Critical Reports on Applied Chemistry Volume 5

Biophysical Methods in Food Research

edited by H.W.-S. Chan

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Editor's introduction

A major problem faced by the food industry is that of controlling the quality of a product. For scientists working in food research, whose objective it is to resolve this problem, there is an overriding need to relate the performance of raw materials to their composition and to processing factors. The major constituents of food are biological in origin and consist mainly of the structural and storage biopolymers, such as proteins and polysaccharides. Apart from the variability and complexity of organization that are present in a given species, most processed foods now consist of a mixture of source materials rather than a single plant or animal species. As well as being composed of chemically inhomogeneous raw materials, the resultant foodstuffs are often multiphasic. Given such complexity and the need to relate structure to function, the techniques used in analytical biochemistry, based largely on separation of soluble species, are insufficient in themselves to advance our understanding of the behaviour of food biopolymers. In recent years, the biophysical techniques used in the study of macromolecular size and dynamics, the physico-chemical methods of determining behaviour of interfaces and the physical methods developed in materials science are increasingly being used to combine with biochemical studies in food research. Such an approach is necessarily multidisciplinary and the interface of different sciences or the meeting of scientists from different backgrounds has led to advances being made by the application of a particular technique in the study of food biopolymers, in some cases for the first time.

In this volume, some of these recent developments in the use of physical and spectroscopic techniques in food research are reviewed. In most cases the techniques have been used only recently to any appreciable extent in food research. The discussion, therefore, takes the form of an introduction to a technique, followed by examples of its application to food science where this is possible. The chief aim of these reports is to generate interest in these techniques so as to widen, if possible, the scope of their application. The reader is therefore given an indication of the potential and likely future development of these methods. Because of the limitations of space, no attempt has been made to discuss all the available physical techniques in detail, nor to catalogue all the applications of a

particular technique. Within the two-dimensional matrix comprising techniques on the one hand and application to commodities on the other, the authors have selected what are, in their view, the most significant developments.

H.W.-S. Chan

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Thermoanalytical methods in food research

D.J. Wright

Notation

Abbreviations

ATP	adenosine triphosphate
DSC	differential scanning calorimetry
DTA	differential thermal analysis
DTG	derivative thermogravimetric analysis
TGA	thermogravimetric analysis
TMA	thermomechanical analysis

Symbols

a, A_o, A_T	transition areas (calories)
c_p	specific heat
C_p	heat capacity

ΔC_p^d	heat capacity change upon denaturation
ΔC_p^m	peak height at transition mid-point
ΔH_{cal}	calorimetric enthalpy (cal mole^{-1})
ΔH_f	heat of fusion
ΔH_{VH}	van't Hoff enthalpy (cal mole^{-1})
ΔT	temperature difference
$\Delta T_{1/2}$	transition width at half-height
E_A	activation energy
k	rate constant
m	sample weight
M	molecular weight
q	heat (calories)
Q	transition heat (cal g^{-1})
R	gas constant
S	calorimeter sensitivity
t	time
T	absolute temperature
T_m, T'_m, T^0_m, T_{max}, T_M	DSC transition temperatures
w	chart width
x	pen displacement
θ	programmed heating rate

1 Introduction

Throughout the food industry, heat must represent one of the commonest processing parameters used during the manufacture of food products. Four basic applications of heat can be identified although one involves heat extraction:

(1) Pasteurization/sterilization—this procedure is used to reduce the level of microorganisms present in a particular product, the aim being to increase the shelf-life. Examples include milk products and canned goods.

(2) Cooking—this operation is basically a means of imparting the desired textural attributes to a product or improving its consumer acceptability, for example, as applied to meat products.

(3) Drying—an operation to reduce the water activity of a product, again with increased shelf-life in mind. Additional benefits afforded include the increased ease of handling, lower costs of storage and transportation and easier formulation in processing. Two examples are dried egg white and soya isolate.

(4) Freezing—the application of heat extraction, generally to prolong storage life by arresting microbial growth or slowing down enzyme-mediated changes. A more specialized use is in the generation of product characteristics in the manufacture of ice cream goods.

The optimization and control of such processing operations, requires an understanding of how heat interacts with the various constituents present in the system. Apart from being expensive, the use of excessive heat can result in deleterious changes in product performance and characteristics.

