Novel Solid State Properties of Drugs, Polymers and Various Chemicals by Thermal and Analytical Techniques

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NOVEL SOLIS STATE PROPERTIES OF DRUGS, POLYMERS AND VARIOUS CHEMICALS BY THERMAL AND ANALYTICAL TECHNIQUES

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DEDICATION

I would like to dedicate my thesis work to my family, my professor and loving friends. Particularly my loving wife Bindu Mantheni for understanding and sacrifices she made for the completion of this dissertation. There is no doubt in my mind that without continuous support and counsel of my prof. Alan T Riga I could have not completed this process. I thank my mom Sumangali Mantheni, my dad Rajender Mantheni, and my sister Deepika Gottimukkala for their unconditional love and support.
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NOVEL SOLID STATE PROPERTIES OF DRUGS, POLYMERS AND VARIOUS CHEMICALS BY THERMAL AND ANALYTICAL TECHNIQUES.

DHRUTHIMAN R MANTHENI

ABSTRACT

I have observed unique variations in AC electrical conductivity of solids by dielectric analysis (DEA or DETA) when studied with respect to temperature and frequency. A wide range of solids were examined for this study e.g. organics, polymers, carbohydrates, API’s (active pharmacy ingredients) and amino acids. Experimental results clearly show novel dielectric behavior of a linear increase in a log ionic conductivity vs. temperature in the pre-melt (20 to 30oC below the melt temperature) and melt transition regions. We have differentiated the solids which show the conductivity variations in pre-melt from those which do not e.g. pre-melt conductivity variations observed with polar polymers such as nyons and acetals vs. not observed in nonpolar polymers such as polyethylene, polypropylenes and long chain alkanes e.g. tetraconsane and pentacosane.

Isothermal dielectric analysis was used to study the cause of this variation in solids which yielded a polarization time property. The effect of various experimental factors on the results such as the effect of heating rate, varying the frequency, and sample size on the dielectric variations in the pre-melt temperature range have been studied. Correlating dielectric with calorimetric analyses gave us a better understanding of solid state properties. Calorimetric analysis was used to assure that the observed variations in the solid state properties are not due to moisture or impurities present in the sample. The ASTM E928-08 “Standard Test Method for Purity by Differential Scanning Calorimetry
(DSC)” was employed to verify the purity of the experimental chemicals used in this study e.g. Acetanilide, Acetophenitidine and Vanillin.

Activation energies were calculated based on Arhennius behavior to better interpret the changes in the solid. As the different chemicals were heat cool cycled they were more amorphous, as evidenced by the decreasing activation energy for charge transfer with an increasing amorphous content. Morphological studies done by scanning electron microscopy (SEM) showed mechanical variations of the solid state in the pre-melt temperature range when treated with dielectric field. These mechanical variations can be related to the changes in the pre-melt temperature range for the solid state.

To explore the cause and effect relationship between structure and enhanced electrical conductivity, we have conducted a number of reactions with and without the AC dielectric field to ascertain if it is affecting the reaction rate or products. The most predominant reaction of all experiments to date was of a reducing amino acid and carbohydrate which showed enhanced reaction rate in the dielectric field. Dielectric analyses of the materials were further investigated by evaluating malaria driven proteins and poly peptides to develop a sensor for detecting malaria.
LIST OF ABBREVIATIONS

APIs – Active Pharmaceutical Ingredients
DSC - Differential Scanning Calorimetry
DEA – Dielectric Analysis
TMA – Thermomechanical Analysis
TG – Thermogravimetry
Ea (k) – Activation Energy (J mol⁻¹)
Ea (δ) – Activation Energy (J mol⁻¹)
Tm - Melting Temperature
Tmp - Peak Melting Temperature
ΔHf- Heat of Fusion
Tg -Glass transition
Tc- Crystallization Temperature
Tcp - Crystallization peak temperature
ΔHc - Heat of Crystallization
MW – Molecular Weight
PXRD – Powder X-ray Diffraction
SEM – Scanning Electron Microscope
IDA – Interdigitated electrode array
τ (Tau) — Polarization time (millisecond)
fc – Tan delta critical peak frequency (Hz)
(ε”/ ε’) – Tan delta (δ)
ε” – Ionic conductivity (Loss factor)
$\varepsilon'$ – Permittivity

pS cm$^{-1}$ – pico Siemens per centimeter

W g$^{-1}$ – Heat Flow

ASTM – American Society for Testing Materials
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CHAPTER I

INTRODUCTION AND INSTRUMENTATION

1.1 Solid State Properties

1.1.1 Electrical properties in solids. Solids can be differentiated into conductors, semiconductors or insulators based on their resistivity or the inverse conductivity properties. Conductors are highly conductive and insulators are non-conductive. Based on these definitions one might think semiconductors to be between these two materials despite semiconductors are far different from conductors, they are a subclass of insulators. The flow of charges through the material is represented by its electrical conductivity. Whereas the ability of a solid to store electricity is depends on its resistance.

Ohm’s law explains relationship between current, voltage and resistance, where resistance is inversely related to conductivity and this explains the conduction nature in solids. In conductors resistance is very low and electric current flowing through them is directly proportional to voltage. Studying microscopic view of ohms law conductors tells that the velocity of charge through material is proportional to its current. In a material ratio of current and voltage is resistance and when this ratio is constant over a wide range of voltage a material is said to be ohmic.
1.1.1 Band theory of conduction. Band theory of solids is another way to study conductors, semiconductors and insulators. In a material electric current is due to motion of valance electrons which can only move from allotted energy state to another energy state. In most of the solids the bonds are formed by completely filling the valance band which leaves no empty space for movement of electrons, unless electrons gain the energy to cross the band gap and get into conduction band. In conductors the valance band is partially filled which gives a lot of space for the movement of electrons and a partially filled valance band is considered to be a conduction band. Given below are the figures which show the difference between the conduction and the insulators (1).

![Figure 1.1. Conductor](image1.png)

![Figure 1.2. Insulator](image2.png)
**1.1.1.2 Polaron theory of conduction.** Polaron is a quasiparticle composed of charge which assists in polarization field. Based on the polaron theory of hopping conduction between localized states, an analytical mobility is obtained. This obtained model is both temperature and electric field dependence. It is assumed that localized states are randomly distributed in the sample.

**1.1.2 Background and significance.** Properties like electrical conductivity, permittivity, dielectric constant, heat of fusion and transition temperatures (e.g. solid-liquid, solid-solid, desorption, crystallization and vaporization) are studied by measuring the electrical and calorimetric solid state characteristics and directly comparing these properties. Therefore, these properties are better described in the comparison rather than individually. The electrical solid state properties are relevant and significant for identifying the ionic conducting materials. The basis of material structure and their electrical properties can contribute greatly to the design and ultimate properties of materials (2). The measured electric conductivity of a drug in close proximity to the melting temperature e.g. 20°C below the melt temperature are highly reactive and can be a source for electro-synthesis, or application of an electric field to initiate a chemical reaction. We are hypothesizing that this newly discovered electrical conductivity in the pre-melt temperature range can be used as a basis for creating a new path to modify chemicals probably at low frequencies. If a chemical has enhanced reactivity due to electrical charging in the pre-melt temperature range; then energy input like ultrasonic input or laser light input should also affect their environments which will lead to blending or modification of known chemicals.
Major pre-melt behavior has been attributed to the solid-solid transition as described by Mesaros et al (3). Temperature dependent variations in the proton NMR of hexa hydro-1,3,5-trinitroso-s-triazine were observed in the premelt solid phase of this chemical and were related to activation of substantial motion of the molecules in the crystal lattice(4). Dielectric thermal analysis of amino acids also exhibited an enhanced electrical linear conductivity increase prior to the melt. Matthews and Riga referred to these new properties as dielectric viscoelastic behavior (5).

Structure property relations need to be identified in order to understand and improve chemical behavior. The electrical properties uniquely define new aspects of the chemicals studied. Excipients, such as mannitol or lactose, have been observed with high electrical conductivity 100°C below their melt temperatures (6). This observation can be used to verify the cause of the instability of the excipients due to the lack of compatibility with the active pharmaceutical ingredient (API).

Modifying materials of construction are needed in the chemical and polymer science world. This can be accomplished by synthesizing or blending various materials to accomplish our ultimate goal of improved performance. Electrical modification of established drugs can yield a drug compound with improved stability, target drug delivery, efficacy, solubility, and reduced side effects. The premelt temperature range defines a new venture in preparing changes in known materials. The fact that we measured a high ionic conductivity of $10^8$ pS.cm$^{-1}$ in a number chemicals tested gives us an opportunity to transform or synthesize new chemicals.

The calibration of the properties to be measured is also significant in collecting material characterization data. Therefore the use of active pharmaceutical ingredients as
standards are implemented to make DSC or DEA more user friendly to the pharmacy community. Our research team accepted the challenge to find APIs which could be used with multiple thermal analytical methods. Therefore we choose a single property per method and studied its variation with a number of known API’s. The latter choice of test materials included the melt temperature for Lidocaine (68°C), Acetanilide (115°C), Acetophenetidin (135°C), and Sulfapyridine (191°C). The factors that affect the premelt temperature range are moisture, heating rate, sample size, particle size, and applied DEA frequency. By studying electrical conductivity of chemicals we can evaluate instrumental variables and their effect on the characterization of materials.

Pharmaceutical materials are often characterized using thermal analytical instruments like DSC and TG. DSC is able to differentiate between different polymorphic structures and, transformations occurred during polymorphic conversions and also polymorphic purity can be determined using varying heating rates in DSC (7). TG is often used to measure residual solvents and moisture. DSC can also be used to determine solubility of pharmaceutical materials in solvents. Analysis of pharmaceutical materials is probably the largest area of application for thermal analysis (8).

DEA analysis of drugs studied has led to a better understanding of the chemistry and molecular mobility that relates to the structure of the drug. This information is of utmost importance during pre-formulation development (9). DEA measures changes in phase transitions and loss of residual solvents as it is subjected to a periodic electric field. Characterization of molecular relaxations or polarization in excipients and APIs are easily measured by DEA. The latter is ultra-sensitive, making it possible to measure conductivity to $10^{-3}$ pS.cm$^{-1}$ and as high as $10^{7}$ pS.cm$^{-1}$ and subsequently detects material
transitions that other techniques can not define significantly to provide useful data. DEA compliments DSC by allowing an evaluation of molecular motion based on tan delta (loss factor/permittivity) electrical properties. DEA gives the pharmaceutical scientist new insight into the nature and behavior of the drugs.

1.2 Instrumentation and Methods

Instrumentations like Thermal analytical (Dielectric, Calorimetric and Mechanical) along with Microscopic, Spectroscopic and X-ray diffraction were used to analyze and characterize a wide range of chemical samples (10,11,12). Thermal analytical techniques are playing a major role in various chemical industries including the pharmaceutical field; the reason behind this is due to their accurate and precise response to thermal stress and small sample size. Dielectric analysis was used to study the electrical properties of the sample and how the properties vary with the respect to temperature, time and frequency. Calorimetry was also employed to study the material thermal properties which gave a better understanding in analyzing the sample properties. Microscopic and Spectroscopic techniques (Infra-Red) were employed to study the synthesis part of the research since it was focusing on the structural detail and there was an attempt to develop a structure property relationship.

1.2.1 Differential scanning calorimetry (DSC). DSC has become a major thermal analytical instrumentation for many industries because of its response to thermal stress. Of all the thermal analytical instruments DSC is most widely used in pharmaceutical industries as an analytical tool to study the physical and energetic properties in pharmaceutical excipients and active ingredients. Developments in DSC such as temperature modulated DSC, robotic systems, DSC spectroscopic
instrumentation, micro-scale test configuration have made it more accurate, user friendly and co-relatable which makes it a forefront thermal analytical tool.

![Figure 1.3. TA Differential Scanning Calorimetry](image)

**1.2.1.1 Instrumentation.** Properties like exothermic and endothermic reactions with respect to temperature and change in heat capacity with time can be studied; these above mentioned properties will reveal physical and chemical characteristics of a sample such as melting point, purity, glass transition temperature. A typical DSC consists of a two stages configuration one for sample and the other for reference as seen in figure 4. Both sample and reference pans are placed on stages in identical environment and both are maintained at same temperature predetermined by the program. 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 4. Based on the DSC cells there are two primary types:
1.2.1.2 *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 4 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 $\Delta T$ between the sample and reference and is indicated in Figure 4 and 5.

