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A study of starch gelatinization using differential scanning calorimetry, X-ray, and birefringence measurements

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ABSTRACT

The order–disorder transition that occurs on heating an aqueous suspension of starch granules has been investigated using differential scanning calorimetry (d.s.c.), X-ray crystallinity, and birefringence methods. Starches from wheat, corn, rice, and waxy maize starches were used for the study. Measurements on dilute suspensions (2wt.% starch), showed that decreases in crystallinity occur both before the birefringence of granules starts to disappear and after all birefringence is lost. At such concentrations the ordered domains in single granules melt over a temperature span of about 10°, indicating that the order–disorder transition is not a highly cooperative process. The crystallinity values of more-concentrated suspensions (~ 50wt.% starch), suggest that a melting process accounts for the two main peaks evident in the corresponding d.s.c. traces. The X-ray data do not support the concept that the specific heat change in the d.s.c. traces is attributable to a glass transition in the initial stages of gelatinization. Firstly, the X-ray measurements do not show that a significant endothermic transition occurs without a corresponding change in crystallinity. Secondly, the X-ray data suggest that the volume expansion functions measured by other investigators using thermomechanical analysis are attributable to the increase in the quantity of amorphous starch polymer with temperature rather than to a glass transition followed by a melt.

INTRODUCTION

When an aqueous suspension of starch granules is heated sufficiently, the granules undergo an order–disorder transition known as gelatinization. In recent years, the mechanism of this transition has been studied extensively[1,2]. Despite such research, many features of gelatinization are incompletely understood at the molecular level.

Starch granules consist of about 90wt.% polysaccharide and about 10wt.% water, together with traces of lipid and protein. The polysaccharide component is typically comprised of about 75wt.% amylopectin and 25wt.% amylose, although these percentages may vary considerably. Amylose is a predominantly linear (1→4)-α-D-glucan, whereas in amylopectin side chains are grafted to the linear polymer by single α-(1→6) linkages at intervals of about 20 monomer units. Amylopectin appears to have a racemose structure[3]. Both amylose and amylopectin are heterogeneous polymers possessing a range of molar masses and degrees of branching.

The precise way in which starch polymers are arranged within granules is uncertain. Although amylopectin is highly branched, the polymer is the predominant

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crystalline component of starch grains[4]. The clusters of short branches in amylopectin are considered to crystallize as double helices and so form the ordered domains[5], a process that may be facilitated by the fact that the molecule has a flat, disc-like structure[6]. A single starch macromolecule may have helical or otherwise ordered segments in one or more microcrystallites and random-coil segments in one or more amorphous regions. Thus native starch particles occur in the form of a semicrystalline polymer of low crystallinity with a fringed-micelle type structure.

In view of the complexity of starch at the molecular and macroscopic levels, structural models have been adopted in an attempt to understand granule behavior. A model in which the granule is considered to be equivalent to a synthetic, semicrystalline polymer appears to be the simplest that is capable of explaining key features of gelatinization. On heating in the presence of diluent, such a structure would be expected to undergo both glass and melting transitions[7]. These transitions have been reported in the starch literature (see for example references[8–14]), although there is disagreement as to the conditions under which they occur. Some of the uncertainty arises from the problems in interpreting differential scanning calorimetry (d.s.c.) data. Under conditions where the volume fraction of diluent is $> \sim 0.5$, a single endothermic peak is observed by d.s.c. However where the volume fraction of polymer is greater, a biphasic endotherm occurs[9]. A change in heat capacity has been reported to accompany the first peak in the biphasic transition[12,13]. Isothermal calorimetry also provides evidence for a change in heat capacity associated with gelatinization[15].

Some investigators suggest that the lower-temperature peak in the biphasic endotherm is a consequence of chain mobilization in the amorphous regions of the granule. With the increase in mobility, the polymer chains are transferred from the glassy state to a rubbery aqueous gel[10–14]. According to the model the glass transition, which is responsible for the change in heat capacity, is located at the leading edge of the first d.s.c. peak. Completion of the glass transition permits the crystalline domains to then undergo a non-equilibrium melting process giving the second endothermic peak. Samples that have undergone the lower-temperature transition are reported to exhibit minimal changes in X-ray crystallinity[12,16], but to have lost birefringence[10].

