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# Effects of thermal history on solid state and melting behavior of amino acids

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**Abstract** Dielectric Thermal Analysis (DETA) of drugs, proteins and amino acids reveals a strongly linear conductivity increase prior to and peaking at the melt, associated with dielectric viscoelastic properties of the material. Premelt onset and peak are shown to depend on thermal history. Comparisons of neat amino acid samples to samples heated to 150 °C; dried in a desiccator; or heated above their melting point and cooled show significant premelt and melt shifts. Melts are also correlated with phase transitions observed by Differential Scanning Calorimetry (DSC). Activation energies attributed to charging in the premelt for amino acids were typically 250 J/mole.

**Keywords** Activation energy · Amino acids · Dielectric thermal analysis (DETA) · Differential scanning calorimetry (DSC) · Phase transitions

## Introduction

Thermal analysis techniques have long been noted for their ability to detect and characterize phase transitions in materials from polymers to proteins. Differential Scanning Calorimetry (DSC) and Thermogravimetry (TG) are particularly useful in measuring these transitions and developing an elemental analysis of a compound of interest. Recent work by Riga et al. shows that Dielectic Thermal Analysis (DETA) can also be used to measure changes in a material due to its phase transitions. Dielectric Thermal Analysis (DETA) of drugs, proteins and amino acids reveals a strongly linear conductivity increase prior to the melt, peaking at the melt, associated with the dielectric viscoelastic properties of the material [1–3].

Anthracene, a highly conjugated fused aromatic ring system with some noteworthy properties in its solid state, premelt temperatures, and through its solid-liquid transition, provides an understanding of these premelt properties as a dielectric viscoelastic process. Anthracene has been observed to form excimers, excited molecules such as dimers, in the solid state and to continue this formation into the amorphous liquid phase. Examination of Anthracene by DETA also discovered a linear electrical conductivity in the premelt temperatures through to the melt. The activation energy ($E_a$) can be calculated from the slope of plots of log conductivity vs. the reciprocal temperature in Kelvin at a cited frequency e.g. 1 or 5 Hz, which had a typical correlation coefficient of 0.999. The $E_a$ for charge formation of Anthracene below and above its melting temperature, 1100 J/mole, was frequency dependent. We further observed linear DETA electrical conductivity from below the melt temperature of a number of pure drugs and organic chemicals, including amino acids, well into their liquid amorphous phases.

The $E_a$ for the premelt charge complex for Sulfapyridine was 990 J/mole, for Acetophenetidin, 1,300 J/mole, and for caffeine, 320 J/mole. Other organic chemicals with known enhanced premelt conductivity behavior include Vanillin, Lidocaine, Acetanilide, Nifenipine and Tolbutamide plus the amino acids.

Amino acids are known to decompose before they melt, breaking into smaller molecular weight compounds, including $CO_2$. This makes their melting points difficult to state precisely, and can make interpreting thermal data related to their phase transitions a challenge [4].

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**Table 1** Amino Acids Ranked by Hydropathy, left to right, based on Kyte and Doolittle [5]

| Amino acids ranked by hydropathy | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| −4.5 | −3.9 | −3.5 | −3.5 | −3.5 | −3.5 | −3.2 | −1.6 | −1.3 | −0.9 | −0.8 | −0.7 | −0.4 | 1.8 | 1.9 | 2.5 | 2.8 | 3.8 | 4.2 | 4.5 |
| Hyrdophilic | | | | | | | | | | | | | Hydrophobic | | | | | | |
| Arg | Lys | Asn | Asp | Gln | Glu | His | Pro | Tyr | Trp | Ser | Thr | Gly | Ala | Met | Cys | Phe | Leu | Val | Ile |

The amino acids provide an interesting study in dielectric premelt behavior, both as chiral pairs (D- and L-) and as hydrophilic and hydrophobic molecular structures. Note that in this paper, the L- isomers are used as the primary data set, but the same patterns were noted in their D-isomers. In 1982, Kyte and Doolittle [5] proposed a simple, graphical system for charting the hydropathy of the amino acids, and, by extension, proteins. The acids are ranked (Table 1) from −4.5 (most hydrophilic) to +4.5 (most hydrophobic); values close to 0 have a relatively neutral hydropathy.

Studies of the most hygroscopic amino acids, such as Lysine, are always confounded by the interaction of the acid with any moisture present in the laboratory environment. Relative humidity (RH) and percent water must be noted and controlled for in any examination of the acid's properties [6]. In observing the DETA premelts of D- and L- Lysine, D- and L- Tryptophan, and D- and L- Cysteine, it became apparent that the onset and peak of the premelt are shown to be dependent not only on the moisture content, but also the thermal history of the material, including the processes used to dry hygroscopic samples.

