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# Thermal properties of polysaccharides at low moisture: 1—An endothermic melting process and water–carbohydrate interactions

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The thermal properties of a broad range of polysaccharides containing 5–25% w/w water have been studied by differential scanning calorimetry and dynamic mechanical thermal analysis (DMTA). Following room temperature conditioning, an endothermic event accompanied by material softening is observed at 45–80°C for all samples except those above their glass transition temperature. The temperature of the event is determined by thermal history and is apparently independent of polymer type or moisture content. The associated enthalpy increases with water content. Variable frequency DMTA analysis suggests a structural melting event rather than a relaxation process. The endothermic event is recovered over the days timescale after heating, and can be annealed to higher temperatures with increasing holding temperature.

Results are interpreted in terms of a dynamic hydration model in which specific energetic water–carbohydrate interactions occur but with a lifetime defined by their local effective microviscosity. The observation of the endotherm below glass transition temperatures suggests that in aqueous polysaccharide glasses, enthalpic structures involving the solvent can be made and broken.

## INTRODUCTION

The nature of water–carbohydrate interactions is a subject of continuing study (Franks, 1991) and is a central aspect of polysaccharide functionality. In (dilute) solutions, the probable similarity between water–water and water–carbohydrate interactions make study of the latter difficult, although combinations of spectroscopic and computational methods are showing some promise (Brady & Ha, 1991; Leeflang *et al.*, 1992). At high carbohydrate concentrations however, numbers of water molecules and carbohydrate residues can be comparable. For example, at 25% w/w water, linear hexose polymers contain one hydroxyl group per water molecule. In this concentration range, therefore, carbohydrate–water interactions should be highly significant and amenable to study.

A second reason for studying polysaccharides at low moisture (defined here as < 25% w/w water) is the considerable interest in the thermal, mechanical and structural properties of this type of material. Of particular interest is the development of experimental data which can be used to test the hypothesis (Slade & Levine, 1991) that kinetic immobilisation below a glass transition temperature (which is subject to plasticisation by water) determines the material properties of low moisture polysaccharides. This model cuts to the heart of water–carbohydrate interactions, as plasticising water is considered to act via entropic/free volume effects and should have no net enthalpic interaction with carbohydrates, i.e. water–carbohydrate interactions are considered to be equivalent to water–water interactions (Slade & Levine, 1991). This model may be regarded as one limiting view of water–carbohydrate interactions, with the other limiting model being that of water 'bound' both energetically and in time to carbohydrate residues. Recent molecular dynamics simulations (Brady & Ha, 1991) suggest very high rates of disruption of water–carbohydrate associations in excess water, although certain modes of association were energetically favoured. However, such simulations have not been reported for low moisture systems.

This paper describes studies of the thermal properties of a broad range of low moisture polysaccharides, with

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moisture content variations induced by conditioning at defined relative humidities. Attention will focus specifically on an endothermic event centred at 50–80°C for materials conditioned at room temperature. This event has been reported previously for amylopectin (Kalichevsky *et al.*, 1992) and gelatinised starch (Shogren, 1992), and is now suggested to be a general probe of water–carbohydrate interactions. Factors which affect the presence, temperature and enthalpy of this event will be described, and implications for models of water–carbohydrate interactions will be discussed. A preliminary account of some of this work has been published (Gidley *et al.*, 1992).

## EXPERIMENTAL METHODS

### Materials

Pullulan, an extracellular polysaccharide produced by the fungus *Aureobasidium pullulans*, was obtained from Sigma Chemical Co. (St. Louis, MO, USA). Pregelatinised waxy maize starch (Amioca) was obtained from National Starch and Chemical Corporation (NJ, USA). All other samples were standard commercial polysaccharides.

### Preparation of pullulan and amylopectin sheets

Solid polymer sheets approximately 2 mm thick were prepared from pullulan and pregelatinised waxy maize starch (referred to hereafter as amylopectin) powders. Samples of pullulan and amylopectin were hydrated to approximately 22 and 18% moisture, respectively, by storing in a sealed, air circulated cabinet with a relative humidity (RH) of 86% at 20°C, for at least 1 month. Excess powder (~ 25 g) was placed in an open brass mould (80 × 100 × 2 mm) which was mounted on a solid brass plate and lined with a sheet of xerox transparency to prevent the sample from sticking. Another lined brass plate was placed on top of the mould, and the whole assembly was placed between two heated plattens of a hydraulic press (maximum load 10 tonnes). The pullulan and amylopectin samples were pressed at 90°C, for 10 min under a load of 10 tonnes. Before releasing the load, the whole assembly was rapidly cooled to room temperature by circulating cold water through the press plattens. The resulting transparent polymer sheets were cut into strips (~ 10 mm wide) using a miniature electric jig-saw (Spirolux) and stored at various relative humidities, ranging from 10 to 86%. The samples were left for at least 1 month, ensuring that constant weight equilibrium had been reached. All the RH chambers were sealed, had air circulated via a fan and were kept at 20°C. Supersaturated salts were used as follows: lithium chloride (10% RH); magnesium chloride (33% RH); sodium bromide (57% RH); sodium chloride (75% RH); and potassium chloride (86% RH). These conditions were also used to manipulate moisture contents in powder samples.