![Diagram of Heat Flux DSC Cell Cross Section](image)

**Figure 1.4.** Heat Flux DSC Cell Cross Section

1.2.1.3 *Power compensation.* Small individual furnaces use different amounts of power to maintain a constant $\Delta T$ between sample and reference and the advantage here include faster heating and cooling, and better resolution.
This type of cell, Figure 5, 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.

![Diagram of Power Compensation DSC Cell Design](image)

**Figure 1.4.2 Power Compensation DSC Cell Design**

1.2.1.4 Sample preparation. Success of DSC experimentation lies in careful sample preparation and appropriate selection of experimental conditions such as heating rate, sample preparation and temperature to which the sample to be heated or cooled, choosing the appropriate pan etc.,. There are several pan types differentiated based on the material they are made of like (aluminum, copper, silver etc.), based on their shape (SFI, flat bottom etc.). Pans may be open or closed or pin-holed based on the type of sample you take closed pans are used when the sample is volatile; pin hole is made to avoid
bursting of pan at extreme temperatures, for moderate sample containment and exposure
to reactive gases. Sealed pans are used to inhibit the heat liberation of sample or its
formed product. Both reference and the sample pan types should be identical. The pan
masses of both sample and reference should match in order to achieve accurate results.

Sample size mainly depends on the density of sample. Average sample size would
be 5 to 10mg in polymers or pharmaceuticals. Reasonable results can be attained with
sample size of less than 5mg. powder sample with low density should be pressed on to
the pan in order to have a better thermal contact. Small sample size have smaller amount
of thermal gradients which make them suitable for thermal analysis. In order to attain
accurate results for energetic parameters such as heat capacity and enthalpy of fusion the
sample should be weighed before and after the run. The sample should be placed in the
center of the pan to have accurate heat flow properties. Size and shape of a particle in
sample may have an effect on the packing which will result in non-reproducibility to
avoid this grinding the sample is necessary.

Heating rate, start and end heating temperatures, isothermal holds, purge gas type
its flow rate, and calibration parameters are important parameters one should consider to
attain a proper result in DSC instrumentation. It is suggested to have start temperature
below and end temperature above the area of interest in order to attain a good linear base
line. Heating rate can be from 1°C/min to 100°C/min based on the sample type typical
polymer samples are heated from 10-20°C/min and pharmaceuticals at 5-10°C/min to
study the purity the sample should be heated at lower heating rates such as 1-2°C/min.
Purge gas type and its flow again depends on sample type.
1.2.1.5 Calibration. Obtained results are reliable only when the instrument is calibrated properly. Comparing results from different instruments and to have reproducibility we need to have well defined calibration procedure and high purity standards. American Society of Testing Materials (ASTM) provides standard procedure for calibration. Standards such as n-octane, indium, tin, lead and bismuth are used to calibrate the instrument. Calibration in temperatures below 0° C is done using n-octane and for above room temperature above mentioned metals are used. Most accurate results are attained when both heating and cooling cycles are calibrated. Calibration should be done close to transition temperature of interest. High purity samples and a clean sensor is required for a good calibration.

1.2.1.6 Single component characterization. The transitions obtained in DSC can be exothermic or endothermic as seen in table 1. Thermal behaviors such as melting, crystallization, boiling, sublimation, dehydration, desolvation, solid-solid transitions, glass transitions and polymorphic transitions are plotted as exothermic and endothermic peaks with differential heating rate versus temperature or time. A typical DSC scan of a sample undergoing properties like glass transition (T_g), exothermic crystallization (T_c), enthalpy of crystallization(ΔH_c), endothermic melting (T_m), enthalpy of fusion(ΔH_f), onset of degradation all the above mentioned properties are shown in figure 6. Important characters like onset of transition (T_o), extrapolated onset (T_e) and peak temperature (T_m) are shown in figure 6.
Table 1.

Enthalpic transitions observed by DSC.

<table>
<thead>
<tr>
<th>Endothermic</th>
<th>Exothermic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fusion</td>
<td>Crystallization</td>
</tr>
<tr>
<td>Vaporization</td>
<td>Condensation</td>
</tr>
<tr>
<td>Sublimation</td>
<td>Solidification</td>
</tr>
<tr>
<td>Deposition</td>
<td>Adsorption</td>
</tr>
<tr>
<td>Desolvation</td>
<td>Solvation</td>
</tr>
<tr>
<td>Decomposition</td>
<td>Decomposition</td>
</tr>
<tr>
<td>Reduction</td>
<td>Oxidation</td>
</tr>
<tr>
<td>Degradation</td>
<td>Degradation</td>
</tr>
<tr>
<td>Relaxation of glass</td>
<td>Curing of resins</td>
</tr>
<tr>
<td>Glass transition</td>
<td>-</td>
</tr>
<tr>
<td>Relaxation of glass</td>
<td>-</td>
</tr>
</tbody>
</table>
1.2.1.7 Melting point. Melting transition is an endothermic process in which the ordered solid state is transformed to disordered liquid or molten state. The amount of heat required by this endothermic process to convert from solid to liquid state can be attained accurately by DSC which is heat of fusion. A pure crystalline compound is expected to give a sharp melting peak. A pure amorphous form would not have a melting but the change in phase is indicated by glass transition temperature. Impurities and crystal defects in a crystalline compound will broaden or shift the peak to lower temperatures. Purity of a sample is determined by shape of the peak, height of the peak and width of the peak.

When the transition begins to deviate from the base line it is considered as onset of melting point \( (T_o) \), as seen in figure 7. Intersecting the extrapolated baseline prior to melting with extrapolated leading edge of the transition gives extrapolated onset melting
point. The area of endothermic transition is proportional to enthalpy of fusion ($\Delta H_f$) which is proportional to amount of energy required to taken to melt the sample, $\Delta H_f$ is affected by selection of baseline. To obtain an accurate baseline for melting endotherm connect the point at which the curve leaves from baseline to point where it rejoins the baseline.

![Graph](image)

**Figure 1.6** Differential Scanning Calorimetric showing onset melting temperature, extrapolated onset of melting, melting peak and enthalpy of fusion

**1.2.2 Dielectric analysis.** Dielectric analysis is a technique used to measure the electrical response of a material with respect to time, temperature and frequency. Molecular motions and structural relaxations present in a sample possessing permanent dipole movements can be determined using a dielectric analyzer (DEA). Dielectric analysis can also detect secondary relaxations in a sample as long as it has a dipole movement. Dielectric analyzer measures capacitance and conductance which gives us the dielectric constant or permittivity which is proportional to capacitance and dielectric loss which is proportional to conductance. Various types of samples like solids, liquids,
powders, gels, thin films etc, can be analyzed using DEA. This is why DEA is used in various chemical industries.

Capacitance and conductance are two major fundamental electrical properties measured by dielectric analyzer with respect to temperature, time and frequency. These two properties mentioned above are contradictory to each other conductance is related to the ability of a sample to transfer electrical energy whereas capacitance is related to the ability to store the electrical energy. Ionic conductivity is associated with the viscosity of the sample because fluidity is identified by the ease with which ionic components can migrate through the sample under the applied electric field. The measured current is divided in to capacitance and conductance. Capacitance and conductance are related to the molecular mobility of a sample which in turn gives us chemical and rheological information like polarization, molecular movement in drugs and polymers. Alternating electrical field is applied to the sample by placing it between the electrodes this creates polarization in sample which leads to oscillation in dipoles in the same frequency as the applied one but with a phase angle shift \(\delta\) seen in figure 8. Measurement of permittivity gives us the capacitance which is related to induced dipoles and alignment of dipoles. Measurement of loss factor gives us conductance which is related to dipole loss factor and ionic conductance. Complex dielectric constant has both imaginary and real parts.

\[ \varepsilon^* = \varepsilon' - i \varepsilon'' \quad \text{or} \quad \varepsilon^* = \sqrt{(\varepsilon')^2 + (\varepsilon'')^2} \]

where \(\varepsilon^*\) = complex dielectric constant; \(\varepsilon'\) = permittivity; and \(\varepsilon''\) = loss factor.
Both the above mentioned permittivity and loss factor are temperature and frequency dependent. Loss tangent tan delta is the ratio of $\varepsilon''$ and $\varepsilon'$. Tan delta values are related to molecular mobility, response time to an electric field, and are related to polarization or relaxation of excited molecules or a measure of charge transfer properties. In alternating electric field a very finite time is required to align dipole in the field of alternating current, when the frequency is moderate dipoles have enough time to orient and their permittivity is high and their loss factor is low. But when the frequency is high the dipoles cannot follow the field by this dielectric constant falls and dispersion is seen in the material. This leads to a phenomenon where there is a sharp increase in dipole loss and resulting in a loss factor peak. With increase in temperature critical frequency value also increases which leads to dispersion.
Polymers have weak dipoles or induced dipoles which lead to dispersion at high frequencies. Whereas few samples like liquid crystals have high permanent dipole movement which shows dispersion at significantly higher frequencies. These samples with high dipole movements have higher permittivity compared to polymers and chemicals.

Cole-Cole plot is a popular curve of \( e'' \) vs. \( e' \), it is a semicircular plot and the highest point or peak of this plot correspond to critical frequency where the dispersion occurs. Cole-Cole plot is a straight line at very low frequencies which is due to conduction and interfacial polarization resulted from accumulation of charges at electrode interface. For a material polarization occurs at higher frequencies when the sample has high conductance. Complex chemicals and polymers often have more than one semicircular arc which means they have different relaxation times and these complex chemicals are analyzed by studying the distribution of their semicircle arcs rather than studying one single arc.

Dielectric thermal analysis is a sophisticated analytical device which is complicated both technically and instrumentally, which demands for a refined understanding of the complex theory. Other electric analytical instruments like cyclic Voltammetry and dielectric spectroscopy have a plain instrumentation which provides information on peak and bulk properties by studying charge transfer kinetics in the sample.

**1.2.2.1 Parameters measured by DEA.** The theory of a dielectric may be illustrated by the time-dependent electrical response of a sample placed on a single surface gold ceramic interdigitated electrode when an alternating (sinusoidal voltage)
electric field is applied. This process produces polarization within the sample, causing oscillation at the same frequency as the electric field but with a phase angle shift delta (δ). This phase angle shift can be measured by comparing the applied voltage to the measured current. The current is then separated into capacitive (e’) and conductive (e’’) components. These two fundamental characteristics (e’ and e’’) of a material measured by means of dielectric analysis are as a function of time, temperature, and sampling frequency. The capacitance is the high frequency permittivity (e’) or dielectric constant. Further electrical conductivity is defined as loss factor (e’’) times the applied frequency (Hz) times a constant 2Π or 6.28.

The capacitive nature of the material allows or has an ability to store an electrical charge and this factor dominates the electrical response at low temperatures. The conductive nature of the material has an ability to transfer an electric charge and this factor becomes very important when the solid material is heated above the melt temperature. These electrical properties are significant since they have been related to molecular activity, allowing for probing the chemistry, and molecular mobility of polymers and pharmaceutical materials. DEA reports three main electrical signals over a wide range of frequencies (e.g. 0.10 to 300,000 Hz). Permittivity (e’) is a measure of the induced dipoles and 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 ion conductivity. Ionic conductivity is associated with the viscosity of the sample because fluidity is identified by the ease with which ionic components can migrate through the sample under the applied electric field. DEA Tan delta is the ratio of the loss factor divided by the permittivity, i.e., Tan delta = e’’/e’. Tan delta values are
related to molecular mobility, response time to an electric field, and are related to polarization (or relaxation) of excited molecules or a measure of charge transfer properties.

1.2.2.2 Calculating polarization times. Under an applied field the dipoles will orient in the direction of the field and the time taken to align is the polarization or relaxation time. DEA can measure a polarization response in an AC electric field at isothermal temperatures or by scanning temperature technique. A Debye plot of tan-delta, a ratio of dielectric loss divided by the relative permittivity, versus frequency can fix the limits of a polarization time. The critical peak frequency in tan-delta peaks is inversely proportional to polarization time. The peak frequency is converted into polarization time with the following equation (9 red book):

- \(\mu = \frac{1}{2} \Pi Fc \times 1000\) (milli second)
- \(\mu\) Polarization time (milli second)
- \(Fc\) Tan Delta Peak Frequency (Hz)
- \(\Pi\) 3.14

1.2.2.3 Working of DEA. DEA can be used with either single surface interdigitated electrode or parallel plate electrode where there are two electrodes involved. To have appropriate results with the sample there are few precautions to be taken like sample preparation, electrode, choosing frequencies, etc.

