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A differential scanning calorimetry study of glass and melting transitions in starch suspensions and gels

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ABSTRACT

Differential scanning calorimetry (d.s.c.) has been used to study the glass and melting transitions that take place when an aqueous suspension of starch granules, or a partially crystalline starch gel, is heated. A baseline shift was found to accompany both the single major d.s.c. endotherms given by relatively dilute suspensions and gels, and the biphasic endotherms obtained from more concentrated suspensions. The shift, which indicates a change in the heat capacity, starts at the same temperature as the endotherm commences and continues to increase in size until the endotherm is completed. This finding seems to differ in certain respects with other reports suggesting the heat capacity change occurs at the leading edge of the first melting peak for suspensions and does not take place during the melting of starch gels. The change in heat capacity of the suspensions and gels does not appear to be due to a glass transition since this transition was detected at lower temperatures. Hence the present study does not support the suggestion that the melting of the polymers in these starch systems is controlled by a glass transition.

INTRODUCTION

Mandelkern[1] has demonstrated that melting of the crystalline material in a large number of polymer–diluent systems obeys the Flory–Huggins expression,

$$\frac{1}{T_m} - \frac{1}{T_m^0} = \frac{R}{\Delta H_u} \frac{V_u}{V_1} (v_1 - \chi_1 v_1^2) \qquad (1)$$

where T_m and T_m^0 are the equilibrium melting points of polymer in the presence and absence of diluent respectively, R is the gas constant, ΔH_u is the enthalpy of fusion per repeat unit, V_u/V_1 is the ratio of the molar volumes of the repeat unit and the diluent, v_1 is the volume fraction of the diluent, and χ_1 is the polymer–diluent interaction coefficient[2]. Ideally Eq. *1* applies to perfect extended-chain crystals undergoing equilibrium melting in a homogeneous macromolecule–diluent matrix[3]. Very slow heating schedules are required to obtain the melting point data. These heating programmes avoid superheating and so assure that the crystalline–amorphous phase transition occurs at the equilibrium temperature. In addition, with slow heating regimes metastable polymer

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crystals tend to perfect themselves and, as a result, give melting parameters which are similar to those for equilibrium crystals[4].

The Flory–Huggins expression has been used in an attempt to understand the order–disorder gelatinization transition that occurs when the temperature of an aqueous suspension of starch granules is raised using the mandatory slow heating rate[5]. Although the analysis is based on an idealized concept of the relatively complex gelatinization process, the model accounts for the relationship between the volume fraction of diluent and T_m. Extension of the Flory–Huggins equation to the case of polymer–diluent–solute systems allows the effects of sugars on T_m to be predicted[6].

With relatively high heating rates, non-equilibrium melting would be expected[4]. Despite this fact gelatinization temperatures, measured by differential scanning calorimetry (d.s.c.) using high heating rates, have been analysed with the Flory–Huggins equation[7,8]. To do this, the assumption has been made that the d.s.c. melting temperature approximately equals the equilibrium melting temperature, or at least follows the same trend with variation in diluent volume fraction[7]. The Flory–Huggins approach has also been modified in an attempt to allow for non-uniform moisture distributions in the sample and to predict the effect of electrolytes on gelatinization[9]. If uneven moisture conditions are present, non-equilibrium melting must take place since the chemical potential of the polymer is not equal throughout the amorphous phase. The same applies to the chemical potential of the diluent.

In recent years, a number of investigators have suggested that it is inappropriate to use the Flory–Huggins equation to analyse gelatinization. It has been reported that the transition is always a non-equilibrium melting process for which the Flory–Huggins treatment has no theoretical basis[10]. Non-equilibrium conditions are present in starch d.s.c. experiments since the sample is not annealed and the free energy associated with the large surface area of the small metastable crystals causes melting below the equilibrium value[4].

