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Preferential solvent interactions and the dissolution of the B-type crystalline polymorph of starch in aqueous solutions

Graham K. Moates, Roger Parker, Stephen G. Ring *

Institute of Food Research, Department of Biochemistry, Norwich Laboratory, Norwich Research Park, Colney Lane, Norwich NR4 7UA, UK

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Abstract

The preferential interactions of starch in aqueous solutions of low-molecular-weight solutes were probed using densimetric techniques which have previously been applied to proteins and nucleic acids. In D-glucitol solutions, the starch was preferentially hydrated, whereas in solutions of urea and guanidinium thiocyanate there was a preferred interaction of the solute with the starch chain. In solutions of D-glucose and glycerol no preferential interactions were observed. The effect of the same solutes on the dissolution temperature, T_m, of the crystalline B-type polymorph of starch was examined by calorimetry. The addition of the hydroxy compounds glycerol, D-glucose and D-glucitol elevated T_m, while the addition of urea and guanidinium thiocyanate caused a depression. For starch–urea–water mixtures, Flory theory predictions of the melting temperature were in good agreement with experiment, but were poor for starch–D-glucitol–water and starch–glucose–water mixtures. The neglect of non-configurational contributions to the entropy of mixing were identified as the principal reason for this lack of agreement.

Keywords: Starch

1. Introduction

Starch occurs naturally as a partially crystalline granular solid [1]. The melting and glass transition behaviour of starch is relevant to its industrial use [2]. Low-molecular-weight components are often used to depress the glass transition temperature, plasticizing the material and modifying its mechanical properties [3]. These compounds also affect the melting and gelatinisation temperatures [4]. Whilst the behaviour of a polymer in the vicinity of its glass transitions is non-equilibrium in nature, the melting behaviour can approach equilibrium [5] and so equilibrium thermodynamic and corresponding statistical thermodynamic approaches [6] can be applied. For starchy materials, water is the most important solvent [2]. It is also important to consider the effect of other low-molecular-weight solutes on dissolution [7] and the phase behaviour of ternary systems [8]. Ternary systems comprising starch, water and a low-molecular-weight solute are suitable models for studying this behaviour. Molecular interactions between each pair of components influence the overall properties of the system. While the interactions of starch with water, and low-molecular-

* Corresponding author. Fax: +44-1603-507723; e-mail: steve.ring@bbsrc.ac.uk

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weight solutes with water, can be studied by thermodynamic measurements on binary systems [9–12] the study of the interactions of starch with low-molecular-weight solutes must be studied in ternary systems as water is invariably present. In ternary systems it is possible to study the balance between the interaction of starch with water and solutes. This can result in a localised redistribution of these components and is known as a 'preferential solvent interaction' [13].

Preferential interactions have been thoroughly studied in synthetic polymer solutions, usually in mixtures of liquid solvents, using techniques such as light scattering [14] and equilibrium centrifugation [15,16]. In the biopolymer field there has been significant interest in the effect of low-molecular-weight solutes on protein stability in aqueous solution and approaches have been developed to probe interactions in protein–solute–water mixtures through the measurement of solution densities [13,17]. The thermodynamic basis of these techniques was clarified by Casassa and Eisenberg [18] in developing a rigorous analysis for light scattering and sedimentation equilibrium experiments on biopolymers in multicomponent systems. The densimetric technique involves measurement of the apparent partial specific volume of the protein, in a solute–water mixture, as a function of protein concentration. The polymer solution is then dialysed against the solute–water mixture. Depending on the relative affinities between the protein, solute and water a redistribution of components occurs which is revealed as a change in the apparent specific volume of the protein. Low-molecular-weight carbohydrates are generally excluded from the domain of proteins, and the protein is said to be preferentially hydrated [13,17]. The effect on protein stability is that the addition of a carbohydrate solute, which does not interact favourably with the protein, leads to the elevation of the denaturation temperature. The solute–protein interaction is formally expressed as a preferential interaction parameter, ξ_3. With water as component 1, the polymer as component 2, and the low-molecular-weight solute as component 3, the preferential interaction parameter, ξ_3 (a measure of the excess of component 3 in the domain of the polymer) is given by [13]

