8852 *Macromolecules* **2002,** *35,* 8852−8859

Reexamination of Phase Transformations in the Starch−Water System

Stanisław L. Randzio,*,‡ Iwona Flis-Kabulska,†,‡ and Jean-Pierre E. Grolier†

Laboratoire de Thermodynamique et Génie Chimique, Université Blaise Pascal, 63177 Aubière, France, and Institute of Physical Chemistry, Polish Academy of Sciences, ul. Kasprzaka 44/52, 01-224 Warsaw, Poland

Received July 12, 2001; Revised Manuscript Received August 26, 2002

ABSTRACT: High-sensitivity temperature-controlled DSC measurements at a low heating rate and creation of differential DSC traces with respect to the reference material (completely dried starch) permitted to revise precisely the actual knowledge on the phase transformations in starch. Five types of well-reproducible transformations have been observed: (1) sharp endothermic transition occurring over the temperature range 319−333 K independent of the water content associated with melting of the crystalline part of the starch granule followed by a helix−coil transformation in amylopectin; (2) water content dependent slow exothermic transition associated with reassociation of the unwound helixes of amylopectin with parts of amylopectin molecules other than their original helix duplex partner, forming physical junctions and creating more general amorphous hydrogen-bonded associations; (3) water content dependent low-temperature exothermic transition associated with recrystallization of partly dehydrated starch; (4) high-temperature endothermic transformation associated with nematic−isotropic transition and small distortions on the main endothermic transition associated with smectic−nematic transition; (5) continuous slow exothermic transformation associated with the softening and sucking in water of the amorphous growth rings of the starch granule.

Introduction

Starch is one of the most important natural macromolecules. Its importance comes basically from the fact that the starch granule is an almost universal form for packaging and storing carbohydrate in green plants.[1] Therefore, an abundant literature exists, sometimes conflicting, with respect to the structure, properties, and processing of starch. Fortunately, the recent introduction of new or improved techniques permits to reexamine the knowledge already acquired and to verify the existing models with a high precision inaccessible in the past. A review of recent studies on internal information regarding architectural organization of starch granules at high resolution obtained by transmission electron microscopy (TEM) and on surface information obtained by both scanning electron microscopy (SEM) and atomic force microscopy (AFM) together with a critical assessment of existing structural models of starch has been presented by Gallant et al.[2] On the other hand, the analysis of the native structure of starch granules performed with small-angle X-ray microfocus scattering has been presented by Waigh et al.[3] The crystallinity and structuring role of water in native and recrystallized starches have been reexamined by ^{13}C NMR spectroscopy equipped with CP-MAS (cross-polarization magic-angle-spinning) accessories,[4] and the time domain NMR spectroscopy was used for the quantitative determination of bound water in starch.[5] Finally, the starch gelation process was observed by FT-IR/ATR spectrometry.[6] The aim of the present contribution is to reexamine with the use of a high-sensitivity temperature-controlled differential scanning calorimeter (DSC) the multistep transitions in the starch−water system during its gelatinization. The calorimetric information is of macroscopic nature, and so when performing an investigation over a large temperature range at various water concentrations using an instrument of high sensitivity, one can obtain a detailed overview of energetic states and follow processes of various kinetics. Such an information correctly related to the information obtained by microscopic techniques is of extreme importance for the deep understanding of the properties and of the behavior of such a complicated natural biopolymer in interaction with water.

It is well-known that biosynthesized native starch granules are semicrystalline and contain densely packed polysaccharides: mainly amylopectin and amylose with a little amount of water. Amylose is a linear array of 1000−10 000 α-(1→4)-D-glucosidic units with a small amount of branching,[1,7] while amylopectin is a highly branched molecule with average molecular weight of the order of 10^8 Da with the same α-(1→4)-D-glucosidic backbone and α-(1→6)-D-glucosidic linkages at the branching points.[8] The molecules of amylopectin are about 10−15 nm in diameter and 200−400 nm long.[1] Some starch granules are almost entirely amylopectin (up to 98%) and others are high in amylose (45−80%), but granules generally are a mixture of amylose and amylopectin with amylose contents of 15−30%.[7] The modern structural techniques permit to reveal and quantify the structural phases in native starches: crystalline A and B forms, partially ordered interfacial and amorphous background forming matrix in which the crystallites are imbedded.[4] The crystalline or semicrystalline properties of native starch are attributed to the short-chain fraction of amylopectin arranged as double helices and packed in crystallites. Like in other polymers, the crystallites have a lamellar structure.[9] However, the lamellae (∼9 nm)[2,3] found in the native starch exhibit some novel features. Their appearance has more in common with a side-chain liquid-crystalline polymer, double helices arranged side by side in a smectic or nematic type structure,[10,11] than with spherulitic struc-

---

† Université Blaise Pascal.

‡ Polish Academy of Sciences.

* Correspondening author: e-mail randzio@ichf.edu.pl; Fax +48-39 12 02 38.