All substances undergo a change or a series of changes when heated, the nature of the change(s) being dependent upon the substance in question. Thermal events include phase transitions (e.g. ice/water), conformational (state) transitions (e.g. order/disorder transitions, denaturation) and mass or compositional changes (e.g. oxidation, decomposition). In addition, heat can bring about changes in the structural or rheological properties of a material, usually as a consequence of conformational transitions. Two examples are the gelatinization of starch and the gelation of proteins. To measure and study these various thermal effects a wide range of so-called thermoanalytical techniques has developed.

2 Thermoanalytical techniques

In theory, any technique which monitors the effect of heat on a given sample parameter can be considered as belonging to the group of thermoanalytical methods. The full definition of thermal analysis as recommended by the Nomenclature Committee of the International Confederation for Thermal Analysis (ICTA) (Mackenzie 1979) is as follows:

'A group of techniques in which a physical property of a substance is measured as a function of temperature whilst the substance is subjected to a controlled temperature programme.'

In addition, the same Committee detailed a list of these thermoanalytical techniques with definitions and recommended abbreviations. Many of these techniques have only very limited application to food research and will not be considered in this article. Those which have found, or are considered to have, widest application are listed in Table 1 together with the properties monitored and the physical parameter measured. Of these, DSC and DTA have attracted most interest and will consequently form the bulk of this article.

Table 1. Thermoanalytical techniques relevant to food research

Property	Parameter	Technique
Stability	Mass	Thermogravimetric analysis (TGA)
State/Phase	Temperature	Differential thermal analysis (DTA)
	Enthalpy	Differential scanning calorimetry (DSC)
Rheological	Stress	Thermomechanical analysis (TMA)

The recent literature contains a number of review articles on thermal analysis (mainly DSC) to which the interested reader is referred for further details or clarification. Barrall and Johnson (1970), McNaughton and Mortimer (1975), and Privalov (1980) review the theory, design and operation of scanning calorimeters whilst the thermodynamic analysis of DSC data is covered by Privalov and Khechinashvili (1974), Sturtevant (1974), Biltonen and Freire (1978), and Privalov (1980), all with specific reference to biological macromolecules. Finally, the application of DSC to particular biopolymers or food constituents (protein, water, lipid) is surveyed by Simatos *et al.* (1975), Mabrey-Gaud (1981) and Wright (1982).

3 Differential scanning calorimetry and differential thermal analysis

3.1 Introduction

As much debate has centred around the relative merits of and distinction between these two techniques, it is perhaps instructive to quote their definitions as proposed by ICTA (Mackenzie 1979).

DTA: 'A technique in which the temperature difference between a substance and a reference material is measured as a function of temperature whilst the substance and reference material are subjected to a controlled temperature programme.'

DSC: 'A technique in which the difference in energy inputs into a substance and a reference material is measured as a function of temperature whilst the substance and reference material are subjected to a controlled temperature programme.'

Two forms of DSC can be identified depending on the mode of measurement: power compensation DSC and heat-flux DSC.

An intermediate type between DSC and DTA was also recognized in the guise of quantitative DTA where the equipment is designed to produce quantitative results, particularly in terms of energy. Schematic representations of all three types are shown in Fig. 1. Briefly summarized, the important differences are as follows: to be quantitative (i.e. for energy measurements) the instrument must measure heat flow either directly, as in heat-flux DSC, or indirectly, as in Boersma DTA (Fig. 1) where the differential temperature observed is strictly proportional to the heat flow. A further option is to measure the compensatory energy flow, in other words that energy required to maintain a T null-balance between sample and reference by compensating for absorption or evolution of heat. This is referred to as power-compensation DSC (Fig. 1). Classical DTA is really only qualitative or at best semi-quantitative because the thermocouples positioned bodily in the sample are subject to variations in thermal resistance of sample and

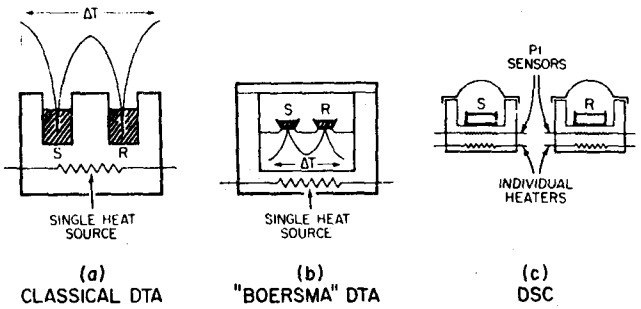

Fig. 1. Operating principles of alternative DSC/DTA systems. (Courtesy of the Perkin-Elmer Corporation.)

apparatus and so there exists no simple proportional relationship between differential temperature and heat flow.