The moisture content of these and all other samples were determined by drying in a vacuum oven at 90°C for 16 h. All moisure determinations were carried out in triplicate and the average calculated.

### Differential scanning calorimetry (DSC)

DSC studies were carried out using a Perkin-Elmer DSC 7 calorimeter equipped with a thermal analysis data station. The instrument was calibrated by standard procedures and the resultant traces were normalised on the basis of sample weight using the data station. Accurately weighed samples (powders) were placed in sealable pans and an empty DSC pan was employed as an inert reference. Samples were heated at a rate of 10°C/min. Enthalpy, onset temperature, and peak temperature were calculated with the Perkin Elmer software programs. The glass transition temperature was taken using the temperature at which the first derivative of the second order transition in specific heat capacity reached a maximum. Pressed samples of pullulan and amylopectin were also measured in the DSC and were found to give the same results as powdered samples of the same moisture content.

### Dynamic mechanical thermal analysis (DMTA)

In DMTA, a material is subjected to a sinusoidal strain at a fixed frequency, and the magnitude and phase of the resulting sinusoidal stress is measured. From this data, three parameters are evaluated: (i) the elastic storage modulus, $E'$, which is a measure of the stiffness and is proportional to the amount of energy stored in the material per cycle; (ii) the loss modulus $E''$, which is proportional to the amount of energy dissipated per cycle; and (iii) the damping or dissipation factor, tan $\delta$, which is the ratio of the loss to the storage modulus.

### DMTA measurements

Transparent bars with dimensions 20 × 10 × 2 mm were prepared from the pressed sheets of pullulan and pregelatinised waxy maize starch and stored at various relative humidities ranging from 10 to 86%, at 20°C for 1 month prior to the measurements. The moisture content of these samples was determined as above. The storage modulus, loss modulus, and tan $\delta$ of the bars were measured as a function of temperature using a Polymer Labs DMTA. The testing was conducted at a constant frequency of 1 Hz (unless otherwise stated), a strain of ×2, and over a temperature range of −110 to 150°C with a heating rate of 2°C/min.

## RESULTS

In order to prepare samples with as high a level of homogeneous mixing between water molecules and carbohydrate residues as possible, polysaccharide powders were equilibrated at constant RH (up to 86%) at least until constant weight was achieved (1–2 weeks) and typically for > 2–3 weeks. Solid polysaccharides having water contents up to ~ 22% w/w were obtained, although for some materials (notably dextran and pullulan) annealing at high RH led to viscous fluids probably because the effective glass transition temperature for the polymer was close to or below the holding temperature (~ 20°C). RH conditioning gave more reproducible thermal behaviour (in the authors' hands) than either adding liquid water to 'dry' polysaccharide (Zeleznak & Hoseney, 1987) or allowing samples to contact water vapour for varying lengths of time (Orford *et al.*, 1989).

### A common endothermic event at 50–80°C

A broad range of polysaccharides were examined by DSC either directly from commercial samples or following RH conditioning. Except for a few materials of very low moisture content (< 5% w/w), all samples showed an apparent endothermic event centred in the temperature range 50–80°C. Illustrative examples are shown in Fig. 1. A further general property was that on immediate reheating, the event was not discernable (Fig. 1(e)). No measurable change in heat capacity was found for any sample as indicated by identical 'baseline' slopes and positions below and above the apparent endotherm. This behaviour appears to be the same as that reported recently for amylopectin (Kalichevsky *et al.*, 1992) and gelatinised corn starch (Shogren, 1992) and has been found in the present study for a range of granular starches, amylopectin, amylose, dextran, pullulan, xanthan gum, pectin, alginate, agar, konjac glucomannan, locust bean gum and guar gum. Similar behaviour has also been noted for proteins such as gluten and elastin (Appelqvist *et al.*, unpublished results).

The peak temperature and width of the endothermic event show some, apparently non-systematic, variation. For room temperature conditioning, peak temperatures were all in the range 45–80°C, with most being 50–75°C. However, sample thermal histories were not strictly controlled in this study, and as discussed later may be the origin of at least some of the peak temperature variations encountered.

The enthalpy associated with the endothermic event did, however, show a significant variation with moisture content. This is illustrated in Fig. 2 which shows DSC thermograms for granular maize starch covering a range of moisture content. No systematic change in peak temperature or width is seen but enthalpy values increase with moisture content. This is as reported for amylopectin by Kalichevsky *et al.* (1992) and Shogren (1992) although no data were given in these studies. In this study, a range of starches (both granular and pregelatinised) together with dextran and pullulan have been analysed by DSC at a range of moisture contents. The variation of endotherm enthalpy with moisture is shown in Fig, 3 for these systems. A systematic increase in enthalpy with moisture content is found, with each system apparently having a different relationship such that at constant moisture, enthalpies increase through the series starch–pullulan–dextran. Enthalpy values were similar for granular and pregelatinised starches at equivalent moisture contents. Results for a range of other polysaccharides at 8–10% w/v water showed most enthalpy values to be in the range 2–3·5 J/g, i.e. similar to pullulan and dextran.

