FE 315 INSTRUMENTAL ANALYSIS

DIFFERENTIAL SCANNING CALORIMETER (DSC)

Differential Scanning Calorimetry (DSC) is a thermal analysis technique used to measure temperature and heat flow associated with important transitions in materials as a function of time or temperature. These measurements provide quantitative and qualitative information about physical and chemical changes that involve exothermic and endothermic processes, or changes in heat capacity. The DSC instrument works by measuring the temperature amount of heat added or extracted from a sample, in comparison to a known reference, to produce a 'thermogram' curve. In a DSC, the difference in heat flow to the sample and a reference at the same temperature, is recorded as a function of temperature or time.

Using thermal analysis, it is possible to understand what is happening in a material during heating, even if there is no visual evidence that a change has occurred. Some measurements that can be made with the DSC are:

- Glass transition temperature
- Melting point
- Crystallisation time and temperature
- Latent heat of melting
- Latent heat of crystallization
- Endothermic and exothermic natures of transitions
- Degree of crystallinity
- Phase changes
- Specific heat capacity

Schematic representation of DSC is like this:

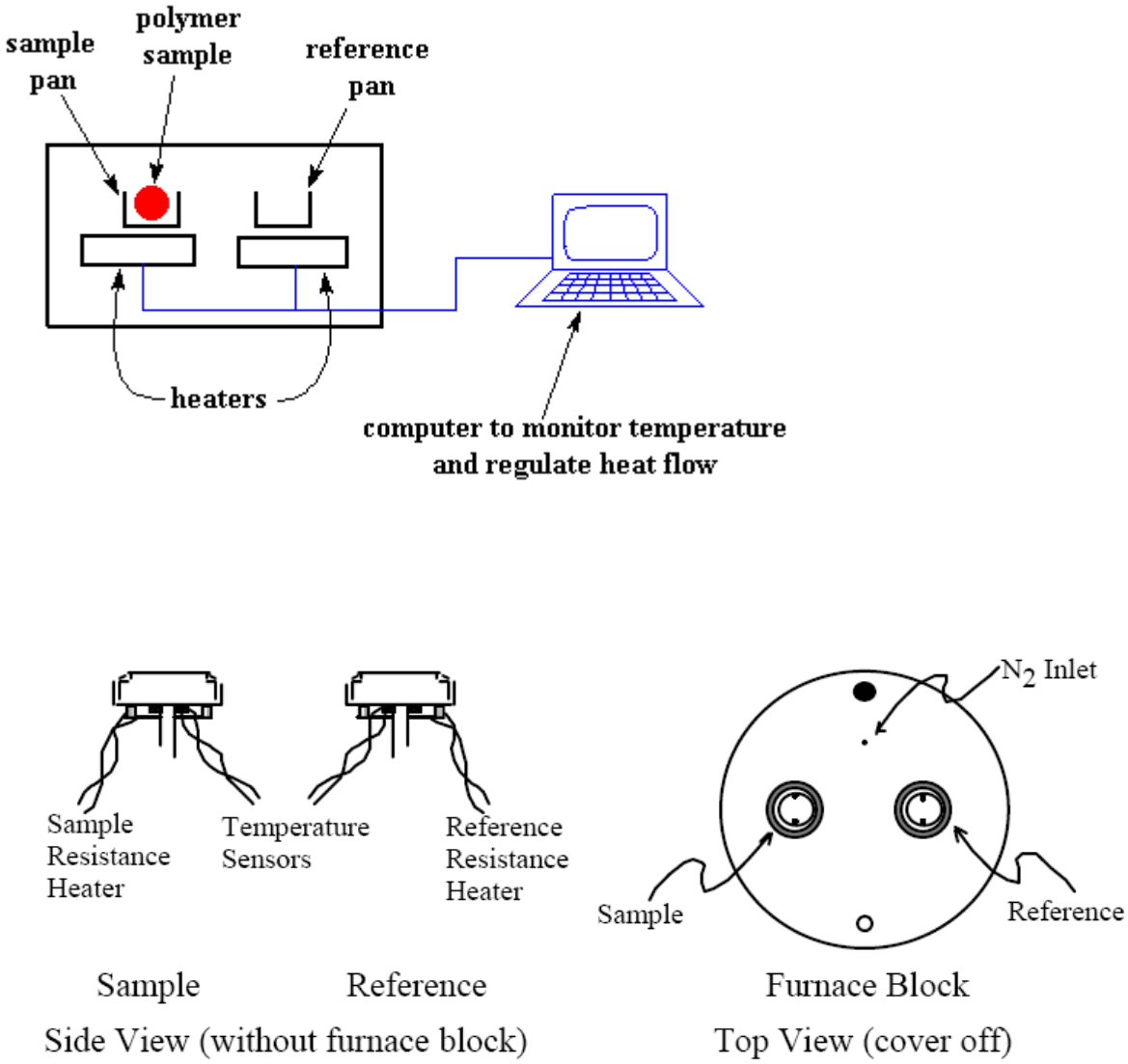

Figure 1. Schematic of DSC

There are two pans in calorimeter furnace: sample pan and the reference pan. Each pan sits on top of a heater. Each pan also has a temperature sensor that indicates what the temperatures of the pans are at any given moment. Currents are applied to the two heaters to increase the temperature at the selected rate. Computer controls the heaters, and the two pans are heated at a specific rate. The sample is sealed into a small aluminum/stainless steel/platinum pan as shown in figure 2. The reference is usually an empty pan and cover. A flow of nitrogen gas is maintained over the samples to create a reproducible and dry atmosphere. The nitrogen

atmosphere also eliminates air oxidation of the samples at high temperatures. The two separate pans, with their two separate heaters, are heated at the same rate as each other. But they do not heat at the same rate? The simple reason is that the two pans are different. One has sample in it, and one doesn't. The presence of sample means there is extra material in the sample pan. Having extra material means that it will take more heat to keep the temperature of the sample pan increasing at the same rate as the reference pan. So the heater underneath the sample pan has to work harder than the heater underneath the reference pan. It has to put out more heat.