There is also a range of high-precision scanning microcalorimeters classified as either heat flow or adiabatic depending upon their mode of operation. These instruments are constructed to allow temperature regulation of the cell surroundings relative to the cells themselves. In this way a more accurate and constant rate of heating can be achieved. For a more detailed discussion of these calorimeters the reader is referred to Privalov (1980).

Before any calorimeter can be used for investigative purposes, it has to be calibrated for both temperature and energy measurement. The latter is generally accomplished by using pure metals of accurately known heats of fusion, such as indium (ΔH_f = 6.79 cal g^{-1}) as standards. An instrument calibration constant is obtained which can then be used in subsequent calculations for determining energies or heats associated with transitions. Similarly the instrument is calibrated for temperature using a number of standards covering the temperature range of interest. These are usually organic compounds such as benzil and β-naphthol ethyl ether. A selection of organic reference materials for both energy and temperature calibration was recently published by Andon and Connett (1980).

The following section discusses the type of information that can be obtained from DSC studies.

3.2 *Characteristics measured by DSC/DTA*

3.2.1 Heat capacity. Heat capacity *per se* and the derived intensive quantity, specific heat, are not often measured or studied in food research or on food components but because they form the basis of enthalpic measurements, it is appropriate to consider the theoretical basis of specific heat determination by DSC.

The form of the output from DSC (and quantitative DTA) is a thermogram depicting differential heat flow (ordinate) against temperature (abscissa) i.e. dq/dt v T. In fact dq/dt represents the calibrated range setting (sensitivity) of the instrument. Any ordinate deflection represents an absorption or evolution of heat by the sample, i.e. an increase or decrease in the enthalpy, H. Thus

$$\frac{dq}{dt} = \frac{dH}{dt}$$

Now, assuming constant pressure, heat capacity

$$C_p = \left(\frac{dH}{dT}\right)_p$$

or

$$C_p = \frac{dq}{dt} \times \frac{dt}{dT}$$

where dT/dt is the programmed heating rate. Thus the ordinate can be calibrated directly in terms of heat capacity, and if the sample mass, m, is known, in terms of specific heat, c_p, since $c_p = C_p/m$. Experimentally, the specific heat of a material is measured relative to a known standard such as sapphire, and in this way, errors inherent in the range calibration and the programmed heating rate of the calorimeter can be eliminated. A schematic representation of specific heat determination is shown in Fig. 2.

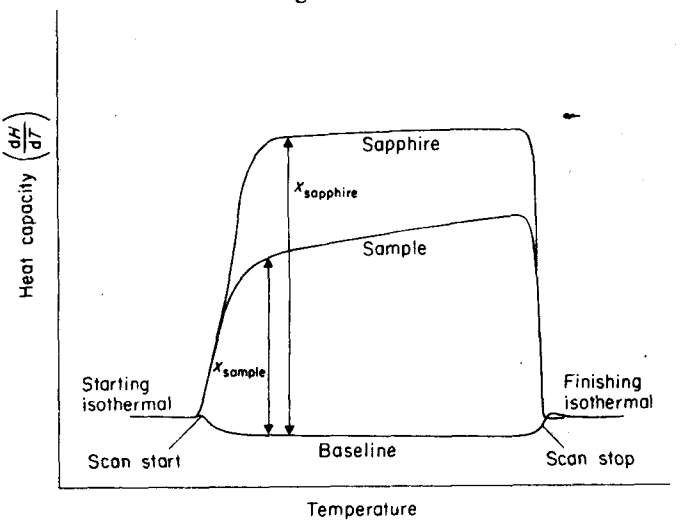

Fig. 2. Determination of specific heat by DSC.

Most substances undergo a change in heat capacity with temperature reflecting a change in structure and therefore a change in their capacity to absorb energy. An example is afforded by the denaturation of a protein, where the abrupt transition from the native to the denatured state is also accompanied by a change in heat capacity (Privalov and Khechinashvili 1974). The enthalpy of the transition itself is sometimes referred to as the excess heat capacity and this will be discussed in more detail in the next section. In addition, Privalov and Khechinashvili (1974) observed that prior to the denaturation transition, there was a steady increase in the heat capacity of a protein. This pre-denaturational change was ascribed to a gradual loosening of the protein structure resulting in a change in the accessibility of side-chain groups to water molecules (Privalov *et al.* 1971).

