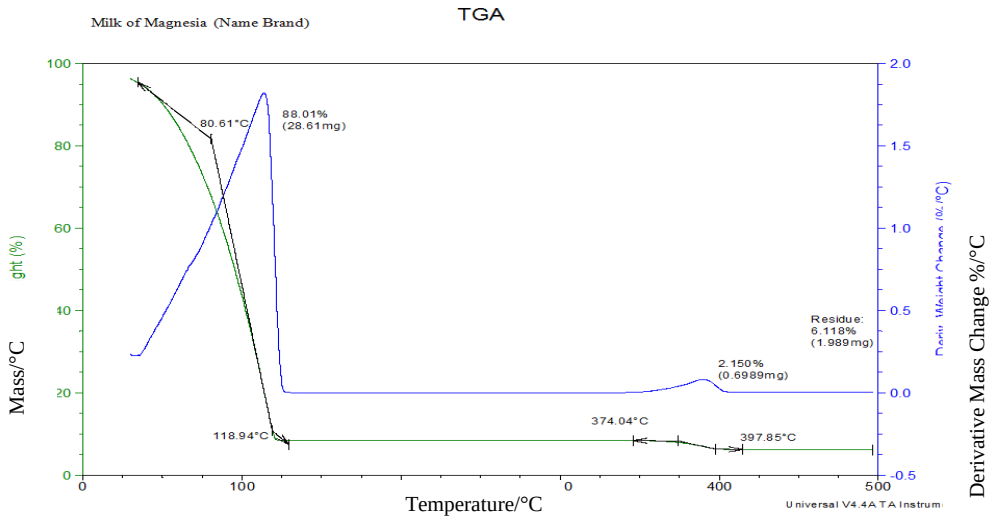

Figure 3 TGA analysis for name brand

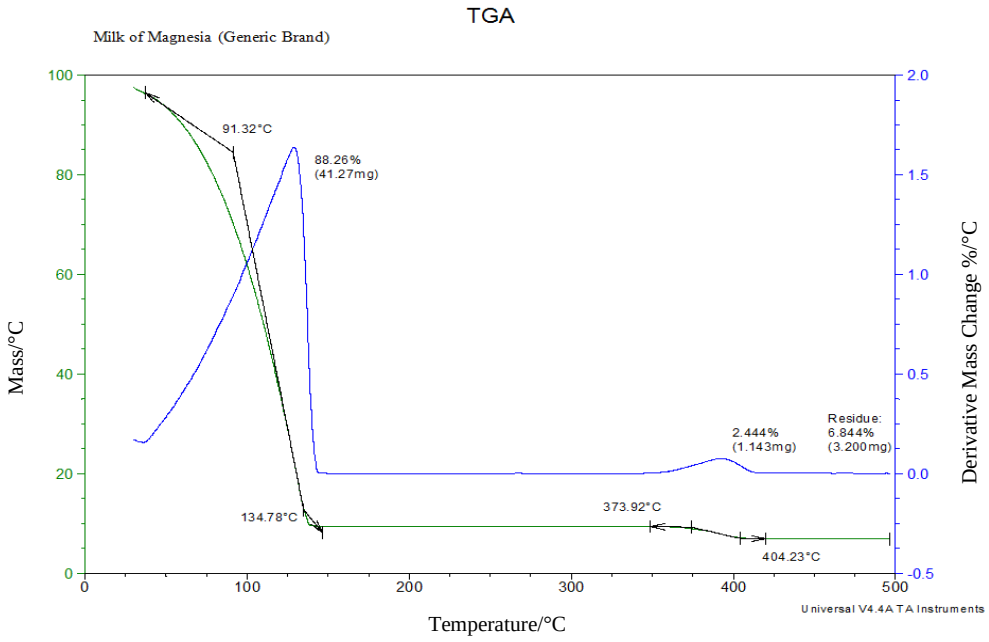

Figure 4 TGA analysis for generic brand

A second water analysis test of the MoM sample is placed into a commercial **Oven** which heats the sample, while measuring the mass of the sample every 0.5 second. The experimental conditions were: ramp 10°C per minute to 500°C in nitrogen. 30-50 mg of sample was used in each run. The results were obtained by subtracting the mass of the pan and sample after testing, from the initial mass of the pan. The difference was the amount of solids left in the pan. From there, the mass of the material left in the pan was subtracted from the initial mass of the sample. The data shows that the amount of water in the commercial brand of milk of magnesia to be 91.8% and the generic brand of milk of magnesia was 90.6%. A third test is the **moisture analyzer** results were virtually identical to the results obtained from the 110° C pan method as seen in Table 4.

In summary, there is reasonable agreement for the total amount of water (3 methods) on average in the two types of MoM, 91.3 w% for the Name Brand and 90.7 w% for the Generic Brand. The amount of bound water by TGA for the Name Brand is 2.2%w and 88.0%w unbound water. The amount of bound water by TGA for the Generic Brand is 2.4%w and 88.3%w unbound water.

	Total Water %w*		
	Oven Test	Analyzer	TGA
MoM Source			
Name Brand	91.8	91.9	90.2
Generic Brand	90.6	90.8	90.7
average values*			

Table 4 Comparison total water content in MoM by three methods

Conclusions

The conventional methods of water analysis, oven and moisture analyzer provided total water. TGA is used to determine the bound and unbound water in MoM which were similar.

CHAPTER IV

DIFFERENTIAL SCANNING CALORIMETRY (DSC)

The DSC will be reviewed in the following manner: Introduction, Optimizing the Experimental Conditions, Calibration and an overview of the most common pharmaceutical applications.

DSC The Technique

Principle DSC is a thermo-analytical technique in which the difference in the amount of heat required to increase the temperature of a sample and reference is measured as a function of temperature. The technique was developed by E.S. Watson and M.J. O'Neill in 1960 [1]

The differences in heat flow occur with the occurrence of two major events:

1) The heat capacity of the sample which increases with temperature (baseline)
2) Transitions that occur in the sample (events superimposed on the heat capacity baseline)

Heat Flow Rate is expressed in a variety of units which can also be normalized for the weight of sample used

1) mW (milli Watts) where W = J/sec

2) W/g

3) mCal/sec cal/sec/g

4) BTU/hr (British Thermal Units/Hour)

Figure 5 Differential Scanning Calorimeter

A DSC consists of a cell, which is the heart of a DSC. The cell is connected with a gas inlet through which different gases are purged depending on the data required, see Figure 5. Based on the DSC cells there are two primary types:

Heat Flux

This consists of a large single furnace which acts as an infinite heat sink to provide or absorb heat from the sample. The advantages generally include a better baseline, sensitivity and sample–atmosphere interaction. Figure 6 is a schematic of a Heat Flux DSC. The key components are the Sample pan (typically an

aluminum pan and lid) which is combined with the Reference pan (always the same material as the Sample pan, aluminum). The Dynamic sample chamber is the environment of the sample pan compartment and the purge gas. Nitrogen is the most common gas, but alternate inert gas is helium or argon. When using an oxidative atmosphere air or oxygen are the gases of choice. The heat flux DSC is based on the Change in Temperature ΔT between the sample and reference and is indicated in Figure 6.

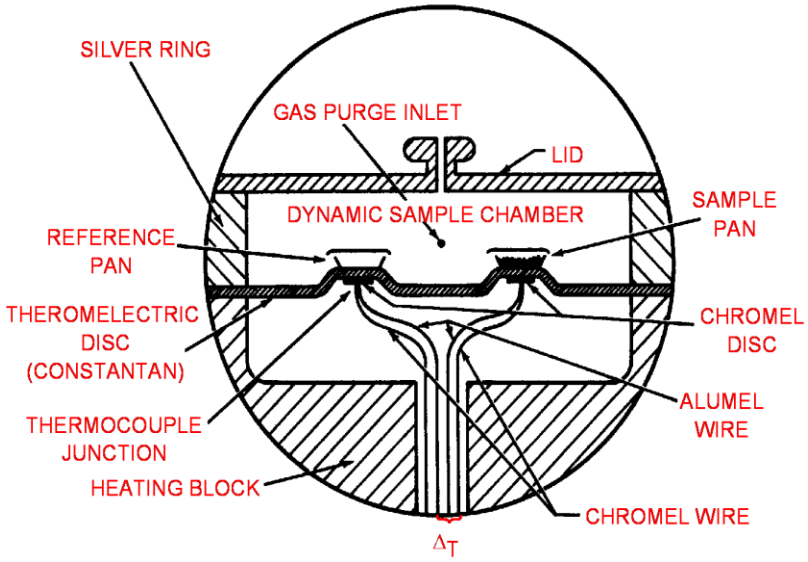

Figure 6 Heat Flux DSC Cell Cross Section

Power Compensation

Small individual furnaces use different amounts of power to maintain a constant ΔT between sample and reference and the advantage here include faster heating and cooling, and better resolution.

This type of cell, Figure 7, with two individually heated with platinum heaters monitors the difference between the sample and reference. Platinum resistance thermometers track the temperature variations for the sample and reference cells. Holes in the compartment lids allow the purge gas to enter and contact the sample and reference.

There are physical differences between the heat flux and power compensated thermal analysis, the resulting fusion and crystallization temperatures are the same. The heat of transition is comparable quantitatively.

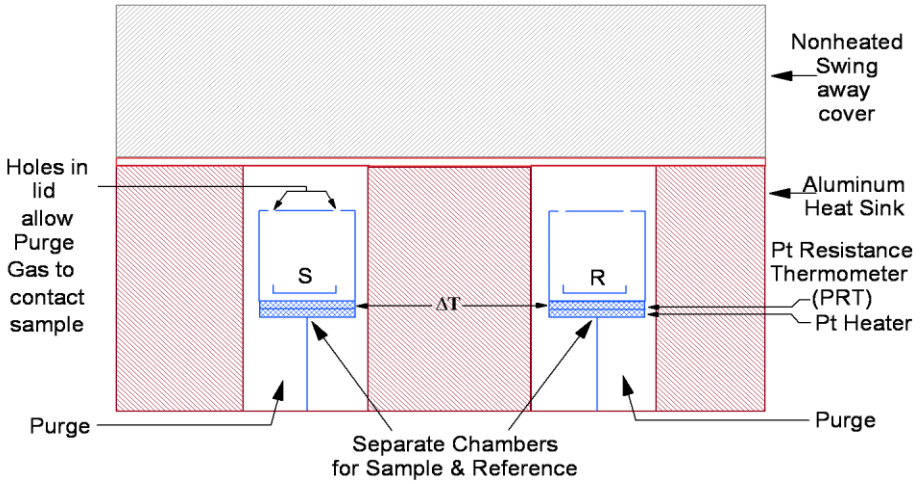

Figure 7 Power Compensation DSC Cell Design

Sample Preparation

Sample Shape

It is recommended that the sample is as thin as possible and covers as much of the pan bottom as possible. Samples in the form of cakes (as in case of polymers) must preferably be cut rather than crushed to obtain a thin sample. Crushing the sample, whether in crystalline form or a polymer, induces a stress, which can in

turn affect the results. In most cases lids should always be used in order to more uniformly heat the sample and to keep the sample in contact with the bottom of the pan. In case where oxidation properties of a sample are to be studied no lid is used and the purge gas is usually oxygen as described in ASTM Standard Test Methods E1858, Oxidative Induction Time or ASTM E2009, Oxidation onset temperature.

Sample Pans

Lightest, flattest pans are known to have the least effect on the results obtained from a DSC. Crimped pans on the other hand provide the highest sensitivity and resolution. Hermetic pans are used where the sample is expected to have some volatile content. These pans prevent evaporation. Two main reasons for the use of these pans are:

- The Tg of a polymer or amorphous material shifts with volatile content
- Evaporation peaks look just like melting endotherm.

Sample Weight

Though 5 to 10 mg is considered to be an appropriate sample weight for a DSC test, selection of the optimum weight is dependent on a number of factors: the sample to be analyzed must be representative of the total sample and the change in heat flow due to the transition of interest should be in the range of 0.1 - 10mW

A recommendation for metal or chemical melting sample is < 5mg. For polymer glass transition Tg or melting sample the mass should be » 10mg. Polymer

composites or blends the sample mass is >10mg. The accuracy of the analytical balance used to measure the sample weight should be accurate to ± 1%.

Experimental Conditions

- Start Temperature

Generally, the baseline should have 2 minutes to completely stabilize prior to the transition of interest. Therefore, at 10°C/min heating rate the run should start at least 20°C below the transition onset temperature.

- End Temperature

Allowing a 2-minute baseline after the transition of interest is considered appropriate in order to correctly select integration or analysis limits. Care should be taken not to decompose samples in the DSC; it not only affects the baseline performance but the cell life.

- Reference Pan

A reference pan of the same type used to prepare the sample should be used at all times. A material in the reference pan that has a transition in the temperature range of interest should never be used.

- Heating Rate

$$\mathbf{dH/dt = Cp * dT/dt + \mathit{f}\,(T,t)}$$

Where dH/dt → heat flow measured by DSC

Cp → heat capacity or weight of the sample

dT/dt → heating rate

$f\,(T,t)$ → time dependant or kinetic component

Heating the samples at low heating rates increases resolution by providing more time at any temperature. Transitions due to kinetic processes (such as crystallization) are shifted to lower temperature at highest cooling rates or higher temperatures at high heating rates.