DEA (TAI 2970) using a single-surface interdigitated gold and ceramic electrode (fig 9) determined electrical conductivity profiles. For each chemical tested, a sample size of approximately 10 to 15 mg was taken so that it covers the entire interdigitated gold ceramic electrode. Samples were heated from room temperature (28°C) to 20°C above the
melting temperature at heating rates of 3°C and 10°C min⁻¹. The experiment was carried out in an inert atmosphere of nitrogen at a flow rate of 50 mL min⁻¹. Conductivity profiles were measured at a frequency range of 0.1 to 105 Hz at all temperatures.

Figure 1.8. DEA cell with single surface gold ceramic electrode

1.2.3 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. This technique is widely applicable to a variety of materials such as pharmaceuticals, polymers, ceramics and metals etc. TMA has been used in pharmaceutical analysis. The 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 2), and recorded as a function of temperature.

Table 2.
Types of TMA probes and resulting measured properties.

<table>
<thead>
<tr>
<th>TMA Probe Type</th>
<th>Information Obtained</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flat probe/ Light load</td>
<td>Coefficient of Thermal Expansion and T_g</td>
</tr>
<tr>
<td>Dilatometer</td>
<td>Coefficient of Thermal Expansion and T_g</td>
</tr>
<tr>
<td>Penetration probe/Significant load</td>
<td>Softening/ T_g, Melting and creep modulus</td>
</tr>
<tr>
<td>Tension accessory</td>
<td>T_g, melting and cure behavior</td>
</tr>
<tr>
<td>Parallel plates</td>
<td>Melting, Viscosity and Gelation</td>
</tr>
<tr>
<td>Flexure accessory</td>
<td>Softening/ T_g and Melting</td>
</tr>
</tbody>
</table>

*T_g = Glass transition temperature

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 inter-laboratory and intra-laboratory comparison and correlation of instrumental temperature scale data within the pharmaceutical community, and would be more relevant to quality control scientists in the pharmaceutical industry.
1.2.4 Thermo-gravimetric 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.

1.2.4.1 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 10 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°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.

![Thermo-gravimetric Analyzer](image)

**Figure 1.9.** Thermo-gravimetric Analyzer

1.2.4.2 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.

1.2.4.3 *Experimental conditions.*

1.2.4.3.1 *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.

1.2.4.3.2 *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.

1.2.4.3.3 *Miscellaneous conditions.* There are generally two limitations of TGA for analyzing materials. 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

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.
1.2.4.4 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.

1.2.4.4.1 Calibration of mass. 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 S₁. Repeating the same operation several times, the mean of S₁ is obtained as S. In this operation it is known that a signal corresponding to S₁ 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.

1.2.4.4.2 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.

Table 3.

Melting Point and Heat of Fusion of Pure Substances

<table>
<thead>
<tr>
<th>Name of pure substance</th>
<th>Melting point /°C</th>
<th>Heat of fusion J/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indium</td>
<td>156.6</td>
<td>28.59</td>
</tr>
<tr>
<td>Tin</td>
<td>231.9</td>
<td>60.62</td>
</tr>
<tr>
<td>Lead</td>
<td>327.5</td>
<td>23.22</td>
</tr>
<tr>
<td>Zinc</td>
<td>419.6</td>
<td>111.4</td>
</tr>
<tr>
<td>Aluminum</td>
<td>660.3</td>
<td>397.0</td>
</tr>
</tbody>
</table>
Table 4.

Standard Substances for Temperature Calibration Verified by ICTAC

<table>
<thead>
<tr>
<th>Substance</th>
<th>Temperature/°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Permanorm</td>
<td>3 259</td>
</tr>
<tr>
<td>GM761 Nickel</td>
<td>353</td>
</tr>
<tr>
<td>Mumetal</td>
<td>381</td>
</tr>
<tr>
<td>Permanorm (for TGA only)</td>
<td>5 454</td>
</tr>
<tr>
<td>Trafoperrn</td>
<td>750</td>
</tr>
</tbody>
</table>

*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°C. A typical example is the TGA of calcium oxalate hydrate, heated to 1000°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 11.
Each step is explained by the following chemical reaction (TA Instruments “Weight Loss Determined from Mass Spectrometry Trend Data in a Thermogravimetric/Mass Spectrometer System” Carlton G. Slough TA Instruments, 109 Lukens Drive, New Castle DE 19720, USA)

At 200°C

$$\text{CaC}_2\text{O}_4\cdot\text{H}_2\text{O} \longrightarrow \text{CaC}_2\text{O}_4 + \text{H}_2\text{O} \uparrow (12.33 \%)$$

At 500°C

$$\text{CaC}_2\text{O}_4 \longrightarrow \text{CaCO}_3 + \text{CO} \uparrow (19.10 \%)$$

At 750°C

$$\text{CaCO}_3 \longrightarrow \text{CaO} + \text{CO}_2 \uparrow (29.84 \%)$$

Calcium oxalate hydrate is a well-known material for the calibration of the TGA.
1.2.4.5 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.

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°C below those temperatures.

1.2.5 Scanning electron microscopy. A characteristic high resolution three dimensional image of sample surface is generated by scanning electron microscopy (SEM). This three dimensional image of sample surface makes it easier to examine the surface structure of the sample.
1.2.5.1 Sample preparation. To have a better image quality under electron microscopy processing of the sample is required, and this required processing technique depends on the type of sample to be analyzed.

1.2.5.1.1 Conductive coating. During the process of electron imaging the specimen is subjected to electron irradiation which leads to accumulation of static electric field on the specimen and to avoid this ultrathin coating of electrically conducting material over the specimen by low vacuum sputter coating or high vacuum evaporation technique. Coating is also applied for samples which are highly conductive because it aids in improving the image contrast. In scanning electron microscopy tungsten, graphite, platinum, gold, gold/palladium etc. are especially employed as coating materials in sample preparation.

There have been developments to scanning electron microscopy to improve the image quality. Gold coating has few disadvantages which lead to loss of information because of which it is considered to be a semi destructive process. It is also not possible to remove the coating without extreme treatment, which leads to destruction of the sample. To avoid this novel and simple technique using potassium cyanide as a removing agent has been reported.

We have used Amray 1830 SEM. Low vacuum sputter coating technique was used to coat gold at 400A on the sample.
1.2.6 Powder x-ray diffration. X-Ray diffraction (XRD) is most widely used in numerous industries for identification of crystallinity in compound by studying their diffraction pattern. Some other specific benefits of XRD are listed below.

- Single phase materials in several samples such as chemicals, ceramics, polymers, etc., are identified.
- Determination of polymorphs.
- Evaluating amorphous and crystalline content in pharmaceuticals or other chemicals.
- Recognition of multiple phases in samples like rocks, minerals, melts, etc.,
- Quantitative determination of different phases is possible by advanced technique called PXRD, which different phases by calculating peak ratios.
In order to interpret the data obtained from XRD you need to know a few basic principles like how the X-rays interact with the sample, possible errors and the different source. The mathematic and physics involved in generating the monochromatic X-rays and X-ray diffraction is not necessary.

1.2.6.1 Sample preparation. The most critical factor in obtaining a quality analytical data relies on preparation of the specimen. In order to have a ideal sample preparation you need to have random distribution of crystals which should be less than 10µm. Specimen should be mounted such a way that there should be no crystallite. Considering the above factors sample preparation is considered to be a significant topic.

1.2.6.2 XRD principle. Sample is exposed to X-rays this interaction leads to secondary wave which is produced by the cones in the sample. According to the mathematic equation Braggs law the produced secondary wave is related to the interplanar spacing in the crystalline sample.

Braggs Law:
\[ n\lambda = 2d \sin\theta \]

Where \( n \) is an integer
\( \lambda \) is the wavelength of X-rays
\( d \) is the interplanar spacing generating the diffraction
\( \theta \) is the diffraction angle

Diffraction maxima are measured along 2θ diffractometer circle for powder samples having infinite amount randomly oriented crystal. According to Braggs law the angle of diffraction is related to the interplanar spacing \( d \), and the intensity of the diffraction is related to the strenght of those diffractions in the specimen. Electronic
detectors are used to measure and record angles and intensities of diffraction. Specialized software is utilized to create plots of 2θ (X axis) vs. intensity (Y axis) for the sample. Units for λ and d are measured in angstroms.

1.2.6.3 *Generate analytical x-rays.* Experimental results are much better as we get closer to the monochromatic beam in our X-ray beam. In X-ray diffraction of most organic and inorganic crystals copper tubes are used. For copper the most strongest radiation (Kα) is 1.54 angstroms. There are low energy and high energy radiations produced by tubes. Kα1, Kα2 and Kβ are the high energy radiations of which we use Kα1 for analytical data. A monochromator or an energy selective detector is used to eliminate Kβ, and Kα2 is electronically removed from X-ray data during processing.

1.3 References


CHAPTER II
STANDARD TEMPERATURE CALIBRATION PROTOCOLS AND MATERIAL
CHARACTERIZATION WITH PHARMACEUTICALS BY THERMAL
ANALYSIS

2.1 Abstract

New test protocols have been developed which describes the temperature and
material characterization calibration of differential scanning calorimeters, dielectric
analyzers, and thermomechanical analyzers with pharmaceuticals over the temperature
range from 25 °C to 250 °C. This study implements the use of pure active pharmaceutical
ingredients (APIs) and an excipient. These test protocols can be blended into a universal
standard protocol for differential scanning calorimetry (DSC), dielectric analysis (DEA)
and thermomechanical analysis (TMA). Calibration is performed by observing the
melting transition temperature of standard pharmaceutical materials within the
temperature range of interest.

Pharmaceutical test specimens 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 that 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 the TMA to test a 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 or contraction is recorded.

The calibration materials used in this test development are: Vanillin, an excipient, Acetanilide, Acetophenetidin, and Sulfapyridine. These test protocols were accomplished based on the ASTM standard test methods for temperature calibration of thermal analytical methods. The $R^2$ correlation value for known standard literature transition temperatures vs. DSC melting peak temperatures, DEA Permittivity melting temperatures and TMA extrapolated onset-melting temperatures for the calibrants was 0.999.

Keywords: DEA, DSC, TMA, IDA, Calibration, Calibrants, APIs, Permittivity, Log Permittivity, Dimension Change and Coefficient of Thermal Expansion.

2.2 Introduction

There are thousands pharmaceutical materials known today (1-3). There is a need for each material that is synthesized and discovered, to be tested and standardized. Calibration typically uses metals e.g., Indium and Zinc to calibrate the three instruments described in this study (4). 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 as well as 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 (5). TMA measures the dimensional variability of a solid polymer or material, and can also be used to characterize powdered API’s (6). To design an efficient procedure for calibration, understanding the thermal analysis of all these instruments is crucial.

2.3 Materials

The following analytical grade >99.9 % pure APIs and an excipient (Vanillin), within the temperature range of interest, were used in this test development as listed in Table 1 with Chemical Abstract Service (CAS) registry numbers. The ASTM E928-08 “Standard Test Method for Purity by Differential Scanning Calorimetry” was employed to verify the purity of the test specimen used in this study (20).

Table 1
Calibration materials and their transition temperatures

<table>
<thead>
<tr>
<th>Calibration Materials*</th>
<th>Literature Transition Temp/ °C (Solid - Liquid)</th>
<th>Chemical Abstract Service registry numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetanilide</td>
<td>113 – 116</td>
<td>103-84-4 (22)</td>
</tr>
<tr>
<td>Vanillin</td>
<td>81 – 83</td>
<td>122-33-5 (23)</td>
</tr>
<tr>
<td>Sulfa pyridine</td>
<td>191 – 193</td>
<td>144-83-2 (24)</td>
</tr>
</tbody>
</table>

*Available from Sigma- Aldrich®
2.4 Instruments

The TAI DSC 2920 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 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.

2.4.1 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 (6).

2.4.2 Sampling. Calibration materials are analyzed by all the three instruments on “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. For DSC and TMA sampling is done by packing the test specimen in a standard aluminum pan.

2.4.3 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 (4), E 2038 (5) and E 1363 (6), respectively.

2.4.4 Experimental procedures for DSC, DEA and TMA.

- Select the calibration material of known transition temperature listed in table 1.

- 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 °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°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 °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 1,000 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.

2.5 Results and Discussion

2.5.1 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: Tm, Tmp, Tc, Tcp, Heat of fusion (J g\(^{-1}\)) and Heat of crystallization (J g\(^{-1}\)) for a pure calibration material.