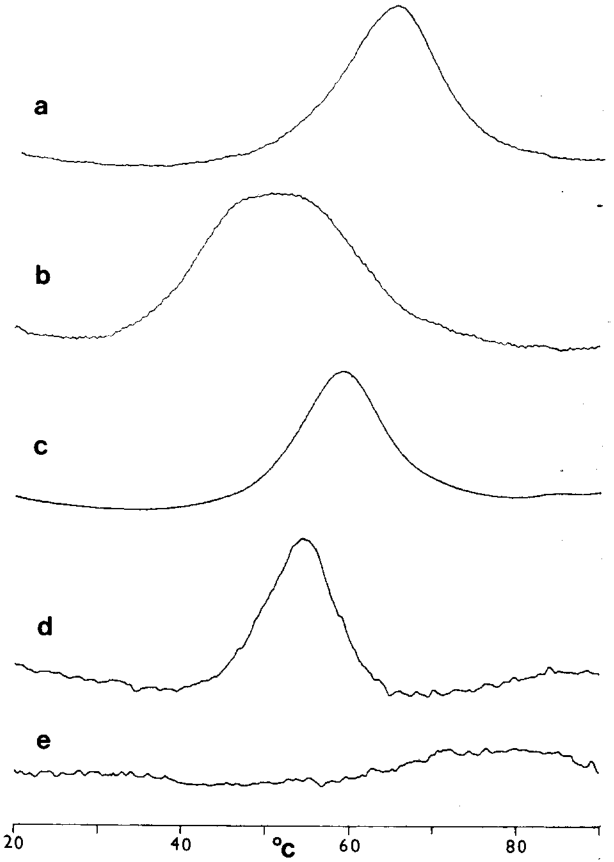

**Fig. 1.**DSC heating thermograms of (a) agar containing 8·9% w/w water, $\Delta H = 2{\cdot}25$ J/g; (b) alginate containing 9·5% w/w water, $\Delta H = 2{\cdot}9$ J/g; (c) locust bean gum containing 10·2% w/w water, $\Delta H = 3{\cdot}2$ J/g; (d) corn starch containing 17·4% w/w water, $\Delta H = 1{\cdot}3$ J/g; (e) an immediate second heating thermogram for sample (d), the slight baseline roll shown was often observed but was not systematic and not reproducible on repeat analyses of the same material.

### Relationship to glass transition

Observation of the endothermic event occurs irrespective of whether or not the system shows evidence for a glass

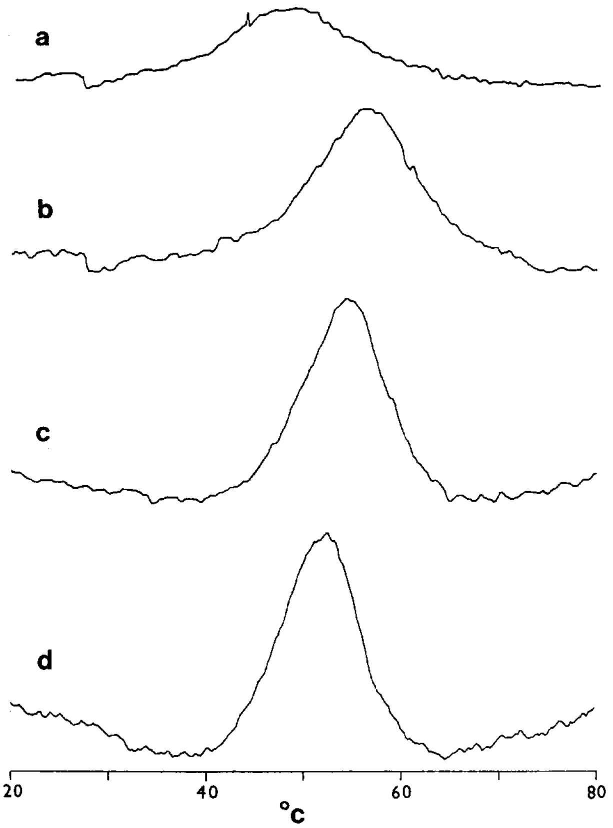

**Fig. 2.** DSC thermograms for corn starch containing (a) 8·8; (b) 14·4; (c) 17·4; (d) 19·9% w/w water, respectively. $\Delta H$ values for the transitions shown were ~0·6, 1·1, 1·3 and 1·4 J/g, respectively.

transition in the DSC experiment. Further evidence for the independence of the endothermic event from glass transition behaviour lies in the apparent invariance of peak temperature with moisture content, i.e. no plasticising action of water is observed. For two of the systems studied (dextran, pullulan), however, clear second order (glass) transitions can be observed by DSC (Gidley *et al.*, 1992), and, therefore, this offers the possibility of probing the relationship between the two types of events. Figure 4 shows DSC thermograms for pullulan at moisture contents where the second order transition is above (10·2% $H_2O$), similar to (13·4% $H_2O$), and below (22·0% $H_2O$) the usual endotherm temperature. This data shows that the endothermic event is not observed if the glass transition temperature is below the usual endotherm temperature. The same effect was also found for dextran, and has recently been reported for gelatinised corn starch (Shogren, 1992).