10.1021/ma011213r CCC: $22.00

tures with chain-folded lamellae as in synthetic polyethylene, and heating starch amylopectin crystallites in the presence of water does not appear to increase or decrease the lamellar long spacing.[12] As yet, there is no definitive evidence that amylose participates in starch crystallites, except that a polymorph can be formed as a result of complexation of amylose with some compounds like lipids, but it is rarely detected as a crystalline material in native starches.[4,13,14] Recent studies on the relationship between thermodynamic and structural properties of low and high amylose starches showed that the amylose content up to 25% does not influence the melting behavior of aqueous dispersions of the investigated starches.[15,16] Most probably amylose, or at least significant fractions of it, are located primarily in the amorphous phase of the granule rather than being incorporated into crystallites.[1] It follows from the recent investigation[2] performed by SEM, TEM, and AFM that the crystalline and amorphous lamellae of the amylopectin are organized into larger, more or less spherical structures, which have been termed “blocklets”. The blocklets range in diameter from around 20 to 500 nm.[2] Possibly, there is no sharp demarcation between the crystalline and amorphous phase of starch granules, and it is generally believed that some or all the starch molecular chains run continuously from one phase to another, which is not difficult to imagine when taking into consideration that the individual molecules are far larger than the crystallites. The interface between the crystalline or semicrystalline phase and the amorphous phase as well as the interorganization of these two phases is very important for understanding the behavior of the starch–water system and has become of great interest in recent investigations.[2,4,17] Anyhow, even if this problem is not completely understood yet, there is no doubt that the starch granule is a highly heterogeneous system. The process of preparation from it in a mixture with water of a homogeneous SOL phase (in the sense of Flory[18]), called gelatinization in the literature, must go through destruction of the native structure in starch granules. This destruction must include melting of ordered regions, both on the crystallite level (inner and surface) and on the level of amylopectin double-helical order. Thus, during gelatinization hydrogen bonds stabilizing the structure of the double helices must be broken and possibly replaced by reassociation of the free ends of the unwound helixes of amylopectin with parts of amylopectin molecules other than their original helix duplex partner, forming physical junctions and creating more general amorphous hydrogen-bonded associations, which normally should be associated with exothermic effects. A recent study performed with synchrotron X-ray diffraction revealed that after the endothermic effect of melting of the starch granule, upon further heating a crystallization of amylose–lipid complexes, was observed, which also should be associated with an exothermic effect.[19] So, the energy balance of the gelatinization process should include both endothermic and exothermic effects. The endothermic effects associated mainly with melting should be rather first-order transitions, the rate of which is simply proportional to the rate of inducing temperature variations. On the other hand, the formation of new complexes in colloidal systems is rather slow, so no sudden evolution of heat can be expected, the thermal effect being spread out over the whole time, and so the thermal power of such a process must be very low.[20] Thus, to record such a process, a high-sensitivity scanning calorimeter should be used with care and skillfulness, allowing all the above processes to be developed over the time frame of the experiment.

Starch and its water pastes and dispersions have been intensively studied by DSC in the past.[21–39] The general view of the gelatinization process of starch as seen from the known temperature scanning calorimetric measurements was interpreted as follows. When heating a starch–water dispersion at around 50% water, an endotherm composed of two overlapping peaks is observed first, and then at higher temperatures a third small single endothermic peak appears. When increasing the water content in the dispersions, the two overlapping peaks progressively converge, and when decreasing the water concentration in the dispersions, the lower temperature peak disappears and the second one is shifted to higher temperatures. An important impact on the interpretation of the calorimetric results and possibly on the performance of further calorimetric investigation in the starch–water system in general was exerted by the analysis made by Donovan.[22] According to this analysis, the lower temperature peak in the overlapping endotherms is a consequence of stripping of polymer chains from the surfaces of crystallites, while the second peak represents melting of crystallites at low diluent volume fractions. On the basis of concepts of Flory[40] for semicrystalline polymers and following Lelievre,[41,42] Donovan described this melting as a regular equilibrium process. This model relates the melting temperature of a semicrystalline polymer to the diluent concentration under equilibrium conditions. Later this approach was reasonably questioned with an argument that starch gelatinization is a nonequilibrium process and that the process is controlled by the requirements for previous softening of the amorphous parts of the granule.[32,43–45] Another explanation is based on the fact that water migrates from one location to another within the sample as gelatinization proceeds and suggests that the two endothermic overlapping peaks correspond to order–disorder transitions occurring at different diluent levels.[46] There are also models for the structure loss during gelatinization which invoke crystalline disruption followed by helix melting at a higher temperature.[47,48] However, comparison of high-resolution ^{13}C CP-MAS NMR spectra, which detect order corresponding to double-helix content, and X-ray diffraction, which detects only those double helices that are packed in regular arrays (crystallites), with DSC thermograms permitted to conclude that the crystalline and molecular order are lost concurrently during gelatinization and that DSC endothermic enthalpy values primarily reflect loss of double-helical order rather than loss of crystalline register.[36] The third endothermic peak appearing at high temperatures was attributed to dissociation of amylose–lipid complexes.[25,31]

It can be seen from the above short review that all existing calorimetric results advocate for the model of gelatinization in starch–water system as being composed of purely endothermic phenomena. There is no mention in the published calorimetric results of any exothermic effects in the course of starch gelatinization. In some studies speculations are presented for the existence in the gelatinization process of such exothermic phenomena as wetting, hydration, and formation of starch–water hydrogen bonds and of other complexes, but they are considered as occurring over the same time

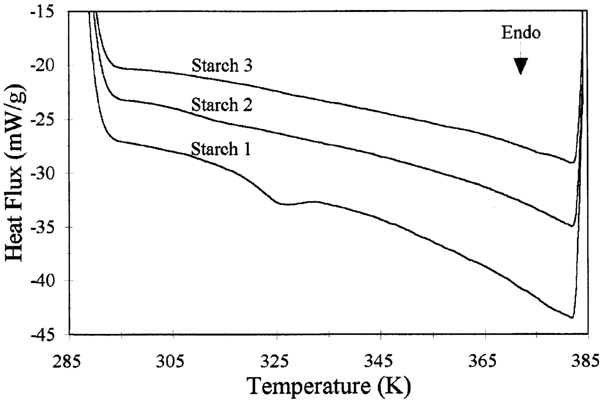

Figure 1. DSC thermograms of wheat starch samples used in the present study obtained at a heating rate 16.67 mK s^{-1}: Starch 1: native starch powder containing 11.8 wt % of water. Starch 2: partly dehydrated starch obtained by drying in a vacuum oven at 333 K for 110 h, still containing 5 wt % of native water. Starch 3: completely dehydrated starch obtained by drying in a vacuum oven at 393 K for 110 h.

frame as the endothermic transitions and being simultaneously and completely compensated by them.[1,22,38] Even some evident slow exothermic phenomena clearly seen on the thermograms presented in the literature[26,32,34–38] were most probably taken only as accidental baseline drifts and were not treated as a significant source of information. In this respect the experiments reported by Biliaderis et al.[32] are a good example. The authors have performed DSC experiments on rice starch at 50% water varying the heating rate from 16.7 to 500 mK s^{-1} (Figure 4 in ref 32). At high heating rates only endothermic effects and endothermic baseline drifts were observed. However, at low heating rates, starting already at a rate 83.3 mK s^{-1}, regular significant exothermic drifts were observed. At the lowest heating rate (16.7 mK s^{-1}) the slow exothermic effects exceeded in amplitude almost twice the fast endothermic effect. Focusing on the endothermic transition, the authors did not pay attention to this problem. However, it seems evident that during fast heating only fast transitions can be observed; the slow phenomena have not enough time to develop. To record the slow transitions and especially the overlapping of slow and fast phenomena, a high-sensitivity DSC technique must be used at low heating rates. Moreover, advanced analysis of DSC traces must be performed such as static baseline corrections, and the use of proper reference materials should be worthwhile. No attention was given previously to those problems in the existing open literature devoted to the starch investigation by DSC.