$$\xi_3 = (\partial g_3/\partial g_2)_{T,\mu_1,\mu_3} = \rho_0(\phi_2^0 - \phi'^0_2)/(1 - \phi_3^0\rho_0) \quad (1)$$

where g_i is the concentration of component i, expressed as grams of component i per gram of water, ϕ_i^0 is the partial specific volume of component i obtained after extrapolation to zero polymer concentration, and ϕ'^0_2 is the value obtained after dialysis against the binary solution of density ρ_0. These quantities can all be determined from density measurements. The apparent partial specific volume, $\phi_2 = (1/\rho_0)(1 - (\rho - \rho_0)/c)$, is determined from a series of density measurements on solutions with a range of polymer concentrations, c (g/mL). The change in chemical potential of the polymer, occurring as a result of mixing with the solute is given by [17]

$$(\partial\mu_2/\partial m_3)_{T,p,m_2} = -(\partial m_3/\partial m_2)_{T,\mu_1,\mu_3}$$
$$[RT/m_3 + RT(\partial \ln \gamma_3/\partial m_3)]_{T,p,m_2} \quad (2)$$

where γ_3 is the activity coefficient of component 3,

$$(\partial m_3/\partial m_2)_{T,\mu_1,\mu_3} = M_2/M_3(\partial g_3/\partial g_2)_{T,\mu_1,\mu_3} \quad (3)$$

m_i is the molality of component i, and M_i is its molecular weight. For polydisperse polysaccharides such as starch it is more convenient if the molecular weight of an anhydrohexose unit, M_{2u}, is used rather than M_2 and so the chemical potential of the polymer in Eq. (2) is the Gibbs free energy of the polysaccharide per mole of anhydrohexose units and is denoted μ_{2u}. Using this experimental approach, the interaction between the polymer and the solute can be characterised in terms of $(\partial\mu_{2u}/\partial m_3)_{T,p,m_2}$ which also provides a convenient link with statistical thermodynamic models of polymer solutions.

The Flory–Huggins theory [6] is well established as a useful, first order theory of polymer solutions. For a ternary system the enthalpic interactions between the various components are expressed as pair interaction parameters χ_{12}, χ_{13}, and χ_{23} with the Gibbs free energy of mixing given by

$$\Delta G_m/RT = n_1 \ln v_1 + n_2 \ln v_2 + n_3 \ln v_3 + \chi_{12} n_1 v_2 + \chi_{13} n_1 v_3 + \chi_{23} n_2 v_3 \quad (4)$$

where n_i and v_i are the number of moles and volume fraction of component i, respectively. If the subscripts 1, 2, and 3 refer to the solvent, polymer and low-molecular-weight solute, respectively, then the change in chemical potential of the polymer with composition may be put in the form

$$\Delta\mu_{2u}/a_{2u}RT = -v_1 - v_3/a_3 + (\chi_{12}v_1 + \chi_{32}v_3/a_3)(v_1 + v_3) - g_{13}v_1v_3 \quad (5)$$

where, $\Delta\mu_{2u} = \mu_{2u} - \mu^0_{2u}$, μ^0_{2u} is the chemical potential in the standard state, $a_{2u} = V_{2u}/V_1$, $a_3 = V_3/V_1$, V_i is the molar volume of component i, and $\chi_{ji} = \chi_{ij}(V_j/V_i)$. Following the notation of Altena and Smolders [8], g_{13} is a concentration-dependent solute–solvent interaction parameter (it should not be confused with the concentrations, g_i, in Eqs. (1) and (3)). The form of Eq. (5) is appropriate to polymers of high molecular weight and, under these conditions, the expression is independent of molecular weight. For the application of Eq. (5), it is necessary to have an estimate of the interaction parameters χ_{12}, χ_{32} and g_{13}. The latter, g_{13}, which characterises the low-molecular-weight solute–water interactions, can be obtained from thermodynamic data on aqueous solutions [8]. For the mixing of components 1 and 3 the excess Gibbs free energy, G^E, is related to the Gibbs free energy of mixing, ΔG_m, through the definition