3.2.2 Temperature and heat of transition. These two parameters probably represent the most important information yielded by DCS analyses, since the more useful and interesting applications of the technique stem from an appreciation of how these parameters vary with experimental or technological conditions. In the area of food research, the observed DSC transitions mainly relate to processes such as protein denaturation, starch gelatinization and fat crystal melting. The reason why these processes are discerned by DSC as a transition or peak is because of their highly co-operative nature, i.e. they all involve near simultaneous rupture of inter- and intra-molecular bonding. The broader the transition observed the less co-operative the underlying process. An idealized thermal transition is depicted in Fig. 3 together with a key to the various parameters normally measured from it. Two problems arise in the analysis of such a thermogram. The first is concerned with the assignment of a temperature characteristic of the process and the second with the construction of a suitable baseline for peak integration and the determination of the transition enthalpy. T_{max}, the temperature at maximum heat flow into the sample is the transition temperature most often quoted. This particular temperature suffers from the disadvantage of being concentration dependent, but where multiple overlapping transitions are present it is usually the only measurable temperature. It serves to identify the transition but not the underlying process. For the latter, the true onset (or melting) temperature is required. An approximation to this is the extrapolated onset temperature (T_m) obtained by constructing a tangent to the leading edge of the transition and measuring the temperature at the point of intersection with the baseline. This is in fact how the calorimeter is normally calibrated for temperature using a pure standard. This construction takes into account the presence of thermal lag in the system resulting from the resistance to heat flow between sample pan and holder. This would then be the true onset temperature for a sharp (isothermal) melting. Where the transition is broad (i.e. occurs over a wide temperature range) an alternative construction has been suggested (McNaughton

and Mortimer 1975) as shown for T'_m in Fig. 3. The slope of this line corresponds to that of the leading edge of the standard temperature calibrant and is constructed from the peak maximum to the baseline. This method however is seldom used. The other variants used include (a) the temperature at zero heating rate (T_m^0), obtained by plotting peak maximum temperature (T_{max}) as a function of heating rate (Rüegg *et al.* 1977a) and (b) the temperature at which 50% conversion has taken place (Velicelebi and Sturtevant 1979). The former again attempts to allow for the presence of thermal lag in the system. The latter, in most instances, is not equivalent to the peak maximum temperature because of changes in heat capacity accompanying the process, but generally speaking they only differ by a degree or so.

It is this heat capacity change that also complicates the determination of the transition enthalpy from the peak area. The difference in heat capacity between pre- and post-transition states (ΔC_p^d in Fig. 3) has to be allowed for in the construction of the transition baseline if a true estimate of the enthalpy is to be obtained. One approach shown in Fig. 3 is to extrapolate pre- and post-transition baselines to the mid-point of the transition and integrate the area enclosed by *ABCDE*. If the shaded areas are approximately equal then the area *ADE* is a

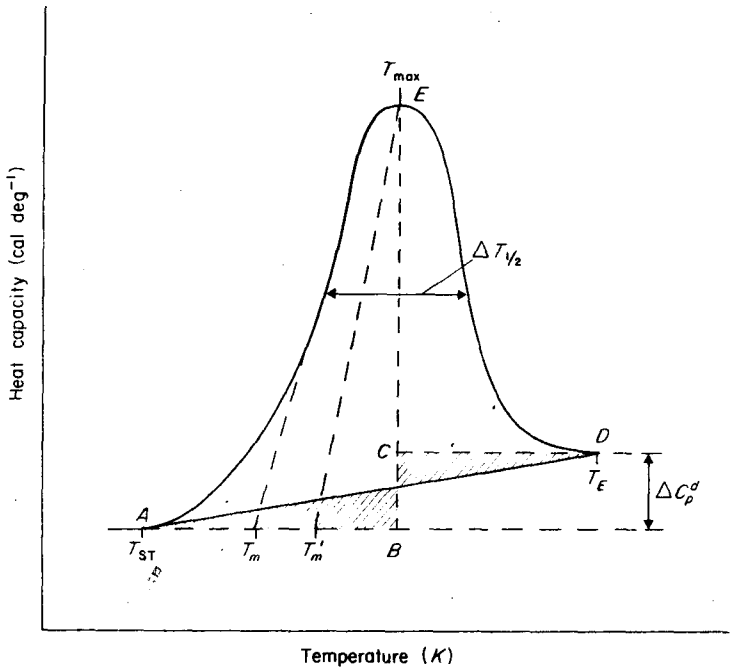

Fig. 3. A DSC transition with associated parameters.

good approximation to this value. In fact it is this latter approach that is most frequently used and in cases where overlapping transitions are present is often the only option.