In contrast, other investigators consider the first d.s.c. peak to be due to stripping of polymer chains from the surfaces of crystallites, while the second peak represents melting at low diluent-volume fractions[9]. Another explanation is based on the fact that water will migrate from one location to another within the sample as gelatinization proceeds and suggests that the peaks correspond to order–disorder transitions occurring at different diluent levels[17]. These models are consistent with reports that X-ray changes occur during the first and second d.s.c. peaks[18] and that the loss of birefringence begins with passage through the first endotherm but is not complete until the second peak has been traversed[19]. However the experiments of Nakazawa *et al.*[20] suggest that, when a sample is held at a temperature above that of the first d.s.c. peak, the resultant starch exhibits an amorphous X-ray pattern but still yields a single, sharp endothermic peak.

Clearly the gelatinization literature contains a number of conflicting results.

Some of the apparent contradictions in the X-ray data may arise because only qualitative measurements have been made. The present paper describes quantitative X-ray crystallinity measurements of the order–disorder transition in starch together with corresponding birefringence and d.s.c. investigations.

MATERIALS AND METHODS

Starch was isolated from wheat, rice, and corn by subjecting a wet-milled sample to repeated sieving and centrifugation[21]. The resultant starch slurry was freeze-dried under standardized conditions to a 10wt.% moisture content[22]. As large and small granules in the bimodal wheat-starch size-distribution gelatinize at different temperatures, and have different crystallinities and d.s.c. endotherms[23], a sample of the large particles was obtained by sedimentation[24] and used for further study. A limited number of measurements were also made on a commercial sample of waxy maize starch.

Aqueous suspensions (2 g), containing 2, 30, 50, or 60wt.% starch, were prepared in glass vials. The heat treatment was applied to the 2wt.% samples by placing the vials directly into a water bath at the designated temperature. The more-concentrated suspensions were first heated at a rate of 1° min^{-1} to the required temperature to promote the more even distribution of water through the sample. All suspensions were held at the final treatment temperature for 60 min, cooled by immersion in an ice–water bath and freeze dried to 10wt.% moisture.

Birefringence was measured on diluted samples[19] obtained after the heat treatment but before freeze drying. Individual granules were examined by microscope. Some particles were observed that had partially lost birefringence, and these were considered to be non-birefringent when calculating the fraction of granules in the sample that displayed birefringence. As granules lose birefringence[19] over a range of 1 or 2°, the method of accounting for any partial birefringence has only a minor effect on the birefringent fraction versus temperature function. A population of > 500 granules was studied to characterize the 2wt.% suspensions. With the more-concentrated suspensions, no attempt was made to establish the exact percentage of birefringent particles.

X-Ray measurements were made using the crystallinity index procedure as described previously[25]. This method compares the crystallinity of an unknown sample with that of crystalline and amorphous references[26]. The intensity of the X-ray diffraction is measured at equal increments over the range of diffraction angles that includes most, if not all, of the crystalline peaks. The intensity data are then normalized so that the area under the curve of intensity versus diffraction angle is the same for all samples. This yields normalized values of the intensities I_c, I_a and I_u, corresponding to the crystalline, amorphous, and unknown samples respectively, at each of the diffraction angle positions, i. A graph of $(I_u - I_a)_i$ versus $(I_c - I_a)_i$ is then plotted for each value of i and the slope multiplied by 100 is the crystallinity index. To make starch crystallinity-index measurements, control samples of starch, which had been suspended in water (2wt.% polysaccharide) without heat treatment and freeze dried, were used as crystalline references. The amorphous references were the 2wt.% samples that had been heated for 60 min at 95°. The amorphous references have smooth curves, with no crystalline peaks, indicating that recrystallization did not occur between thermal treatment of the samples and freeze-drying. The X-ray intensities were recorded from 12 to 26° 2Θ at intervals of 0.25Θ using CuK$_\alpha$ radiation, an Ni filter, a voltage of 42 kV and a current of 30 mA.

D.s.c. measurements were made by using a Perkin–Elmer 2C calorimeter with thermal analysis data-station. The instrument was calibrated by standard procedures[10]. Starches 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. An empty pan was used as inert reference. A heating rate of 10° min^{-1} was used in the d.s.c. experiments.