$$G^E = \Delta G_m - RT(x_1 \ln x_1 + x_3 \ln x_3) \quad (6)$$

where x_i is the mole fraction of component i. For 1 mole of mixture this is related to the Flory model by the relation

$$\Delta G_m/RT = x_1 \ln v_1 + x_3 \ln v_3 + g_{13}x_1v_3 \quad (7)$$

The terms $x_1 \ln v_1$ and $x_3 \ln v_3$ are Flory–Huggins estimates of the configurational entropy, the entropy which arises from the number of ways of arranging the solute and solvent molecules on the lattice [6]. The term $g_{13}x_1v_3$ is nominally an enthalpic term, though it also includes a contribution from the non-configurational entropy, which arises from specific nearest-neighbour interactions [6]. The study of ΔG_m is well established in aqueous solution thermodynamics and has been measured for commonly encountered systems with precision [10–12]. χ_{12} was determined from a study of the effect of water content on the melting of B-type spherulites [19] using the melting model of Flory, as described later. The remaining parameter, χ_{32}, can be determined from the preferential interaction measurements described above. The value of χ_{32} was estimated, using Eq. (5), by calculating the value of $(\partial\mu_{2u}/\partial m_3)_{T,p,m_2}$, a quantity which is related to the preferential interaction parameter through Eqs. (1)–(3).

In the study of starch, the Flory–Huggins model has been used to model melting both in water [19,20] and in aqueous solutions [7]. The theory of the effect of a solvent on the equilibrium melting temperature, T^0_m, of a crystalline polymer [6,19,20] gives

$$1/T_m = 1/T^0_m + R/\Delta H_u V_u/V_1(v_1 - \chi_{12}v_1^2) \quad (8)$$

where ΔH_u is the heat of fusion per repeating unit, V_u and V_1 are the molar volumes of polymer repeating unit and solvent, respectively, v_1 is the solvent volume fraction and χ_{12} is the Flory–Huggins interaction parameter. Eq. (8) may be generalised to the dissolution of a crystalline polymer in the presence of a solvent and low-molecular-weight solute giving [7]

$$1/T_m - 1/T^0_m = R/\Delta H_u V_u/V_1\{v_1 + v_3/a_3 + g_{13}v_1v_3 - [\chi_{12}v_1 + (\chi_{32}v_3)/a_3](v_1 + v_3)\} \quad (9)$$

where the interaction parameters are as defined previously. Lelievre [7] used this theory to model gelatinisation of starch in maltose–water solutions. Another application of Flory theory to ternary systems is in the prediction of liquid–liquid phase separation as described by Altena and Smolders [8]. In the polysaccharide literature, reference can be found to preferential interactions of dextran in ethanol–water mixtures, characterised by ultrasonic measurements of the adiabatic compressibility, [21] and modelling of its ternary phase diagram using the Flory model [22,23].

In the first part of the present study we examined the interaction of solutes—glycerol, D-glucitol, glucose, urea and guanidinium thiocyanate—with the starch chain using the approach outlined above. We then went on to examine the effect of these solutes on the dissolution of the B-type crystalline polymorph of starch. There have been a number of studies on the effect of low-molecular-weight solutes on the gelatinisation of partially crystalline starch granules [4,7] and the crystallisation of starch from aqueous dispersions [24]. It was found that most low-molecular-weight carbohydrates elevate the gelatinisation temperature of starch granules. It was suggested that Eq. (9) gave insight into the factors which affect gelatinisation in these mixed systems although the differences between the idealised systems to which Eq. (9) is applicable and the more complex gelatinisation process were recognised. More particularly, the starch granule was only partially crystalline and gelatinisation was a non-equilibrium process. To overcome these difficulties we have examined the dissolution of highly crystalline spherulites [19] in the different solute–water mixtures. Finally, the extent to which the Flory model can predict the melting behaviour of spherulites in ternary mixtures was examined.