The latest research on the mechanism of gelatinization has tended to focus on the glass transition that occurs when a partly crystalline polymer such as starch is heated[10–14]. The glass transition takes place at a temperature, T_g, defined by the point below which changes in the position and orientation of amorphous polymer molecules cease[4]. The glass transition explanation of gelatinization is based on the fact that water is an effective plasticizing agent and reduces T_g (ref. 15). According to the model[14], initially there is an imbalance of moisture contents inside (10%, w/w) and outside (100%, w/w) the starch granules. It is suggested that on heating, the plasticizer (water) content inside the granule increases dynamically and a major glass transition and subsequent superimposed crystalline melt take place[14]. The relatively low initial hydration of the amorphous regions is believed to give a kinetically metastable condition where the effective T_g is higher than the equilibrium T_m of the crystalline domains[14]. Consequently, the apparent melting temperature is thought to be elevated and to be observed only after softening of the amorphous regions at T_g (ref. 14). It is further considered that, in contrast, in a gel (say 50% starch, w/w) a uniform moisture content exists and T_g has a value of about − 5° (ref. 16). When the fully hydrated and matured crystalline junctions in such a gel are heated, the junctions are reported to melt at the equilibrium T_m without a heat capacity change[16].

D.s.c. provides a means of studying both the glass and melt transitions in starch–water systems. When a concentrated system containing about 50% (w/w) starch granules is heated, two major peaks are evident in d.s.c. traces in the 50–90° range[7]. According to some investigators[14], extrapolated d.s.c. baselines[14] and derivative thermal curves[12] reveal a characteristic shift in heat capacity at the leading edge of the first d.s.c. peak. These investigators consider the heat capacity change is due to a glass transition and that this transition controls melting. However, other scientists[17] report that T_g is considerably lower than T_m, as would be expected by comparison with synthetic polymers[4]. There is some theoretical support for a lower T_g value[18]. Furthermore, it is well established that starch undergoes annealing below the melting temperature[19], yet annealing only takes place above T_g (ref. 19). Advocates of the glass transition explanation of gelatinization consider these apparent discrepancies due to the fact that in limited water situations, the effective T_g depends on the instantaneously operative conditions of moisture content, temperature, and time[14].

Other evidence has been reported in support of a glass transition. Thermomechanical analysis (t.m.a.) appears to show several stages of expansion in the gelatinization region, as would be expected if softening of amorphous polymers occurs[12]. A difficulty in interpreting such t.m.a. traces is that the volume expansion depends largely on the amount of water taken up by the starch and only secondarily on thermal expansion of the amorphous regions of the granule[14]. In any case, recent X-ray crystallinity measurements suggest these stages are attributable to melts, rather than to a glass transition[20] followed by melting. Other evidence for plasticization of the amorphous regions of granules is based on qualitative X-ray observations, which have been reported to indicate that a d.s.c. endothermic peak occurs in the absence of an order–disorder phase change[21]. However, quantitative X-ray crystallinity measurements do not support this conclusion[20].

Since a re-evaluation of previous t.m.a.[12] and X-ray[21] experiments indicates they do not support amorphous softening, the major evidence for a glass transition in the gelatinization region is that the heat capacity of a starch–water suspension is lower than that of gelatinized starch. In view of the key importance of this experimental finding to understanding the mechanism of gelatinization, further investigations of the heat capacity change accompanying the melting of starch polymers, in granules and in gels, were required. The objective of the present study was to use d.s.c. to make such measurements.

MATERIALS AND METHODS

Starch was isolated from wheat, rice, and corn by subjecting wet-milled samples to repeated sieving and centrifugation[22]. The resultant starch slurries were freeze-dried under standardized conditions to 10% (w/w) moisture[23]. A commercial sample of waxy maize starch was used for some experiments.

D.s.c. measurements were made using a Perkin–Elmer DSC 2C calorimeter equipped with a thermal analysis data station. The instrument was calibrated by standard procedures[12]. The heating rate was 10° $\min^{-1}$ in the polymer melting experiments and 2° $\min^{-1}$ in the experiments on frozen systems. The sample size was about 20 mg and an empty d.s.c. pan was used for the inert reference. All traces were normalized to a basis of 1 mg of starch using the thermal analysis data station. Measurements were made at least in triplicate.