The relationship between enthalpy and peak area can be established as follows:

$$\frac{d\Delta H}{dT} = \Delta C_p$$

Integrating with respect to temperature, T,

$$\Delta H = \int_{T_2}^{T_1} \Delta C_p dT \text{ or } \int_{Tst}^{Te} \Delta C_p dT = \text{Area } ADE$$

provided the ordinate is calibrated in terms of heat capacity.

Methods employed for peak integration include area measurement by planimetry (Velicelebi and Sturtevant 1979) and image analysis, and computer-based data analysis involving deconvolution of overlapping peaks (Donovan and Ross 1975).

The final peak parameter shown in Fig. 3 is $\Delta T_{1/2}$, or the peak width at half-peak height, and is essentially a guide to co-operativity: the smaller the value of $\Delta T_{1/2}$, the more co-operative the process concerned. However, it is also to be found in the expression

$$\Delta H_{VH} = \frac{4RT_M^2}{\Delta T_{1/2}}$$

where ΔH_{VH} is the van't Hoff enthalpy for a two-state process and T_M is the temperature at the transition mid-point. It is possible, by comparing the value of ΔH_{VH} with the calorimetrically determined enthalpy ΔH_{cal}, to obtain an indication of how closely a given process approximates to a two-state transition. For a true two-state process, i.e. one in which there are only two thermodynamically stable states

$$\frac{\Delta H_{VH}}{\Delta H_{cal}} = 1.$$

The use of $\Delta T_{1/2}$ in the van't Hoff expression is only an approximation based, as it is, on the assumption that the transition peak itself can be approximated by a triangle. For more accurate work, the following expression should be applied:

$$\Delta H_{VH} = \frac{4RT_M^2 \Delta C_p^m}{Q}$$

where ΔC_p^m is the peak height at the transition mid-point and Q is the total peak

area (cal g^{-1}). Since $\Delta H_{cal} = MQ$, the ratio of the two enthalpies becomes

$$\frac{\Delta H_{cal}}{\Delta H_{VH}} = \frac{MQ^2}{4RT_M^2 \Delta C_p^m}$$

Privalov (1969) has calculated this ratio for a number of proteins. That for lysozyme is close to two indicating the presence of two independent transitions or two independent co-operative regions in the molecule. For pancreatic trypsin inhibitor, the ratio equalled 0.5 and was explained on the basis of a dimer, rather than monomer, being the co-operative unit.

3.2.3 Kinetic parameters. A number of methods have been proposed for determining reaction kinetics from DSC/DTA curves. Some of these have been reviewed by Reed *et al.* (1965) and Pope and Judd (1977). It should be emphasized that most of the procedures involve assumptions which should be validated for the system under investigation. For example, Kissinger (1957) derived the following expression:

$$\frac{\mathrm{d}\,(ln\ \theta/T_{max}^2)}{\mathrm{d}\,(1/T_{max})} = \frac{-E_A}{R}$$

where θ is the programmed heating rate $(\mathrm{d}T)/\mathrm{d}t$, T_{max} is as defined previously and E_A is the activation energy. This expression is only correct, however, if T_{max} corresponds to the temperature of maximum reaction rate and as has already been pointed out, this is not necessarily the case. In most of the other procedures an expression is derived which allows the calculation of rate constants from the DSC peak characteristics (Borchardt and Daniels 1957; Beech 1969).

If, in a small time interval, it is assumed that the number of moles of compound a reacting is directly proportional to the observed enthalpy change, then

$$\mathrm{d}a = \mathrm{d}H \times \text{constant}.$$

The constant of proportionality is obviously equal to $1/H$ where H is the enthalpy change per mole of a.
Differentiating with respect to time, t, gives

$$\frac{\mathrm{d}a}{\mathrm{d}t} = \frac{1}{\Delta H} \cdot \frac{\mathrm{d}H}{\mathrm{d}t} \qquad (1)$$

From the general rate equation for a 1st order reaction,

$$\frac{\mathrm{d}a}{\mathrm{d}t} = k(a_0 - a_t)$$

where k is the 1st order rate constant, a_0 is the number of moles of a initially and a_t

is the number of moles of a reacted after time t (or temperature T, since dT/dt = constant). Substituting into equation (1) and rearranging,

$$k = \frac{dH}{dt} \cdot \frac{1}{\Delta H} \cdot \frac{1}{(a_0 - a_t)} \tag{2}$$