2. Experimental

Materials.—Short chain amylose was prepared by lintnerisation of potato starch granules in dilute HCl as described [25]; the material was purified by crystallisation from a 20% w/w aqueous solution at 2 °C. The crystalline product was characterised by ion-exchange chromatography (Dionex) and had a weight-average degree of polymerisation of 22. A 20% w/w aqueous solution of the short chain amylose was prepared by dissolution at 120 °C , the solution was cooled from 80 to 2 °C at a rate of 5 °C h^{-1} and left for 16 h [26]. The main factor affecting crystallisation was the presence of small amounts of aggregated material which was removed by filtration of the hot amylose solution (60 °C) through a 0.45 μm Millipore filter. The spherulitic amylose of the B-type crystalline polymorph was collected by centrifugation and washed with water to remove soluble material. The crystallinity of spherulitic amylose has previously been characterised by wide-angle X-ray diffraction [26]. The observed diffraction pattern, of the B-type, was very sharp and extended to wide angles. Estimates of crystallinity can be obtained both from the size of the dissolution endotherm and from the separate contributions of amorphous and crystalline material to the observed diffraction pattern. From the comparative size of the dissolution endotherm and the known crystallinity of starch samples, as determined by X-ray methods, the crystallinity of the spherulitic material was estimated to be $>$ 75% [27]. Amylopectin was prepared from waxy–maize starch as described [25]. Urea, guanidinium thiocyanate, glycerol, glucose and D-glucitol were obtained from Sigma, Poole, Dorset, UK.

Calorimetry.—Samples ($\sim$10 mg) containing $\sim$20% by volume of spherulites in the solute–solvent mixture were dispensed into aluminium pans (30 μL capacity and nominal maximum working pressure 2 atm.), quickly sealed and weighed. Differential scanning calorimetry (DSC) was performed with a Perkin–Elmer DSC7 calorimeter, equipped with a robotic sample handling system, over the temperature range 30–110 °C at a scanning rate of 10 °C min^{-1}. The machine was calibrated for temperature using the melting of β-naphthyl ether and indium. Satisfactory sealing of the pans was verified by checking pan weights after scanning. The dissolution temperature, T_m, was obtained from the peak maximum of the endothermic transition using the standard Perkin–Elmer software.

Preferential solvent interaction.—Amylopectin was used to study preferential solvent interactions. As the technique uses dialysis a polymeric form of starch was required. The use of amylopectin avoids problems of gelation and aggregation under the conditions used in this study. The apparent specific volume of amylopectin dissolved in water containing the low-molecular-weight solute of interest was determined as a function of amylopectin concentration in the range 5–20 mg

mL^{-1} at 25 °C . In the customary terminology this quantity is called the specific volume determined at 'constant molality' [13]. The amylopectin solution was then dialysed against a solution of the low-molecular-weight solute of the same composition. Dialysis was carried out using 14.3 mm Visking tubing (Medicell) which was cleaned in two changes of distilled water. Dialysis was carried out against the appropriate solutions, in stoppered containers for 24 h (20 h at 2 °C , then 4 h at 20 °C), to ensure equilibration. The total amount of solution, before and after dialysis, was checked by weighing to ensure there was no loss of solvent. After equilibration the apparent specific volume of the amylopectin was again determined as a function of concentration, its new value reflecting the redistribution of solvent components. This apparent specific volume is known as the value determined at 'constant chemical potential' [13]. Solution densities were measured using an Anton Paar DMA 60 density meter equipped with a DMA 601 cell. The apparatus was calibrated against pure water and 1.0 M KCl, with the temperature of the measuring cell maintained to better than ± 0.02 °C . The experimental procedures of Lee et al. [13] were followed, with particular care being taken to avoid evaporative loss during dissolution of amylopectin at 100 °C. Amylopectin concentration in solution after dialysis was determined spectrophotometrically from the absorbance of the polyiodide amylopectin complex [1].