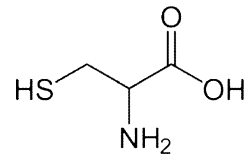


**Fig. 1** Skeletal structure of Cys

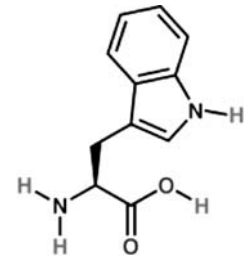


**Fig. 2** Skeletal structure of Trp

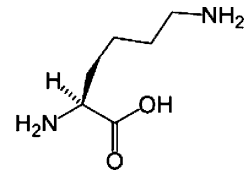


**Fig. 3** Skeletal structure of Lys

## Experimental

The experimental design involved the comparison DETA profiles of neat amino acid samples (D- and L- Cysteine, D- and L- Lysine and D- and L- Tryptophan) to samples heated to 150 °C prior to DETA, samples dried in a desiccator and samples heated above their melting point and cooled. These DETA profiles were also correlated with phase transitions observed by DSC and TG. The three amino acids were chosen to represent three different hydropathys: hydrophilic (Lysine); neutral (Tryptophan); and hydrophobic (Cysteine). They also represent three different chemical structures (Figs. 1, 2, 3).

A TA Instruments (TAI) 2970 DEA was used to determine the electrical conductivity profile of the amino acids. For each amino acid, a sample of approximately 10 mg was placed on a single surface gold ceramic interdigitated electrode in an isolated nitrogen rich dry atmosphere. The samples were ramped at a rate of 10 °C/min from room temperature (24 °C) to just above melting. Conductivity measurements were recorded at controlled interval frequencies ranging from 0.10 to 10,000 Hz for all temperatures.

A TAI 2920 DSC was used to characterize melting, and crystallization properties of the samples. Aluminum pans and lids were prepared with samples weighing between 7 and 14 mg and subjected to a cool and heat series cycling between −50 °C and 150 °C at a rate of 10 °C/min in an isolated nitrogen atmosphere. Heat flow (W/g) values versus time and temperature were generated.

A TAI 2950 TGA was used to measure the percent mass loss of the amino acids when heated to temperatures below the melting point, but above the boiling point of water. Samples were loaded into aluminum pans and heated in an

isolated nitrogen environment to 150 °C. Isothermal conditions were then maintained to ensure reaction completion.

Samples dried in a desiccator were stored under a 250 mm glass vacuum desiccator filled with desiccant for a minimum of 48 h.

## Results and discussion

The comparison of dielectric premelt behavior for the three amino acids showed a clear difference between the samples taken directly from the bottle (neat), those heated by TGA prior to dielectric analysis, and those dried in a desiccator. Although it was expected that dielectric profiles would differ based on moisture content for the more hydrophilic amino acids (e.g. Lysine), the studies also showed differences for the hydrophobic acids. In this case, the differences cannot be accounted for by moisture content but seem to be a function of the thermal history itself.

Cysteine, the most hydrophobic of the three amino acids, with a TGA-determined moisture content of less than 1 percent (Fig. 4), had the largest measured difference in premelt peak (Fig. 5). For both Cysteine and Lysine (Fig. 6), the desiccated sample peaked earliest. For Lysine, the desiccated sample was also closest to the literature melting point (224.6 versus 224.5). All the values are listed below in Table 2.

Tryptophan, the hydropathically neutral amino acid, is an interesting case because its neat and heat treated samples showed no premelt peak (Fig. 7). Absence of a premelt peak is typically associated with a greater amorphous content; in this case, it is possible that desiccation encouraged crystallization of the sample.

This brief, preliminary look at three amino acids of different hydropathy subject to three different thermal histories suggests a rich line of future inquiry into the effects of those histories on the melting behavior of the amino acids. As they are known to decompose as they melt,