Effects of heating rate

Shown below are the DSC curves of Acetophenetidin Figure 8 and Phenacetin Figure 9. The Acetophenetidin DSC at 0.5^{o}C/min and 10^{o}C/min showed no effect of heating rate. If there were some minor eutectic in this sample then they would have been detected at the lower heating rate. The melting temperature of pure drugs or chemicals will have the same extrapolated onset temperature or the melting point as seen in Figure 8 at two varying heating rates. The DSC Curve for Phenacetin viewed in Figure 9 at heating rates of 1.0, 5.0 and 20^{o}C/min yielded the same Tm of 135^{o}C ±1^{o}C.

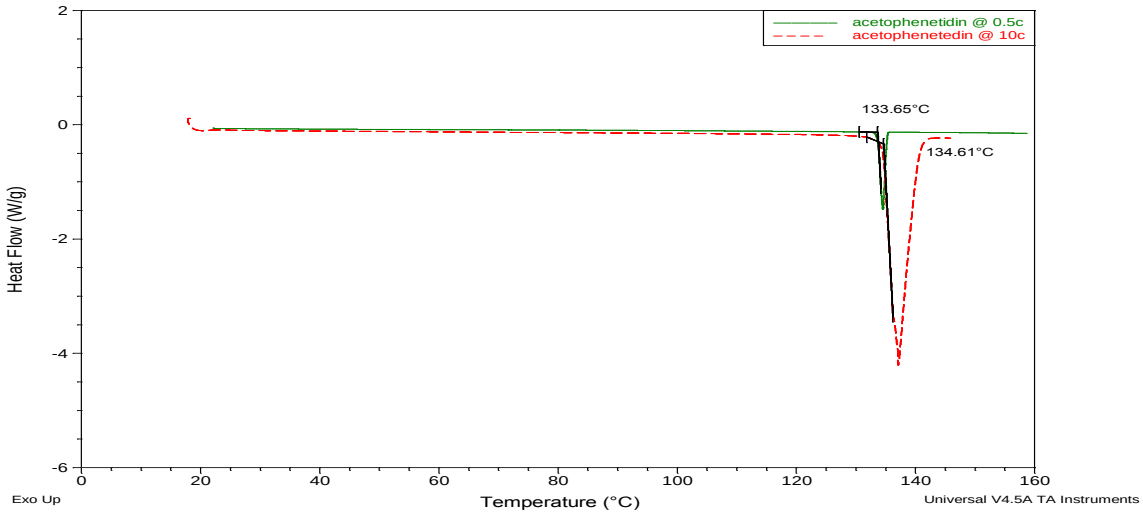

Figure 8 DSC curves of Acetophenetidin

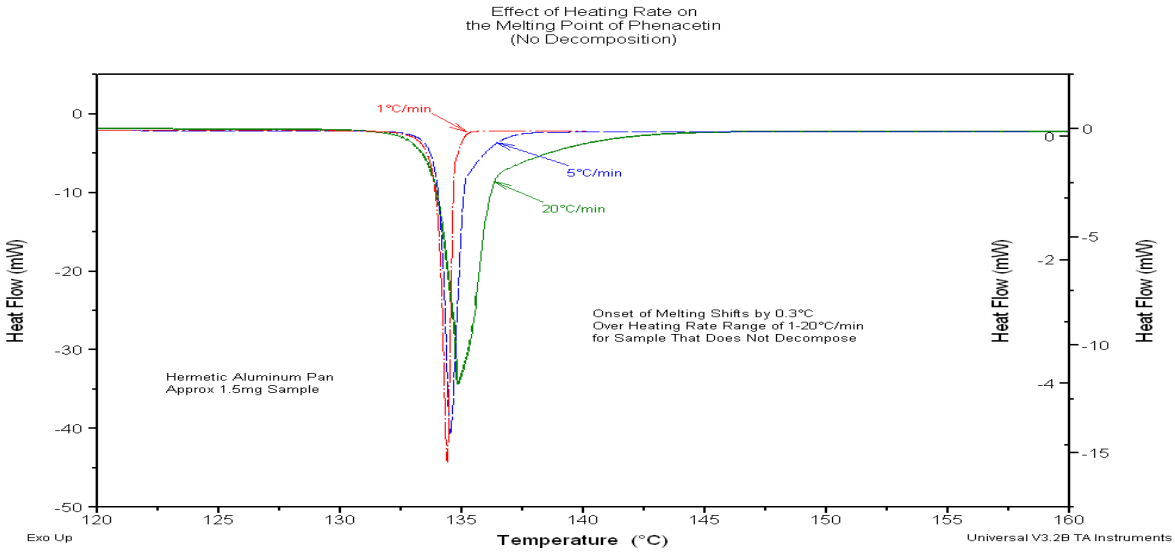

Figure 9 DSC curves of Phenacetin.

If you use multiple heating rates then start with 1.0 and 10°C/min. The two main conclusions can be drawn from the figures 8-9 are:

- Melting is a thermodynamic process and the onset of melting does not change significantly with heating rate.
- Evaporation, desolvation and decomposition are kinetic processes that will move to higher temperatures as heating rate increases.

Purge Gas

Nitrogen being a relatively poor thermal conductor increases sensitivity whereas helium which is a good conductor of heat to or from the sample increases resolution. Table 5.

Condition	To Increase Sensitivity	To Increase Resolution
Sample Size	Increase	Decrease
Heat Rate	Increase	Decrease
Ref Pan Weight	Increase	No Effect
Purge Gas	Nitrogen*	Helium*

*Instrument should be calibrated with the same purge gas as used to run a sample

Table 5 Summary of DSC experimental conditions

DSC is used in studying the melting, crystallization, glass transition, oxidation and decomposition of pharmaceuticals. By selecting different parameters useful data such as the purity, polymorphic transitions can be obtained. A typical DSC curve could give glass transition temperature, melting temperature, crystallization temperature and decomposition temperatures. Examples of the transitions in a DSC curve is shown in figure 10: The sigmoidal shaped glass transition curve (1st on the left of Figure 10) is typical but can vary in shape based on amorphous molecular weight and base line stability. The crystallization curve is exothermic and as shown here is due to post crystallization observed with polylactic acid (PLLA), next is the melting of e.g. the PLLA, the curing or cross linking of a monomer which is exothermic and lastly the oxidation or decomposition/degradation curve which is also exothermic.

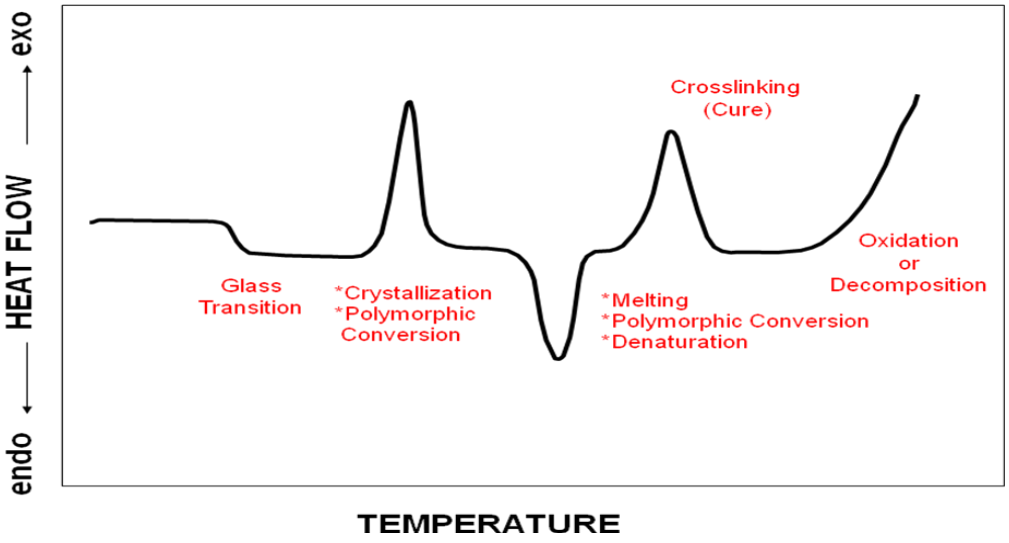

Figure 10 Transitions in a DSC Curve

DSC Calibration

Calibration of DSC is done using Indium metal. Calibrating an instrument with a metal when pharmaceuticals are to be studied appears to be not appropriate. To overcome this, an effort has been made to calibrate DSC with pharmaceuticals. A detailed discussion is available in Chapter 6. A calibration curve using indium is shown in Figure 11. The true melting temperature of indium metal is 156.7^{o}C and the observed in calibration is 157.4^{o}C. It is 0.7^{o}C high and the instrument values must be adjusted down to accommodate the true melting temperature.

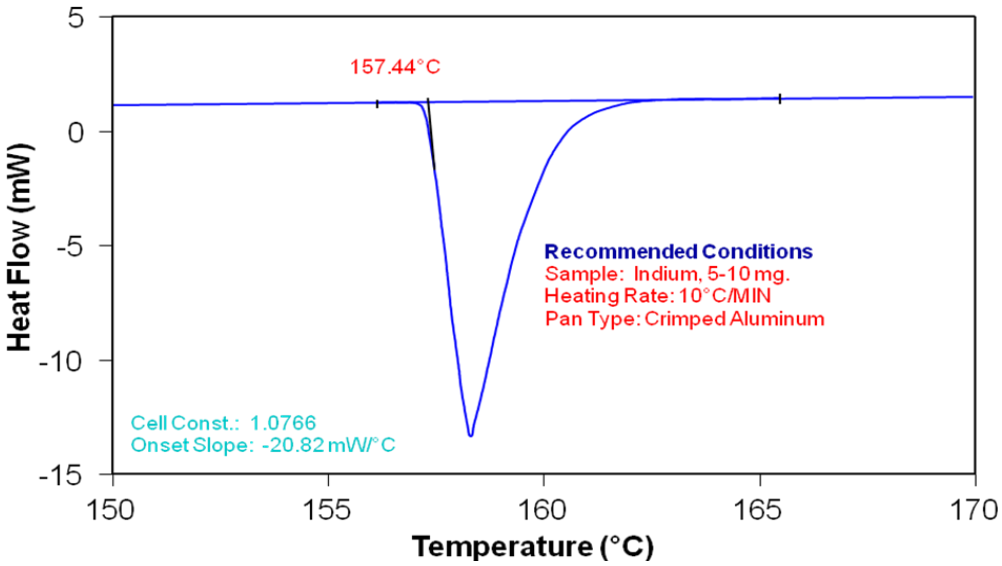

Figure 11 DSC Calibration curve of indium

DSC – Applications

Glass Transition Temperature (Tg)

The glass transition is due to the presence of amorphous structures in the sample. It is detected by DSC based on a step-change in molecular mobility that results in a step increase in heat capacity and heat flow rate. Amorphous materials flow, they do not melt and hence no DSC melt peak. The physical and reactive properties of amorphous structure are different than crystalline structure. The physical and reactive properties of amorphous structure are significantly different at temperatures above and below Tg. The glass transition temperature, Tg, is a second order pseudo transition [2]. It constitutes a parameter of high interest in the study of amorphous and

semi-crystalline drugs since amorphous drugs are more bio available and soluble. [3-5].

Glass Transition Size (⊿Cp)

The ΔCp at Tg is a measure of the flexibility associated with the Tg. A larger value implies a more rubbery material, e.g., polybutadiene. Stiffer polymers like polystyrene have a lower value. .

Crystallization temperature (Tc)

The Tc of many drugs has been determined in our lab based on a DSC that can program heat and cool. The difference in Tm to determine the Tc is a measure of super cooling, e.g. Vanillin has a 50^{o}C super cooling temperature while indium melts and crystallizes at the same temperature or super cooling is zero ^{o}C

Crystallinity (based on J/g and adjusted to %)

The Crystallinity measured by comparing successive heat and cool DSC runs on a drug will yield the change in crystallinity by comparing the Heat of Crystallization to the Heat of Fusion x100. This % crystallinity by this method was 78% for Acetophenetidin, 20% for Sulfapyradine and 0% for Lidocaine. This implies that Lidocaine remains amorphous for a period of time.

Polymorphic Transitions.

Sulfanilamide Polymorphs: It was observed that sulfanilamide polymorphs are stable and do not show transition among its forms at heating rates between 1 and 10°C/min. Shown below is an example of one of the polymorph heated at 1 and 10°C/min.