To study gelatinization, granules were weighed into O-ring stainless steel pans designed to withstand high pressures and suppress the volatilization of solvent. Water was added from a microsyringe, any excess water was removed by evaporation, and the pans were then sealed. Samples were allowed to equilibrate for at least 1 h before measurements were made. When calculating the starch content of the samples so prepared, no account was taken of the 10% (w/w) water the starch initially contained, unless otherwise stated.

To make gels, pans containing starch that had been gelatinized by heating to 145° were stored for about 4 days at 5° to encourage nucleation, followed by 40° for at least 24 h to encourage crystal growth and annealing[16]. The melting of the crystalline polymer domains so formed was then measured.

RESULTS AND DISCUSSION

Fig. 1 shows that the d.s.c. trace for a 30% (w/w) starch suspension slopes and exhibits one main endothermic peak. There is also a minor peak at about 105° attributable to lipid–amylose complexes[24]. On cooling the sample and rescanning, a sloping trace with a small peak due to the lipid inclusion complex occurs. All these results are in agreement with those of previous investigators[7–9]. The thermal analysis data station allows the difference between the two traces to be computed. This procedure clearly demonstrates a baseline displacement in the region of the endotherm as also shown in Fig. 1. Hence, as concluded elsewhere[17,25], the heat capacity of a starch–water suspension is not the same as that of the corresponding gelatinized sample[17]. The baseline shift is relatively small, as would be expected from isothermal calorimetry measurements[25]. With more concentrated starch systems two main endothermic peaks occur[7–9], as may be seen in Fig. 2. No inclusion peaks are evident in this figure since waxy starch was used. The trace giving the difference between the first and second scans is not completely flat prior to gelatinization, because the change in the heat capacity of the native and gelatinized granules with temperature is not exactly the same.

The relationship between the course of the heat capacity change and the biphasic endotherm can be studied using an adaption of the method of Slade and Levine[14]. The procedure entails heating the sample to a given temperature, cooling to the starting point, and rescanning to a temperature of about 145°. The difference between the two scans in then computed. The maximum temperature reached in the partial initial scan is first kept below that where the endotherms begins. In subsequent runs this temperature is raised until the end point of the d.s.c. run is reached. A plot of the relative magnitude

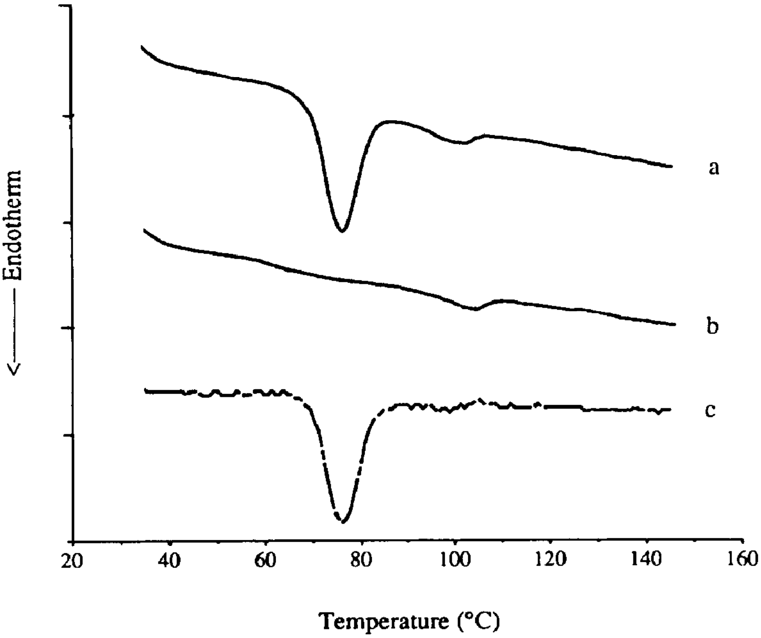

Fig. 1. D.s.c. traces for 30% (w/w) rice starch. Curve *a*, conventional scan; *b*, rescan of sample immediately after cooling to 20°; *c*, difference between scans *a* and *b*. Heat flow axis: 250 μJ s^{-1} per division.