## Recovery and annealing of endotherm

As illustrated in Fig. 1, the endothermic event is not observed by DSC on immediately reheating through the same temperature range. This behaviour was seen for every material examined in this study. However, on storage of heated materials at room temperature (i.e. significantly below the temperature of the DSC endotherm), a slow recovery of endothermic behaviour was observed on subsequent DSC thermograms. For starches and the related oligomer maltoheptaose, both endotherm enthalpy and temperature gradually increased following storage over many days such that typically 60–70% of the enthalpy was recovered following storage for 7 days with peak temperatures 2–5°C lower than observed on initial heating in the DSC (Gidley *et al.*, 1992). There were no obvious variations with moisture content in the range 9–18% w/w with respect to endotherm recovery.

The recovery process was studied more systematically for pullulan, particularly with respect to the differential between holding and glass transition temperatures. Figures 5 and 6 show DSC thermograms for pullulan containing 8·2 and 12·4% w/w water, respectively, with glass transition temperatures of 105–110°C and 70–75°C, respectively. A significant difference in the rate of endotherm recovery is found with no obvious event detectable after 10 days storage at 40°C for the 8·2% w/w

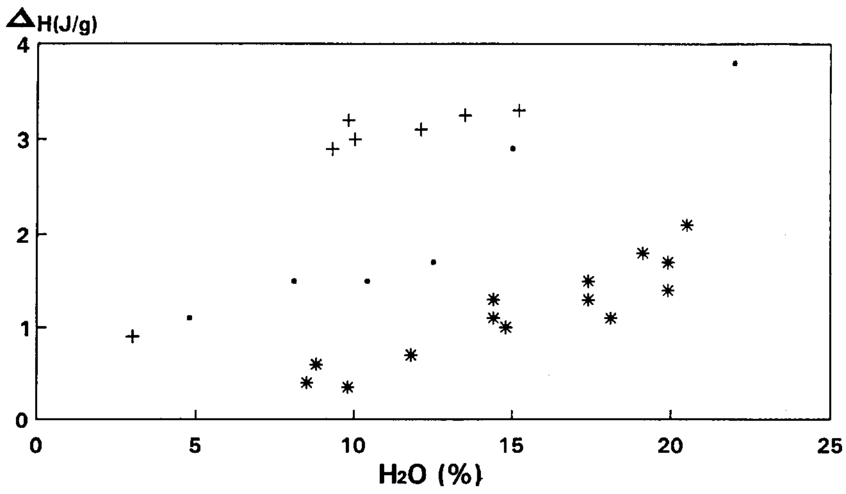

**Fig. 3.** Variation of endotherm enthalpy with moisture content for a range of (✱) starches, (■) pullulan, and (+) a range of dextrans.