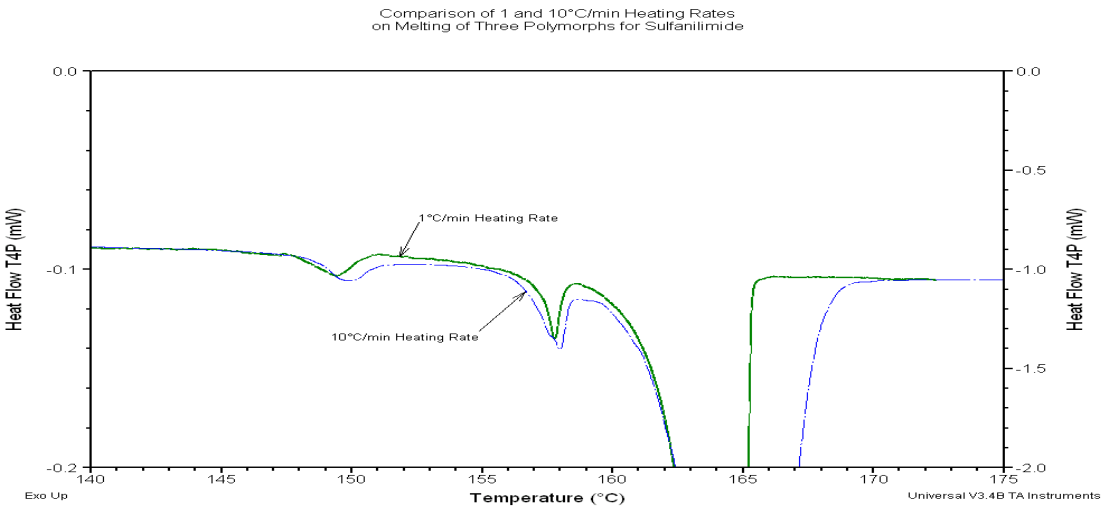

Figure 12 DSC curves of Sulfanilamide Polymorphs

DSC of Polymorphs of Tolbutamide: Tolbutamide A (Form 1) and B (Form 3): When tolbutamide polymorphs were observed by DSC a significant difference was seen in their behavior. The difference is due to their structures which were observed by scanning electron microscope (SEM). The DSC curves, Figure 13 and 14, are shown below along with the SEM

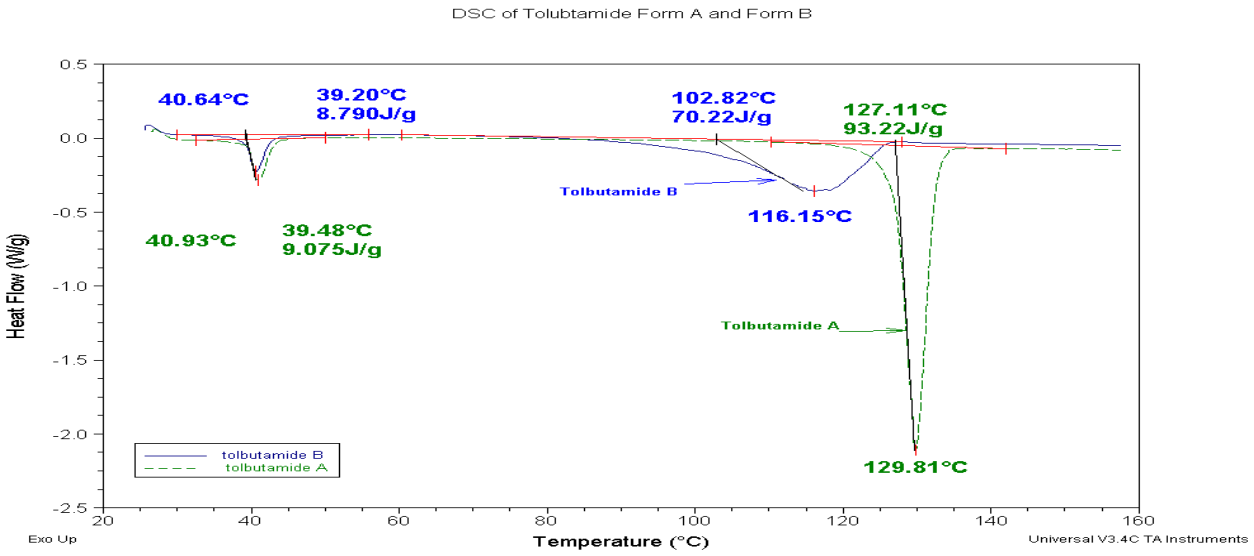

Figure 13 DSC of Polymorphs of Tolbutamide: Tolbutamide A (Form 1) and B (Form 3)

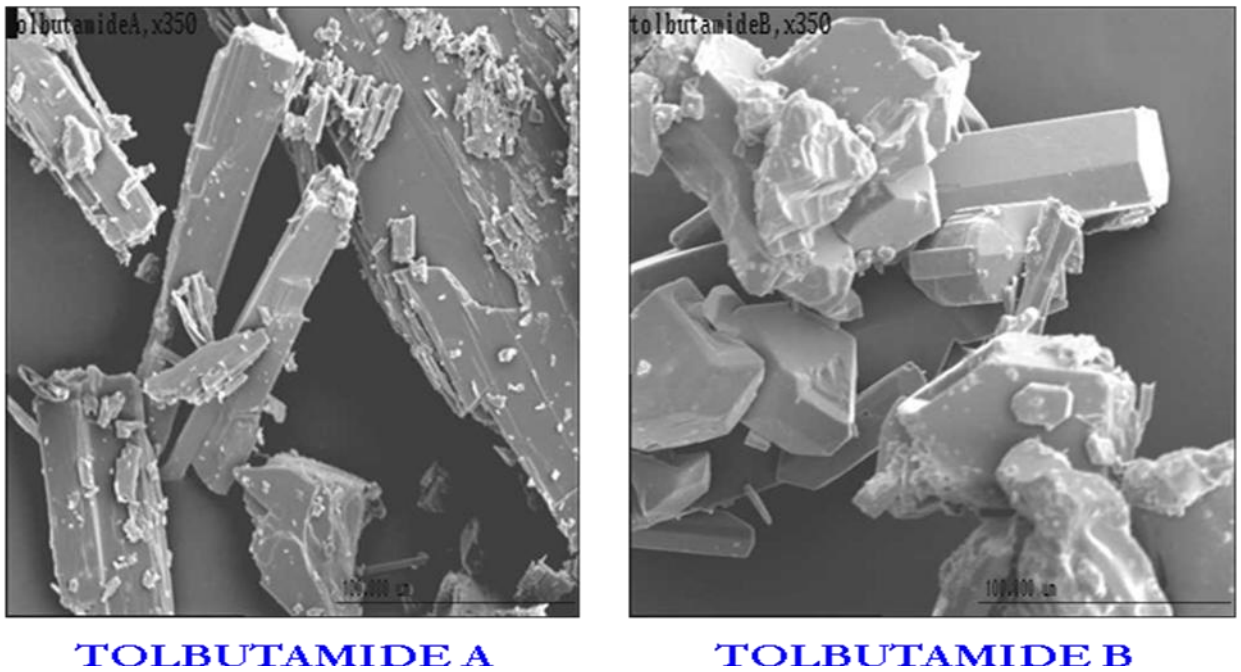

Figure 14 SEM of Tolbutamide polymorphs

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CHAPTER V

THERMOMECHANICAL ANALYSIS

TMA is a thermal analysis technique used to measure changes in the physical dimensions (length or volume) of a sample as a function of temperature and time under a non oscillatory load. [1] This technique is widely applicable to variety of materials such as pharmaceuticals, polymers, ceramics and metals etc. TMA has been used in pharmaceutical analysis. [2-3] Variables considered while performing the thermal mechanical analysis are applied load, gas environment, temperature range and heating rate as well as TMA probe type. The tests are run in a heating mode at a desired heating rate and temperature range of interest. Probe displacement profiles are subsequently analyzed in terms of coefficient of thermal expansion, softening and melting temperatures, and glass transition temperatures. Information obtained based on the different TMA probe types are shown in (Table 6), and recorded as a function of temperature.

TMA Probe Type	Information Obtained
Flat probe/ Light load	Coefficient of Thermal Expansion and Tg
Dilatometer	Coefficient of Thermal Expansion and Tg
Penetration probe/Significant load	Softening (Tg),Melting and creep modulus
Tension accessory	Tg, melting and cure behavior
Parallel plates	Melting, Viscosity and Gelation
Flexure accessory	Softening (Tg) and Melting

**Tg =Glass transition temperature*

Table 6 Types of TMA probes and resulting measured properties.

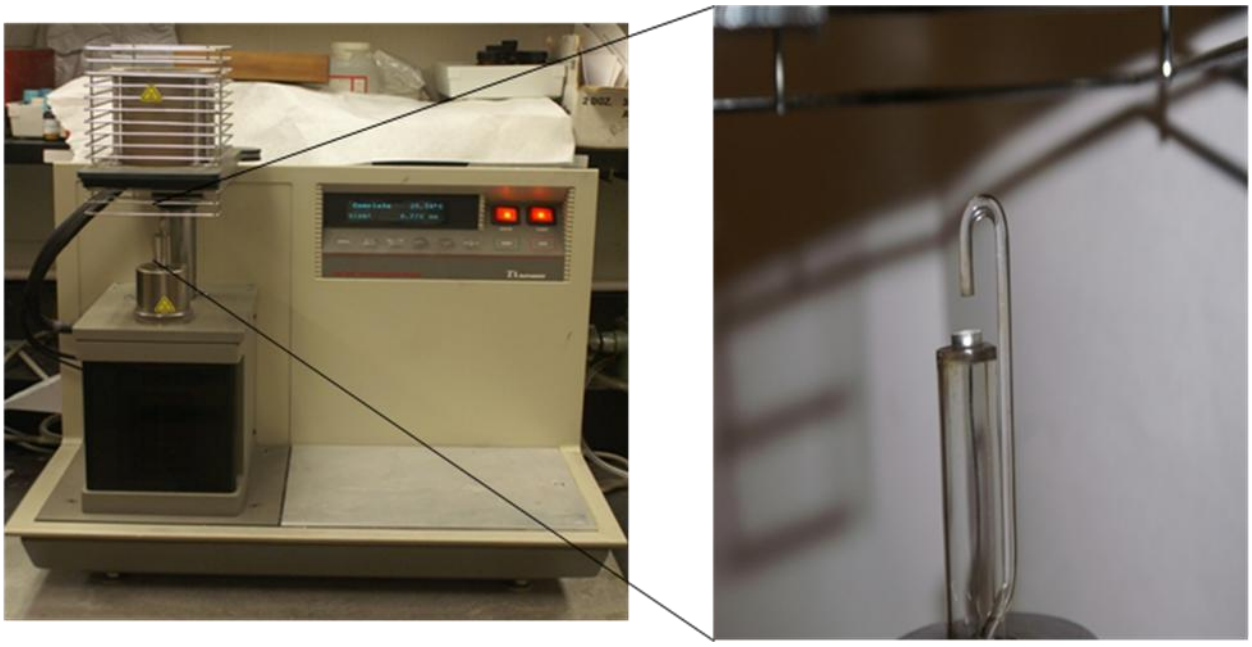

Figure 15 Thermomechanical Analyzer and blow of the quartz probe with a DSC pan/sample on the quartz stage.

TMA consists of a quartz stage, a quartz probe, furnace which sits on top of the stage, equipped with inlet for purge gas, thermocouple adjacent to the stage and a LVDT (linear variable differential transformer) attached to the probe, which measures the difference in the dimensions caused under the probe.

Sample preparation: The use of TMA in the pharmaceutical industry is limited to polymers. In order to examine powdered samples, the sample is packed into a flat DSC pan. The dimension of the sample is measured by TMA in millimeters.

Experimental Conditions: The TMA is operated under the following conditions and includes the heating rate at 10^{o}C/min, applied stress of 0.1 N; flat tip quartz expansion probe with outer diameter 0.125 mm, gas purge nitrogen at 50 mL/min, sample in a DSC pan and the probe is applied onto the packed crystalline powder, and the sample size in the DSC pan is 100 mgs..

Calibration: Calibration of TMA is done using an Indium metal. Calibrating an instrument with a metal when pharmaceuticals are to be studied does not sound appropriate. To overcome this, an effort has been made to calibrate TMA with pharmaceuticals. A detailed discussion is available in Chapter 7. A calibration curve using indium is shown in the figure below:

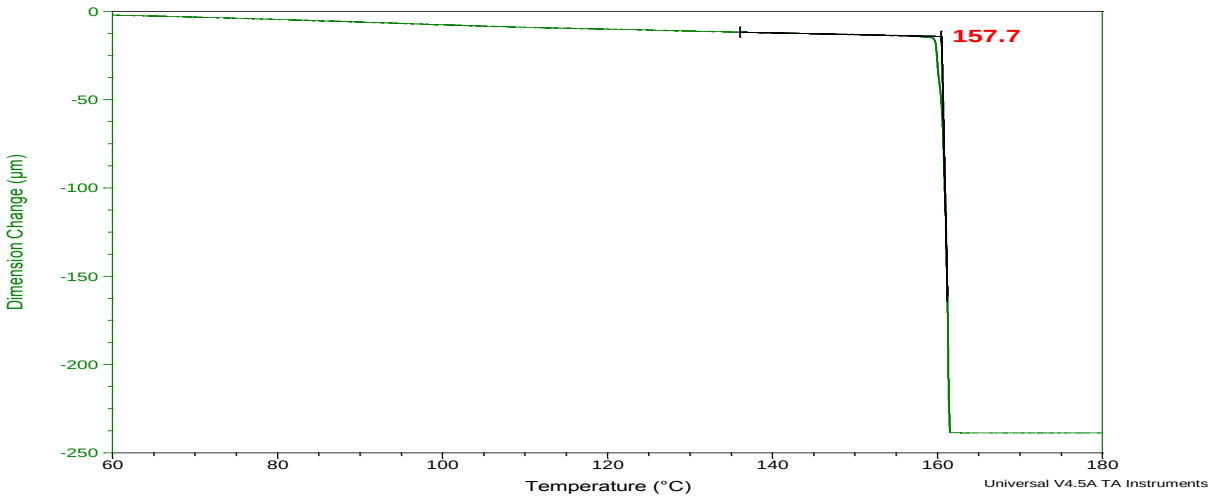

Figure 16 TMA Curve of Indium

This was the draft DSC curve Figure 16 with an indium melting point of 157.7^{o}C. The TMA calibration was applied and adjusted the Tm by TMA to 156.7^{o}C.

TMA Applications

TMA is used to obtain the melting temperature, softening temperature, coefficient of thermal expansion (CTE) and glass transitions (Tg) of materials. An example of determining the softening temperature is discussed in the next chapter.