Experimental Section

Materials. Wheat starch powder was from PROLABO, France, catalog no. 21 146 290, with amylose content 21.0 ± 0.2%, lipid content 0.27 ± 0.01%, and protein content 0.36 ± 0.01%. Three types of starch samples were used in calorimetric measurements: (1) native (starch 1), (2) dried in a vacuum oven at 333 K for 110 h (starch 2), and (3) dried in a vacuum oven at 393 K for 110 h (starch 3). The content of water in the native starch was 11.8 wt % as determined on the basis of mass loss after heating a weighted sample in a vacuum oven at 393 K for 110 h. Thus, it was assumed that the samples of starch 3 did not contain any water. After heating for the same period of time at 333 K, 5 wt % of native water still remained in the starch sample (starch 2). Respective DSC traces of the three types of starch samples are presented in Figure 1. One can see that the two dried samples do not demonstrate any sharp transition, while the native starch demonstrates an endothermic transition occurring over the temperature range from 319 to 333 K.

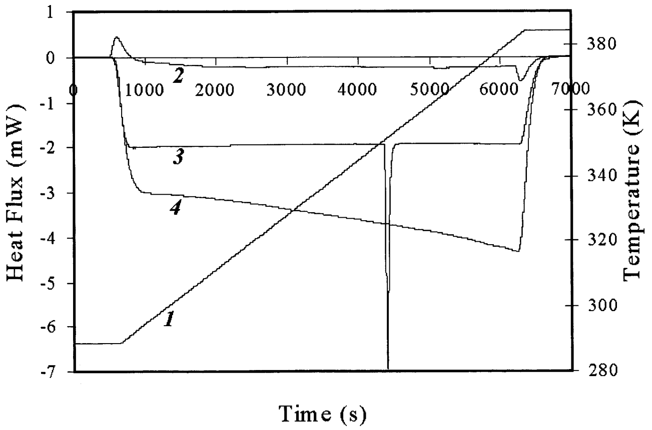

Figure 2. An example of DSC traces used for calibration purposes: (curve 1) linear temperature rise at a rate 16.67 mK s^{-1}, (curve 2) instrument baseline obtained with empty vessels, (curve 3) with a sample of naphthalene, and (curve 4) with a sample of completely dehydrated starch (starch 3).

To prepare a water dispersion or a paste, a given amount of bidistilled and demineralized water was added to a weighted sample of either native (starch 1) or modified (starch 2) starch powder, and the mixture was carefully blended manually until a homogeneous phase was obtained.

Methods. A sample of 130–150 mg of prepared starch–water paste or dispersion was placed in a stainless steel calorimetric vessel, and then the vessel was closed and placed in the calorimeter (micro-DSC, SETARAM, Caluire, France). The reference calorimetric vessel remained empty. After a thermal stabilization during around 2000 s at 282.6 K, an initial static (isothermal steady state) baseline was recorded for at least 200 s, and then the temperature scanning was performed up to 384.1 K at a rate of 16.67 mK s^{-1}. After the end of the temperature scanning a return to the static steady state at the high-temperature limit was recorded. In the case of any difference between the initial (low temperature) and the final (high temperature) static baselines, a small linear correction of recorded traces was performed in such a way that after the correction both at the initial and at the final static states the heat flux signals (calorimetric outputs) were always reduced to zero. In such a way *corrected DSC traces* were obtained. In the same manner the calorimetric output signals were recorded and treated for the calibration purposes: (1) with the two calorimetric vessels remaining empty (curve 2 in Figure 2), (2) with a standard substance for calibration of temperature and heat flux scales (curve 3 in Figure 2), and (3) with a sample of completely dehydrated powder starch (starch 3) (curve 4 in Figure 2). One can see in Figure 2 that the calorimetric signal with empty vessels (instrumental baseline signal) is very small and is practically not dependent on temperature. However, the calorimetric signal with a sample of completely dehydrated starch rather strongly and almost linearly depends on temperature. This temperature dependence is caused mainly by the thermal vibrations of the starch molecular backbone. Because the aim of the present study is to reexamine the phase transformations in the starch–water system and because as many others we think that these transformations arise from starch–water interactions, rather than from changes in the starch itself, we decided to use the calorimetric signal obtained with the completely dried starch as a reference baseline. The procedure of subtraction and scaling of obtained *differential DSC traces* (DT) is described by eq 1

$$\mathrm{DT} = \frac{\mathrm{CT} - \mathrm{EBL}}{m_{\mathrm{s}}} - \frac{\mathrm{SCT} - \mathrm{EBL}}{m_{\mathrm{st}}}\frac{m_{\mathrm{sts}}}{m_{\mathrm{s}}} \qquad (1)$$

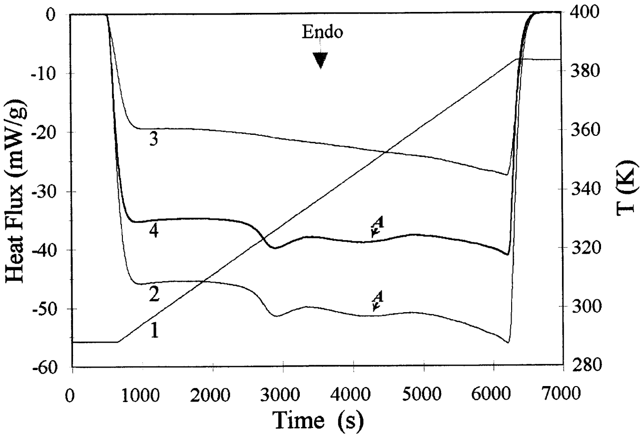

Figure 3. An example of creation of a differential DSC trace: (curve 1) linear temperature rise at a rate 16.67 mK s^{-1}; (curve 2) difference between the corrected DSC trace (corrected for a small static baseline drift; CT in eq 1) obtained with a sample of 52.6 wt % (full water content: remaining 5 wt % native + added) water–starch (starch 2) paste and the instrumental baseline (curve 2 in Figure 2, EBL in eq 1) divided by the mass of the sample (m_s); (curve 3) difference between the corrected DSC trace (corrected for a small static baseline drift; SCT in eq 1) obtained with a sample of starch powder completely dehydrated at 393 K (starch 3) and the instrumental baseline (curve 2 in Figure 2, EBL in eq 1) divided by the mass of the sample (m_{st}); (curve 4) = 2 − 3(m_{sts}/m_s) = differential DSC trace representing the pure thermal effects of interaction of water with starch at 52.6 wt % water concentration (DT in eq 1).