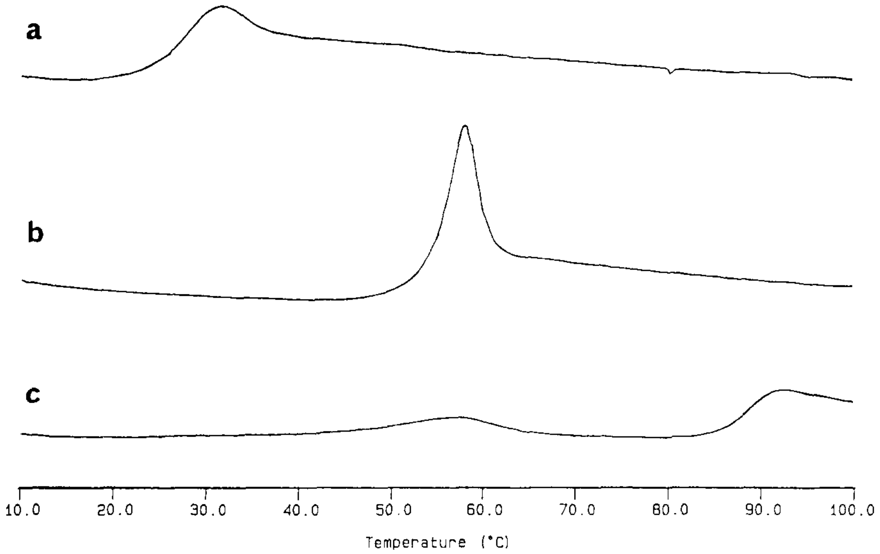

**Fig. 4.** DSC thermograms for pullulan containing (a) 22·0; (b) 13·4; and (c) 10·2% w/w water, respectively.

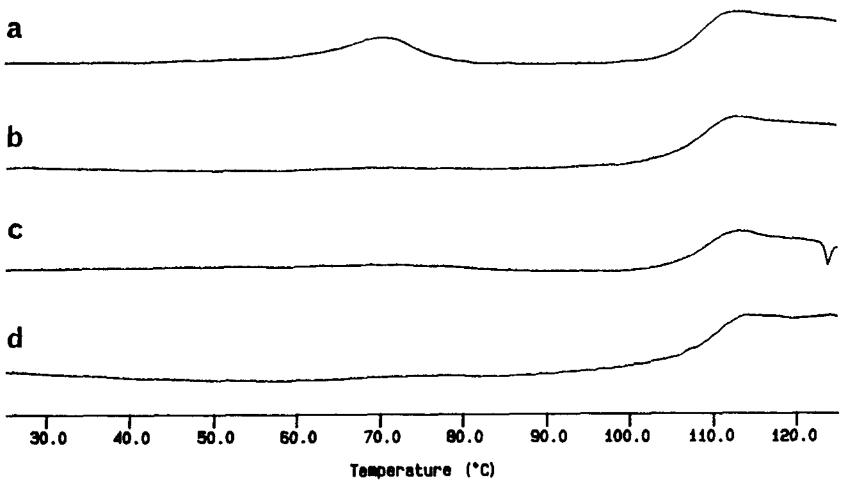

**Fig. 5.** DSC thermograms for pullulan containing 8·2% w/w water (a) on initial heating to 150°C; (b) after heating to 150°C and storage at 40°C for 2 days and then reheating to 150°C; (c) as (b) but with storage for 6 days; and (d) as (b) but with storage for 10 days.

water system (Fig. 5: holding temperature 65–75°C below $T_g$) whereas substantial recovery of endothermic behaviour is seen after 2 days storage for the 12·4% w/w water system (Fig. 6: holding temperature 30–40°C below $T_g$).

The effect of storage temperature prior to first heating was studied for a pullulan sample containing 8·2% w/w water; the results are shown in Fig. 7. Glass transition temperatures were found to be unaffected by storage temperature as would be expected, but endotherm parameters showed systematic variation. For a 7 day holding period at each temperature, endotherm enthalpies were similar but peak widths decreased with increasing storage temperature above 20°C. Temperatures for the endotherm were essentially constant following storage at −20, 5 or 20°C but increased markedly with holding temperatures of 40°C and above until at 90°C the endotherm event was superimposed on the glass transition. Holding at temperatures close to or above the glass transition resulted in loss of the endotherm (Fig. 4). Prior to holding at constant temperature for 7 days, all samples had been stored at room temperature. It appears (Fig. 7) that subsequent storage at lower temperatures does not lead to a reduction in endotherm temperature. This suggests that observed endotherm temperatures may reflect maximal temperatures encountered in the history of the sample, and is a possible cause for the apparently non-systematic variation in endotherm temperatures (in the range 45–80°C) observed in this study. The behaviour illustrated in Fig. 7 can be considered to be a kind of annealing over the 7 day timescale employed. Other preliminary experiments suggest that effective annealing can also occur on much shorter timescales (min or h).

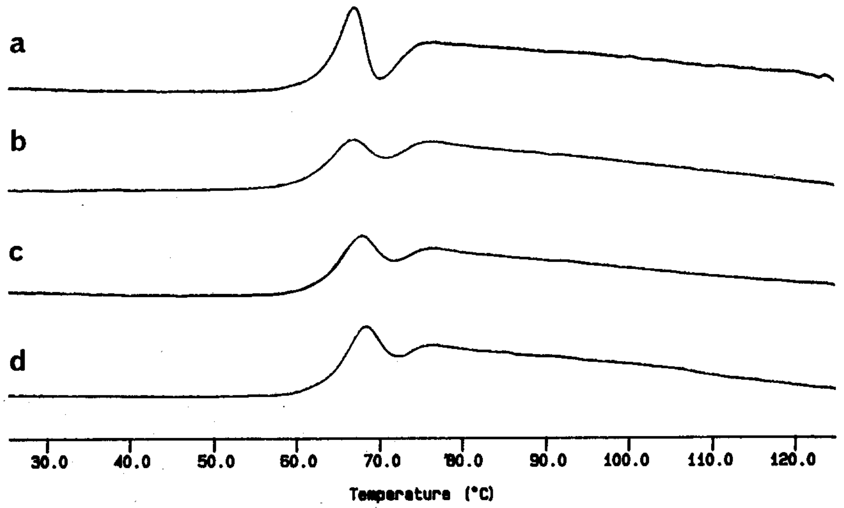

**Fig. 6.** DSC thermograms for pullulan containing 12·4% w/w water (a) on initial heating to 150°C; (b) heating to 150°C following 40°C storage for 3 days after initial heating to 150°C; (c) as (b) but with storage for 4 days; and (d) as (b) but with storage for 6 days.

## Mechanical properties

Dynamic mechanical thermal analysis (DMTA) is a convenient probe of mechanical properties for low moisture polysaccharide systems (Pizzoli *et al.*, 1991; Kalichevsky *et al.*, 1992). Figure 8 shows results for a pullulan sample containing 4·8% w/w water. The major event (110–130°C) involves a fall in $E'$ and a peak in $E''$ (and consequently a peak in tan $\delta$) and occurs in the region of the glass transition by DSC. $E'$ and $E''$ behaviour in this region during a first and second heat were closely similar, as would be expected for a glass transition. In the temperature range of the endotherm observed by DSC (~ 50°C), there is a slight fall in $E'$ and a small rise in $E''$ leading to a rise in tan $\delta$.