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CHAPTER VI

SOLID STATE MECHANICAL PROPERTIES OF CRYSTALLINE DRUGS AND EXCIPIENTS SUBSTANTIATE NEWLY DISCOVERED DIELECTRIC VISCO-ELASTIC CHARACTERISTICS

Abstract

Thermal mechanical analysis (TMA) of crystalline drugs and excipients in their pre-melt temperature range corroborate their newly found linear dielectric conductivity properties with temperature. TMA of crystalline active pharmacy ingredients (APIs) or excipients shows softening at 30-100^{o}C below the calorimetric melting phase transition, which is also observed by dielectric analysis (DEA). Acetophenetidin melts at 135^{o}C as measured calorimetrically by differential scanning calorimetry (DSC), but softens under a low mechanical stress at 95^{o}C. At this pre-melting temperature, the crystals collapse under the applied load and the TMA probe moves vertically at a rapid rate. The mechanical properties yield to a soft structure and a dimensionally slow disintegration to a sharp mechanical change at the melt temperature. In order to incorporate these findings into a structure-property relationship, several United States Pharmacopeia (USP) melting-point

standard drugs were evaluated by TMA, DSC and DEA, and compared to the USP standard melt temperatures. The USP standard melt temperature for vanillin (80^{o}C) [1], Acetophenetidin (135^{o}C) [2], and caffeine (235^{o}C) [3] are easily verified calorimetrically via DSC. The combined thermal analysis techniques allow for a wide variety of newly discovered physical properties of drugs and excipients.

Introduction

Most of the active pharmaceutical ingredients (APIs) known today are crystalline. An amorphous material can be substantially more soluble than the corresponding crystalline material, and thus more readily bioavailable, but the crystalline structure is often preferred because of better shelf stability [4]. Because of the dichotomous nature of the two morphologies, there are myriad drug applications that could possibly benefit from a combination of the two morphologies, and also many where the presence of a particular morphology could be detrimental to the formulation.

In past studies, methods used to determine the extent of crystallinity of APIs and excipients included use of solution calorimetry and others [5], but these methods did not incorporate multi-dimensional analysis and do not completely describe the morphology of the drug compounds and excipients through the various stages of dissolution.

This lack of a complete description of the physical characteristics of APIs and excipients exemplifies the fundamental gap between the current techniques used and the extent of information required to truly define the morphology of these compounds. As such, it is essential to develop methods by which we can use multiple instruments to more accurately map the physical properties of the drugs and excipients. Our approach is to use a multi-instrumental approach, where we show that the crystalline APIs soften well below their melting temperatures, and that they also show differences in their electrical properties, which can indicate a potentially significant discovery in the properties of APIs. To pilot this study, three common USP standards are used: vanillin USP (80^{o}C) [6-

8], acetophenetidin USP (135^{o}C), and caffeine USP (235^{o}C). The above mentioned APIs were characterized by three thermal analytical techniques.

Thermomechanical analysis (TMA), because of its known usefulness in studying the elastic and viscous properties of many materials including polymers [9] and pharmaceutical APIs and excipients [10-12], is used to measure changes in the physical dimensions (length or volume) of a sample as a function of temperature and/or time under a non-oscillatory load. In this study, TMA will be used to determine the coefficient of thermal expansion and the glass transition temperature of the three USP ingredients.

We also characterize the ingredients by DSC, which has been used extensively to study glass transition temperature (T_g), crystalline melting temperature (T_m), heats of fusion (ΔH_f), and thermal stability of materials including pharmaceuticals and polymers [13-15]. DSC will provide both qualitative and quantitative data on the three USP ingredients based on the endothermic (heat absorbing) and exothermic (heat evolving) processes.

Finally, dielectric analysis (DEA) is used to measure the dielectric properties of the three USP ingredients [16]. DEA has been used extensively to characterize a wide variety of materials such as polymers, food products, pharmaceuticals, and proteins, which may be in the form of solids, liquids or gels [16]. As DEA is a thermal analysis tool, it compliments DSC by allowing a measurement of molecular motion initiated by the alternating current (A.C) electric field.

Methods

A TAI 2970 DEA (TA Instrument) was used to determine the electrical conductivity and Tan delta curve for each drug studied. For each solid powdered drug, a sample of approximately 20 mg was placed on a single surface gold ceramic interdigitated sensor. The samples were ramped at a rate of 10 ^{o}C min^{-1} in a purged nitrogen flow at 60 mL min^{-1} from room temperature to 30 ^{o}C above the melting temperature of the drug. The gold ceramic interdigitated sensors were calibrated by the instrument, and were used to evaluate the electrical properties of the drugs. The conductivity measurements were recorded at controlled interval frequencies ranging from 0.10 to 10,000 Hz for all temperatures.

Calibration

Calibration of the TA Instrument (TAI) TMA 2940 was performed based on ASTM method E1363. At the transition temperature of the test specimen, there is a change in dimensional stability and a measured change in the coefficient of thermal expansion is recorded by the instrument. From the TMA thermal curve recorded, extrapolated onset temperature is calculated by extending the pre-transition portion of the curve which to the point of intersection with a line drawn tangent to the steepest portion of the curve which describes the probe displacement. [3]

Calibration for the DSC instrument TAI 2920 was accomplished based on Standard Test method for temperature calibration, adapted from ASTM method E967.

Calibration for the DEA instrument TAI 2970 was done based on the standard test method for temperature calibration of DEA using the prescribed fixtures. The sensor is calibrated to a conductance of zero. At the thermodynamic melt transition temperature, an abrupt change in DEA permittivity is observed. The temperature observed for this transition is recorded by the instrument. From the resultant DEA thermal curve, the following parameters are measured to determine the property variation associated with the transition: frequency, permittivity, log permittivity, temperature, derivative of permittivity and log permittivity with respect to temperature.

Experimental Protocol

TMA Protocol

The TAI 2940 was used to evaluate the pharmaceutical samples. The probe in this study is a flat probe made of quartz which has an expansion coefficient of $0.6 * 10^{-6}$ K^{-1} which is negligible when compared that of the samples studied. [12] TMA tests are run in heating mode at controlled heating rates. The sample was added to a standard DSC aluminum pan and weighed, then crimped and heated at 5°C/min in nitrogen at a flow rate of 50 mL min^{-1}. The length of the sample measured by the TAI software varied from 0.90 mm to 1.30 mm. Probe displacement profile is recorded using a linear variable differential transducer (LVDT). Subsequently, the data is analyzed in terms of thermal expansion coefficients, softening temperatures, and/or the glass transition temperature.

DSC Protocol

The TAI 2920 was used for DSC studies. 10 mg of sample was weighed into an aluminum pan and then crimped. The sample was heated at 10°C/min in nitrogen at a flow rate of 50 mL min^{-1} under open pan.

DEA Protocol

To generate DEA data, the TAI 2970 was used varying frequency ranging from 0.1 to 1000 Hz. The sample was spread on the surface of a gold ceramic interdigitated (IDA) electrode and ramped in the dielectric analyzer at 5°C min^{-1} with nitrogen gas purge at a flow rate of 50 mL min^{-1}.

Results

Acetophenetidin was characterized by TMA, DEA and DSC. Corroboration of the newly discovered DEA properties in the pre-melt of two drugs and an excipient is based on the TMA premature softening, prior to melting (TMA rapid vertical dimensional change at the rate of -12.5µm/°C); see Figure 17. Onset of TMA softening detected occurred for Acetophenetidin at 95°C and melting at 134°C, i.e., 39°C below the melting and dimensionally a weak structure. The latter corresponds to the DEA rapid rise in conductivity from 10^{-2} to 10^{5} pS cm^{-1} [17]. The DSC curve T_m 135°C and ΔH_f 176 J g^{-1}. The pre-melt enhanced conductivity, probably based on the Polaron theory of conduction in an AC electric field, also contributes to the orders of magnitude dimensional change below the melt temperature by TMA and DEA. The DEA curve cited the onset temperature of dielectric conductivity of 127°C. The DSC showed no enhanced activity but only the classic melting and crystallization properties as given in Figure 17 .Vanillin

was also characterized by TMA, DEA and DSC. Onset of TMA softening occurred for vanillin at 72^{o}C and melting at 84^{o}C, i.e., 12^{o}C below the melting temperature. The DSC curve Tm 81^{o}C and ΔH_f 149 J g^{-1}. The DEA curve cited the onset temperature of dielectric conductivity of 60˚C. The DSC showed no enhanced activity but only the classic melting and crystallization properties as given in Figure 18.

Caffeine's T_{os} was 176 and the T_m was 226˚C as measured by TMA. The DSC T_m was 237˚C; the ΔH_f was measured as 65 Jg^{-1}. The difference in the T_m by DSC and TMA can be explained by the sublimation property of caffeine. In TMA which is an open system, there is more availability for caffeine to sublime as where in DSC the sample is confined, which gives the better melting temperature for caffeine and minimizes sublimation.

The DEA T_{dc} was 219 or 18˚C below the melting temperature. A plot of the T_{os} vs. the T_{dc} had a correlation R^2 of 0.95 for these drugs or excipient. This correlation held for many of the other drugs or chemicals studied for example acetanilide (melting 114^{o}C).

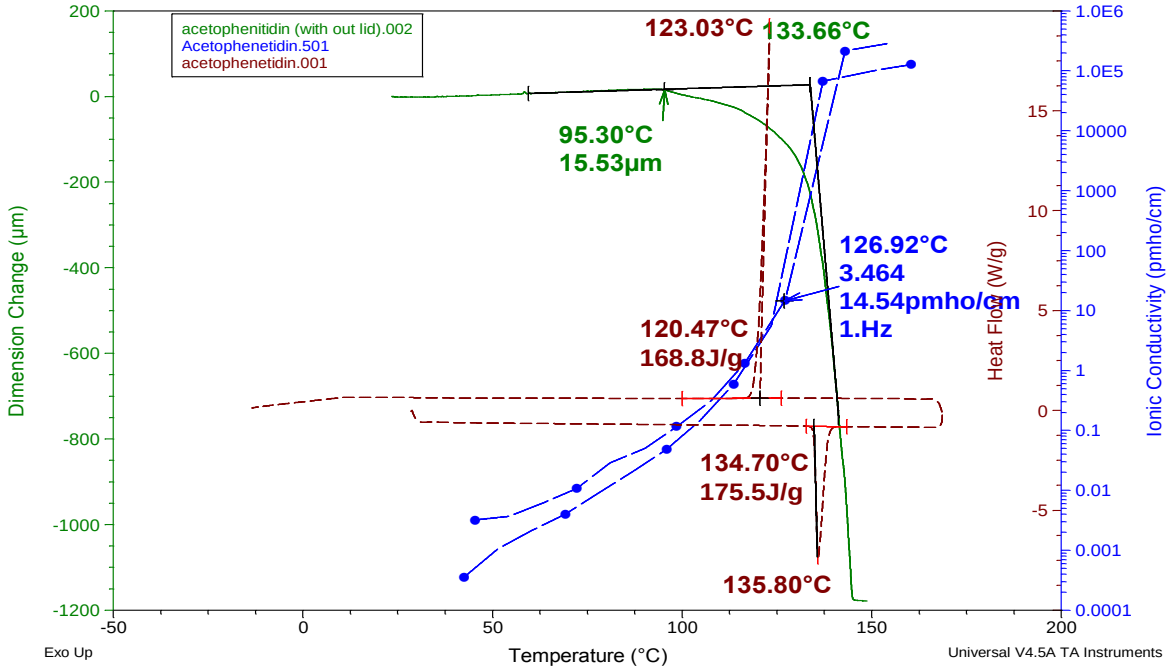

Figure 17 Acetophenetidin run by TMA (solid line), DSC (dash line), and DEA (dotted line)

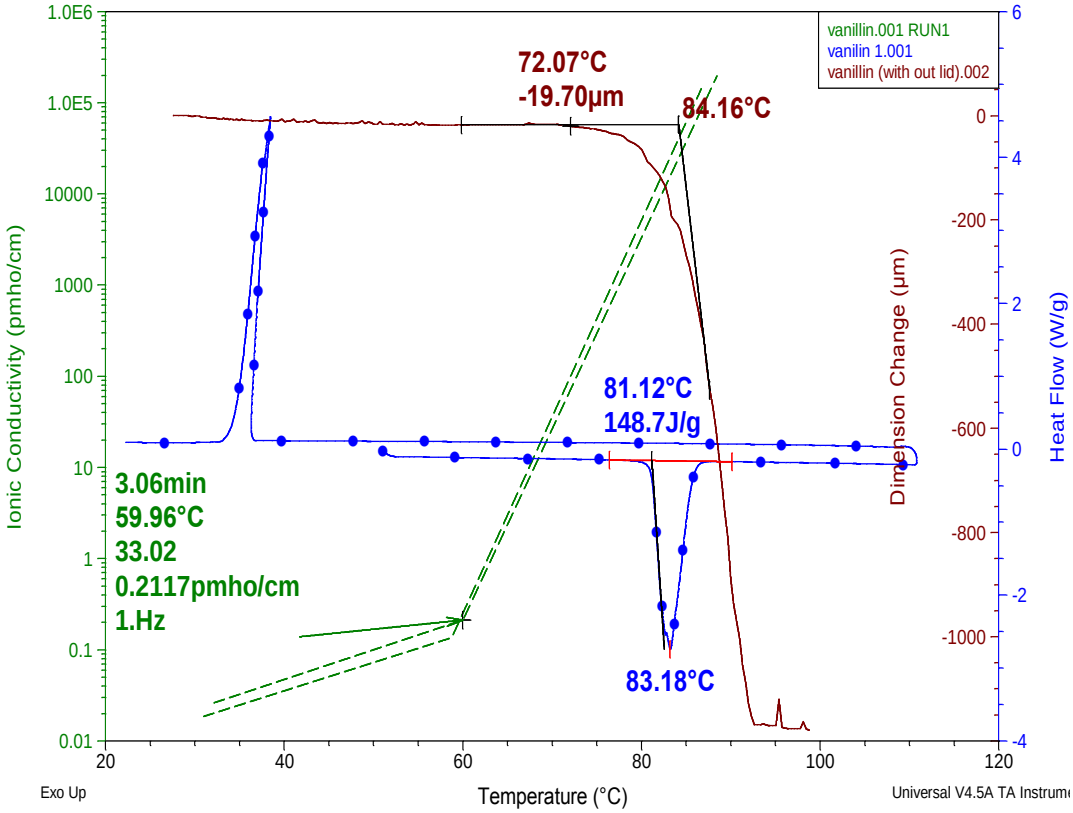

Figure 18 Vanillin run by TMA (solid line), DSC (dotted line) and DEA (dash line).