where CT is a corrected DSC trace obtained with a sample of a starch–water mixture, m_s is the mass of the sample which corresponds to CT, EBL is the corrected DSC trace obtained with empty vessels (curve 2 in Figure 2), SCT is a corrected DSC trace obtained with a sample of completely dried starch (starch 3), m_{st} is the mass of the sample of completely dried starch which corresponds to SCT, and m_{sts} is the mass of starch in the sample of the starch–water mixture which corresponds to CT, recalculated for the completely dried mass. Figure 3 presents an example of the applied procedure with the use of starch 2 + water mixture (52.6 wt % of total water content). From Figure 3 one can see that on the differential DSC trace it is much easier to distinguish various transformations (for example, the beginning of the slow exothermic effect in point A), because such a differential DSC trace represents mainly a heat flux caused by the interaction of water with starch induced by temperature variations; all other temperature effects such as thermal vibrations of the starch molecular backbone being included in the reference trace after subtraction are not present in the differential DSC trace. This procedure of obtaining the differential DSC traces was preferred over placing the reference material directly in the calorimetric reference vessel in order to avoid any doubts with respect to the behavior of the reference material and so to the symmetry of the differential calorimetric system.

Both temperature and heat flux calibrations of the micro-DSC were performed and verified with naphthalene (PROLABO, T_{fus} = 351.4 K, $\Delta_{fus}H$ = 149.2 J g^{-1}) and with azobenzene (ACROS ORGANICS, T_{fus} = 340.25 K, $\Delta_{fus}H$ = 120.96 J g^{-1}). The calibrations were performed with five various masses of each calibrating substance in order to verify the linearity of the measuring system with respect to the magnitude of the thermal effect. The dispersion of calibrating results was within ±1%.

Results

A series of differential DSC traces obtained for various water concentrations are presented in Figure 4. The samples for these investigations were prepared from starch 2. With a precision of ±0.5 wt % the water concentrations correspond to the full water content. A complete set of results obtained with the use of native starch (starch 1) are presented in Figure 5. Here also the numbers given at the respective differential DSC traces correspond to the full water content (native + added) in the investigated mixtures.

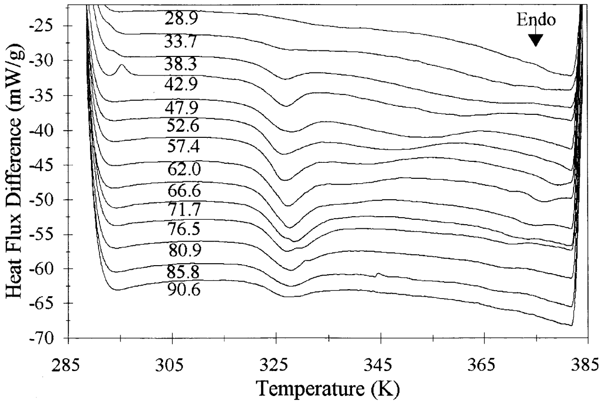

Figure 4. Differential DSC traces obtained for partially dehydrated starch (starch 2) at various concentrations of water (full water content in wt %).

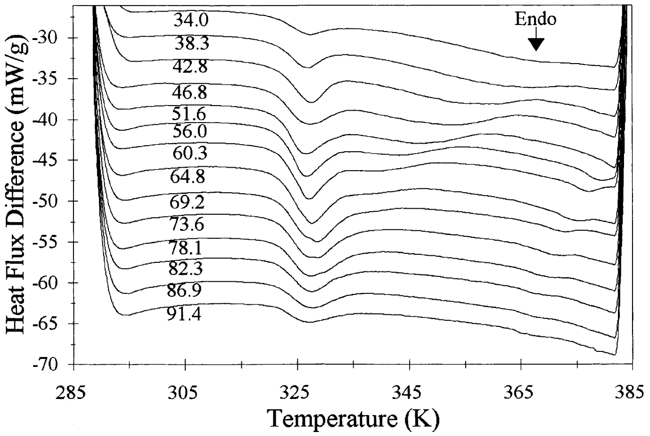

Figure 5. Differential DSC traces obtained for native starch (starch 1) at various concentrations of water (full water content in wt %).

Discussion

The role of water in the behavior of starch and in other natural biopolymers is of extreme importance. The improved experimental procedures of temperature-scanning calorimetric measurements and the new method of exploring recorded DSC traces (creation of differential DSC traces) introduced and explored in the present study permitted to extract experimental information which is directly related to the influence of water on the behavior of starch as a function of temperature. The most important result obtained in this study with the use of the new procedure is the exothermic nature of the second transition out of the two main transitions observed in the middle temperature interval; both of them were previously[22–39] interpreted as endothermic. Such a confusion could happen in the past because of the overlapping of the transitions resulting from the water–starch interaction with the almost linear heat flow coming from the increase of the heat capacity of the starch backbone as a function of temperature. This is clearly seen in Figure 3, where from the original full DSC trace (curve 2) obtained for 52.6 wt % water–starch paste one could erroneously deduce that there