RESULTS AND DISCUSSION

The typical results in Fig. 1 show that, in the initial stages of gelatinization of 2wt.% starch suspensions, the decrease in crystallinity is larger than the corresponding loss in birefringence. For example, when ~5% of the granules have lost birefringence, the crystallinity is reduced by ~20%. Figure 1 also indicates that reductions in crystallinity occur at temperatures below the point at which granules first lose birefringence. When suspensions were kept at 5° below this point for more than the standard time of 1 h, crystallinity increases were recorded while the birefringence appeared to remain constant. For example, in the case of corn starch, the crystallinity value increases to 112% after a treatment time of 190 h. Rice and wheat starches give a similar trend. According to the definitions used in the thermal characterization of synthetic polymeric materials[7], this is not strictly an annealing process in which crystal improvement occurs below the melting temperature, but is rather a recrystallization process which entails melting followed by renewed crystallization. Such recrystallization processes have been found in starch by other investigators[13,27,28]. However the phenomenon is more noticeable at higher temperatures, perhaps because at lower temperatures the presence of the greater number of crystallites tends to constrain the amorphous regions into conformations that are incompatible with crystal formation[13].

Figure 1 also shows that decreases in crystallinity occur after all granules have lost birefringence. Clearly birefringence measurements, which are widely used to monitor polymer melting[29], provide only an approximation of the final melting point of starch polysaccharides. Birefringence and crystallinity reflect different structural attributes in starch[30]. The fact that starch is birefringent implies there is a high degree of molecular order within the granule[2,30]. Partial melting of crystallites appears to occur without disrupting this orientation sufficiently to cause birefringence to disappear. The decrease in crystallinity that takes place after all birefringence is lost may reflect the disordering of small-scale structures that are not aligned with respect to one another or to the geometry of the granule[31].

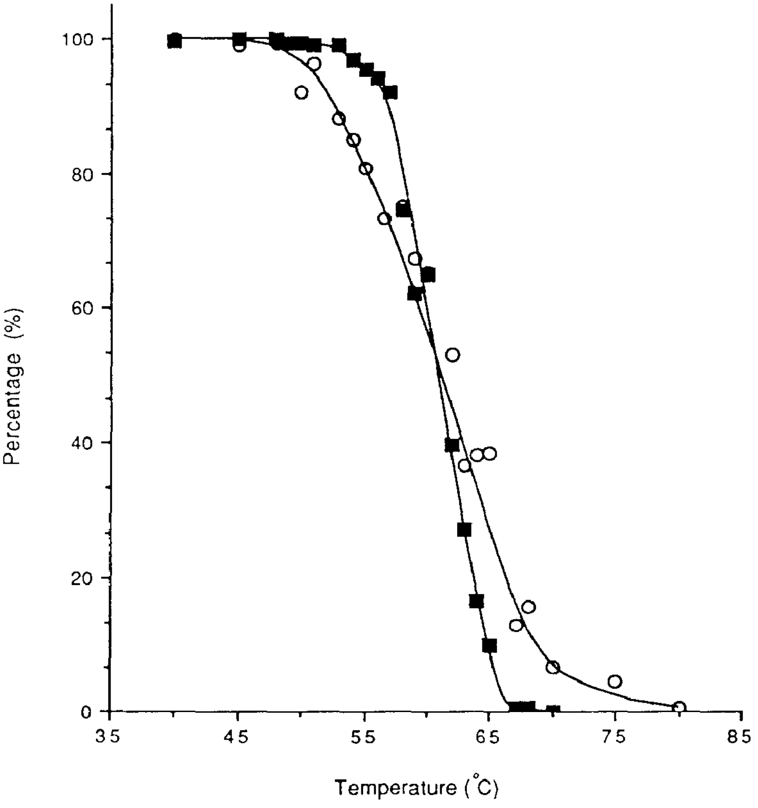

Fig. 1. The birefringence ■ and crystallinity ○ of a 2wt.% corn starch suspension as a function of temperature.

Light microscopy suggests that, in the presence of sufficient diluent, individual granules lose birefringence, and so gelatinize, over a narrow temperature-range[19] of 1 or 2°. For this reason, the transition, within any given starch particle, has been considered to be a highly cooperative process in which all the crystallites melt at about the same temperature[17]. The data in Fig. 1 suggest that, for a population of granules, the temperature range over which crystallinity losses occur is about 10° broader than the range over which birefringence disappears. Accordingly the melting range for a single granule may be more like 10°. Nevertheless, polymer theory[7] suggests the melting would be cooperative in that in a semi-crystalline polymer, amorphous regions are maintained by crystallites which function as cross-links. These cross-links hold the amorphous regions in a metastable strained state. With a strained state there is a higher melting temperature. On partial melting, the strain relaxes and the melting temperature of the remaining crystallites is decreased. Thus the crystallites melt over a narrower range than occurs when no strain is present[7]. In addition, with a reduction in the number of crosslinks, a flux of diluent into the amorphous zones takes place, which would be expected to depress the melting temperature further[17].