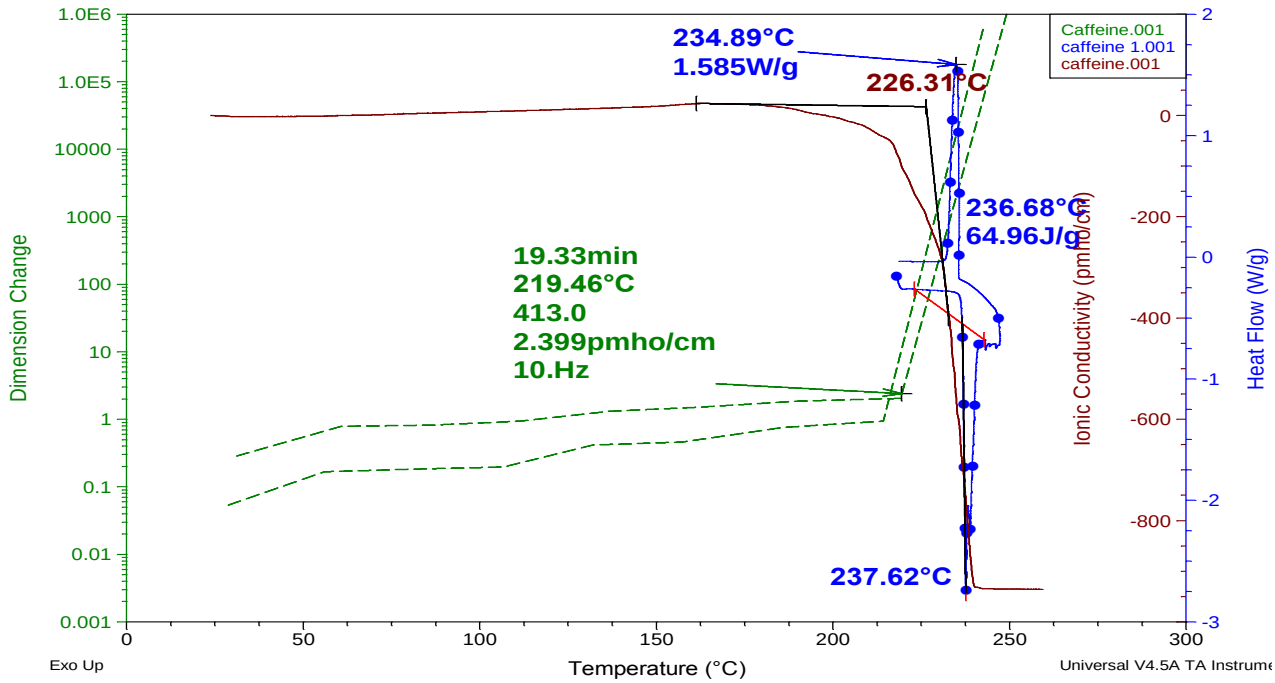

Figure 19 Caffeine run by TMA (solid line), DSC (dotted line) and DEA (dashed line).

Drug	TMA		DSC			DEA
	T_{os} (^oC)	T_m ^oC	T_m (lit) ^oC	T_m ^oC	T_c ^oC	T_{dc} ^oC
Vanillin	**72**	**84**	**81-83**	**81**	37	**60**
Acetophenetidin	**95**	**134**	**134-136**	**135**	123	**127**
Caffeine	**176**	**226**	**235-237**	**237**	234	**219**

Table 7 Onset of softening temperature observed by TMA melting temperature determined by DSC, the literature temperature of the APIs and excipient and the onset of dielectric permittivity change.

T_{os}: Onset softening temperature by TMA

T_m: Melting temperature

T_m (lit): Literature melting temperature

T_c: Temperature of crystallization

T_{dc}: Onset of Conductivity by Dielectric change

Conclusions

DSC melting temperature (T_m) of the APIs correlates well with the DSC published values. TMA shows the softening at lower temperature (T_{os}) than the T_m. At this temperature the electrical properties were observed using DEA which showed an increased in conductance which confirms the softening. The relationship between T_{os} and T_{dc} is linear and the correlation coefficient (R^2) was greater than 0.9

It is our conclusion that we have measured unique dielectric visco-elastic properties of drugs. They appear to be related to thermally induced formation of excimers or charge transfer complexes prior to melting in the solid state. Our nomenclature for this unique structural formation is the creation of a Dielectric Visco-Elastic Material observed by TMA, DEA and not DSC.

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CHAPTER VII

UNIVERSAL STANDARD PROTOCOLS FOR TEMPERATURE AND MATERIAL CHARACTERIZATION

CALIBRATION WITH PHARMACEUTICALS BY THERMAL ANALYSIS

Abstract

New test protocols have been developed which describes the temperature and material characterization calibration of Differential Scanning Calorimeters, Dielectric Analyzers, and Thermal mechanical analyzers with pharmaceuticals over the temperature range from 25°C to 250°C. This study implements the use of pure Active Pharmacy Ingredients (APIs) and Excipients. These test protocols can be blended into a universal standard protocol for Differential Scanning Calorimetry (DSC), Dielectric Analysis (DEA) and Thermal Mechanical Analysis (TMA) employing devices from a variety of commercial companies. Calibration is performed by observing the melting transition temperature of standard Pharmaceutical materials within the temperature range of interest.

Pharmaceutical test specimens (calibrants) of known melting properties are evaluated in a closed system typically in a nitrogen atmosphere over a specific temperature range. While calibrating DSC, a thermodynamic transition i.e. change in heat flow is marked by

absorption (or release) of energy by the calibrants resulting in an endothermic (or exothermic) peak in the heating (or cooling) curve is recorded. Similarly, the test calibrants are evaluated by DEA using an interdigitated electrode array (IDA) over a specific temperature range. At the melt transition temperature, there is an abrupt change in DEA permittivity which is recorded by the instrument. A quartz stage and probe are used in TMA to test the sample and then evaluated for the melting properties of the calibrants. At the transition temperature of the test specimen, there is a change in dimensional stability and a measured change in the coefficient of thermal expansion is recorded. These melt transition temperatures of test specimens obtained from DSC; DEA and TMA data are compared directly to the known literature melt transition temperatures.

The calibration materials used in this test development are: Vanillin (melting temperature, Tmp, 81-83°C), Acetanilide (Tmp, 113-116°C), Acetophenetidin (Tmp, 132-138°C), Benzoic Acid (122°C), and Sulfapyridine (Tmp, 191-193°C). These test protocols were accomplished based on the Standard Test Method for temperature calibration of DSC, DEA and TMA, ASTM method E967 [1], E 2038 [2] and E 1363 [3], respectively. The preliminary R^2 coefficient of correlation for known literature transition temperature vs. DSC melting peak temperature, DEA Permittivity melt temperature and TMA extrapolated onset melt temperature for the calibrants was 0.98. These new pharmaceutical based test protocols, permit interlaboratory comparison and intralaboratory correlation of instrumental temperature scale data within the pharmaceutical community, and will be implemented in our chemical pharmaceutical research.

Introduction

There are thousands pharmaceutical materials known today. There is a need for each material that is discovered or synthesized to be tested and standardized. Calibration typically uses metals e.g., Indium and Zinc to calibrate the three instruments described in this study. In order to make DSC, DEA and TMA more user friendly in the pharmaceutical community, we have implemented the use of analytical grade APIs and excipients for temperature and material characterization calibration. Calibration is performed by observing the melting transition temperature of standard pharmaceutical materials within the temperature range of interest. DSC is used to determine the glass transition temperature, melting temperature, heat of fusion and heat of crystallization of pure materials. These first and second order thermodynamic transitions can also be delineated using pure drug samples. DEA is typically used to measure the ionic conductivity and dielectric properties of a broad range of materials. [2 and 4] TMA measures the dimensional stability of a solid polymer or materials can also be used to characterize powdered APIs. To design an efficient procedure for calibration, understanding the thermal analysis of all these instruments is crucial.

Differential Scanning Calorimetry

DSC is a thermal analysis technique used to measure the changes in the heat flow of a sample, which involves exothermic or endothermic processes, as a function of time and temperature. It is widely applicable to a variety of materials such as pharmaceuticals, polymers, ceramics, metals, food and inorganics etc. [5] DSC is a well established tool for pharmaceutical or material analysis, and provides information regarding melting

temperatures, heats of fusion and crystallization temperatures, glass transition, drug and excipient interaction, thermal stability of pure materials or APIs which is crucial for pre-formulation and drug dosage design of pharmaceuticals.

The experimental variables considered while performing DSC are the sample size, environment, heating rate and pan type (open pan, closed pan etc.). The performance of DSC is dependent on all these experimental variables. [6] The heat of fusion (J/g) is associated with the DSC examination of the crystalline API (run 1). The thermal analysis curve represents a physical chemical property of the sample drug, i.e., melting. Cooling of the active pharmaceutical ingredient sample after melting through the crystallization yields the temperature and heat of crystallization (J/g) and is needed to further characterize the APIs.

Dielectric Analysis

Dielectric analysis is a material characterization technique used to measure quantitative thermal dielectrical information on wide variety of materials which include solid, liquids, films and polymers [7]. It can be used e.g., to determine the polymer viscosity, thermal transitions of polymers, characterization of food products and pharmaceuticals [7]. As DEA is a thermal analysis tool it compliments DSC by allowing a measurement of molecular motion initiated by the A.C. electric field.

Principle of dielectric analysis

An applied sinusoidal voltage on a sample placed on a single surface gold ceramic interdigitated electrode creates an alternating electric field, producing polarization in the sample which oscillates at the same frequency as the electric field. There is a phase angle

shift ä measured by comparing the applied voltage to the measured current which is separated into capacitive (e') and conductive (e'') components.

- *Capacitance* - High frequency permittivity (e') or dielectric constant.
- *Electrical conductivity (pS cm^{-1})* - Loss factor (e") * applied frequency (Hz) * a constant.

From known geometrical constants, such as electrode arrangement and electrode spacing of the IDA electrode, desired electrical properties of the test sample or material can be recorded. Such electrical properties are ionic conductivity, dielectric constant, dielectric loss angle, dissipation factor, dipole relaxation time, permittivity (e'), loss factor (e") and tangent delta (e''/e'). These properties are recorded as a function of time, temperature and frequency during the course of the experiment by varying and measuring these independent parameters. [8]

- *Permittivity (e')* is a measure of the alignment of molecular groups (dipoles) in the electric field.
- *Loss factor (e'')* is a measure of the energy required to move the molecular groups or ions and is proportional to ionic conductivity.
- *Tan delta* is the ratio of the loss factor divided by the permittivity; Tan delta = e''/e'.

Thermomechanical Analysis

TMA is a thermal analysis technique used to measure changes in the physical dimensions (length or volume) of a sample as a function of temperature and time under a non oscillatory load. [9] This technique is widely applicable to variety of materials such

as pharmaceuticals, polymers, ceramics and metals etc. TMA has been used in pharmaceutical analysis. [10-11] Variables considered while performing the thermal mechanical analysis are applied load, gas environment, temperature range and heating rate as well as TMA probe type. The tests are run in a heating mode at a desired heating rate and temperature range of interest. Probe displacement profiles are subsequently analyzed in terms of coefficient of thermal expansion, softening and melting temperatures, and glass transition temperatures. Information obtained based on the different TMA probe types are shown in (Table 8), and recorded as a function of temperature.

TMA Probe Type	Information Obtained
Flat probe/ Light load	Coefficient of Thermal Expansion and Tg
Dilatometer	Coefficient of Thermal Expansion and Tg
Penetration probe/Significant load	Softening (Tg),Melting and creep modulus
Tension accessory	Tg, melting and cure behavior
Parallel plates	Melting, Viscosity and Gelation
Flexure accessory	Softening (Tg) and Melting

**Tg =Glass transition temperature*

Table 8 Types of TMA probes and resulting measured properties.

The focus of this study is to extend the selection of calibration materials from metals to pharmaceuticals i.e., APIs for the three instruments described in this study. Primarily by introducing a modified ASTM standard where the temperature is calibrated with a current NIST (National Institute of standards and Technology) standard material in

addition to the use of APIs. Our overall focus is to calibrate the temperature axis of DSC, DEA and TMA with the melting temperatures of APIs and excipients. These test protocols permit interlaboratory comparison and intralaboratory correlation of instrumental temperature scale data within the pharmaceutical community, and would be more relevant to quality control scientists in the pharmaceutical industry.

Materials

The following analytical grade >99.9 % pure APIs and excipients, within the temperature range of interest, were used in this test development as listed in Table 9 with Chemical Abstract Service (CAS) registry numbers.