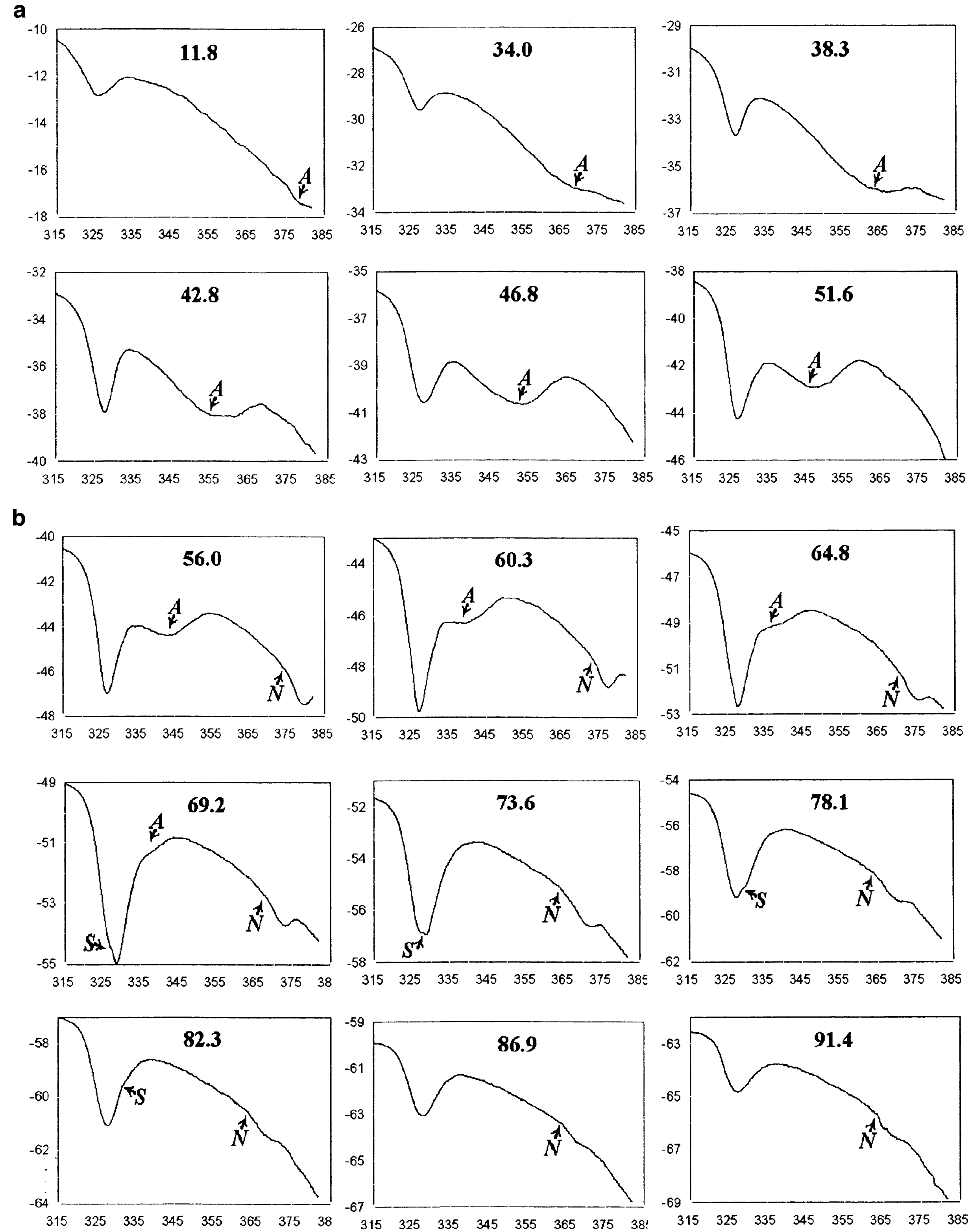

Figure 6. Individual differential DSC traces obtained for starch 1: (a) at low full water contents (in wt %); (b) at high full water contents (in wt %) (A, beginning of the slow exothermic transition; N, beginning of the high-temperature endothermic transition; S, distortion caused most probably by the smectic−nematic transition). *X*-axes are in (K), *Y*-axes are in (mW/g).

are two successive endothermic transitions. However, after subtraction from that DSC trace the DSC trace for the completely dried starch (curve 3) with appropriate scaling (eq 1), one can easily notice that the second transition has a slow exothermic nature (curve 4, see also that DSC trace in Figure 4 in a more sensible reduced scale), while the first one is clearly endothermic. This is confirmed for other water contents, both for the modified starch (starch 2, Figure 4) and for the native starch (starch 1, Figure 5). From those results one can see that the temperature interval of the first (endothermic) transition (319−333 K) is practically independent of the water content in the investigated mixtures, while the water concentration has very important influence on the second (exothermic) transition. For the low water contents the exothermic transition starts at

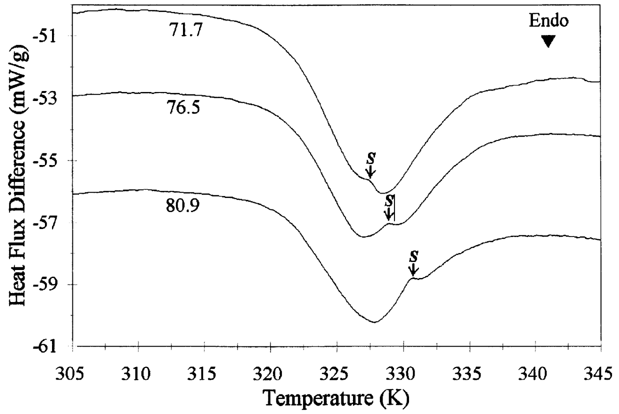

Figure 7. Differential DSC traces obtained with native starch (starch 1) demonstrating distortions caused most probably by the smectic−nematic transition (S).