These features are not seen on a subsequent reheat, thus suggesting strongly that they have an origin common to that of the observed endothermic event in DSC. It can thus be concluded that the DSC data are not due to any technique artefact, and that there is a small (compared with the glass transition) but detectable mechanical 'softening' which occurs in the same temperature region as the endothermic event.

Thermal and mechanical transitions below the glass transition are a common phenomenon in synthetic polymer systems (Sperling, 1986) and are usually associated with local motions, e.g. of sidegroups (Smith & Boyd, 1992). The origins of such transitions lie in relaxation processes rather than structural melting effects (Hodge & Berens, 1982). In other words, the

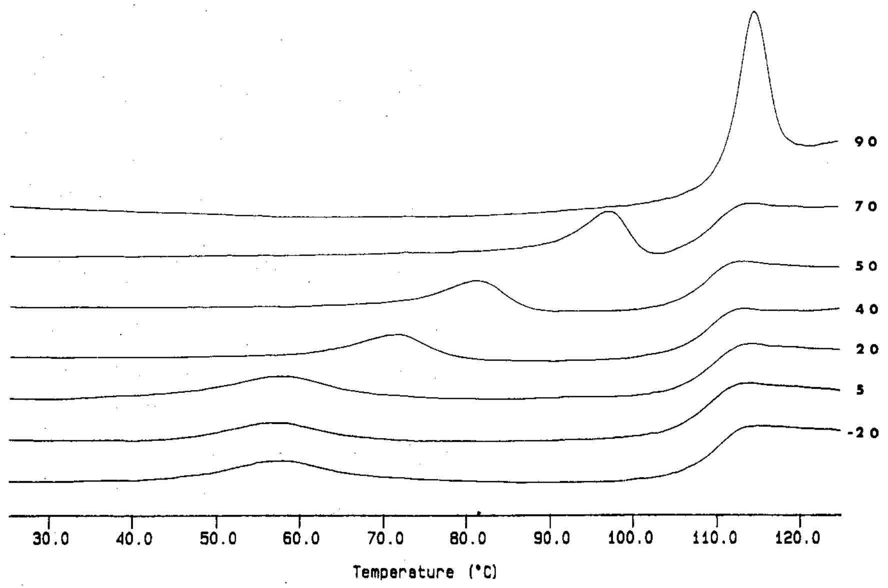

**Fig. 7.** Effect of holding temperature prior to heating in the DSC. Samples of pullulan containing 8·2% w/w water were held at the indicated temperatures (°C) for 7 days, and then examined by heating from 5 to 150°C. For holding temperatures of −20 to 70°C, endotherm enthalpies were similar (2·0 ± 0·3 J/g) and showed no systematic variation.

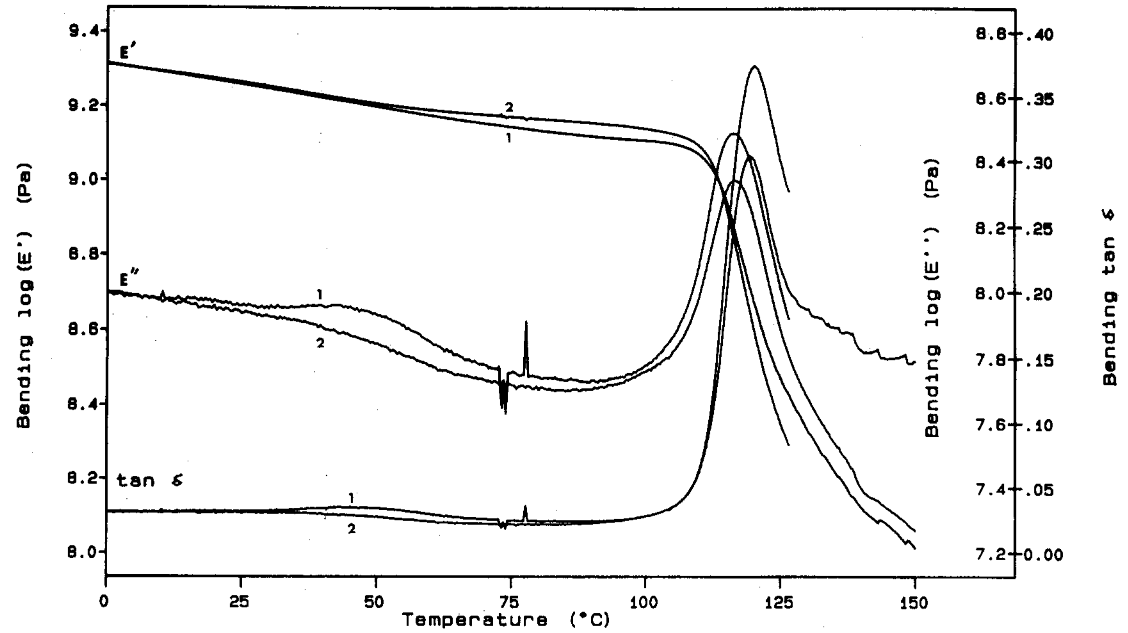

**Fig. 8.** DMTA analysis for pullulan containing 4·8% w/w water heated up to 150°C on two consecutive scans, labelled 1 and 2, respectively.