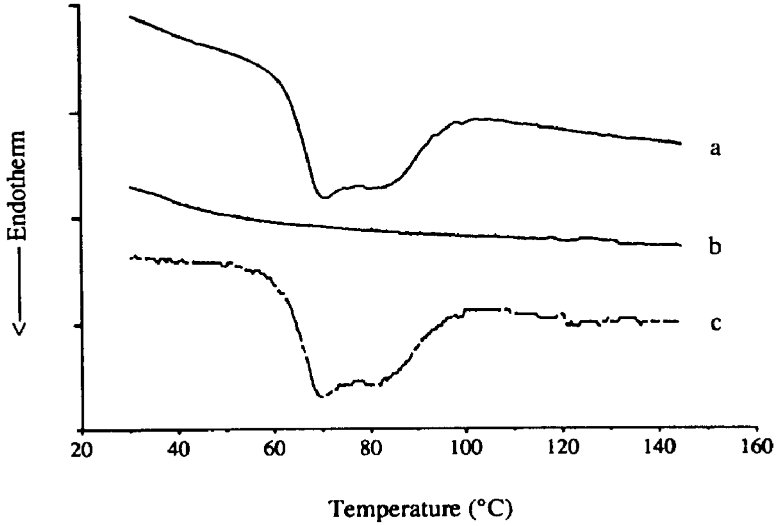

Fig. 2. D.s.c. traces for 50% (w/w) waxy maize starch. Curve *a*, conventional scan; *b*, rescan of sample immediately after cooling to 20°; *c*, difference between scans *a* and *b*. Heat flow axis: 100 μJ s^{-1} per division.

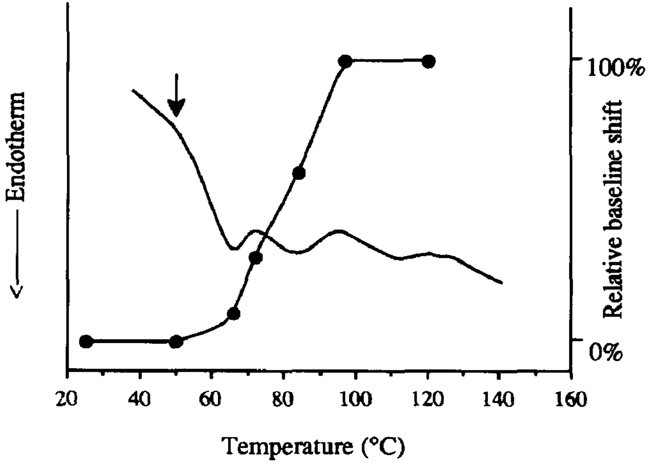

Fig. 3. Conventional d.s.c. trace for a 50% (w/w) corn starch–water mixture (——) and the corresponding baseline shift (-●-).

of baseline shift prior to the rescan *vs.* temperature (Fig. 3) clearly demonstrates that the heat capacity change starts at the same point as the endotherm and increases in size until the phase transition is complete.

An earlier report suggested that the heat capacity change is located exclusively at the leading edge of the first peak in the d.s.c. trace on the grounds that after heating to any given temperature in this region, rescanning gives a baseline difference between the first and second scans[14]. In addition, rescanning after heating to the peak maximum yields a comparatively symmetrical endotherm with a relatively flat baseline, which was considered to represent the melting transition of microcrystalline regions of fully water-plasticized starch[14]. However, the data in the report[14] appear to show that when the initial scanning temperature is increased further the baseline difference at the start of the two scans continues to increase. This would be anticipated if, as reported in the present study, the heat capacity of the crystalline and amorphous starch polymer is not the same. The heat capacity change would also be expected to change as the entire endotherm is traversed if, for all granules, T_g approximately equals T_m. The reason for this is that granules have a range of stabilities and some do not commence to melt until the trailing edge of the d.s.c. endotherm is almost complete[26]. However, the baseline difference[14] would not be expected to increase on the grounds that there is more amorphous polymer due to gelatinization prior to the rescan, even if the glass transition of amorphous polymer in the ungelatinized granules were located at the leading edge of the first endothermic peak. This is because the amorphous material in gelatinized starch has a glass transition temperature below the melting point of ice[14].