Tm = Extrapolated onset melt temperature
Tmp = Melting peak temperature
Tc = Extrapolated crystallization temperature
Tcp = Crystallization peak temperature
\(\Delta H_f\) = Heat of fusion
\(\Delta H_c\) = Heat of crystallization

The DSC thermal curves of Acetanilide (see Fig. 1), Acetophenetidin (see Fig. 2) and Vanillin (see Fig. 3) are described respectively. These heat-cool-heat plots show changes in the heat flow (W g\(^{-1}\)) with respect to time and temperature.

**Figure 2.1.** Acetanilide DSC curve showing Tmp = 116.31 °C, Tcp = 81.50 °C; Heat of Fusion for first endothermic peak \(\Delta H_f = 143.9\) J g\(^{-1}\) and \(\Delta H_f = 123.3\) J g\(^{-1}\) for second endothermic peak; Heat of Crystallization, \(\Delta H_c = 112.1\) J g\(^{-1}\).
Figure 2. 2. Acetophenetidin DSC curve showing Tmp = 136.25 °C, Tcp = 126.18 °C; Heat of fusion for first endothermic peak $\Delta H_f = 163.1 \text{ Jg}^{-1}$ and $\Delta H_f = 152.8 \text{ Jg}^{-1}$ for second endothermic peak; Heat of crystallization, $\Delta H_c = 151.9 \text{ Jg}^{-1}$.

Figure 2. 3. Vanillin DSC curve showing Tmp = 83.08 °C, Tcp = 38.33 °C; Heat of fusion for first endothermic peak $\Delta H_f = 149.0 \text{ Jg}^{-1}$ and $\Delta H_f = 137.0 \text{ Jg}^{-1}$ for second endothermic peak; Heat of crystallization $\Delta H_c = 116.2 \text{ Jg}^{-1}$.

A summary of the DSC melting and crystallization properties of calibration materials is cited in Table 2. Table 2 describes the Melting peak temperatures (Tmp), Crystallization peak temperatures (Tcp), Heat of fusion ($\Delta H_f$, Jg$^{-1}$), Heat of crystallization ($\Delta H_c$, Jg$^{-1}$) and % Crystallinity of calibration materials evaluated by DSC.
Table 2

DSC Melting and Crystallization Properties of Calibration Materials

<table>
<thead>
<tr>
<th>Calibration materials</th>
<th>Melting peak Temperatures/</th>
<th>Cryst peak</th>
<th>Temp/</th>
<th>ΔHf (J g⁻¹)</th>
<th>% Crystallinity</th>
<th>ΔHc (J g⁻¹)</th>
<th>(ΔHc/ΔHf) *100</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tmp °C</td>
<td>1st Peak</td>
<td>2nd Peak</td>
<td>1st Peak</td>
<td>2nd Peak</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetanilide</td>
<td>116.31</td>
<td>116.58</td>
<td>81.50</td>
<td>144</td>
<td>123</td>
<td>112</td>
<td>78</td>
</tr>
<tr>
<td>Acetophenetidin</td>
<td>136.25</td>
<td>136.61</td>
<td>126.18</td>
<td>163</td>
<td>153</td>
<td>152</td>
<td>93</td>
</tr>
<tr>
<td>Vanillin</td>
<td>83.08</td>
<td>82.45</td>
<td>38.33</td>
<td>149</td>
<td>138</td>
<td>116</td>
<td>78</td>
</tr>
<tr>
<td>Sulfapyridine</td>
<td>192</td>
<td>T_g-(62)</td>
<td>123</td>
<td>174</td>
<td>-</td>
<td>57</td>
<td>33</td>
</tr>
</tbody>
</table>

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

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

- 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 and 1000 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, and 1000 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 curve of acetanilide showing the permittivity transition temperature for a single frequency (1000 Hz) run is described in Fig. 4. Fig. 5 describes the acetanilide derivative of permittivity transition temperature for a single frequency run when plotted vs. temperature. Fig. 6 describes the DEA Acetanilide curve showing Log permittivity transition temperature at 10Hz. Fig. 7 describes the DEA acetanilide curve showing Derivative of log permittivity transition temperature at 1 Hz.
Figure 2.4. DEA curve of Acetanilide showing permittivity transition temperature (114.12 °C) for single frequency (1,000 Hz) run.

Figure 2.5. DEA curve of Acetanilide showing Permittivity and Derivative of Permittivity transition temperature (114.12 °C) for a single frequency (1000Hz) run.
Figure 2.6. DEA curve of Acetanilide showing Log permittivity transition temperature (115.11 °C) at 10 Hz.

Figure 2.7. DEA curve of Acetanilide showing Log permittivity and Derivative of Log permittivity transition temperature (113.23 °C) 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: Table 3: Acetanilide; Table 4: Acetophenetidin; Table 5: Vanillin; and Table 6: Sulfa pyridine.

Table 3

DEA Permittivity and Log Permittivity transition temperature of single (1000 Hz) and set of frequencies (1, 10, 100, 1000 Hz) of Acetanilide Tmp =113-116 °C

<table>
<thead>
<tr>
<th>Frequency/ Hz</th>
<th>Permittivity Transition Temperatures/ °C</th>
<th>Log Permittivity Transition Temperatures/ °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000 Hz</td>
<td>114.2</td>
<td>113.0</td>
</tr>
<tr>
<td>1 Hz</td>
<td>115.6</td>
<td>113.3</td>
</tr>
<tr>
<td>10 Hz</td>
<td>115.1</td>
<td>113.5</td>
</tr>
<tr>
<td>100 Hz</td>
<td>113.9</td>
<td>114.0</td>
</tr>
<tr>
<td>1000 Hz</td>
<td>114.1</td>
<td>114.0</td>
</tr>
<tr>
<td>Average</td>
<td>114.6</td>
<td>113.7</td>
</tr>
<tr>
<td>Std Deviation</td>
<td>0.80</td>
<td>0.34</td>
</tr>
<tr>
<td>% Relative Error</td>
<td>0.70</td>
<td>0.26</td>
</tr>
</tbody>
</table>
Table 4

DEA Permittivity and Log Permittivity Transition Temperature of Single (1000 Hz) and Set of Frequencies (1, 10, 100, 1000 Hz) of Acetophenetidin Tmp = 132-138 °C

<table>
<thead>
<tr>
<th>Frequency/ Hz</th>
<th>Permittivity Transition Temperatures/ °C</th>
<th>Log Permittivity Transition Temperatures/ °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000 Hz</td>
<td>131.0</td>
<td>131.0</td>
</tr>
<tr>
<td>1 Hz</td>
<td>132.5</td>
<td>132.3</td>
</tr>
<tr>
<td>10 Hz</td>
<td>132.5</td>
<td>132.7</td>
</tr>
<tr>
<td>100 Hz</td>
<td>132.6</td>
<td>132.5</td>
</tr>
<tr>
<td>1000 Hz</td>
<td>132.7</td>
<td>132.7</td>
</tr>
<tr>
<td>Average</td>
<td>132.6</td>
<td>132.6</td>
</tr>
<tr>
<td>Std Deviation</td>
<td>0.09</td>
<td>0.23</td>
</tr>
<tr>
<td>% Relative Error</td>
<td>0.66</td>
<td>0.17</td>
</tr>
</tbody>
</table>
Table 5

DEA Permittivity and Log Permittivity Transition Temperature of Single (1000 Hz) and Set of Frequencies (1, 10, 100, 1000 Hz) of Vanillin Tmp = 81-83 °C

<table>
<thead>
<tr>
<th>Frequency/ Hz</th>
<th>Permittivity Transition Temperatures/ °C</th>
<th>Log Permittivity Transition Temperatures/ °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000 Hz</td>
<td>81.0</td>
<td>80.9</td>
</tr>
<tr>
<td>1 Hz</td>
<td>82.3</td>
<td>80.8</td>
</tr>
<tr>
<td>10 Hz</td>
<td>82.0</td>
<td>80.9</td>
</tr>
<tr>
<td>100 Hz</td>
<td>81.3</td>
<td>80.8</td>
</tr>
<tr>
<td>1000 Hz</td>
<td>81.0</td>
<td>80.9</td>
</tr>
<tr>
<td>Average</td>
<td>81.5</td>
<td>80.7</td>
</tr>
<tr>
<td>Std Deviation</td>
<td>0.90</td>
<td>0.18</td>
</tr>
<tr>
<td>% Relative Error</td>
<td>1.09</td>
<td>0.22</td>
</tr>
</tbody>
</table>
### Table 6

DEA Permittivity and Log Permittivity Transition Temperature of Single (1000 Hz) and Set of Frequencies (1, 10, 100, 1000 Hz) of Sulfapyridine Tmp = 191-193 °C.

<table>
<thead>
<tr>
<th>Frequency/ Hz</th>
<th>Permittivity Transition Temperatures/ °C</th>
<th>Log Permittivity Transition Temperatures/ °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000 Hz</td>
<td>192.0</td>
<td>191.0</td>
</tr>
<tr>
<td>1 Hz</td>
<td>192.7</td>
<td>191.5</td>
</tr>
<tr>
<td>10 Hz</td>
<td>192.8</td>
<td>191.0</td>
</tr>
<tr>
<td>100 Hz</td>
<td>191.3</td>
<td>190.8</td>
</tr>
<tr>
<td>1000 Hz</td>
<td>191.0</td>
<td>191.4</td>
</tr>
<tr>
<td>Average</td>
<td>191.9</td>
<td>191.1</td>
</tr>
<tr>
<td>Std Deviation</td>
<td>0.9</td>
<td>0.35</td>
</tr>
<tr>
<td>% Relative Error</td>
<td>0.47</td>
<td>0.18</td>
</tr>
</tbody>
</table>

#### 2.5.3 For TMA.

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

- From the TMA thermal curve recorded, extrapolated onset temperature is measured. This 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 (6).
The thermal curves of Acetanilide (see Fig. 8) and Acetophenetidin (see Fig. 9) recorded by the instrument are described showing the extrapolated onset melting temperature.

**Figure 2.8.** TMA of Acetanilide curve showing the extrapolated onset melt temperature (115.51 °C).

**Figure 2.9.** TMA of Acetophenetidin curve showing extrapolated onset melt temperature (134.27 °C)
Figure 2.10. TMA of Vanillin curve showing extrapolated onset melt temperature (83.46 °C).

A summary of TMA observed extrapolated onset temperatures of calibration materials and literature transition temperatures are cited in Table 7.
Table 7

Summary of TMA Extrapolated Onset Temperatures Of Calibration Materials And Literature Transition Temperatures

<table>
<thead>
<tr>
<th>Calibration Materials*</th>
<th>TMA Extrapolated Onset Temperatures/ °C</th>
<th>Literature Transition Temperatures °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetophenetidin</td>
<td>134.27</td>
<td>132 – 138</td>
</tr>
<tr>
<td>Acetanilide</td>
<td>115.51</td>
<td>113 -116</td>
</tr>
<tr>
<td>Vanillin</td>
<td>83.46</td>
<td>81-83</td>
</tr>
<tr>
<td>Sulfapyridine</td>
<td>192.78</td>
<td>191-193</td>
</tr>
</tbody>
</table>

A summary of DSC, DEA and TMA melting temperatures for the calibrants (API’s) studied vs. the standard literature melting temperatures values are given in Table 8.
Table 8

Summary of DSC, DEA and TMA Results

<table>
<thead>
<tr>
<th>Materials*</th>
<th>DSC Melting</th>
<th>DEA Transition</th>
<th>TMA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Literature</td>
<td>Temperatures/ °C</td>
<td>Temperatures/ °C</td>
</tr>
<tr>
<td>Calibration</td>
<td>Transition</td>
<td>Tmp °C</td>
<td>°C</td>
</tr>
<tr>
<td>Temps/ °C</td>
<td>1st Peak</td>
<td>2nd Peak</td>
<td>Permittivity</td>
</tr>
<tr>
<td>Acetophenetidin 132- 138</td>
<td>136.25</td>
<td>136.61</td>
<td>133.6</td>
</tr>
<tr>
<td>Acetanilide 113 – 116</td>
<td>116.31</td>
<td>116.58</td>
<td>114.6</td>
</tr>
<tr>
<td>Sulfapyridine 191 – 193</td>
<td>192</td>
<td>T_g= 62</td>
<td>191.9</td>
</tr>
<tr>
<td>Vanillin 81 – 83</td>
<td>83.08</td>
<td>82.45</td>
<td>81.5</td>
</tr>
</tbody>
</table>

*Available from Sigma Aldrich®, **CAS=Chemistry Abstract Service registry number.
2.6. 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. The three drugs plus the excipient, studied for this standard protocol present outstanding list of viable temperature standards. Their observed melting transition temperature best correlates with the known literature transition temperature values. All, but Sulfapyridine, yielded melting-crystallization - melting properties that enhance the overall characterization of those drugs. The $T_g$, on the 2\textsuperscript{nd} heat cycle, of Sulfapyridine at 62°C supplied additional important thermal characterization data. 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 value for known standard literature temperatures vs. DEA permittivity melting temperature for of single
(1000 Hz) and set of frequencies (1, 10, 100, 1000 Hz) was 0.99. The \( R^2 \) correlation value for known standard literature temperatures vs. TMA extrapolated onset temperatures was 0.999. The \( R^2 \) correlation value for known standard literature temperatures value vs. DSC melting peak temperatures was 0.999. 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. (See Fig. 11) 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. These new pharmaceutical based test protocols, permit inter-laboratory comparison and intra-laboratory correlation of instrumental temperature scale data within the pharmaceutical community, and will be implemented in our chemical pharmaceutical research.