temperature of observation depends on the sampling time used experimentally (effective kinetic control) rather than being invariant (effective thermodynamic control). DMTA analysis at a range of frequencies was carried out on a sample of amylopectin containing 10·5% w/w water with the results shown in Fig. 9. The characteristic fall in $E'$ and peak in tan $\delta$ in the region 100–150°C corresponds to the DSC-determined glass transition and shows the expected effect of observation frequency in raising detected transition temperatures (Sperling, 1986). The region corresponding to the endothermic event in DSC (50–60°C), however, showed no systematic change in the temperature at which tan $\delta$ starts to rise, although the extent of the rise is apparently depressed at higher frequencies.

Therefore, this suggests very limited frequency dependence for the event at 50–60°C, more characteristic of a structural melting (i.e. analogous to a phase transition) than a relaxation process.

## DISCUSSION

### Features of the endothermic event

Results described above suggest the following general features for the endothermic event common to all low moisture polysaccharides (and at least some proteins).

1. The temperature of observation shows no systematic dependence on polymer type or water content, but seems to be determined primarily by thermal history.
2. The enthalpy of the transition is determined by the water content although each polymer family may have a different dose–response curve.
3. No event is seen above the DSC-detected glass transition.
4. Recovery of endothermic behaviour is on the h/days timescale and is quicker for holding temperatures nearer the glass transition (for polymers which show such behaviour).
5. Detectable softening of low moisture polysaccharides occurs in the same temperature range as the DSC endotherm, and the effect of DMTA observation frequency on softening temperature is negligible.

### On possible mechanisms

As the endothermic event is observed for a wide range of biopolymers, and the associated enthalpy increases with moisture content, a central role for water is indicated. A second contributing feature appears to be a significantly immobilised biopolymer matrix. This is particularly evident in the loss of the endotherm when the glass transition is lower than the characteristic observation temperature of the endotherm. Other systems which do not show glass transition behaviour are considered to be immobilised by other mechanisms (Gidley *et al.*, 1992) and will be discussed elsewhere.

Many of the features found for polysaccharides with a well-defined glass transition (e.g. pullulan) are similar to those described for synthetic polymers below their glass transitions (Berens & Hodge, 1982). This includes the increase of endotherm temperature following annealing (Fig. 7), and the effect of the difference between

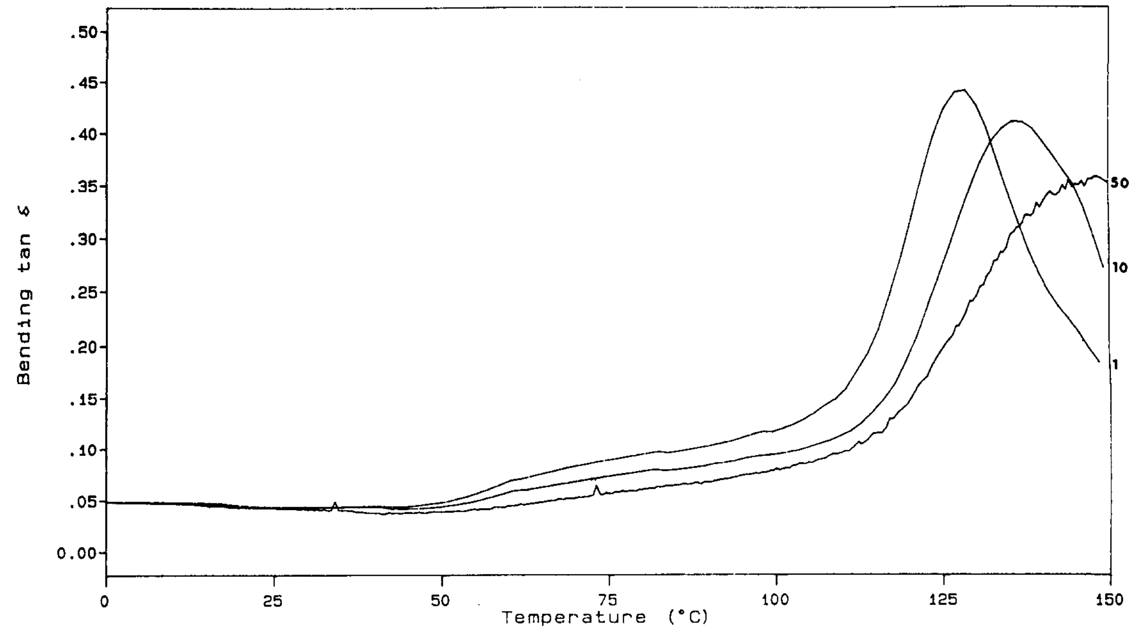

**Fig. 9.** Effect of frequency (1, 10 or 50 Hz) on tan δ determined by DMTA for amylopectin containing 10·5% w/w water. The high temperature peak is assigned to the glass transition with the temperature increasing with frequency. The initial rise in tan δ at ~ 50°C shows limited frequency dependence.