Calibration Materials*	**Transition Temperature (°C) (solid - liquid)**	**CAS # References**
Acetophenetidin	132 – 138	62-44-2 [12]
Acetanilide	113 – 116	103-84-4 [13]
Vanillin	81 – 83	122-33-5 [14]
Sulfapyridine	191 – 193	144-83-2 [15]

**Available from Sigma- Aldrich®*

Table 9 Calibration materials* and their transition temperatures.

Instruments

The TAI DSC 2920 as well as Mettler DSC 823^{e} was used to measure the heat flow properties of calibration materials as a function of time and temperature. The test specimens of desired temperature range from 25 to 250°C were heated at a rate of 5°C

min^{-1} with nitrogen gas purge of 50 mL min^{-1}. Open or closed pans were used in this study.

The TA Instruments (TAI) Dielectric Analyzer 2970 was used for dielectric analysis of the calibration materials (test specimen), possessing dielectric properties that undergo solid-liquid or solid-solid transition, over a wide range of frequencies from 0.1 to 1000 Hz and temperatures from 25 to 250°C. The test specimen was ramped at a rate of 5°C min^{-1}, nitrogen gas purge at a flow rate of 50 mL min^{-1} and liquid nitrogen cooling when necessary. The single surface gold ceramic interdigitated (IDA) electrodes were utilized.

The TAI TMA 2940 was used to measure the dimensional change (μm) of the calibration materials as a function of temperature. The test specimens, packed into a DSC aluminum pan were studied over a desired temperature range from 25 to 250°C and heated at a rate of 5°C min^{-1} with a nitrogen gas purge of 50 mL min^{-1}. Sample height was typically of 0.9 to 1.3 mm was used in the study.

Hazards

This test protocol involves the use of hazardous materials, operations and instruments. It is the responsibility of the user to take care and establish appropriate safety practice and to determine the applicability of regulatory limitations prior to use, adaptation of ASTM method E1363. [3]

Sampling

Calibration materials are analyzed by all the three instruments on an "as received" basis. Since sample size is very small, care should be taken, so that the test specimens are homogeneous and representative of the sample. While performing DEA, the test

specimen must cover the entire surface of the IDA electrode. The thickness of the test specimen should be at least 1.5 times of the IDA electrode spacing. The sampling for TMA and DSC is done by packing the test specimen in a standard aluminum pan.

Calibration

Temperature signal from the instrument must be calibrated accurately over the desired temperature range, to obtain consistent results from different experimental conditions. Therefore, calibration is a basic process for any instrument in order to obtain accurate results. Calibrate the permittivity and temperature sensors of the DEA instrument using the procedure described by the manufacturer in the operator's manual. This test protocol was performed and developed by using Standard Test Method for temperature calibration of DSC, DEA and TMA, ASTM method E967 [1], E 2038 [2] and E 1363 [3], respectively.

Experimental procedures for DSC, DEA and TMA

- Select the calibration material of known transition temperature listed in table 9.
- For DSC, weigh 10 mg of test material into a clean, dry aluminum pan. The sample size for DEA is 20 to 40 mg. Care should be taken, so that the test specimen covers the entire surface of the IDA electrode. Sample height of 0.9 to 1.3 mm was used for TMA analysis.
- Load the test material into the instrument chamber e.g., (DSC, DEA and TMA), and purge the instrument with dry nitrogen gas (99.99% purity purge gas) at constant flow rate of 50 ml min^{-1} throughout the experiment.

- Set the initial temperature of the instrument to a value about 30^{o}C below the estimated transition temperature of the test material, and allow it to equilibrate for 5 min at that temperature.
- Initiate a temperature program at a constant heating rate of 3^{o}C min^{-1} to a temperature 20°C above the estimated melt transition temperature of the test material.
- **Note:** When a DSC is used, run a Heat-Cool-Heat cycle for each test material. Cool the test material at 3^{o}C min^{-1} through the crystallization exotherm until the baseline is re-established below the crystallization temperature.
- **Note:** when a DEA is used, initiate the measurement of permittivity at a test frequency of 1000 Hz and a set of frequencies (1, 10, 100, 1000 Hz). Record permittivity and log Permittivity, on a linear scale, as a function of temperature
- Record the accompanying thermal curve by using the instrument software.
- Repeat the procedure described above for other calibration materials chosen.

Results and Discussion

For DSC

At a thermodynamic transition temperature, i.e. change in heat flow is marked by absorption (or release) of energy by the calibrants resulting in an endothermic (or exothermic) peak in the heating (or cooling) curve is recorded.

- From the resultant DSC thermal curve, measure the temperatures for the desired points on the curve: T_m, T_{mp}, T_c, T_{cp}, Heat of fusion (J/g) and Heat of crystallization (J/g) for a pure calibration material.

Tm = Extrapolated onset melt temperature

Tmp = Melting peak temperature

Tc = Extrapolated crystallization temperature

Tcp = Crystallization peak temperature

ΔH_f = Heat of fusion

ΔH_c = Heat of crystallization

The DSC thermal curves of Acetanilide (Figure 20), Acetophenetidin (Figure 21) and Vanillin (Figure 22) are described respectively. These heat-cool-heat plots show changes in the heat flow (W/g) with respect to time and temperature.

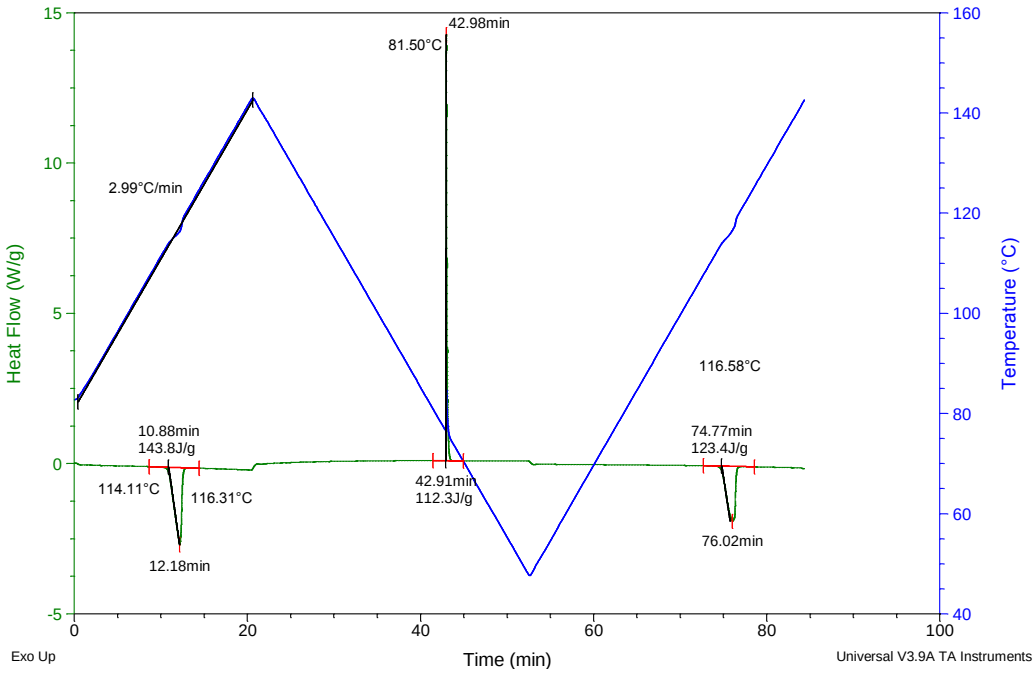

Figure 20 Acetanilide DSC curve showing Tmp (116 °C), Tcp (81 °C), Heat of Fusion (144 J/g) for first endothermic peak and (123 J/g) for second endothermic peak and Heat of Crystallization (112 J/g).

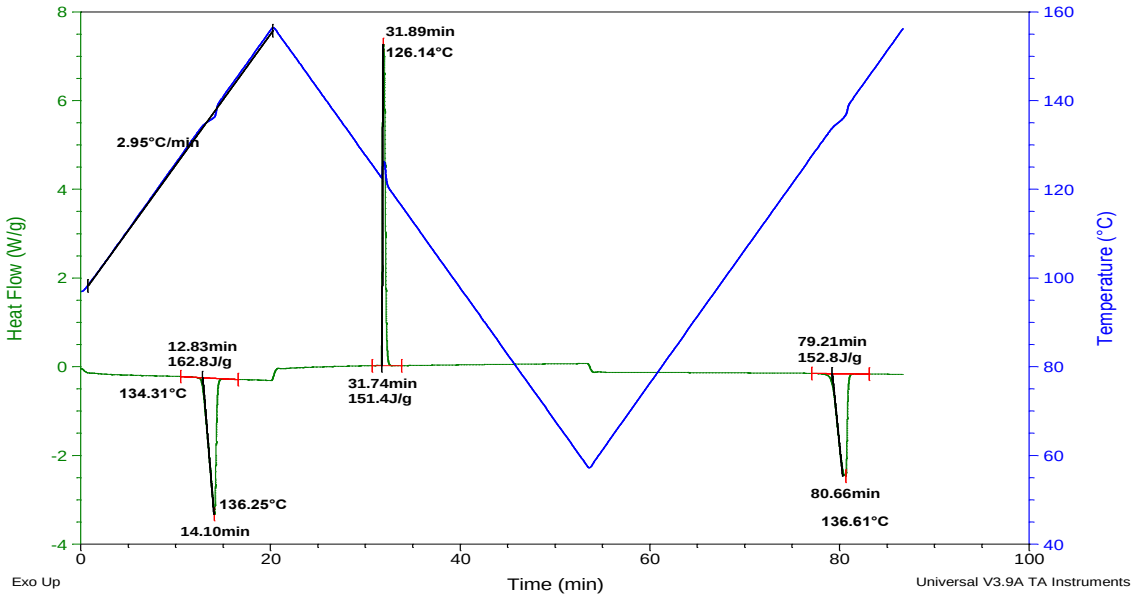

Figure 21 Acetophenetidin DSC curve showing Tmp (136 °C), Tcp (126 °C), Heat of fusion (163 J/g) for first endothermic peak and (153 J/g) for second endothermic peak and Heat of crystallization (151J/g).

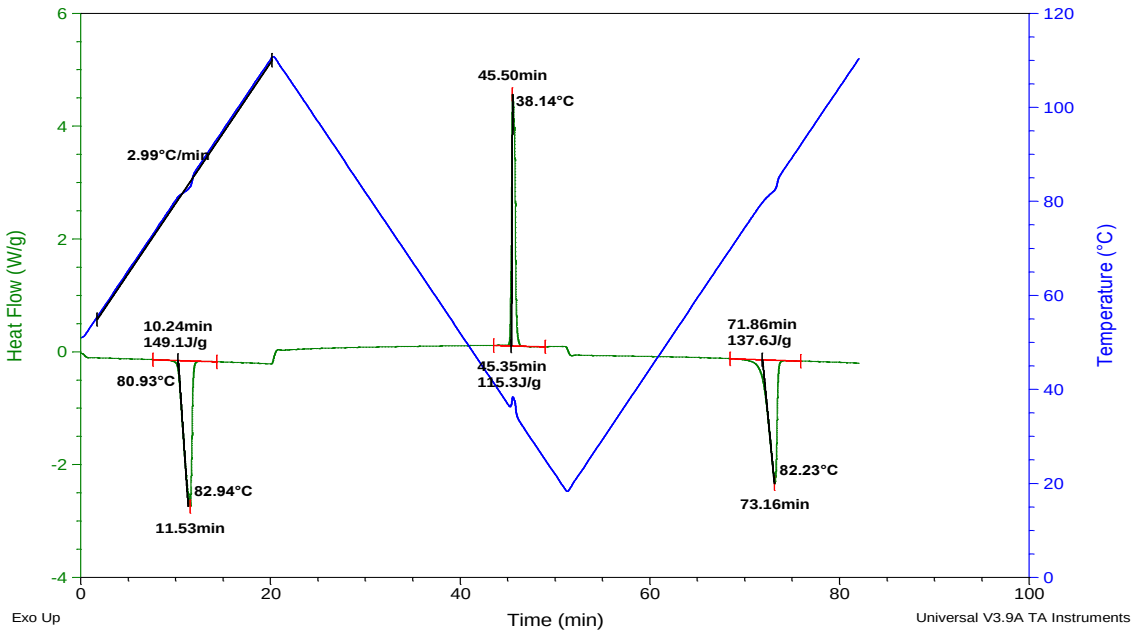

Figure 22 Vanillin DSC curve showing Tmp (83 °C), Tcp (38 °C), Heat of fusion (149 J/g) for first endothermic peak and (138 J/g) for second endothermic peak and Heat of crystallization (115 J/g).

A summary of the DSC melting and crystallization properties of calibration materials is cited in Table 10.

Calibration Materials*	**Melting peak Temperature (Tmp) °C**		**Crystallization peak Temperature (Tcp) °C**	**ΔH_f (J/g)**		**ΔH_c (J/g)**	**% Crystallinity ($\Delta H_c/\Delta H_f$) *100**
	1st Peak	**2nd Peak**		**1st Peak**	**2nd Peak**		
Acetanilide	**116**	**116**	**81**	**144**	**123**	**112**	**78**
Acetophenetidin	**136**	**137**	**126**	**163**	**153**	**151**	**93**
Vanillin	**83**	**82**	**38**	**149**	**138**	**115**	**77**

Note: Tmp = Melting peak temperature. Tcp=Crystallization peak temperature.

Table 10 DSC melting and crystallization properties of calibration materials.