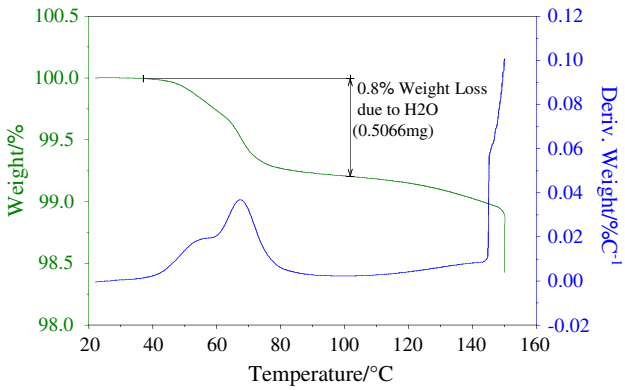


**Fig. 4** Moisture content of L-Cysteine by TGA

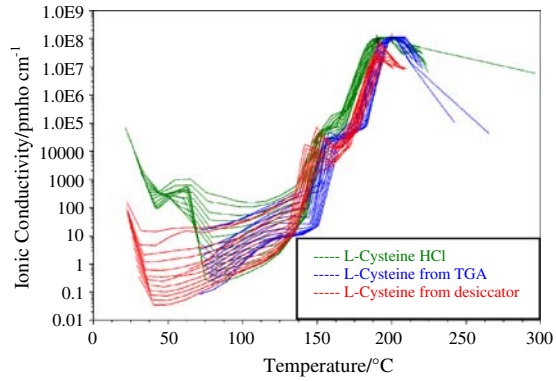


**Fig. 5** L-Cysteine comparison of premelt

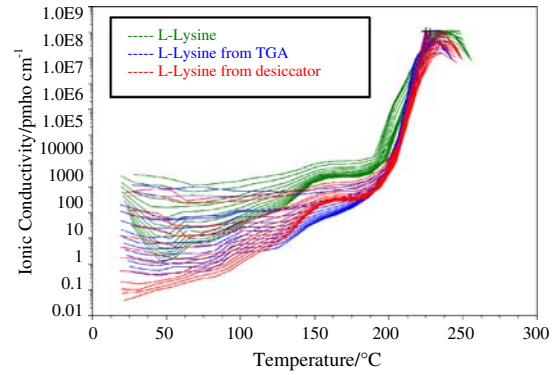


**Fig. 6** L-Lysine comparison of premelt

**Table 2** Comparison of melting points for neat, heat treated, and desiccated samples

| Amino acid | Melting Point (°C) | | | |
|---|---|---|---|---|
| | Lit. | Neat | Heated | Desiccated |
| Cysteine | 260–261 | 188.9 | 200.0 | 181.0 |
| Lysine | 224.5(d) | 227.9 | 225.3 | 224.6 |
| Tryptophan | 289(d) | N/A | N/A | 268.5 |

Note that (d) indicates a decomposition point

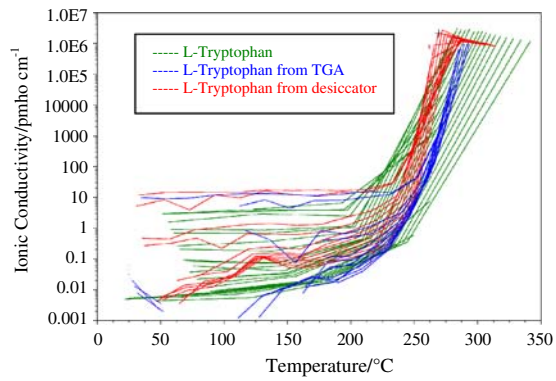


**Fig. 7** Tryptophan comparison of premelt

treatments that increase their stability at high temperatures could be valuable for laboratory studies. In particular, desiccated samples seem to yield sharper, more defines dielectric curves and may be more crystalline in content.

The study also offers additional evidence that DETA is a sensitive and significant tool [7] for analyzing phase transitions in tandem with the more traditional DSC and TGA temperature scans.

## References

1. Riga A, Alexander K. Electrical conductivity analysis/dielectric analysis differentiate physical chemical properties of drugs and excipients. Am Pharm Rev. 2006;6:45–50.
2. Riga A, Cahoon J, Pialet JW. Characterization of electrorheological processes by dielectric thermal analysis. In: Riga AT, Judovits L, editors. Materials characterization by dynamic and modulated thermal analytical techniques, ASTM STP 1402. West Conshohocken, PA: American Society for Testing and Materials; 2001. p. 139–56.
3. Cahoon J, Riga A, Lvovich V. Characterization of organic surfactants and dispersants by frequency-dependent dielectric thermal analysis and electrochemistry. In: Riga AT, Judovits L, editors. Materials characterization by dynamic and modulated thermal analytical techniques, ASTM STP 1402. West Conshohocken, PA: American Society for Testing and Materials; 2001. p. 157–73.
4. Wesolowski M, Erecińska J. Relation between chemical structure of amino acids and their thermal decomposition. J Therm Anal Calorim. 2005;82(2):307–13.
5. Kyte J, Doolittle RF. A simple method for displaying the hydropathic character of a protein. J Mol Biol. 1982;157(1):105–32.
6. Presswala L, et al. Discovery of bound and unbound waters in crystalline amino acids revealed by thermal analysis. J Therm Anal Calorim. 2008;93(1):295–300.
7. Matthews ME, et al. Dielectric classification of d- and l- amino acids by thermal and analytical methods. J Therm Anal Calorim. 2008;93(1):281–7.