3. Results

Starch–solute interactions.—The interaction of the solutes with starch was probed through the measurement of solution densities and the calculation of the apparent specific volumes of amylopectin, ϕ_2 and ϕ'_2 at constant molality and constant chemical potential, respectively, at 298 K. These data are shown in Fig. 1 for the aqueous solutions 2.0 M urea, 2.0 M guanidinium thiocyanate and 4.1 M D-glucitol. The partial specific volume of the amylopectin, ϕ_2, is in the range 0.644–0.650 mL g^{-1} in the solutions examined. This value is comparable to a value of 0.627 mL g^{-1} for the partial specific volume of glucose in aqueous solution at 298 K [28]. For urea and guanidinium thiocyanate, $\phi'_2 < \phi_2$, indicating that on dialysis and solute redistribution, there is an excess of solute within the domain of the macromolecule, indicating a preferred interaction between these solutes and amylopectin. For D-glucitol, although the effect is smaller, the reverse is observed indicating a preferential interaction of the amylopectin for water. In similar experiments with 4.2 M D-glucose and 2.3 M glycerol a difference between ϕ_2 and ϕ'_2 was not observed (data not shown). Extrapolation of ϕ_2 and ϕ'_2 to zero concentration yields the quantities ϕ_2^0 and ϕ'^0_2 which are shown in Table 1. From these data and the apparent partial specific volume of the solute v_3, the preferential interaction parameter ξ_3 was calculated using Eq. (1). The solution densities ρ_0 and other parameters characterising the solutes and their aqueous solutions are collected together in Table 2.

The chemical potential change, $(\partial\mu_{2u}/\partial m_3)_{T,\,p,\,m_2}$, induced by addition of the solute

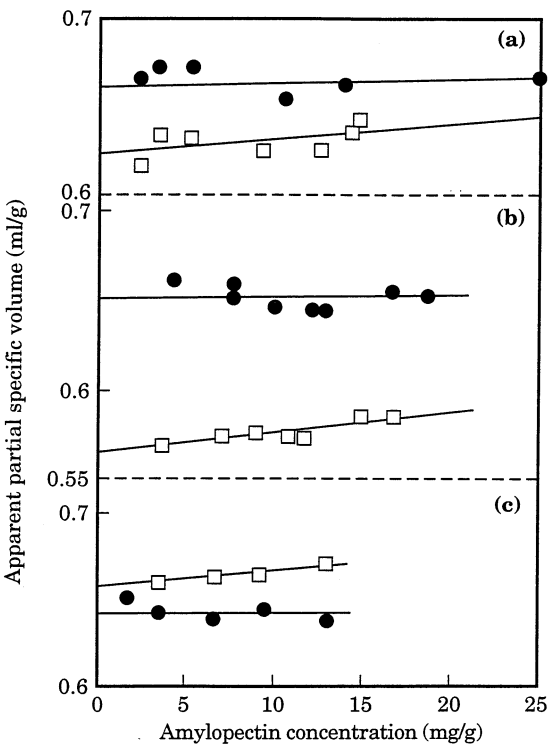

Fig. 1. Apparent specific volume of amylopectin as a function of concentration (ϕ_2, constant molality, ●; ϕ'_2, constant chemical potential, □) in aqueous solution of: (a) 2.0 M urea, (b) 2.0 M guanidinium thiocyanate, and (c) 4.1 M D-glucitol.