Table 10 describes the Tmp (°C), Tcp (°C), Heat of fusion (J/g) Heat of crystallization (J/g) and % crystallinity of calibration materials evaluated by DSC.

For DEA

At the thermodynamic melt transition temperature, an abrupt change in DEA permittivity is observed. The temperature observed for this transition is recorded by the instrument.

- From the resultant DEA thermal curve, following parameters are measured to determine the property variation associated with the transition; frequency, permittivity, log permittivity, temperature, derivative of permittivity and log permittivity with respect to temperature.

- Plot the DEA thermal curves of test specimens in the following manner:

  a) Plot permittivity vs. temperature and first derivative of the resultant curve

     A single frequency (1000 Hz) and a set of 4 frequencies i.e. (1, 10, 100 and1000 Hz) were used in evaluating each calibration material.

  b) Plot log of permittivity vs. temperature and first derivative of the resultant curve

     A single frequency (1000 Hz) and a set of 4 frequencies i.e., (1, 10, 100, and1000 Hz) were used in evaluating each test material.

  c) For both these calibration processes use the first derivative to determine the inflection point of the original thermal curve and this inflection point is used as first onset point. The second point is typically above the known transition temperature on the original thermal curve where the slope is constant.

  d) Employ the instrument software to determine the onset temperature and the calibrated temperature.

The DEA curves of Acetanilide showing permittivity transition temperature for a single frequency (1000 Hz) run is described in Figure 23. Figure 24 describes the acetanilide derivative of permittivity transition temperature for a single frequency run when plotted vs. temperature.

Figure 25 describes the DEA Acetanilide curve showing Log permittivity transition temperature at 10Hz.

Figure 26 describes the DEA acetanilide curve showing Derivative of log permittivity transition temperature at 1 Hz.

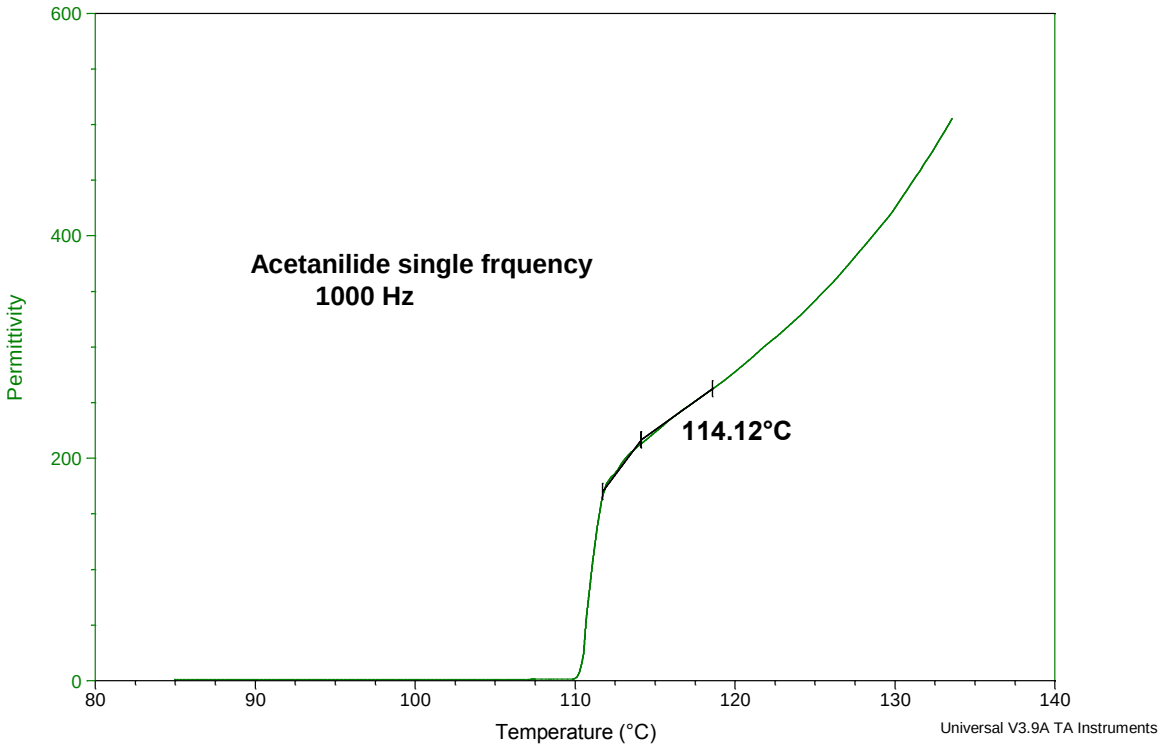

Figure 23 DEA curve of Acetanilide showing permittivity transition temperature (114 °C) for single frequency (1000 Hz) run.

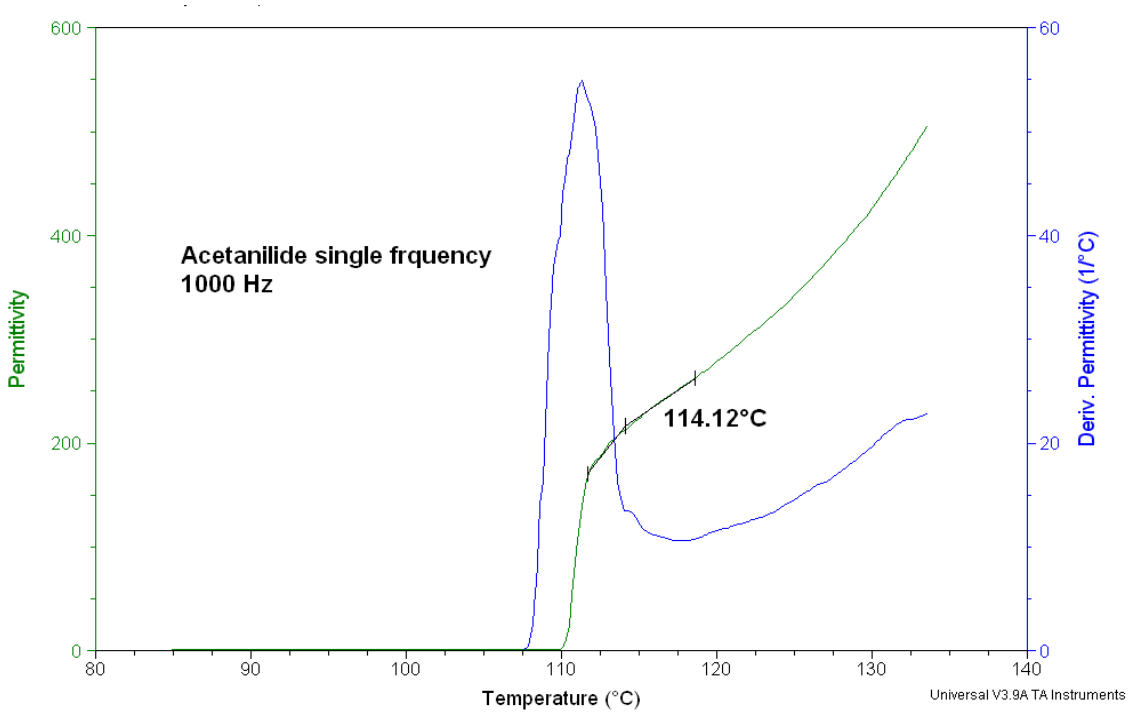

Figure 24 DEA curve of Acetanilide showing Permittivity and Derivative of Permittivity Transition temperature for single frequency (1000Hz) run.

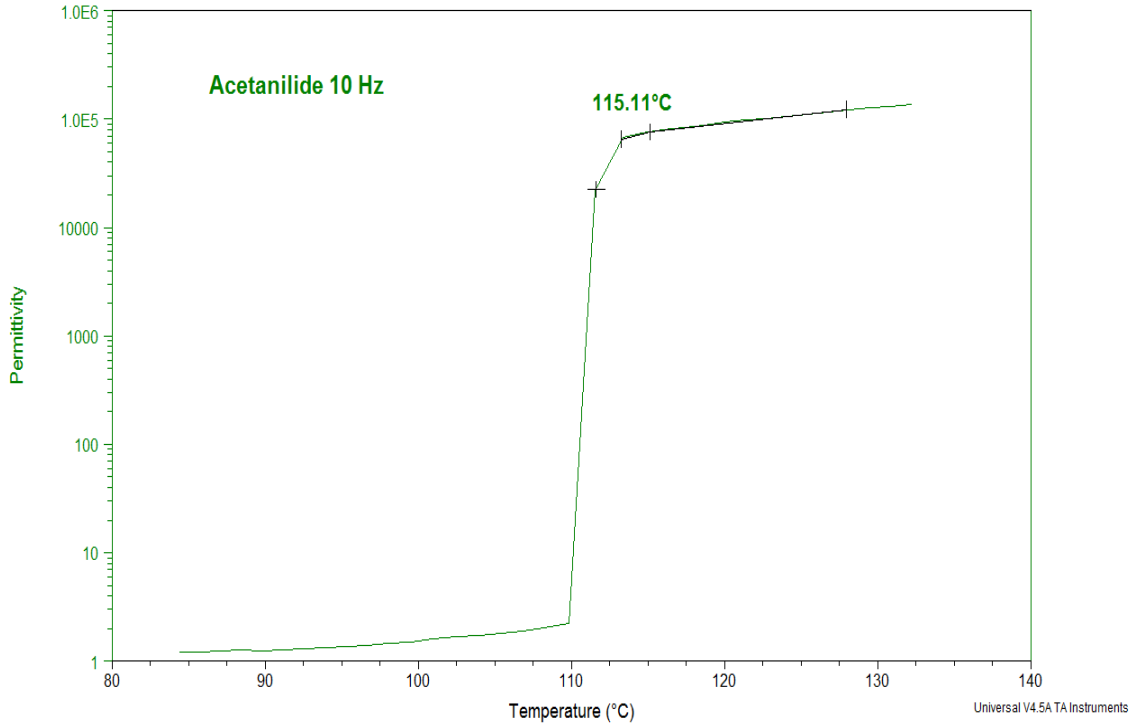

Figure 25 DEA curve of Acetanilide showing Log permittivity transition temperature at 10 Hz frequency.

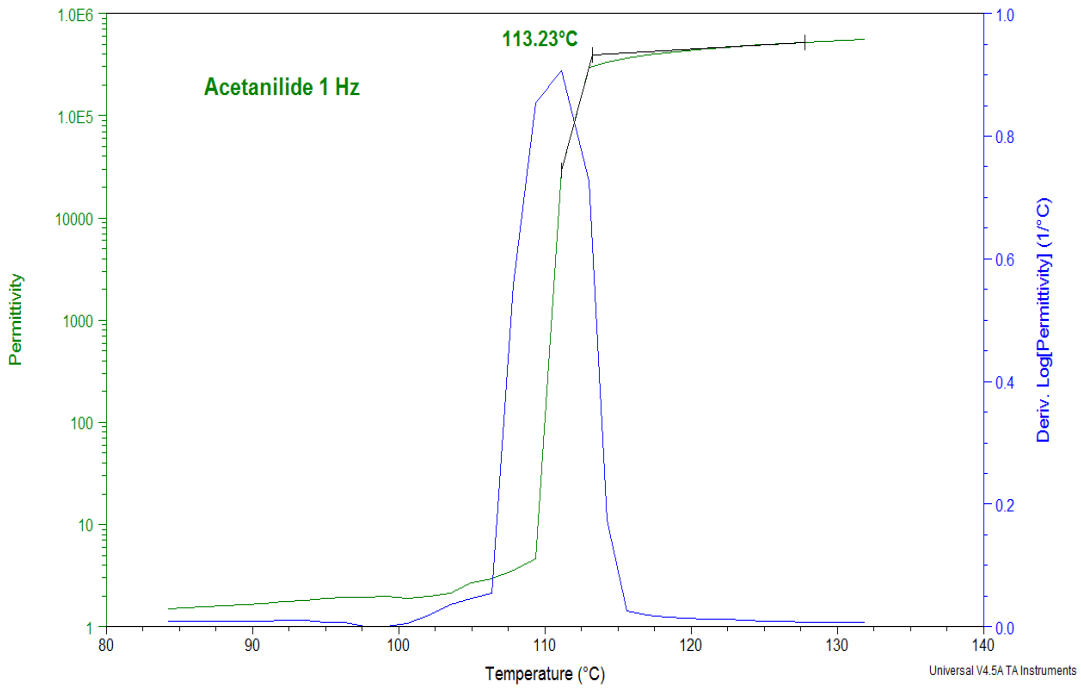

Figure 26 DEA curve of Acetanilide showing Log permittivity and Derivative of Log permittivity transition temperature at 1 Hz frequency.

A summary of frequency used i.e. (single or set of 4 frequencies); permittivity and Log permittivity transition temperature obtained from the DEA thermal curves of the calibration materials are listed in: Acetanilide, Table 11; Acetophenetidin Table 12; Vanillin, Table 13; and Sulfapyridine, Table 14.

Frequency (Hz)	Permittivity Transition Temperature °C	Log Permittivity Transition Temperature °C
1000 Hz	114	113
1Hz	116	113
10 Hz	115	113
100 Hz	114	114
1000 Hz	114	114
Average	115	113
Std Deviation	0.89	0.55
% Relative Error	0.77	0.48

Table 11 Acetanilide Tmp (113-116) °C; DEA Permittivity and Log Permittivity Transition Temperature of single and set of frequencies.