The following ASTM methods were also reviewed in the course of preparing this text:

- E691 Standard Practice for Conducting an Interlaboratory Study to Determine the Precision of a Test Method (27).

We would like to acknowledge the ASTM International for their support and funding this ASTM International Graduate Student Project grant 2010.

2.7 References


22. Chemical Abstract Service CAS no. 103-84-4, Acetanilide

23. Chemical Abstract Service CAS no. 122-33-5, Vanillin

24. Chemical Abstract Service CAS no. 144-83-2, Sulfapyridine


CHAPTER III
SOLID STATE STUDIES OF DRUGS AND CHEMICALS BY DIELECTRIC
AND CALORIMETRIC ANALYSIS

3.1 Introduction

In 2005 there were number of drugs evaluated by Dielectric Analysis and Differential Scanning Calorimetry in a preliminary study by Riga and Alexander. Various active pharmaceutical ingredients (APIs) were analyzed by DETA, the resulting log conductivity vs. temperature curve in OC revealed new electrical signatures of each drug (1). A very complete review by Rolf Hilfiker, F Blatter, and M Raumer on the relevance of solid state properties for pharmaceutical products but they did not include dielectric properties which were initiated in 2005 (2). The bio pharmaceutics classification system was reviewed and did not include any electrical properties to understand the effect of solid state properties (3). A well reviewed paper in 2006 on solid state pharmaceutical compounds and impact of ICH on Q6 guidelines did not include any information on dielectric properties (4).

Thermal analysis studies in pharmaceuticals uses instrumentation like DSC, DTA, and TG. They were employed to study solid dispersion systems, time for drug disintegration, prediction of drug- excipients compatibility, and analysis of enantiomers,
and recemates (5). Introduction of DETA as part of thermal analysis of drugs was initiated by Dr. Riga. This opened a new door for studying many more properties of drugs and other chemicals, like premelt behavior and the determination of the amorphous and crystalline content in a solid dosage form.

A crystalline solid drug had a conductivity of about $10^{-2}$ pS/cm when the drug melted the liquid amorphous drug had a conductivity of $10^6$ pS/cm and organic salts had a conductivity of $10^8$ pS/cm. Multiple chemicals as drugs, organics, amino acids and carbohydrates undergo 3 to 4 orders of electrical conductivity change prior to melting. In the solid state, the premelt variations detected by DETA and were not observed by DSC. Riga and Alexander identified this premelt as related to its defect structure. Later Riga and Mantheni discovered that the premelt was either due to formation of modified zwitter ions or an excimer (David Grant) (6). The novel Premelt behavior was related to electrical conductivity variations and not permittivity values, i.e. no premelt activity was recorded in the permittivity vs. temperature plot.

Determining amorphous and crystalline phases in a drug is a very challenging assignment. Properties like bioavailability, feasibility, stability depend on the amorphous content of the solid dosage form. Amorphous phase in drug was studied using DSC, x ray diffraction, and calorimetry (7). These studies couldn’t tell how much amorphous and crystalline content is present in the solid dosage form. The research of Maheswaram in our laboratories revealed the amount of amorphous and crystalline content in the solid state by DETA and DSC. The amorphous content was determined by the conductivity – activation energy in the solid state (8).
Pre major melt behavior has been attributed to the solid-solid transition as described by D V Mesaros. Temperature dependent variations in the proton NMR of hexa hydro -1, 3, 5trinitroso-s-triazine were observed in the premelt solid phase of this chemical and were related to activation of substantial motion of the molecules in the crystal lattice(9).

Dielectric thermal analysis of amino acids also exhibited an enhanced electrical linear conductivity increase prior to the melt. Matthews and Riga referred to these new properties as dielectric viscoelastic behavior. Tryptophan a hydropathically neutral amino acid as a neat and heat treated sample showed no premelt behavior. Cystine the most hydrophobic amino acid of all the three amino acid studied has the largest difference in premelt activity (10). Anthracene a hetero cyclic organic chemical forms an excimer while undergoing fusion this might be one of the causes for the premelt variations. Premelt charge transfer complexes in organics were studied using DETA and DSC by Riga and Alexander (11). The Activation energy is calculated for all the chemicals using an Arrhenius plot, where the slopes of the plot log conductivity vs. 1/T (K) *1000 at a particular frequency is considered and multiplied by constant, Arrhenius factor 19.2, yielding an activation energy (J/mole).

Dielectric analysis, a thermal analytical instrument can be used to study the changes in materials due to the dielectric Visco-elastic transition as well as other fundamental electrical properties. Those measured are permittivity (e’), which is related to dipole content, loss factor (e’’) which is the energy required to align the dipoles, AC conductivity which is (loss factor or e’’ * frequency * constant) in pS/cm, activation energy in J/mole (using Arrhenius plot).
Correlation of DETA and DSC plots highlights the solid state properties which can clearly explain where the onset of increase conductivity is initiated (fig 4). Given below is the list of chemicals tested along with their melting temperatures (Table 1).

3.2 Results and Discussions

We have observed multiple unique solid state variations in the chemicals tested by DEA. The phenomenon observed is attributed to the electrical change (ionic conductivity) not a crystalline change in the solid state. In order to better understand the process we are measuring the effect of the applied critical frequency, the sample mass taken and heating rate, on this behavior of the suspected dipolar character of the chemical studied. Here a wide variety of crystalline materials were used to define and characterize their melt temperature profile. Examples are drugs, sugars, amino acids, and organics, and contrarily linear polymers (LDPE, HDPE), and long chain alkanes did not show this electrical behavior. Arrhenius plot was used to calculate activation energy in the premelt state. The activation energy for materials varied from 35 J/mol (naphthalene) to 1600 J/mol (acetophenetidin), the rest are listed in table 1.

Anthracene a polycyclic organic compound, composed of three fused rings undergoes dimerization when heated through its melt temperature (fig 1). The dimerization and excimer formation is relevant in the DEA heating curve since there is no abrupt transition seen in the further heating profile. DEA electrical conductivity vs. 1/T K is linear in the premelt through to the fusion as seen in the (fig 2). Anthracene is our model for excimer or zwitter ion formation in interpreting our DEA results. For example, Sulfapyridine undergoes a strong linear ionic conductivity change in the premelt observed below its melt temperature in the superimposed DSC fusion curve (fig 3). An
Arhennius plot for Sulfapyridine (log conductivity vs. 1/T K * 1000) has a linear correlation coefficient fit for the curve of 0.965 a better choice of premelt values yielded a correlation coefficient of 0.999 (fig 4).

![Figure 3.1: Anthracene showing Excimer formation](image)

Table 1

List of Drugs and Chemicals and Their Corresponding Activation Energies in the Premelt Temperature Region

<table>
<thead>
<tr>
<th>Chemical Type</th>
<th>Activation Energy</th>
<th>J/m</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anthracene</td>
<td>800</td>
<td></td>
</tr>
<tr>
<td>Naphthalene</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>Sulfapyridine</td>
<td>950</td>
<td></td>
</tr>
<tr>
<td>Lidocaine</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>Caffeine</td>
<td>300</td>
<td></td>
</tr>
<tr>
<td>Acetophenetidin</td>
<td>1600</td>
<td></td>
</tr>
<tr>
<td>Dextrose</td>
<td>180</td>
<td></td>
</tr>
<tr>
<td>Arginine</td>
<td>340</td>
<td></td>
</tr>
<tr>
<td>Histidine</td>
<td>350</td>
<td></td>
</tr>
</tbody>
</table>
Figure 3.2. log conductivity vs. 1/T (K)*1000 for Anthracene (2.035 melt, 2.075 premelt)

Figure 3.3. Sulfapyridine DSC & DEA curve overlay and comparing conductivity in crystalline and amorphous samples
The DEA profile of polymers and two long chain alkanes clearly shows the onset of increase in ionic conductivity was initiated after the melt temperature of the sample (Fig 5), and it is obviously due to formation of a highly conductive amorphous liquid form. Comparing these results to any other samples studied will noticeably delineate those that are undergoing the solid state behavior variation as observed for acetanilide (Fig 6). The reason behind these polymers and long chain alkanes not showing the premelt electrical activity is probably due to their high molecular weight or their short and long chain linear structures. The Arrhenius plots for these chemicals show that the ionic conductivity increase in the premelt region is very linear. Conductivity values in the premelt region were taken and plotted against $1/T \ K \ *1000$ which gave a coefficient of correlation of approximately 0.99. Dextrose a carbohydrate is shown in (Fig 7) and an amino acid, Histidine, is shown in (Fig 8).
Figure 3.5. DEA and DSC overlay for high density polyethylene showing increase in conductivity after melt.

Figure 3.6. DEA and DSC overlay for Acetanilide showing increase in conductivity before melting in premelt region.
Figure 3.7. Amino acid Histidine showing ionic conductivity increase in the pre melt region.

Figure 3.8. Carbohydrate Dextrose showing ionic conductivity increase in the premelt region.
The experimental variables studied were the sample mass, heating rate, and applied frequency. A fourfold increase in mass (5 to 20 mg) caused a 5°C decrease in electrical onset (Fig 9). The heating rate variation from 3 to 10°C/min, a 3.3 increase in heating rate caused a decrease of 20°C in the onset temperature for ionic conductivity (Fig 10). A 10,000 fold increase in frequency (0.1Hz to 1000Hz) caused a 7°C increase in the conductivity onset (Fig 11). These three factors tested are apparently not effective variations in dielectric analysis for the samples studied. The observed changes recorded are not paralleling DSC studies because of much higher sample surface area in DEA compared to DSC. An increase in heating rate in DSC shifts the curve to higher temperatures, while in DEA it shifted to lower temperatures. The interdigitated electrode has more points of contacts than DSC pan. DEA can differentiate surface and bulk changes which cannot be seen in the DSC. The 5 to 20°C change in the onset of conductivity by the three variables studied here are significant but not relevant since the rate of change of conductivity with temperature is the defining property (activation energy).
Figure 3.9. DEA and DSC overlay showing the effect of frequency on the onset of conductivity in the premelt region. 0.1Hz (97.84°C) to 1000 Hz (104.79°C).

Figure 3.10. Acetanilide DEA curves showing the effect of sample mass on onset of increase in conductivity in the premelt region (red 5mg low sample mass, green 20mg high sample mass)
3.3 Conclusions

We observed unique dielectric viscoelastic, properties (based on DEA and Thermal mechanical analysis, Shravan NATAS 2010) in a variety of chemicals like carbohydrates, amino acids, APIs, and organics. Our studies have shown that polymers and long chain alkanes do not exhibit the premelt behavior. Molecular weight may be a factor in prohibiting pre-melt behavior. This dielectric viscoelastic property observed in chemicals and drugs does suggest new synthesis routes. Activation energy, $E_a$ (J.m$^{-1}$) varied based on source of chemicals from 36 J.m$^{-1}$ (e.g. naphthalene) to 1600 J.m$^{-1}$ (e.g. acetophenetidin) at 1 Hz applied frequency (surface analysis). The significance of the absolute value of the $E_a$ is that it ranks the chemical’s amorphous content. For the various crystalline and amorphous phases in the chemicals studied, the amorphous content is inversely related to activation energy (Maheswaram, Mantheni, and Riga 2010 (8)). Work is in progress to define other factors that will affect the premelt activity and the basis for...
pre-melt activity. We are searching for reactive species that can produce new products for
the future e.g. a reaction of a sweetener and a fatty acid for the continuous presence of the
sweetener.