Table 1
Apparent partial specific volumes, preferential interaction parameters and chemical potential gradients for amylopectin and solutes in aqueous solutions

Solute	ϕ_2^0 (mL g^{-1})	$\phi_2'^0$ (mL g^{-1})	ξ_3 (g g^{-1})	ϕ_3^0 (mL g^{-1})	$(\partial m_3/\partial m_2)_{T,\mu_1,\mu_3}$	$(\partial\mu_{2u}/\partial m_3)_{T,p,m_2}$ [a]
2.0 M urea	0.660	0.622	0.155	0.726	0.424	−417
2.0 M GTC	0.650	0.564	0.493	0.778	0.685	−370
4.1 M D-glucitol	0.644	0.658	−0.106	0.674	−0.097	34 (29)
4.2 M glucose	0.645	0.645	0.0	0.638	0.0	0.0

[a] Units: J (kg water) (mol of anhydrohexose units)$^{-1}$ (mol of solute)$^{-1}$.

was calculated using Eq. (2) and expressed in J (kilogram of water) (mol of anhydrohexose units)$^{-1}$ (mol of solute)$^{-1}$ (Table 1). For this calculation, it is necessary to have values of the activity coefficients of the solutes in order to calculate $\partial \ln \gamma_3/\partial m_3$. For urea and guanidinium thiocyanate activity data were obtained from the literature [10,11]. For D-glucitol, as far as we are aware there are no literature data; for this reason two values of $(\partial\mu_{2u}/\partial m_3)_{T,p,m_2}$ are shown, the first of which neglects the contribution of the activity coefficient of the solute, the second assumes that D-glucitol has a similar activity coefficient to D-glucose for which data are available [12].

Dissolution of starch crystallites.—The dissolution of spherulites of the B-type crystalline polymorph of starch was probed by DSC. In the absence of solutes at volume fractions of water >0.8, the peak of the endothermic transition occurred at 342 K. This compares with a value of 347 K determined in previous studies [19], and the difference was attributed to small differences in chain length and polydispersity of the amylose used in the two studies. The effect of low-molecular-weight solutes on the observed dissolution temperature was studied as a function of their volume fraction, v_3. The solutes examined were D-glucose, D-glucitol, glycerol, urea and guanidinium thiocyanate. Fig. 2 shows some typical DSC melting endotherms for the spherulites in these mixtures. The melting peaks are typically symmetrical, though those for D-glucitol (Fig. 2(ii)) show some asymmetry.

With increasing volume fraction of solute, v_3, the hydroxy compounds increased the dissolution temperature T_m (Fig. 3). For example, at a volume fraction of solute of 0.3, the observed elevation in the dissolution temperature T_m was ~ 18 K for D-glucose and D-glucitol. At a fixed volume fraction of the solute of 0.3 the effect of glycerol was less with a 4–5 K elevation in T_m.

Turning to the situation where there is a preferential interaction of starch with the solute, the addition of the urea and guanidinium thiocyanate to aqueous dispersions of the B-type crystallites caused a progressive depression in T_m (Fig. 3). This effect was more marked with guanidinium thiocyanate with an observed depression of ~ 17 K at a volume fraction of solute of 0.1.

Considering the D-glucitol, urea and guanidinium data, there appears to be a correlation between the preferential solvent interactions and the effect of the solute on the melting of starch. In cases where there is a preferential interaction of the solute the melting temperature is depressed and, conversely, in cases of preferential hydration the addition of the solute results in an elevation of the melting temperature. The present measurements on glucose and glycerol do not conform with this correlation.