D.s.c. traces of starch samples containing 13–22% (w/w) water have been reported to show a shift in heat capacity due to a classical glass transition[17]. When the water content of the samples is increased, the temperature of the heat capacity change decreases[17]. The transition is reversible in that starch polymer may be heated above, and then cooled below, T_g and on reheating the glass transition reoccurs[17]. Such a transition was confirmed in the present study, as shown in Fig. 4. As would be expected[4], T_g is considerably below the corresponding melting point of starch, which in this sample could not be detected by heating to 145°. The value of the heat capacity change due to the glass transition shown in Fig. 4 is 0.1 J deg^{-1}g^{-1}; similar magnitudes were obtained at the other moisture levels studied. This is lower than found for some synthetic polymers[4]. However, starch is a heterogeneous, partly crystalline polysaccharide, so direct comparison with the data for synthetic polymers is difficult. In addition, the glass transition is broad and hard to measure accurately at low plasticizer concentrations. Wide glass transition regions at low plasticizer levels have been attributed to the effect of cross linking, by crystalline domains, in polymer–diluent systems[27].

Since d.s.c. suggests the softening of amorphous polymer has already occurred, it appears that the heat capacity change accompanying melting in granules does not indicate a concomitant glass transition. In a further experiment designed to support this conclusion, a rice starch sample (50%, w/w) was annealed for 1 week at 50° and then gelatinized. The temperature of the peak maximum increased, by about 10°, to 86° and

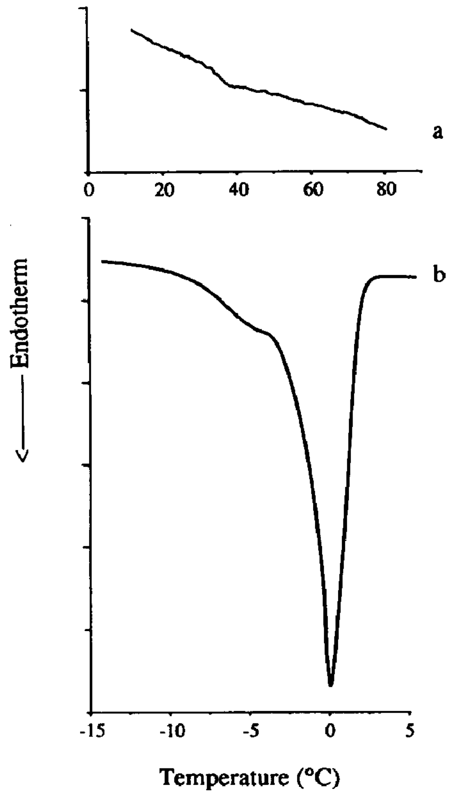

Fig. 4. Glass transitions. Curve *a*, heat flow for a rice starch containing 8% (w/w) added water, *i.e.* 17% (w/w) total water; *b*, heat flow curve for a 50% (w/w) wheat starch gel heated from −15° to 5°. Heat flow axis: *a*, 75 μJ s^{-1} per division; *b*, 400 μJ s^{-1} per division.

the sample still exhibited a heat capacity change during melting (data not shown), despite the fact that T_g must have been exceeded for annealing to have occurred.

In the concentration range studied, the value of the heat capacity change accompanying gelatinization was found to be about 0.3 J $deg^{-1}g^{-1}$. This is comparable to that for globular proteins undergoing denaturation, where the heat capacity shift is thought to be due to the exposure to water of hydrophobic regions of proteins originally buried in the core of the molecule[28]. However, isothermal calorimetry suggests the heat capacity change is -0.06 ± 0.13 (ref. 25). The reason for the discrepancy is uncertain. The explanation for the heat capacity change in starch samples has yet to be established. Shiotsubo and Takahashi propose that the change may be attributable to the displacement of the hydrogen bonds between starch crystalline micelles and water molecules[25].