Frequency (Hz)	Permittivity Transition Temperature °C	Log Permittivity Transition Temperature °C
1000 Hz	131	131
1 Hz	132	132
10 Hz	133	133
100 Hz	133	132
1000 Hz	133	133
Average	133	132
Std Deviation	0.89	0.8
% Relative Error	0.66	0.6

Table 12 Acetophenetidin Tmp (132-138) °C; DEA Permittivity and Log Permittivity Transition Temperature of single and set of frequencies.

Frequency (Hz)	Permittivity Transition Temperature °C	Log Permittivity Transition Temperature °C
1000 Hz	81	81
1 Hz	82	81
10 Hz	82	82
100 Hz	81	81
1000 Hz	80	80
Average	81	81
Std Deviation	0.83	0.7
% Relative Error	0.98	0.86

Table 13 Vanillin Tmp (81-83) °C; DEA Permittivity and Log Permittivity Transition Temperature of single and set of frequencies.

Frequency (Hz)	Permittivity Transition Temperature °C	Log Permittivity Transition Temperature °C
1000 Hz	192	191
1 Hz	193	191
10 Hz	193	191
100 Hz	191	190.8
1000 Hz	191	191
Average	192	191
Std Deviation	1	0.089
% Relative Error	0.5	0.04

Table 14 Sulfapyridine Tmp (191-193) °C; DEA Permittivity and Log Permittivity Transition Temperature of single and set of frequencies.

For TMA

At the transition temperature of the test specimen, there is a change in dimensional stability and a measured change in the coefficient of thermal expansion is recorded by the instrument.

- From the TMA thermal curve recorded, extrapolated onset temperature is measured. This is calculated by extending the pretransition portion of the curve

which to the point of intersection with a line drawn tangent to the steepest portion of the curve which describes the probe displacement. [3]

The thermal curves of Acetanilide (Figure 27) and Acetophenetidin (Figure 28) recorded by the instrument are described showing the extrapolated onset melting temperature.

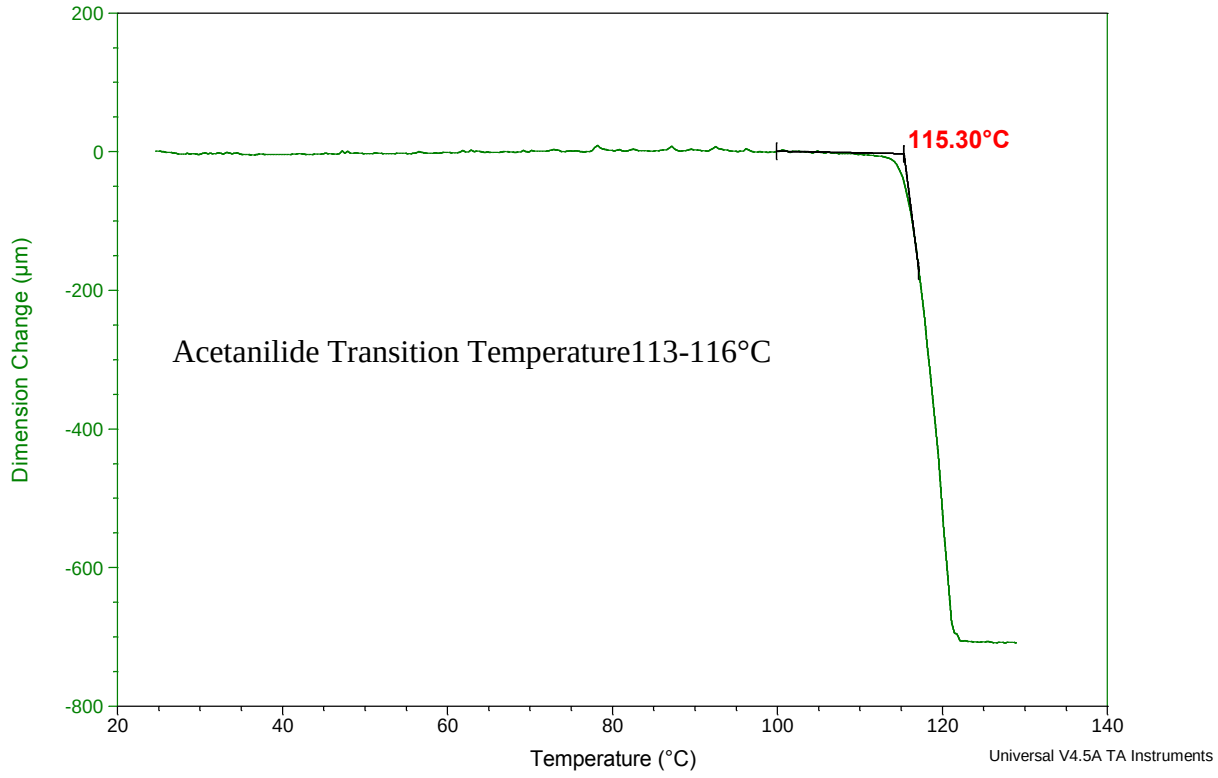

Figure 27 TMA of Acetanilide curve showing the extrapolated onset melting temperature.

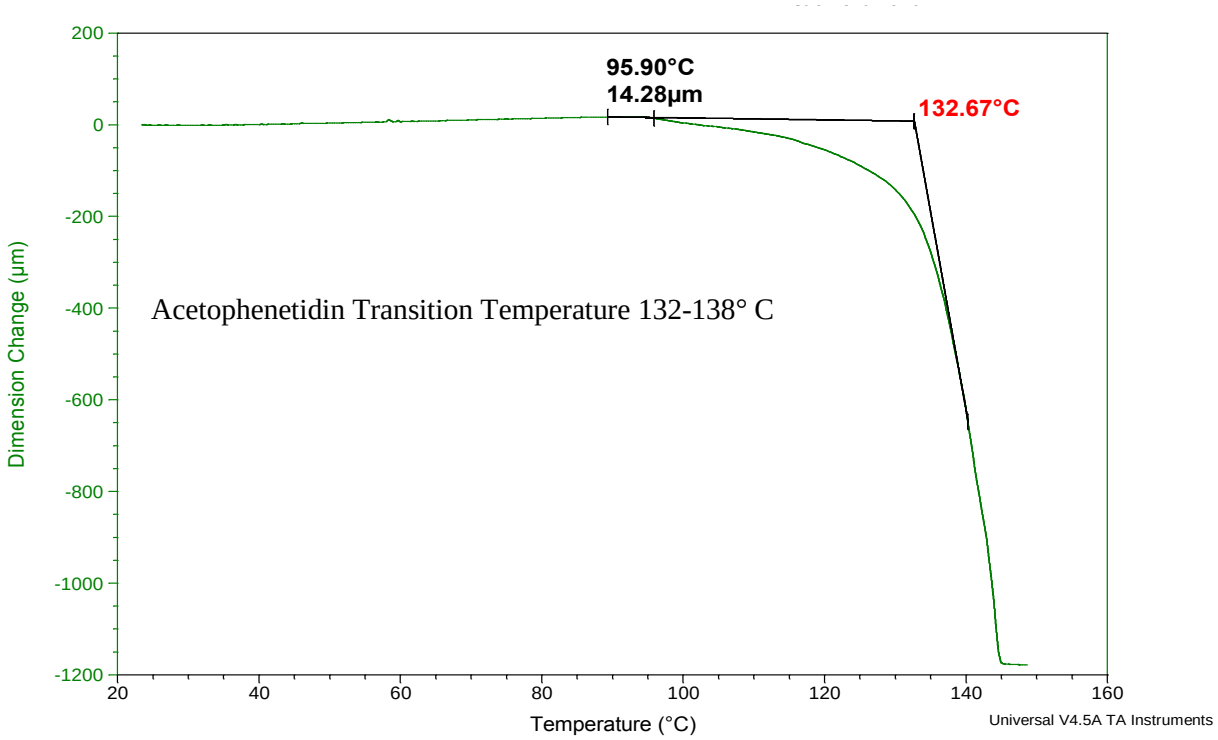

Figure 28 TMA of Acetophenetidin curve showing extrapolated onset melt temperature.

A summary of TMA extrapolated onset temperatures of calibration materials and literature transition temperatures are cited in Table 15. A summary of the melting temperatures for the calibrants (APIs) versus the known standard literature melting temperature values are given in Table 16.

Calibration Materials*	TMA Extrapolated Onset Temperature (°C)	Literature Transition Temperature (°C)
Acetophenetidin	133	132 - 138
Acetanilide	115	113 -116
Vanillin	84	81-83
Sulfapyridine	193	191-193

Table 15 Summary of TMA extrapolated onset temperatures of calibration materials and literature transition temperatures.

Calibration Materials*	Literature Transition Temperature (°C) (CAS**)	DSC Melting Peak Temperature (Tmp °C)		DEA Transition Temperature °C		TMA Extrapolated Onset Temp (°C)
		1st Peak	2nd Peak	Permittivity	Log Permittivity	
Acetophenetidin	132- 138	136	137	133	132	133
Acetanilide	113 - 116	116	116	115	113	115
Sulfapyridine	191 – 193	192	192	192	191	193
Vanillin	81 - 83	83	82	81	81	84

**Available from Sigma Aldrich®; **CAS=Chemistry Abstract Service registry number.*

Table 16 Summary of DSC, DEA and TMA results

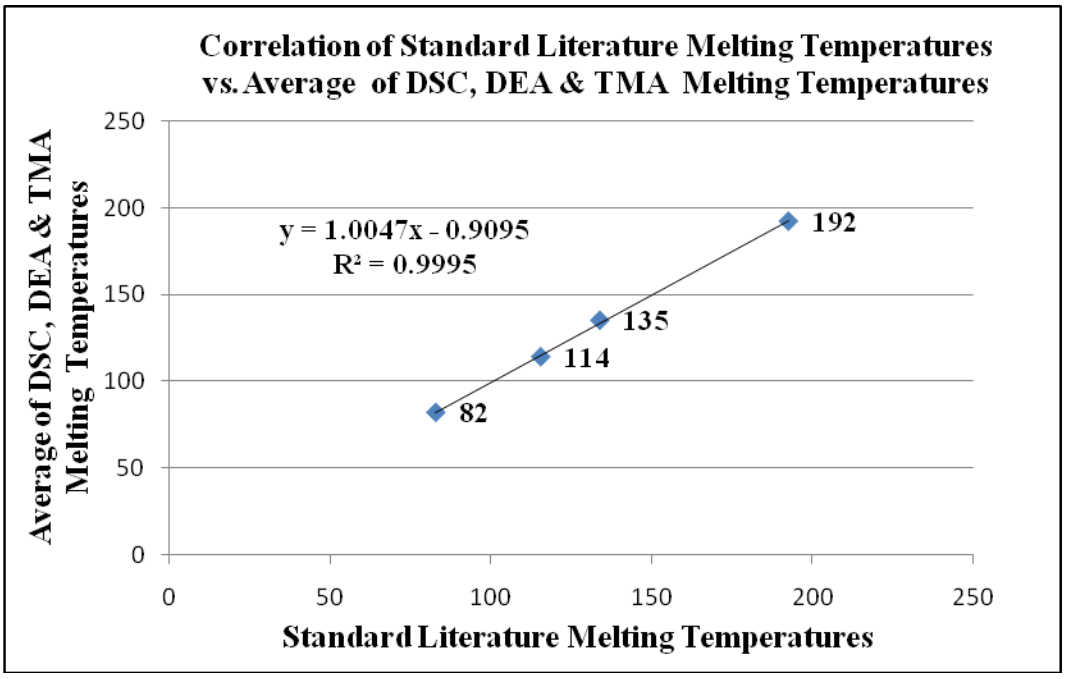

Figure 29 Correlation graph of Standard Literature Melting Temperature versus average of DSC, DEA & TMA Melting Temperature of calibration Materials.

Conclusions

Statistical analysis of the results from the three methods (DSC, DEA and TMA) indicates a high correlation with known literature values. Acetanilide, Acetophenetidin and Sulfapyridine were quality API standards for calibration. Vanillin is a quality excipient calibrant. Acetanilide observed melting transition temperature best correlates with the known literature transition temperature values. Permittivity is the most appropriate property to calibrate the temperature of DEA as it deals only with dipole variations. The ionic conductivity, though an interesting material property, is a function of multiple properties, that are dipolar and ionic. The R^2 correlation coefficient value for known standard literature temperature value vs. DEA permittivity melt temperature for a set of frequencies and 1000 Hz was 0.99. The R^2 correlation value for known standard literature temperature vs. TMA extrapolated onset temperature was 0.98. The R^2 correlation value

for known standard literature temperature value vs. DSC melting peak temperature was 0.99. The average melting temperature (Tm) for DSC, DEA and TMA correlated with the melting temperatures (Tm) of the known literature values with an R^2 of 0.999. (Figure 29) It is our quest to infuse these new ASTM type standard test protocols into the pharmaceutical industry for drug pre-formulation and dosage design of pharmaceuticals.

Referenced ASTM Standards

- E794-01 Standard Test Method for Melting and Crystallization Temperatures by Thermal Analysis
- E473-00 Standard Terminology Relating to Thermal Analysis
- E691-99 Standard Practice for Conducting an Interlaboratory Study to Determine the Precision of a Test Method
- E967-97 Standard Practice for Temperature Calibration of Differential Scanning Calorimeters and Differential Thermal Analyzers
- E1142-97 Standard Terminology Relating to Thermo physical Properties
- E1325-02 Standard Terminology Relating to Design of Experiments
- E2038-99 Standard Test Method for Temperature Calibration of Dielectric Analyzers
- E2039-99 Standard Practice for Determining and Reporting Dynamic Dielectric Properties

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13. CAS no. 103-84-4, Acetanilide
14. CAS no. 122-33-5, Vanillin