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CHAPTER IV

VALIDATING THE BASIS OF PRE-MELT BEHAVIOR IN POLAR AND NON-POLAR CHEMICALS

4.1 Introduction

The history of dielectric studies in solids goes back to the 18th century, and even to ancient Greeks, and yet there is a lack of satisfactory solution for theoretical understanding the ionic charge transport properties (1). In 2005 Riga and Alexander evaluated a number of API standards using DEA and DSC which revealed unique ionic conductivity behavior (2). Based on this article we have initiated a study to determine the charge transfer mechanism the basis of the unique solid state electrical conductivity behavior. Ionic conductivity in solids is low when compared to the melted sample. There is an unusual linear rise in ionic conductivity in the solid pre-melt temperature range. The polar behavior in the pre-melt temperature range is only related to ionic conductivity (loss factor a measure of the energy to move ions) and not to the permittivity electrical property (alignment of dipoles), we have also studied the effect of instrumental variables (3).

Similar studies have been made in Schottky diodes and polymers where they have observed an increase in ionic conductivity with respect to temperature and frequency (4,
5). Solid state stability can be evaluated by examining its ionic conductivity. Also residual moisture can be identified by investigating pre-melt behavior. There is a 5 to 7 order magnitude change in conductivity in the solid state in the pre-melt range.

Riga and Alexander originally interpreted the ionic conductivity variations in the solid state were considered to be due to defects in the structure (2), but later our studies show that this behavior is due to structural variations (3). Isothermal Dielectric analysis was used to study the tan-delta property which identifies the polarization time in a polar material (6). Studying polarization times at a particular temperature gave us a clear picture behind the variation in the ionic conductivity in the pre-melt vs. melt temperature range (7).

The Activation energy is calculated for all the chemicals in the pre-melt using an Arrhenius plot, where the slopes of the plot log conductivity vs. 1/T (K) *1000 at a particular frequency is considered and multiplied by constant, Arrhenius factor 19.2, yielding an activation energy (J/mole). Activation energy, Ea (J/m) varied based on source of chemicals from 36 J/m (e.g. naphthalene) to 1600 (e.g. Acetophenetidin) at 1 Hz applied frequency (surface analysis). The significance of the absolute value of the Ea is that it ranks the chemical’s amorphous content. For the various crystalline and amorphous phases in the chemicals studied, the amorphous content is inversely related to activation energy (3, 8).

Studying polymers like HDPE (high density polyethylene) and LDPE (low density polyethylene) and long chain alkanes like tetraconsane and pentacosane which do not exhibit the variations in ionic conductivity in the pre-melt behavior. Though there was noted a significant change in ionic conductivity only after the melt in the amorphous
phase. We assumed it to be due to long chain molecules or high molecular weight (higher viscosity) which is preventing the increase in ionic conductivity in solids. In later studies the polar polymers, like acetal and nylon, gave us a better perceptive of the reason behind the pre-melt behavior.

Dielectric Analysis (DEA) - is the electrical analog of DMA in which the current (phase and magnitude) resulting from a sinusoidal imposed voltage are measured. Because mechanical and electrical properties are highly sensitive to small changes in chemical internal structures, DMA and DEA are more sensitive to low energy transitions, for e.g., the glass transitions of filled thermoset systems, than are other thermal analysis methods. The static stress modes of the DMA further allow long term strength forecasts to be made using the creep or stress relaxation methods at elevated temperatures (3,9).

A wide range of chemicals were studied for this analysis in order to better understand the conductivity behavior in the pre-melt and to differentiate chemicals which show variations in pre-melt from those which do not. Given below is the list of chemicals which were analyzed for this study.
Table 1

List of Chemicals Examined for the Study

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetanilide</td>
<td>Active Pharmaceutical Ingredient (API)</td>
</tr>
<tr>
<td>Acetophenitidine</td>
<td>Active Pharmaceutical Ingredient (API)</td>
</tr>
<tr>
<td>Sulphapyridine</td>
<td>Active Pharmaceutical Ingredient (API)</td>
</tr>
<tr>
<td>Lidocaine</td>
<td>Active Pharmaceutical Ingredient (API)</td>
</tr>
<tr>
<td>Anthracene</td>
<td>Organic Chemical</td>
</tr>
<tr>
<td>Pentacosane</td>
<td>Long Chain Alkane</td>
</tr>
<tr>
<td>Tetraconsane</td>
<td>Long Chain Alkane</td>
</tr>
<tr>
<td>Polyethylene (High and low density)</td>
<td>Polymer polyolefin</td>
</tr>
<tr>
<td>Nylon 6 (Amorphous and homopolymer)</td>
<td>Polymer polyamide</td>
</tr>
</tbody>
</table>

4.2 Experimental

We have used thermal analytical instrumentations like dielectric thermal analysis (scanning and isothermal), and differential scanning calorimetry for studying a wide range of chemical samples like polymers, carbohydrates, amino acids, organics, etc. We have also studied the effect of key instrument variables on results we have obtained.

4.2.1 Testing Conditions for Scanning and Isothermal Dielectric Thermal Analysis. DEA 2970 by TA instruments was used for scanning and isothermal analysis of a sample. Interdigitated single surface gold plated ceramic sensor (fig. 1) was used for the study. DEA 230/2 by Netzsch with interdigitated aluminum electrode embedded in polyimide was also used for isothermal evaluation.
The sample can be studied by heating the sample over a range of temperature (scanning) or can be studied at a particular temperature (isothermal) at different frequencies (from 0.1Hz to 300,000 Hz).

In our analysis we have studied both scanning and isothermal dielectric analysis. In scanning we have heated the sample to 30°C above the melting point and in isothermal we have selected three temperature regions, two in the solid state before melt and one after melt.

1. solid state 60-70°C below the melt temperature (below pre-melt)
2. solid state 20-30°C below the melt temperature (pre-melt)
3. liquid state 10°C above melt temperature (melt)

For all the studies the instrument was supplied with nitrogen gas in order to maintain inertness in the chamber and the heating rate as 10°C/min.

Figure 4.1. Single surface gold plated ceramic sensor

Capacitance and conductance are two major fundamental electrical properties measured by dielectric analyzer with respect to temperature, time and frequency. These two properties mentioned above are contradictory to each other conductance is related to the ability of a sample to transfer electrical energy whereas capacitance is related to the ability to store the electrical energy. Ionic conductivity is associated with the viscosity of the sample because fluidity is identified by the ease with which ionic components can
migrate through the sample under the applied electric field. The measured current is divided into capacitance and conductance. Capacitance and conductance are related to the molecular mobility of a sample which in turn gives us chemical and rheological information like polarization time, molecular movement in drugs and polymers.

Under an applied field the dipoles will orient in the direction of the field and the time taken to align is the polarization or relaxation time. DEA can measure a polarization response in an AC electric field at iso thermal temperatures or by scanning temperature technique. A Debye plot of tan-delta, a ratio of dielectric loss divided by the relative permittivity, versus frequency can fix the limits of a polarization time. The critical peak frequency in tan-delta peaks is inversely proportional to polarization time. The peak frequency is converted into polarization time with the following equation 1 (6, 10):

\[ \mu = \frac{1}{2} \pi Fc \times 1000 \text{ (millisecond)} \]  

(\text{eq : 1})

| \( \mu \) | Polarization time (millisecond) |
| \( Fc \) | Tan Delta Peak Frequency (Hz) |
| \( \pi \) | 3.14 |

4.3 Results and Discussions

Most of the samples, e.g. amino acids, carbohydrates, polar polymers, polar drugs and excipients in the pre-melt temperature region which is below the melt temperature in the solid state exhibit unique variations in electric conductivity behavior (2, 3, 7). That is, a rapid linear rise in ionic conductivity vs. temperature below the DSC melts temperature at a variety of frequencies that have an \( R^2 \) of 0.99. Results from scanning dielectric spectroscopy clearly show the conductivity in the pre-melt is increasing to \( 10^4 \) to \( 10^6 \) pS.cm\(^{-1}\). Isothermal dielectric spectroscopy gives us additional information behind this unique variation in conductivity. Acetophenididine an API in figure 2 shows the above
The mentioned increase in conductivity in the solid state premelt temperature region at 30°C below the DSC Tm melt temperature. Figure 2 is an overlay of DSC and DEA which shows the melting of Acetophenitidine at 135-137°C and DEA at a number of frequencies (8.0 to 30,000Hz) which shows the conductivity variations with temperature. The conductivity rises linearly at 101°C which is considerably below the melt temperature and establishes the sample is still solid. The conductivity after melt continues to increase and is constant if the sample is heated well into the liquid phase.

**Figure 4.2.** DEA (green) and DSC (blue) overlay of Acetophenitidine showing unusual conductivity in pre-melt.

There are also chemicals like tetracosane (C_{24}H_{50}), pentacosane (C_{25}H_{52}), LDPE, and HDPE which do not show this variation in their premelt temperature region. Isothermal dielectric studies also demonstrated the cause and affect of the polar variations. Figure 3 which is an overlay of LDPE DSC and DEA is a clear difference from figure 2 which shows linear variations below the melt. In figure 3 you see that the
linear increase in conductivity is after melt temperature of polyethylene which is seen in
the DSC curve at 128-133°C. The cause for the increase after melt is the sample
transitions from solid to liquid and conductivity is significantly higher in the liquid phase.

We have also studied the effect of moisture in the sample which is known to
increase conductivity in the sample. The conductivity variations in Sulphapyridine were
measured during the premelt. It exhibited changes in conductivity at 170°C and the melt
temperature of this sample is 191°C. A second run was made but this time same amount
of sample was used but this sample was heated to 130°C and was maintained at that
temperature for about an hour which insures that the moisture, if any present in the
sample, would be removed and then again heated to above melt temperature. But this did
not affect the unusual behavior of conductivity in pre-melt region. Figure 4 shows the
overlay of both studies which yielded that the Sulphapyridine sample which heated at
130°C for one hour showed conductivity variations at 153°C which was less than melt temperature.

Figure 4.4. DEA (red heated to remove moisture, green as sampled) and DSC (blue as sampled) overlay of Sulphapyridine showing the limited affect of moisture on the pre-melt.

Next studied was the effect of impurities in the sample if any were present. ASTM method E928 was used to determine the purity of samples. Purity test is based on the Van’t Hoff melting point depression equation (11, 12).

\[ T_s = T_o - \left( \frac{R \cdot T_m \cdot 2\chi}{\Delta H \cdot F} \right) \]

(eq: 2)

\( T_s \) = Specimen temperature,
\( T_m \) = Melting temperature of 100% pure material,
\( R \) = Gas constant (8.314 J mol\(^{-1}\) K\(^{-1}\)),
\( \chi \) = Mole fraction of impurity,
\( \Delta H \) = Heat of fusion J mol\(^{-1}\),
\( F \) = Fraction melted.
All the samples studied were checked by this DSC ASTM method which showed a 99.97% purity or greater, for Acetophenitidine, Sulphapyridine and Acetanilide. Acetophenitidine is shown in figure 5.

![DSC of Acetophenitidine showing the purity of sample](image)

**Figure 4.5.** DSC of Acetophenitidine showing the purity of sample

Studying the tan delta property by isothermal dielectric spectroscopy gave us a clearer understanding behind the variations occurring in the premelt temperature region. Tan delta is a property which can be used to calculate the polarization times (eq: 1). When a sample is placed in an electric field usually the dipoles in it are aligned in the direction of the applied field and the time taken for this alignment is the polarization time. We have studied polarization times in three different regions: two in the solid state (below pre-melt and in pre-melt) and one in the liquid state (melt). Figure 6 is an overlay of tan delta peaks of acetanilide in below premelt, premelt and melt region. Acetanilide below pre-melt temperature at 60°C does not have any peak which one concludes that there is no polarization in this region whereas in the premelt we see a peak at 0.5 Hz,
converting this to a polarization time it is 318 milliseconds. Acetanilide in the melt has a peak at 9300 Hz which is at a higher frequency than the previous one and calculating the polarization time it is $1.7 \times 10^{-2}$ milliseconds. The latter is less than the premelt polarization time. The sample is in the liquid state and polarizes faster with higher mobility than in the solid state.

The isothermal DEA of a drug salt, Lidocaine.HCl, was evaluated for the presence of its polarization time (fig 7). This chemical polarization time was critically determined at 35, 65 and 85°C. The melting temperature of Lidocaine.HCl was 72–74°C by DSC. The DEA isothermal temperature below the melt by 43°C was 35°C and no peaks were observed for the Tan Delta ($\varepsilon''/\varepsilon'$) plot vs. log frequency in Hz. The DEA at 65°C (9°C below the melt) yielded a polarization time of 159 ms in the premelt temperature range. The polarization time at 85°C or 11°C above the Tm was 0.031 ms and 0.0032 ms for two tan delta peaks. Therefore, the Tan Delta plots for a non-salt and a drug salt were similar.