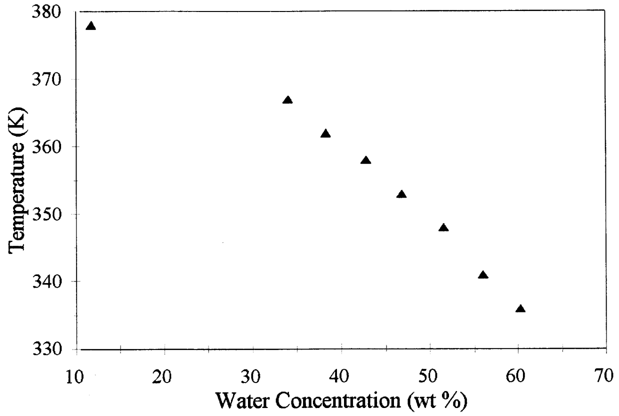

Figure 8. Water content dependence of the temperature of the beginning of the exothermic transformation.

elevated temperatures and could not be even recorded completely over the temperature interval of this study. With an increase of the water concentration the beginning of this transition is shifted to lower temperatures, and with further increase of the water content this exothermic transition overlaps and finally converges with the endothermic one. This mechanism can be observed on the global presentations of obtained results in Figures 4 and 5. However, an inspection of the individual DSC traces is even more conclusive with this respect. In Figure 6a,b are presented separately differential DSC traces obtained for the starch−water mixtures prepared with the use of native starch (starch 1). From this presentation one can see that even for the native water content (11.8 wt %, without any water added) this exothermic transition has a tendency to start near 378 K (point A). At adding water up to 34 wt % the beginning of this transition is shifted to 367 K. Further increase of the water content decreases almost linearly (see Figure 8) the temperature of the beginning of the exothermic transition until its overlapping with the endothermic one near 60 wt % water. The appearance of the exothermic effect after the sharp endothermic transition is a logic effect of melting of the crystalline part followed by a helix−coil transition in amylopectin: the free ends of the unwound helixes of amylopectin can reassociate with parts of amylopectin molecules other than their original helix duplex partner, forming physical junctions and creating more general amorphous hydrogen-bonded associations.[49] It also possible that extracted from the granules into surrounding water amylose can associate or amaylose−lipid complexes can crystallize.[19] All those processes after the endothermic helix−coil transition must be exothermic, and this is clearly observed in the results obtained in the present study. Unfortunately, the overlapping of the endothermic and exothermic transitions made difficult the integration in order to get the enthalpic contributions in the observed transitions. An attempt has only been made to determine the enthalpy change during the endothermic transition in the samples prepared with the starch 2 powder. The result is presented in Figure 9. One can see that up to 76.5 wt % water this specific enthalpy (per gram of the completely dried starch) increases and demonstrates an exponential water content dependence; at higher water contents this dependence has a small positive gradient and a small negative curvature. The integration of that parts of the differential DSC traces which correspond to the exothermic transition is rather impossible for the moment.

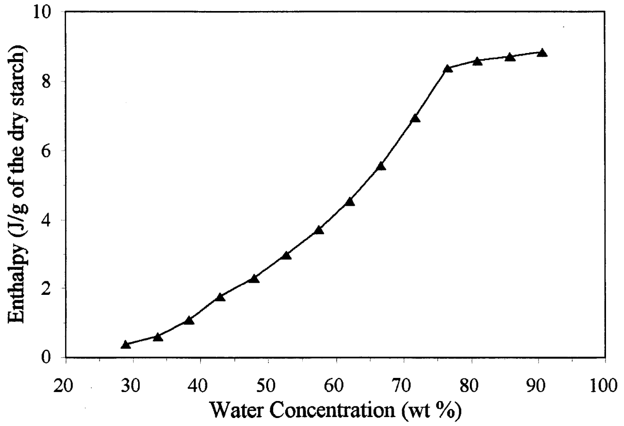

Figure 9. Water content dependence of the enthalpy of the main endothermic transition occurring over the temperature range from 319 to 333 K.

When carefully inspecting the present results one can also notice on the differential DSC traces that the increase of the water content correlates with a continuous slow exothermic effect appearing almost from the very beginning of the heating. At the native water content the differential heat flux is related significantly only to the reactive heat capacity increase in this system with temperature, the heat capacity of the starch backbone was already subtracted with the reference DSC trace, and the heat capacity of water is almost independent of temperature (less than 1% dependence). At the highest water contents, even when taking into consideration the large contribution of the heat capacity of the added water which is practically independent of temperature, one can notice a prevalence of the continuous exothermic effect. Most probably, this slow continuous exothermic process is related to the amorphous part of the starch granule; it is possible that the amorphous growth rings suck in water and expand in size.[2,4,17,49] A similar slow continuous exothermic effect was recently observed on the nonreversing signal (heat flux) obtained with a temperature-modulated DSC for a 1:2 starch−water suspension.[50] The same TMDSC study reveals that at certain experimental conditions the nonreversing signal (heat flux) shows an exothermic effect which follows the main endothermic transition while the reversing signal (heat capacity) shows practically only almost linear variations with temperature (Figure 2 in ref 50). Similar observations can be concluded from recent results reported by Pyda on measurement and modeling of heat capacity in starch and starch−water system.[51] When comparing the results of measurements on samples prepared with the use of partly dehydrated starch (starch 2, Figure 4) with the results of similar measurements performed on samples prepared with the native starch (starch 1, Figure 5), one can notice an important difference consisting in the appearance of a sharp exothermic transition at low water content added to the partly dehydrated starch powder (Figure 4). At the same time the differential DSC traces at the lowest water concentrations where the exothermic peaks did not yet appear demonstrate the main endothermic transition (the temperature range from 319 to 333 K) much smaller than the similar transition observed with the native starch (Figure 5). This can lead to the conclusion that the partial dehydration caused a partial reversible rupture of the crystalline part of the starch granules. After adding a small amount of water, there was not enough kinetic freedom for recrystallization to be possible, but at heating this hindrance has disappeared, which has allowed the recrystallization. It results from the present study that for the water contents higher than 50 wt % the recrystallization was possible without heating. When comparing this observation with the results obtained for dehydrated and native starches (Figure 1), one can come to a conclusion that dehydration of native starch causes its amorphization at least over the investigated temperature range. However, such a conclusion requires further studies in this respect and a comparison with a concept of a plasticizer effect of water.[33]