The typical data in Fig. 2 indicate that, when starch suspensions are more concentrated, substantial amounts of crystallinity remain even when the temperature reaches 95°. Control experiments with waxy maize confirmed that the values of the residual crystallinity were not an artifact caused by the presence of lipid–amylose complexes. After heating at 95° for 1 h, the waxy maize crystallinities were found to be 21, 11, and 8% for the 60, 50, and 30wt.% suspensions respectively. In further control experiments, the possibility that crystallization processes were responsible for the residual crystallinity was explored. Such crystallization might be expected since the kinetics are not necessarily unfavourable[11] and since there are few constraints on the polymer in the amorphous zones as substantial regions of granules are disordered[13]. A 30wt.% suspension of rice starch was heated at 95° for periods of 10, 20, 30, 40, 50 and 60 min. The crystallinity values after this thermal treatment were found to be 24, 26, 20, 21, 26 and 21%, respectively. Thus recrystallization cannot be detected and does not account for the residual crystallinity at 95° shown in Fig. 2. One explanation for the residual crystallinity may be that due to non-equilibrium conditions, uneven moisture levels in the sample cause incomplete melting to occur[17]. This explanation is supported

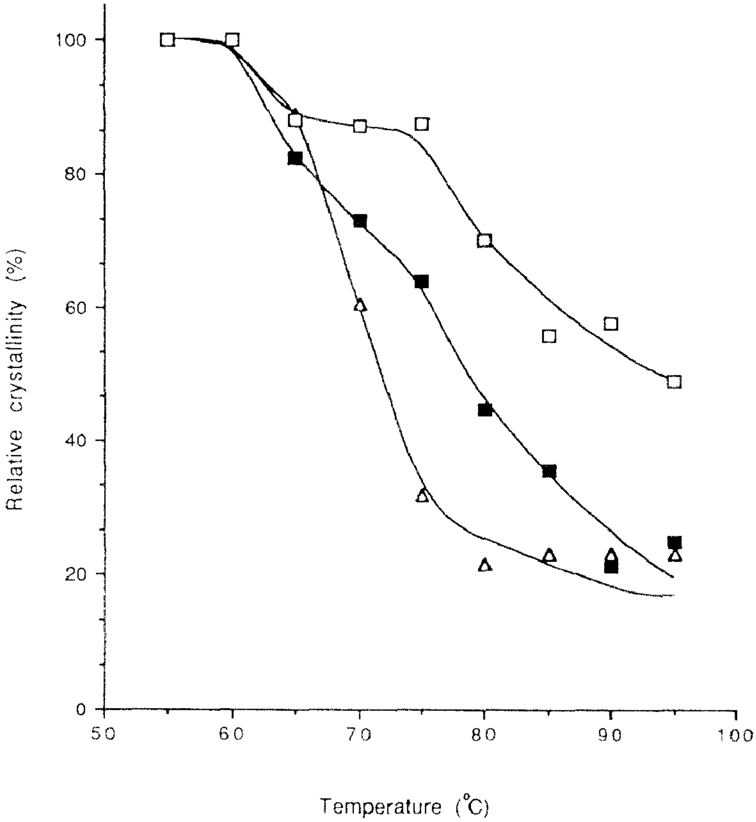

Fig. 2. X-Ray crystallinity versus temperature for 30wt.% ▲, 50wt.% ■, and 60wt.% □ rice starch.

by the fact that some birefringent granules were observed in the concentrated suspensions heat-treated at 95°.

The relationship between loss of crystallinity and temperature for the 50% and 60% suspensions suggests that a two-step process is occurring. There appears to be an initial decline in crystallinity, followed by an inflection leading to an intermediate plateau section, followed by a region of rapid crystallinity loss. The shape of the relative amorphous content (i.e. 100 – %crystallinity) versus temperature function is similar to that reported for volume expansion curves obtained by thermomechanical analysis[10,20]. The initial volume expansion in the latter traces has been attributed to a glass transition, the plateau to a partial melting and structural perfecting region, and the final abrupt increase to a melt[10]. As an increase in amorphous material equates with a volume expansion[32], the X-ray data presented here suggest that the initial change in thermomechanical analysis traces may reflect a melt rather than a glass transition.