annealing and glass transition temperatures on the rate of endotherm development (Figs 5 and 6). A theory relating sub-$T_g$ endotherm temperatures and heat capacity maxima to thermal history has been developed for glassy synthetic polymers (Hodge & Berens, 1982). The reference point in this treatment is the glass transition temperature. However, in this study, similar temperatures and enthalpies in the range 50–70°C were also observed for samples which show melting rather than glass to rubber transitions on heating (e.g. agar, κ-carrageenan, granular starches). For the two systems studied which showed glass transition behaviour (pullulan, dextran), there was no systematic relationship between glass transition and endotherm temperatures, and similar $T_g$ values were observed at equivalent moisture content whereas endotherm enthalpy values differed significantly (Fig. 3). These results, together with the finding (Fig. 7) that endotherm enthalpy values for pullulan did not increase significantly with annealing temperature, would not be predicted by the model developed for glassy synthetic polymers (Hodge & Berens, 1982). The rate of development of the endothermic event clearly indicates that long time relaxation processes are important, and in this respect as well as others mentioned above, the model of glassy synthetic polymers is a useful reference point. However, the more general nature of the observed event suggests that the search for an endothermic mechanism should focus on a type of structural melting or phase transition involving water and a relatively immobilised biopolymer.

Although kinetic/relaxation behaviour does not appear to be dominant on DSC or DMTA timescales (i.e. up to 10 s), two features associated with the endotherm, namely recovery and annealing, do have a significant kinetic component. Thus, recovery of endothermic behaviour takes at least many hours, and annealing of endotherm temperature to higher values is a relatively slow event. It would be predicted that with decreasing thermal scan rates, a point would be reached where endotherm annealing occurs continuously during the scan leading to its non-observation. The relevant timescales for annealing are yet to be determined but it is clear that thermal history (both temperature and time) would need to be carefully controlled in further experimentation.

A further experimental observation which has a bearing on possible mechanisms is the peak width associated with the endotherm in DSC, which is usually of the order of 10°C at half height but can be as little as 2–3°C, particularly near to a glass transition (e.g. Figs 6 and 7). This implies a high level of cooperativity which increases with the flexibility of the polymer. The rate of recovery of endotherm sharpness is slow for storage temperatures significantly below endotherm temperatures (Fig. 6), but may be more rapid at elevated temperatures (Fig. 7).

Taking the above factors into consideration, the following features of the underlying mechanisms are suggested.

(1) The energetic association which is disrupted involves water molecules and hydrophilic groups on the biopolymer. Many of these associations would be expected to be bridging two adjacent (intra- or intermolecular) residues.
(2) Observation of the endotherm requires a long

range connectivity between associating units which takes a long time (days) to develop.

(3) Increasing but restricted polymer mobility leads to a more rapid achievement of endotherm behaviour and greater cooperativity of structural disordering.

Clearly, more experimental information, particularly at the molecular level, is required to arrive at a complete description of the underlying mechanism(s). The importance of doing so lies in the very general nature of the endothermic event. One of the difficulties involved will be that sample thermal history and observation time/temperature regimes will need to be closely controlled.

### General features of water–polysaccharide interactions

The evidence and possible mechanisms discussed above point to specific enthalpic associations between water and carbohydrates. This is in accord with both molecular modelling (Brady & Ha, 1991) and bulk physical properties (Galema & Hoiland, 1991) of carbohydrate solutions. Such associations are not permanent for solutions, having lifetimes on the picosecond timescale (Brady & Ha, 1991) and therefore leading to a lack of temporally 'bound' water. This leads to a description which may be termed 'dynamic hydration' (Gidley *et al.*, 1992) where a major factor which will determine the lifetime of any hydration-like associations will be the effective local microviscosity. With increasing concentration of carbohydrate residues, a point will presumably be reached where lifetimes of association are sufficiently long to be detected by physical techniques. This appears to be the regime in which the current work has been performed.

The interactions between water and polysaccharides which are proposed to lead to the observed endotherm are present irrespective of whether a glass transition is observable for the system (although not above the temperature of the glass transition). This reflects the diversity of 'immobilisation' mechanisms available to polysaccharides, of which glass formation is only one (Gidley *et al.*, 1992). Perhaps more surprising is the fact that an endothermic 'melting' event akin to a first order phase transition is observed at temperatures significantly below that of the glass transition. This has two connotations with respect to glass-forming polysaccharide systems. Firstly, it suggests that although water may have an effective plasticising action in that increasing levels depress glass transition temperatures, the mechanism by which this is achieved may not be purely entropic (or free volume) in nature, but may also involve dilution of water–carbohydrate energetic associations. Secondly, it is difficult to reconcile a completely glassed system with the presence of an apparent phase transition. A plausible model might be that glass formation reflects effective prevention of backbone (glycosidic) reorientation, whereas water–carbohydrate interactions between residues can still be formed or disrupted.

Further work is clearly required to pursue some of the concepts raised above, which hopefully will lead to a better understanding of the nature of water–carbohydrate interactions in general, and at low moisture in particular.

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