The present results obtained in the region of high water concentrations (above 55 wt %) are also interesting because of the systematic and reproducible appearance near 365−375 K (points N in Figure 6b), depending on the water content, of a small endothermic transition followed always by a small exothermic effect. Although such high-temperature small endothermic transitions were attributed previously to a rupture of a kind of amylose−lipid complexes,[25,31] a comparison of the present results with the results of pressure and temperature scanning calorimetric investigations on liquid crystals[52] leads rather to another conclusion: this can be a nematic−isotropic transition. After that transition the last molecules of amylopectin liberated from the nematic order react with water or with other liberated molecules of amylopectin, giving rise to a small exothermic effect, observed on the differential DSC traces (see especially Figure 6b). Only after that process the starch−water mixture is a real homogeneous SOL phase in the sense of Flory[18] or a pure isotropic phase in the sense of the liquid-crystal theory.[49] This conclusion is in agreement with a liquid-crystalline approach to the gelatinization of starch.[49] It also follows from the present results that most probably the starch−water mixtures with the water contents less than 56% even at temperature near 383 K have still some nematic order, because for those mixtures the nematic−isotropic transition was not observed over the temperature range under investigation. With respect to the liquid-crystalline approach of the gelatinization of starch it is also worth noting small, but regular and reproducible, distortions (points S in Figures 6b and 7) on the peaks of the main endothermic transition of the differential DSC traces for the water contents near 70−80 wt %, both for the native starch (starch 1, Figure 5) and for the partly dehydrated starch (starch 2, Figure 4). A closer look at these regular distortions observed on three differential DSC traces obtained with the native starch (starch 1) is presented in Figure 7. Similar distortions have been previously observed in scanning calorimetric and transitiometric investigation of a liquid crystal with three smectic and one nematic phases.[53,54] Thus, the present results could confirm the existence in the starch−water mixtures also of smectic−nematic transition related to the helix−coil transition and helix−helix dissociation or to a transformation of A-type to B-type unit cells of amylopectin, as it was observed before in the WAXS patterns and in high-resolution ^{13}C CP-MAS NMR spectra.[36,49]

Conclusion

High-sensitivity temperature-controlled DSC measurements at a low heating rate and creation of differential DSC traces scaled with respect to the reference material (completely dried starch) permitted to revise the actual knowledge on the phase transformations in the wheat starch in the course of its gelatinization and to determine exact evolution of thermal effects associated with the water−starch interactions over the temperature range from 283 to 384 K under atmospheric pressure. From the obtained results of extremely good reproducibility it follows that:

(i) The second transformation out of two associated transitions reported in the literature as being endothermic has in reality a clear exothermic nature. This exothermic transformation is strongly water content dependent: at low water contents it starts at elevated temperatures, at higher water contents it shifts to lower temperatures, and near 70 wt % of water it converges completely with the main endothermic transition which occurs over the temperature range from 319 to 333 K independent of the water content. The appearance of the exothermic effect after the sharp endothermic transition is a logical effect of melting of the crystalline part followed by a helix−coil transition in amylopectin: the free ends of the unwound helixes of amylopectin can reassociate with parts of amylopectin molecules other than their original helix duplex partner, forming physical junctions and creating more general amorphous hydrogen-bonded associations. All those processes after the endothermic helix−coil transition must be exothermic, and this is clearly observed in the results obtained in the present study. This observation and its interpretation are in close agreement with the recent results obtained with WAXS patterns and high-resolution ^{13}C CP-MAS NMR spectra.[49]

(ii) Comparison of the high-temperature endothermic transitions (near 365−380 K depending on the water content) and small but well-reproducible distortions on the peaks of the main endothermic transition for the water contents near 70−80 wt %, with the results of scanning calorimetric investigation of liquid crystals,[52−54] permits to conclude that at least part of the starch granule is in the liquid-crystalline state, and on heating it transforms to the isotropic (or pure SOL) phase passing through smectic−nematic and nematic−isotropic transitions. This is in agreement with the liquid-crystalline approach to the gelatinization of starch.[10,11,49]

(iii) The increase of the water content correlates with an important continuous slow exothermic effect appearing almost from the very beginning of the heating. Most probably, this slow continuous exothermic process especially observed at high water contents is related to the softening and sucking in water of the amorphous growth rings of the starch granule.[2,4,17]

(iv) Low-temperature sharp exothermic peaks obtained with samples prepared with partly dehydrated starch demonstrate that partial dehydration caused a partial reversible rupture of the crystalline part of the starch granules. After adding water in small amounts below 50 wt %, there was not enough kinetic freedom for recrystallization to be possible, but at heating this hindrance could disappear and the recrystallization was possible. This conclusion and observation that dried starches did not show melting can lead to a general conclusion that dehydration of native starch causes its amorphization, at least over the investigated temperature range.

Acknowledgment. We acknowledge the financial support by the French program ALIMENT POUR DEMAIN and gratefully thank PROTIAL and CLEXTRAL, partners in this program, for stimulating discussions, encouragement, and friendly partnership. We also acknowledge the stimulating comments of the reviewers. I.F.K. thanks the French Ministère de l'Education Nationale, de la Recherche et de la Technologie, for a postdoc scholarship.