**Figure 4.6.** Overlay of DEA tan-delta peaks of acetanilide at 60°C (below pre-melt), 100°C (pre-melt) and 125°C (after melt).
Figure 4.7. Overlay of DEA tan-delta peaks of lidocaine HCL at 35°C (below pre-melt), 65°C (pre-melt) and 85°C (after melt).

We have studied LDPE and other samples which did not show rise in conductivity in premelt region. Studies reveal that these do not have any tan delta peaks in all three phases we have studied below premelt, pre-melt and melt region see figure 8.

Figure 4.8. Overlay of DEA tan-delta values of polyethylene at 100°C (pre-melt) and 140°C (melt).
4.4 Conclusions

Results obtained from scanning and isothermal DEA confirm that there are electrical variations in the premelt temperature range. We observed unique dielectric viscoelastic properties in a variety of chemicals like carbohydrates, amino acids, polar polymers, APIs, and organics. Our studies have shown that non-polar polymers and long chain alkanes do not exhibit the ionic conductivity premelt behavior. However polar polymers do exhibit conductivity changes in the pre-melt temperature range. Dipoles in the samples respond to an electric field and mechanical forces. Under an applied field the dipoles will orient in the direction of the field and subsequent measurement of conductivity should theoretically prove presence of dipoles. This alignment of dipoles leads to a non-adiabatic hopping charge carriers in the material which yields an increase of the conductivity (Polaron theory of conduction i.e. the hopping model) (13, 14).

Comparing the results obtained from DEA screening of Sulfapyridine and heated Sulfapyridine (with out moisture), we do see a rise in ionic conductivity before melting which implies that the same effect is not due to moisture. Calorimetric purity analysis showed that the variations in conductivity are not due to impurities as all the chemicals were 99.97% or higher purity.

4.5 References


CHAPTER V
CHARACTERIZATION OF MORPHOLOGICAL CHANGES IN THE CHEMICAL PRE-MELT BY SEM TECHNIQUES

5.1 Abstract

Detailed morphology of a number of solid chemicals was studied by scanning electron microscopy (SEM). A closer evaluation of the solid chemical was viewed at 50 to 800 X magnification. All of the specimens were gold sputter coated because of the particle charging i.e. they were conducting. The morphology of the specimen was viewed in three different samplings: As-received chemical at room temperature; chemicals examined by DEA through the pre-melt by applying an electrical field; and chemical heat treated external to the DEA (no electrical field). The SEM evaluation includes a number of drugs, amino acids, and carbohydrates but reported here are model examples of two drugs, acetophenitidine and sulphapyridine as well as an amino acid, L-arginine. The samples heat treated along with the applied AC dielectric field in the DEA system produced various morphological changes like softening of the crystal edges (acetophenitidine), brittle fracturing of crystal edges producing distinct smaller crystals (sulphapyridine), and enhanced fractures and brittleness in the solid crystals (L-arginine). Samples heat treated to the same temperature range without the electrical field did not
exhibit these morphological changes. These mechanical changes in the crystal form as viewed by SEM are co-relatable with the enhanced electrical conductivity observed by DEA prior to the melting in the solid state.

5.2 Introduction

Implementation of SEM images has greatly aided the interpretation of the premelt properties. One has to view the DEA conductivity profile followed by the SEM to understand what is happening in the premelt temperature range, 20-30°C below the melting temperatures as determined by DSC. In general the onset of the premelt change is identified as the rapid rise of the conductivity vs. the temperature (fig 1, 2,). As you will notice in all three of these DEA curves with temperature there is a flat conductivity region and entering the premelt temperature region material rises in conductivity linearly with temperature, conductivity vs. temperature with the $r^2$ equal to 0.99 (1). The melt temperature by DEA and DSC is obvious where the material endothermically melts has a melt temperature, a peak melting temperature and a heat of fusion. The melt in DEA instrumentation is evaluated while the material undergoes a rapid increase in conductivity with temperature through the premelt and the melt in to the amorphous phase. The latter for most polar materials undergoes a conductivity variation to ultimately $10^5$ pS/cm.
Figure 5.1. Sulphapyridine DEA and DSC overlay showing premelt conductivity behavior at 170 to 180°C

Figure 5.2. DEA and DSC overlay of Acetophenitidine showing premelt conductivity behavior at 101 to 115°C
5.3 Methodology

The samples for this study were analyzed by a DEA 2970 by TAI and an Amray SEM 1820. The three samples employed to study are:

1. Chemical as received from a commercial company.
2. Chemical heat treated with an applied AC dielectric field on DEA.
3. Chemical heat treated without a dielectric field.

The DEA experimental conditions to prepare the SEM samples were, as cited before in chapter 2. The samples were heated to premelt temperature range which is 10-30°C below the DSC melt temperature, and were isothermally maintained at premelt temperatures for 30 minutes (Tm extrapolated onset and Tmp peak melting temperature or Tm-Tmp). Another set of samples were heated in the DSC isothermally for 30 minutes at a premelt temperature which was also used in the DEA (no dielectric field is applied in this instrument).

In analysis of samples by SEM the pedestal was coated with silver paint to insure adhesion of the sample to the pedestal. The sample was later sprinkled on the pedestal and allowed to dry for 5 minutes. This drying step was followed by sputter coating with gold. The coating was 4000 angstroms thick this application avoided sample charging.

5.4 Results and Discussions

Comparing the morphology of chemicals (drugs and amino acids) under SEM protocols produced insight into the effect of the dielectric field in the premelt temperature range of the samples studied. Sulphapyridine was examined as-received for the first case. Cited below are figures 3 and 4 describing the nature of the sulphapyridine crystals which are about 75000 to 5200µ² with a smooth surface texture.
Figure 5.3. As received sulphapyridine crystal in SEM at magnification 745.

Figure 5.4. As received sulphapyridine crystal at magnification 292.
Figures 5 and 6 were sulphapyridine crystals exposed to a dielectric field which were heated to the premelt temperature range in the DEA. The large crystal evident in the image was rectangular or square with a smooth surface about 80,000 to 4600μ². The crystal images also exhibit smaller crystals with an area of 1000 to 130μ². The interpretation of the origin of the smaller crystals is due to the dielectric field in the premelt. The larger crystals have brittle fractures probably on the high field edges producing the smaller fragments. It is apparent that brittle failure occurred under DEA sensitized conditions. The mechanical change is due to the electric field and not only heating in the DEA.

Since conductivity is based on pS per unit area and the area has definitively changed the conductivity would result in a dramatic increase observed by the DEA scan.

Figure 5.5. Sulphapyridine crystal heat treated on DEA to pre-melt temperature range 175°C at magnification 183
Figures 7 and 8 are images of sulphapyridine heated on a hot plate at pre-melt temperature for 30 minutes. The sulphapyridine crystals show similar size and surface which was seen in the as-received crystals. We did not observe any 1000 to 200 $\mu^2$ crystals that were evident due to the applied dielectric field below the melting in solid state.
Figure 5.7. Sulphapyridine crystal from sample which was heat treated on a hot plate without the electrical field at magnification 180.

Figure 5.8. Sulphapyridine crystal from sample which was heat treated on hot plate without electrical field at magnification 170.
Figures 9 and 10 represent L arginine crystals from as received sample which varies from are 138,000 to 20,000µ² with a surface texture on some of the crystals and a few crystals have a smooth surface texture. There are a few surface elevations observed in most of the crystals.

Figure 5.9. As received L-arginine SEM crystals at magnification 100.
Figure 11 and 12 are images of L-arginine which is heated to its pre-melt temperature range in the DEA with an applied electric field. The crystals in the DEA treated samples have shown clear changes from the above non treated sample. The crystals here show clear cracks on the surface which are not visible in the regular sample and also very small crystals are observed which might be due to brittleness in the solid exposed to the DEA. These small crystals may be the edges of crystals. We have also seen softening of a few crystals as seen in figure 10.
Figure 5.11. Crystals from sample L-arginine heated to pre-melt (215°C) on DEA at magnification 130.

Figure 5.12. Crystals from sample L-arginine heated to pre-melt (215°C) on DEA at magnification 110.
Figures 13 and 14 are images of acetophenitidine as received and at lower magnification in figure 13 we see crystal structures and clusters of small crystals. Figure 14 at a higher magnification when compared to figure 13 show a variety of crystal shapes and structures.

**Figure 5.13.** As received acetophenitidine crystals at lower magnification of 46

**Figure 5.14.** As received acetophenitidine crystals at magnification 226.
Crystals in figures 15 and 16 are an acetophenitidine sample which was exposed to the dielectric field and heat on the DEA. These images clearly show the softening of the crystals on the edges.

**Figure 5.15.** Crystal of acetophenitidine sample heated on DEA to pre-melt temperature 110°C at magnification 378

**Figure 5.16.** Crystal of acetophenitidine sample heated on DEA to pre-melt temperature 110°C at magnification 605
5.5 Conclusions

From the above obtained results we clearly see that applying the dielectric field to the sample has lead to morphological changes in the crystal structure in the pre-melt temperature range. Morphological changes in the crystal structure are correlated to the conductivity variations in the pre-melt temperature range observed in the DEA. Along with polarization of particles in the pre-melt temperature range morphological changes may also lead to variations in conductivity. Britteness in the sample which leads to smaller crystals will have a higher conductivity as conductivity is inversely proportional to area. Softening of the material will lead to higher molecular mobility which aids in polarization as measured by DEA. The softening of the compounds is also seen in the analysis by TMA as shown in figure 9 in chapter II (2).

It is our interpretation that qualitatively the nature of the morphological changes recorded by the SEM is related to the mechanical properties of the crystals and their particle size.

Table 1

<table>
<thead>
<tr>
<th>Sample</th>
<th>Pre-melt temp (°C)</th>
<th>Crystal size (µm²)</th>
<th>Tg (°C)</th>
<th>Tm (°C)</th>
<th>Observed affect by DEA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetophenitidine</td>
<td>100 – 110</td>
<td>2000-2000</td>
<td>1-10</td>
<td>133</td>
<td>Softening of crystals, edges rounded</td>
</tr>
<tr>
<td>Sulphapyridine</td>
<td>170 – 180</td>
<td>70,000-5000</td>
<td>50-60</td>
<td>192</td>
<td>Edges brittle and fracture</td>
</tr>
<tr>
<td>L-Arginine</td>
<td>210- 220</td>
<td>138,000 – 20,000</td>
<td>65-70</td>
<td>238</td>
<td>Cracks in the crystal, fracture of edges, and softening</td>
</tr>
</tbody>
</table>
As the apparent Tg and the crystal size increase more significant mechanical changes are noted by SEM.

5.6 References


CHAPTER VI
APPLICATIONS OF DIELECTRICS IN PREMELT TEMPERATURE RANGE

6.1 Introduction to Synthesis in Dielectric Field

Application of the novel dielectric properties in the premelt to modify chemical entities studied in this project or to synthesize new compounds or to enhance the chemical reaction rate. We have investigated the premelt properties with a wider range of compounds. These compounds include additional solid drugs, organics, carbohydrates and amino acids. Application of the proposed scheme in characterizing additional solids will lead to validation and establishment of statistical data with other methods. A preliminary reaction of an organic acid and base chemical in the premelt temperature range with and without the DEA electric field should reveal the affect of DEA on the reactivity of these two chemicals. DSC, FTIR, and other microscopic instrumentations will be used to characterize any new reaction products. It is anticipated that the reaction rate of these two chemicals will be enhanced while under the influence of the AC dielectric field. Next, investigate the premelt properties in a wider range of compounds. These additional compounds include: amino acids, and additional carbohydrates as well as, organics containing polar functional groups. Application of the proposed scheme in
characterizing additional solids will lead to validation and establishment of statistical established data with other methods.

6.1.1 Acid Base Reaction

6.1.1.1 Methodology. A calibrated DSC will be used to check the heat flow properties in samples. Measured amounts of samples will be taken separately to examine them by DSC, and later the reactants will be blended together to evaluate their heat flow properties. The DEA was initially calibrated according to the instrument fixtures; later the single surface gold ceramic sensor will be calibrated to assure that it is clean reacting surface. Reactants will be measured and analyzed separately on DEA to check their electrical properties. Later selected reactants will be measured in calculated amounts and spread over the calibrated electrode to run the experiment. Reactants will be heated to an appropriate temperature so that at least one of the reactant reaches its premelt exited state. After completion of DEA the sample from the sensor will be examined by the DSC to compare the difference between heating the reactants in an aluminum pan and heating them on a DEA gold electrode at various frequencies and in an electric field. Verification of the reaction between the organic acid and base will be accomplished by synthesizing the proposed reaction in a standard organic synthesis manner.