Another thermal analysis technique with which crystallinity measurements may be compared is d.s.c. However the small size of the d.s.c. samples precludes their use for X-ray measurements[12] and a direct comparison is not possible. In the absence of diluent, the loss of crystallinity may be considered to be equivalent to an enthalpy change[7]. In the presence of diluent, the enthalpy change reflects the net effect of an endothermic melt, together with an exothermic heat of hydration of the disordered polymer[2]. Any change in heat capacity will also be apparent in the d.s.c. trace. If, in the first instance, the assumption is made that a given decrease in crystallinity gives rise to a constant enthalpy change, then the crystallinity versus temperature relationships may be converted into a form that is comparable with d.s.c. output. Since d.s.c. measures dH/dt, where H is the heat, the first derivative of the X-ray crystallinity curves is taken.

The 30wt.% starch suspensions give a single peak in the derivative trace; see the typical data in Fig. 3. Given the different thermal treatments received by the samples, the range over which the order–disorder transition occurs in the X-ray experiments would be expected[14] to shift to about 8° lower than found in the corresponding d.s.c. runs. Thus there is broad agreement between the derivative X-ray traces and the d.s.c. outputs for

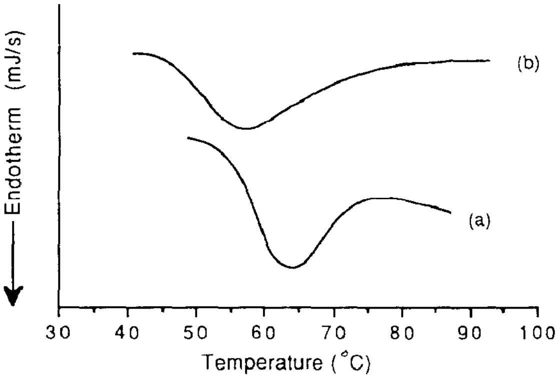

Fig. 3. D.s.c. trace (*a*) for a 30wt.% corn starch suspension and (*b*) the corresponding trace calculated from X-ray crystallinity data.

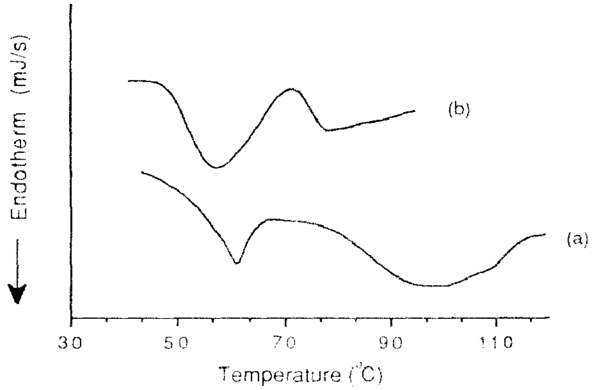

Fig. 4. D.s.c. trace (*a*) for a 60wt.% corn starch suspension and (*b*) the corresponding trace calculated from X-ray crystallinity data.

30wt.% suspensions. The X-ray data for the 60wt.% suspensions clearly give a biphasic effect (see Fig. 4). The transition again shifts to a lower temperature-range than found by d.s.c. With low-moisture systems, non-equilibrium water gradients resulting from high heating rates would be expected to cause a greater fraction of granules to gelatinize at high temperatures[2]. Thus as shown in Fig. 4, more particles gelatinize at lower temperatures in the X-ray than in the d.s.c. experiments.

Qualitative X-ray results have been reported to show that, with concentrated starch suspensions, the leading peak in d.s.c. traces occurs without a crystallinity change[12,16]. This has been taken as evidence for a glass transition in the initial stages of gelatinization. The quantitative data given in Fig. 4 do not support this concept. Furthermore, the X-ray results are in accord with the gelatinization model of Evans and Haisman[17]. This model suggests that the biphasic order–disorder transition is due to water gradients within the sample and requires that crystallinity is lost in parallel with the progress of gelatinization as detected by d.s.c.[2]. Figure 4 indicates this is the case, yet qualitative X-ray measurements have been reported to rule out this model[16]. Thus this section of the present study serves to emphasize the problems of estimating crystallinity changes by visual assessment of X-ray traces.

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