Considerable evidence indicates that the glass transition in starch gels is at a temperature below the melting point of the frozen solvent[14,29]. The d.s.c. trace in Fig. 4 is consitent with previous work and shows the presence of the shoulder, due to a glass transition[14], that precedes and is superimposed on the ice melt. Fig. 5 shows that, in contrast to a previous report, melting of the crystalline regions in a starch gel gives rise to a baseline shift. Hence the heat capacity change occurring during the melting of the crystalline material in starch gels is not due to a glass transition.

A key feature of the glass-transition explanation of gelatinization is that an imbalance of water contents inside (10%, w/w) and outside (100%, w/w) the granule is considered to occur[14]. Accordingly, the water content inside the granule is not thought to reach the level required to fully plasticize the amorphous regions of the granule below the gelatinization temperature. However, there is extensive evidence demonstrating that

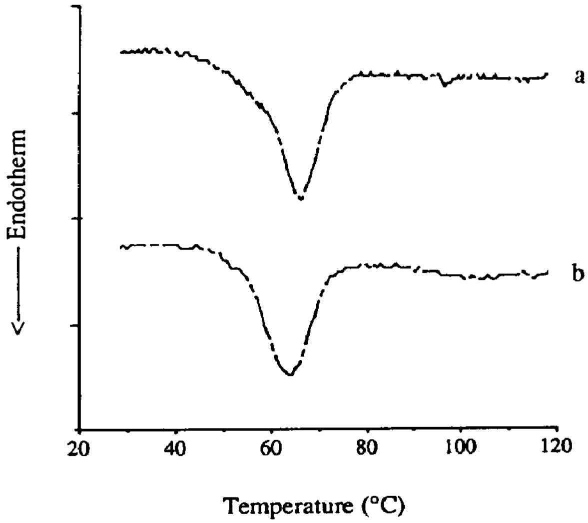

Fig. 5. D.s.c. traces for 50% (w/w) starch gels. Curve *a*, rice; *b*, corn. Each trace represents the difference between a conventional scan and a rescan of the same sample. Heat flow axis: 100 $\mu J\ s^{-1}$ per division.

the water content does reach 27% (w/w), at which point T_g < ambient temperature[17], in the time required to prepare samples for d.s.c. work. Firstly, the gel phase of native starch granules is penetrable by molecules having molecular weights up to about 1000 (ref. 30). Secondly, n.m.r. studies demonstrate that the boundaries of starch granules present no barrier to water diffusion into the granule[31], and it can be calculated that water diffuses into the granule in a matter of seconds[32]. Thirdly, tracer dilution measurements show the water content of intact granules to be about 45–50% (w/w) on a dry basis[9,33]. BeMiller and Pratt[34] found a range of water contents (28–33%, w/w) for granular starch in aqueous suspension at room temperature. Finally, experiments using deuterium show that the hydroxyl hydrogens in the amorphous regions of native starch particles exchange readily[35]. In contrast, there appears to be no evidence that the water content of starch granules remains as low as 10% (w/w) when they are suspended in water. However, the possibility that melting is controlled by a glass transition cannot be ruled out solely by the presence of sufficient water in the granule to decrease T_g below the melting temperature, since the water may be present without plasticization occurring[36].

The results of the present study indicate that gelatinization is due to an order–disorder melting transition rather than to a superheated glass transition immediately followed by a melt. Hence the Flory–Huggins expression might be expected to be an appropriate description if polymer is annealed sufficiently and equilibrium melting conditions apply. However, it may not be possible to obtain the requisite equilibrium crystals in a highly branched polymer such as amylopectin, since the branch points may limit crystal growth[4]. With rapid heating regimes, common under industrial conditions, melting is a non-equilibrium process[4]. In this case application of the Flory–Huggins expression will predict trends in melting behavior that may be useful in practice but the values of the parameters obtained do not agree with the equilibrium case and have limited theoretical basis.

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