6.1.1.2 Results and Discussions. Preliminary reaction of acetanilide and benzoic acid on a DEA revealed a new product being formed at 68-70°C by DSC (fig: 23, 24, 25) (note the Tm of acetanilide is 116°C and benzoic acid 122°C) with an enhanced dielectric reaction rate (conductivity per temperature, pS/cm°C) based on a specific frequency range from 10 to 50 Hz. The DSC test results confirm the formation of a new product or a
eutectic with a significantly lower, melt temperature than either reactant. The formation of a eutectic based on its solid state dielectric properties is probable.

Figure 6.1. Heat-Cool run of Benzoic acid on DSC.

Figure 6.2. Heat-Cool-Heat run of Acetanilide on DSC
6.1.3 Conclusions. From the results obtained through DEA we have observed that heating both the reactants on the gold interdigitated electrode with an applied voltage and frequency gave a lower melting compound which had a higher ionic conductivity. The result from DSC indicates that the material became more amorphous by heating it in a dielectric analysis system. More testing of the reactants and products should be done on IR, GCMS, and NMR to confirm the state of the end product. Proof of our hypothesis, electric field will produce varying materials will be obtained by instrumental analysis.

6.1.2 Dimer synthesis.

6.1.2.1 Introduction to dimer synthesis. Anthracene and substituted anthracene like 9-methyl anthracene, 9-cayano anthracene were used for dimer synthesis. When anthracene in solvent is exposed to UV light for a period of time dimers are formed as seen below (1).
6.1.2.2 Methodology. Benzene was used as a solvent to dissolve anthracene or substituted anthracene whose dimers were desired to be synthesized. Later the solvent was taken in a test tube and exposed to UV light. The solution was purged with nitrogen gas in order to keep an inert atmosphere. As the solvent was exposed to UV light for some time a thin layer of dimers were formed on the surface of the test tube or deposited in the bottom as the dimers do not dissolve in benzene. Later the deposited dimers were collected and purified by washing them with benzene several times.

Anthracene or substituted anthracene were taken as powders and placed on the DEA parallel plate sensor which was heated to its premelt temperature and the ran isothermally for about 30 min but this did not synthesized any dimers.

6.1.2.3 Results and discussions. The prepared dimers of anthracene or substituted anthracene were analyzed by color and their melting points. Usually dimers have a higher melting point than their monomers. Figure 5 and 6 shows the DSC curves of anthracene and anthracene dimers where dimers melt at about 264°C which is 40°C higher than anthracene melting which is 220°C.
Figure 6.5. Anthracene DSC curve showing peak at 220°C

Figure 6.6. Anthracene dimers DSC showing melting at 263°C
Synthesis for substituted anthracene dimers is similar to anthracene. But it is difficult to obtain a pure compound because of the substituted group on the 9\textsuperscript{th} position which causes strain in the compound and it tends to go back to the monomer form. This can be clearly differentiated by color. Figures 7 and 8 show microscopic figures where dimers are white in color whereas monomer of 9 methyl anthracene is yellow in color.

![Figure 6.7: Microscopic image of 9-methyl Anthracene as received (yellow)](image)

![Figure 6.8: Microscopic image of 9-methyl Anthracene dimer (white).](image)
6.1.2.4 Conclusions. Our aim was to prepare dimers using dielectrics in the premelt we have tried to make dimers of anthracene and substituted anthracene by providing the energy in form of dielectrics in the pre-melt. But this did not enough created dimers in both the cases. Our conclusion is that the energy provided in form of AC frequency is not sufficient ot make dimers in anthracene.

6.1.3 Maillard reaction

6.1.3.1 Introduction to Maillard reaction. Maillard reaction is a non enzymatic thermal reaction. This food reaction is a complex series of reactions between reducing sugars and amino acids. It is mostly seen in all kinds of foods when heated. The Maillard reaction is responsible for the color, flavor and nutritive nature in the food. When the food is heated reducing sugars break and the aldehyde group in the sugar reacts with the amino acid to give brown color (2). Every food has different kinds of sugars and amino acids which react to give a unique flavor to the food when heated. In order to see a maillard reaction food or samples should be heated above 154°C.

6.1.3.2 Methodology and results. Our aim was to see if we could enhance the Maillard reaction using Dielectrics in the pre-melt temperature range. We have selected lactose which is a reducing sugar and amino acid selected was L-arginine which is a non essential amino acid which is most commonly found in the diet. Both samples are white in color and melt above 200°C. Certain factors were considered in selecting these two samples as they both show premelt behavior at around 170°C. This would be an ideal temperature to reach the premelt of both the samples. Both the samples were taken in 1:1 ratio and heated for an hour on hot plate and on DEA (where AC electric field is applied)
at a temperature of 170°C. Later the change in color has been compared for both samples which are shown below in figures 9, 10 and 11.

Figure 6. 9. Mixture of L-arginine & lactose

Figure 6. 10. Mixture of L-arginine and lactose after heating for 30 min on hotplate
6.1.3.3 Conclusions. Comparing the color changes in both DEA heated and hot plate heated samples it clearly shows that dielectrics can be used to enhance the maillard reaction. As seen in the SEM images of L-arginine and other chemicals in chapter V, DEA is leading to morphological changes in premelt which may be a reason to enhance the reaction. We did not find success in other reactions as the energy provided was not sufficient to enhance reaction rate or for the reaction to occur.

6.2 Characterization of Human Cytokines and Their Receptors for Sensor Development

6.2.1 Introduction. Thermal analytical techniques like DEA, DSC, and TGA were employed in characterizing human cytokines and their receptors which are related to the disease malaria. Cytokines and cytokine receptors serve as important protein...
biomarkers for chronic and infectious diseases (3). Cytokines are small regulatory proteins secreted by immune system they participate in anti-inflammatory and pro-inflammatory process. We have detected a unique dielectric signature pattern by DEA for all cytokines which will aid in sensor development. Malaria affects over 500 million people worldwide leading to 1 to 2 million deaths each year. Proper diagnosis of infection will result in reduction of morbidity, mortality, and of drug resistant parasites (4).

Properties like permittivity and loss factor were considered to study the molecular mobility in cytokines. Debye plots were used to study the polarization times in cytokines and receptors. These above mentioned properties aided in identifying signature patterns in all the cytokines studied (5).

6.2.2 Methodology. In an ongoing series of studies to understand the thermal analytical behavior of cytokines and their receptors the cytokines IFNγ, TNFα, IL-1α, IL-2, IL-4, IL-6, IL-10 and soluble receptors and antagonists TNF I, TNF II, IL-1Ra, and sIL-2α receptor were analyzed. DEA 2970, DSC 2920, and TG 2950 by TAI were employed for the analysis and characterization of the cytokines. DEA was performed at wide range frequencies of 0.1 Hz to 300,000 Hz at 37°C to study their behavior at human body temperature. Cytokines were also heated to 250°C to study the interference associated with protein. In TG cytokines and cytokine receptors were studied at 37°C for an hour and later were heated to 150°C. The cytokines were held at 37°C to ensure the reaction and were heated to 150°C to study the mass loss in cytokines. A 100% water loss was obtained for all samples by TG. In DSC the samples were studied by cooling the sample to -40°C from room temperature and then heating it to 125°C. DSC was
employed to characterize the thermal behaviors such as melting, crystallization properties and oxidative behavior.

In all the instrumentations sample size was from 5-40 µl, heating rate was 5ºC/min or 10ºC/min as required and instruments were purged with nitrogen gas during analyses to ensure an inert atmosphere.

**6.2.3 Results.** DEA was used to produce the dielectric spectrum of each cytokine. The spectra are distinguishable for each cytokine. Given below figure 12, 13 and 14 showing how cytokines can be categorized depending on distribution of peaks, charge obtained and frequency level.

![Debye plot](image)

**Figure 6.12.** IL-10 Debye plot (tan vs. log frequency), exhibiting several peak frequencies
A trend analysis was conducted using the values calculated by the DSC versus water analysis to observe any outliers to serve as another way to profile the cytokines. Given below is figure 15 which is an overlay of DSC curves of cytokines IL-4, 6 and 10.
We did not observe any minimum heat value for cytokines, but moderate heat of crystallization. This results differentiated cytokines into two classes IL-1 and 2 showed similar results which were different from IL-4,6 and 10.

![DSC overlay of IL-4 (blue, dotted line), IL-6 (green, solid line), & IL-10 (red, hatched line).](image)

**6.2.4 Conclusion.** DETA, TG, and DSC can be used to develop differentiating dielectric signatures for human cytokines based on the hydration pattern of the proteins. The temperature, level of conductivity, and peak frequencies determine the strength of interaction between proteins and the different types of water. As observed, intrinsic dielectric properties distinguish protein structure. The cytokines follow the trend from earlier studies conducted on other cytokines associated with malaria and differ the same way from the soluble cytokine receptors. Overall, the data show that distinct and reproducible differences in dielectric spectra can be obtained by analyzing proteins in
solution. Such differences will be crucial in the sensitivity and specificity of sensors designed for malaria diagnosis.

### 6.3 Dielectrics for Drug Delivery

#### 6.3.1 Introduction to drug delivery by dielectrics.

This study encompasses the significance of dielectrics in characterization and evaluation of drugs which further aid in drug delivery. We have driven commercially available drugs through a wide range of biological membranes in-vitro by macroesis (delivering large molecular size drugs using an AC electric field). Isothermal and scanning dielectric analysis as function of frequency, temperature and time was used to study the molecular mobility and transport properties. This new technique of the drug delivery system enhances benefits over systemic oral therapies, in which clinically sufficient quantities of the active ingredient do not reach the intended target organ and/or use of the drugs result in serious side effects. An optimally-tuned low-voltage applied AC electrical field has been found capable of inducing polarization and delivering micro and macromolecules through a biological membrane. The relationships between factors such as delivery time, AC voltage amplitude and frequency, and transported drug concentration were investigated. The throughput of the drug was confirmed by analytical techniques like ELISA, UV analysis, atomic force microscopy, dental spectrophotometer.

#### 6.3.2 Methodology.

We have worked with various biological membranes like the pig skin, enamel from human and bovine teeth, and keratin tissue from cow hoof. Our study was to study the variables like polarization frequency, and time by DEA which aid in drug delivery. Later we analyze the drug through put and employ modification to the study to enhance the throughput of the drug.
Horse radish peroxidase (HRP) a macromolecule was used to deliver through the pig skin. HRP was the choice of drug as 90% of drugs available commercially have a smaller particle size when compared to HRP. Our next study was to deliver carbamide peroxide into human teeth which aids in teeth whitening. We have also conducted studies to deliver fluoride into human teeth which aids in mineralization of calcium and strengths the teeth.

ELISA was performed to study the throughput of HRP through pig skin tissue. Dental spectrophotometry was used to study the carbamide peroxide penetration into human teeth whitening. XRF spectrophotometry was used to determine the penetration depth of fluoride in cow teeth.

A lyophilized HRP powder was dissolved in a required amount of water for the study. 35% Carbamide peroxide gel was used in teeth whitening and 1% pedia fluoride gel was used for fluoride delivery into teeth. DEA analysis was made on all to be used samples for drug delivery. Debye plots from DEA analysis gave us the polarization frequencies which were further used in the drug delivery process.

**6.3.3 Results and conclusions.** In the delivery of HRP through the pig skin we have measured a bimodal reproducible through put of 63% and 92% by UV analysis and verified by ELISA testing. For teeth whitening we have improved whitening of teeth by 2 shades which were tested by a dental spectrophotometer and in delivering fluoride we have pushed fluoride to a 200µ depth.

From the above obtained results we have concluded that macroesis can be developed for drug delivery through biological membranes.
6.4 References


CHAPTER VII

FUTURE DIRECTIONS

- Future directions for my thesis would include studying more about morphological changes in the pre-melt.
- Study how the crystal structure changes in the pre-melt by X-Ray diffraction.
- Studies on cytokines and peptides should be made to develop a signature pattern for biological compounds which will further help in rapid and ease of disease diagnosis.
- There are more thermal reactions like Maillard reaction e.g. carmallization which might be enhanced using dielectrics.
- As the energy we have provided using DEA is not sufficient for several reactions modifying the instrumentation to apply higher frequency AC field which yields higher energy may enhance the reaction in the solid state.
- Linear and prominent increase in conductivity in the solid state can be used in developing novel technologies.
• Other electrochemical instrumentations can be used to correlate the changes seen by DEA, TMA and SEM.