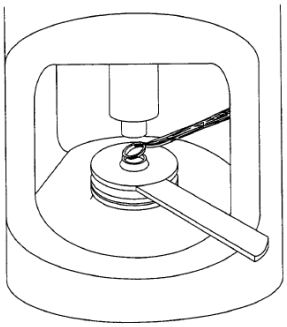

Figure 2. Inserting the DSC pan into the sealing press

The computer will plot the difference in heat output of the two heaters against temperature or time. On the *x*-axis, the temperature/time is plotted and on the *y*-axis, difference in heat output of the two heaters at a given temperature is plotted. During the heating of a sample, for example, from room temperature to its decomposition temperature, peaks with positive and negative enthalpy change (ΔH) may be recorded; each peak corresponds to a heat effect associated with a specific process, such as crystallization or melting (Fig. 3). The area under the peak is directly proportional to the enthalpic change and its direction indicates whether the thermal event is endothermic or exothermic. The heat flow difference between the sample and the reference can be either positive or negative. In an endothermic process, such as most phase transitions, heat is absorbed and, therefore, heat flow to the sample is higher than that to the reference. Hence enthalpy change is positive. Endothermic processes include protein denaturation, dehydrations, reduction reactions, and some decomposition reactions. In an exothermic process, such as crystallization, some cross-linking processes, oxidation reactions, and some decomposition reactions, enthalpy change is negative.

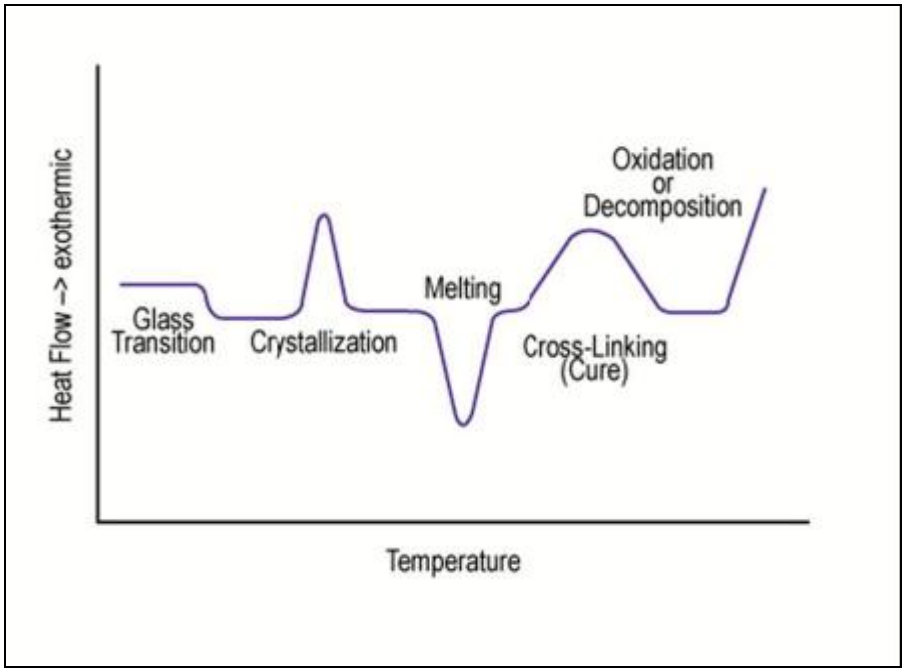

Figure 3. DSC profile

Experimental:

Purpose

The main purpose of this experiment is to learn the basic concepts of the differential scanning calorimeter, to illustrate its importance in food science and to also to determine enthalpy of denaturation and denaturation temperature of a protein sample.

Theory

For proteins, the thermally induced process detectable by DSC is the structural melting or unfolding of the molecule. The transition of protein from a native to a denatured conformation is accompanied by the rupture of inter- and intra-molecular bonds. Analysis of a DSC thermogram enables the determination of two important parameters : transition temperature peak (T_p) or maximum (T_{max}) or denaturation (T_d) temperature, and enthalpy of denaturation (ΔH). The denaturation temperatures are measures of the thermal stability of proteins, although they are influenced by the heating rate and protein concentration. The ΔH value, calculated from the area under the transition peak, is correlated with the content of ordered secondary structure of a

protein. The ΔH value is actually a net value from a combination of endothermic reactions, such as the disruption of hydrogen bonds and exothermic processes, including protein aggregation and the break up of hydrophobic interactions. Heat denaturation of small globular proteins is generally carried out under conditions preventing aggregation., i.e. dilute solution and far from the isoelectric point.

Procedure

- Dissolve a sample of powdered protein in a suitable buffer.
- Weigh an empty DSC pan
- Place dissolved protein sample into the weighed empty pan
- Weigh sample containing DSC pan and determine the amount of sample
- Seal the pan using sealing apparatus
- Place sample pan and reference pan into furnace
- Obtain a plot of heat flow vs temperature by using software programme
- Determine enthalpy of denaturation (ΔH) and denaturation temperature (T_d) from DSC curve

Note: A detailed procedure for the operation of the DSC software will be provided in the lab

Questions

1. Explain the effects of sample mass and heating rate on DSC thermograms.
2. What are the sources of errors in DSC?
3. State another technique that could be used to determine the thermal properties of materials