Since

$$\frac{A_0}{a_0} = \frac{A_T}{a_T} = \Delta H$$

where A_0 is total area of peak (in calories) and A_T is area up to temperature T, equation (2) becomes

$$k = \frac{dH}{dt} \cdot \frac{1}{A_0 - A_T}$$

dH/dt is simply the height of the curve above the baseline and is equivalent to Sx/w where S is the machine sensitivity (cal sec^{-1}), x is the recorder pen displacement (cm) and w is the chart width (cm). Thus k, the rate constant, can be determined by measuring peak height and residual areas at a series of temperatures, and from the Arrhenius plot of the temperature dependence of the rate constant, the activation energy and pre-exponential factor can be obtained.

3.3 Applications

DSC has been applied to the study of all three major food biopolymers, protein, starch and fat or oil, either in isolation or as part of a complex food system. The purpose of this section is to demonstrate how this technique has been applied in these areas and indicate the type of information it can yield. It is not intended as an exhaustive review of the field.

3.3.1 Protein studies. The effect of heat on the majority of proteins is to convert them from an ordered (native) state to a disordered (denatured) state. The intramolecular bonding is disrupted, the polypeptide chains unfold and if conditions are conducive, aggregation normally ensues. The initial unfolding is usually a highly co-operative phenomenon and is accompanied by a significant uptake of heat, seen as an endothermic peak in the DSC thermogram. The subsequent aggregation reactions are exothermic in nature and if they occur in the temperature range of the denaturation transition, can complicate the determination of the denaturation enthalpy. Furthermore, since aggregation reactions preclude the possibility of reversible processes, a rigorous or quantitative thermodynamic analysis is not possible. In the most precise work, this problem is overcome by careful choice of experimental conditions, namely, dilute solutions

and extremes of pH (to maximize repulsive forces between chains) and the use of high precision calorimeters. It was this approach that Pfeil and Privalov (1976a, b and c) used in their study of the denaturation of globular proteins. Using lysozyme as an example, they were able to demonstrate the existence of two distinct thermodynamic states, corresponding to the native and denatured states of the protein, and the absence of any thermodynamically stable intermediate states. Thus the denaturation of lysozyme could be correctly described as a two-state transition, allowing the application of equilibrium thermodynamics.

In a previous study of five globular proteins (including lysozyme), Privalov and Khechinashvili (1974) concluded that the two-state model of denaturation was a close approximation to the real process. They determined the average value of $\Delta H_{cal}/\Delta H_{VH}$ to be 1.05 ± 0.03 which they interpreted as indicating the existence of a few intermediate states, albeit ones which were thermodynamically highly unstable. These studies also led them to interpret the denaturation enthalpy in terms of the intramolecular bonding present in these proteins. They concluded that the enthalpy of denaturation was largely attributable to the heat of rupture of intra-chain hydrogen bonds (determined as 1.7 kcal per mole of hydrogen bonds) and that its observed temperature dependence was a result of an exothermic contribution from the cluster formation of water molecules around newly exposed non-polar groups of the proteins. This ordering effect of non-polar groups on water structure decreased with increasing temperature with the result that the apparent enthalpy of denaturation increased with increasing temperature.

As this latter example illustrates, DSC can provide fundamental information on the denaturation of proteins. More importantly perhaps, DSC can be used to study the behaviour of proteins by monitoring changes in the DSC profile of denaturation. In this way the interaction of proteins with a range of other compounds has been investigated, including polysaccharides (Imeson *et al.* 1977), sugars (Donovan *et al.* 1975; Back *et al.* 1979), polyhydric alcohols (Back *et al.* 1979) and monohydric alcohols (Velicelebi and Sturtevant 1979). Sugars and polyols stabilized proteins against thermal denaturation, an effect attributed to their ability to alter the structure of the water and, as a consequence, the strength of the hydrophobic interactions. On the other hand, alcohols had a pronounced destabilizing effect on proteins. Again this was attributed to modification of the water structure, in this case, leading to a decreased contribution of hydrophobic interactions to the stabilization of the native state. A variation on the theme of protein-carbohydrate interaction was demonstrated by Mattiasson and Borrebaeck (1981) in their DSC study of lectin-carbohydrate interactions. They observed a significant stabilization of Concanavalin A in the presence of hapten sugars and also in the presence of the glycoprotein horse radish peroxidase. The glycoprotein was unaffected by its interaction with Concanavalin A, a finding interpreted as indicating that the interaction was solely via the carbohydrate moeity of the enzyme.