References and Notes

(1) French, D. In *Starch: Chemistry and Technology*; Whistler, R. L., Bemiller, J. N., Paschall, E. F., Eds.; Academic Press: New York, 1984; pp 183−247.
(2) Gallant, D. J.; Bouchet, B.; Baldwin, P. M. *Carbohydr. Polym.* **1997**, *32*, 177.
(3) Waigh, T. A.; Donald A. M.; Heidelbach, F.; Riekel, C.; Gidley, M. J. *Biopolymers* **1999**, *49*, 91.
(4) Paris, M.; Bizot, H.; Emery, J.; Buzaré, J. Y.; Buléon, A. *Carbohydr. Polym.* **1999**, *39*, 327.
(5) Le Botlan, D.; Rugraff, Y.; Martin, C.; Colonna, P. *Carbohydr. Res.* **1998**, *308*, 29.
(6) Iizuka, K.; Aishima, T. *J. Food Sci.* **1999**, *64*, 653.
(7) Zobel, H. F. In *Starch: Chemistry and Technology*; Whistler, R. L., Bemiller, J. N., Paschall, E. F., Eds.; Academic Press: New York, 1984; pp 285−307.
(8) Whistler, R. L.; Daniel, J. R. In *Starch: Chemistry and Technology*; Whistler, R. L., Bemiller, J. N., Paschall, E. F., Eds.; Academic Press: New York, 1984; pp 153−182.
(9) Oestergetel, G. T.; van Bruggen, E. F. J. *Carbohydr. Polym.* **1993**, *21*, 7.
(10) Waigh, T. A.; Jenkins, P. J.; Donald, A. M. *Faraday Discuss.* **1996**, *103*, 325.
(11) Waigh, T. A.; Hopkinson, I.; Donald, A. M.; Butler, M. F.; Heidelbach, F.; Riekel, C. *Macromolecules* **1997**, *30*, 3813.
(12) Jenkins, P. J.; Cameron, R. E.; Donald, A. M.; Bras, A. M.; Derbyshire, G. E.; Mant, G. R.; Ryan, A. J. *J. Polym. Sci., Part B: Polym. Phys.* **1994**, *32*, 1579.
(13) Gernat, C.; Radosta, S.; Anger, H.; Damaschun, G. *Starch/Staerke* **1993**, *45*, 309.
(14) Morrison, W. R.; Law, R. V.; Snape, C. E. *J. Cereal. Sci.* **1993**, *18*, 107.
(15) Noda, T.; Takahata, Y.; Sato, T.; Suda, I.; Morishita, T.; Ishiguro, K.; Yamakawa, O. *Carbohydr. Polym.* **1998**, *37*, 153.
(16) Matveev, Y. I.; van Soest, J. J. G.; Nieman, C.; Wasserman, L. A.; Protserov, V. A.; Ezenitskaja, M.; Yuryev, V. P. *Carbohydr. Polym.* **2001**, *44*, 151.
(17) O'Sullivan, A. C.; Perez, S. *Biopolymers* **1999**, *50*, 381.
(18) Flory, P. J. *J. Am. Chem. Soc.* **1941**, *63*, 3083.
(19) Le Bail, P.; Bizot, H.; Ollivon, M.; Keller, G.; Bourgaux, C.; Buléon, A. *Biopolymers* **1999**, *50*, 99.
(20) Hurd, C. B. *Chem. Rev.* **1938**, *22*, 403.
(21) Stevens, D. J.; Elton, G. A. H. *Starch/Staerke* **1971**, *23*, 8.
(22) Donovan, J. W. *Biopolymers* **1979**, *18*, 263.
(23) Wooton, M.; Bamunuarachchi, A. *Starch/Staerke* **1979**, *31*, 201.
(24) Biliaderis, C. G.; Maurice, T. J.; Vose, J. R. *J. Food Sci.* **1980**, *45*, 1669.
(25) Kugimiya, M.; Donovan, J. W.; Wong, R. Y. *Starch/Staerke* **1980**, *32*, 265.
(26) Eliasson, A.-C. *Starch/Staerke* **1980**, *32*, 270.
(27) Eberstein, K.; Höpcke, R.; Konieczny-Janda, G.; Stute, R. *Starch/Staerke* **1980**, *32*, 397.
(28) Longton, J.; Le Grys, G. A. *Starch/Staerke* **1981**, *33*, 410.
(29) Ghiasi, K.; Hoseney, R. C.; Varriano-Marston, E. *Cereal Chem.* **1982**, *59*, 258.
(30) Shiotsubo, T.; Takahashi, K. *Agric. Biol. Chem.* **1984**, *48*, 9.
(31) Biliaderis, C. G.; Page, C. M.; Slade, L.; Sirett, R. R. *Carbohydr. Polym.* **1985**, *5*, 367.
(32) Biliaderis, C. G.; Page, C. M.; Maurice, T. J.; Juliaano, B. O. *J. Agric. Food Chem.* **1986**, *34*, 6.
(33) Zeleznak, K. J.; Hoseney, R. C. *Cereal Chem.* **1987**, *64*, 121.
(34) Liu, H.; Lelievre, J.; Ayoung-Che, W. *Carbohydr. Res.* **1991**, *210*, 79.
(35) Münzig, K. *Thermochim. Acta* **1991**, *193*, 441.
(36) Cooke, D.; Gidley, M. J. *Carbohydr. Res.* **1992**, *227*, 103.
(37) Svensson, E.; Eliasson, A.-C. *Carbohydr. Polym.* **1995**, *26*, 171.
(38) Rolee, A.; LeMeste, M. *Cereal Chem.* **1999**, *76*, 452.
(39) Ahmad, F. B.; Williams, P. A. *Biopolymers* **1999**, *50*, 401.
(40) Flory, P. J. *Principles of Polymer Chemistry*; Cornell University Press: Ithaca, NY, 1953; pp 563−576.
(41) Lelievre, J. *J. Appl. Polym. Sci.* **1974**, *18*, 293.
(42) Lelievre, J. *Polymer* **1976**, *17*, 854.
(43) Slade, L.; Levine, H. In *Proceedings of the 13th North American Therm. Anal. Soc. Conf.*; McGhie, A. R.; Ed.; NATAS: Philadelphia, 1984; p 64.
(44) Maurice, T. J.; Slade, L.; Sirett, R. R.; Page, C. M. In *Properties of Water in Foods*; Simatos, D., Multon, S. L., Eds.; Nijhoff M. Publishers: Dordrecht, 1985; p 211.
(45) Yost, D. A.; Hoseney, R. C. *Starch/Staerke* **1986**, *38*, 289.
(46) Evans, I. D.; Haisman, D. R. *Starch/Staerke* **1982**, *34*, 224.
(47) Blanshard, J. M. V. In *Starch Properties and Potential*; Galliard, T., Ed.; Wiley: Chester, 1987; pp 16−54.
(48) Tester, R. F.; Morison, W. R. *Cereal Chem.* **1990**, *67*, 551.
(49) Waigh, T. A.; Gidley, M. J.; Komanshek, B. U.; Donald, A. M. *Carbohydr. Res.* **2000**, *328*, 165.
(50) Lai, V. M.-F.; Lii, C.-Y. *Cereal Chem.* **1999**, *76*, 519.
(51) Pyda, M. *J. Polym. Sci., Part B: Polym. Phys.* **2001**, *39*, 3038.
(52) Randzio, S. L. *Pure Appl. Chem.* **1991**, *63*, 1409.
(53) Randzio, S. L. *Chem. Soc. Rev.* **1996**, *25*, 383.
(54) Randzio, S. L. *Thermochim. Acta* **1997**, *300*, 29.

MA011213R