Flory theory prediction of melting temperatures in ternary mixtures.—To use the Flory–Huggins approach for describing the phase behaviour of ternary mixtures it is necessary to estimate the interaction parameters g_{13}, χ_{12} and χ_{32}. The concentration-dependent solute–water interaction parameter, g_{13}, was calculated from literature data using Eqs. (6) and (7) (Table 3). In the absence of literature data on D-glucitol, g_{13} was assumed to be the same as for glucose. This assumption is expected to be of sufficiently good to allow a comparison of the behaviour of D-glucitol with that of urea and GTC but not for a comparison with the behaviour of glucose. For consistency with

Table 2
Properties of solutes and their aqueous solutions[a]

Solution	m_3	ρ_0	M_3	V_3	v_3	a_3	$(\partial \ln \gamma_3/\partial m_3)$
2.0 M Urea	2.20	1.030	60.06	43.6	0.089	2.42	−0.058
2.0 M GTC	2.44	1.050	118.2	91.9	0.184	5.09	−0.185
4.1 M D-glucitol	8.23	1.241	182.2	122.8	0.502	6.80	0.023[b]
4.2 M Glucose	8.33	1.276	180.2	114.9	0.488	6.37	0.023

[a] m_3, molality, mol kg^{-1}; ρ_0, density, g cm^{-3}; M_3, solute molecular weight; V_3, solute molar volume, mL; v_3, solute volume fraction; a_3, V_3/V_1; $(\partial \ln \gamma_3/\partial m_3)$, partial differential of the natural logarithm of the solute activity coefficient with respect to solute molality.
[b] Estimated from glucose data.

our ternary mixture melting data, the starch–water interaction parameter, χ_{12}, was estimated using starch–water melting data [19] and the Flory model, Eq. (8). This earlier melting study used spherulites which were prepared under near identical conditions to those in the present study. This yielded values for T^0_m, ΔH_u and χ_{12} of 536 K, 22.0 kJ mol^{-1} and 0.57 (in the temperature range 350–370 K), respectively. V_u and V_1 were taken to be 105.9 and 18.07 mL mol^{-1}. Values of χ_{12} close to 0.5 are reasonable on the basis of alternative measurements which show that water is close to being a Θ-solvent for starch [1]. The value indicates that water is a poor solvent for the α-(1→4)-glucan chain. The starch–solute interaction parameter, χ_{32}, was determined iteratively (using a Microsoft Excel spreadsheet) to calculate $(\partial \mu_{2u}/\partial m_3)_{T,p,m_2}$ using Eq. (5). Solute molality m_3 is related to volume fraction through the equation $m_3 = 1000 v_3/V_3 v_1 \rho_1$, where it is assumed that the partial molar volume of the solute, V_3, and that of water are independent of concentration. The values of χ_{32} were calculated at a polymer concentration of about 0.1 mg mL^{-1} ($v_2/v_1 = 7 \times 10^{-5}$). In each case χ_{32} was found to be negative (Table 3).

Fig. 4 shows a comparison of predictions of the melting temperature made using Flory theory with experiment for starch–urea–water mixtures. Agreement between experiment and theory is good in this case. The various contributions to the melting point depression (see Eq. (9)) are shown in Fig. 5. The bar chart shows the change in the magnitude of the various terms when 0.1 of the volume fraction of water is replaced by urea. The first and second terms arise from the entropy of mixing [6]. Removing water and replacing it with urea, a larger molecule, is unfavourable en-

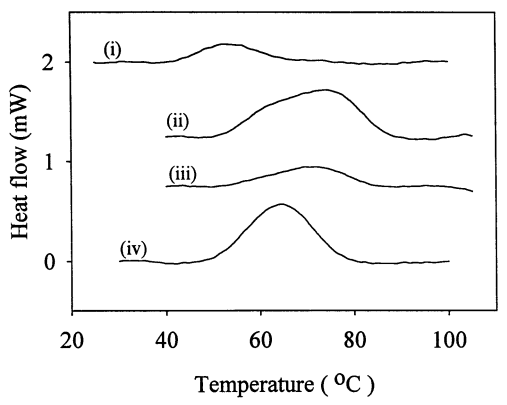

Fig. 2. The DSC melting endotherms of the B-type crystalline polymorph of starch in mixtures with: (i) guanidinium thiocyanate, (ii) D-glucitol, (iii) glucose, and (iv) urea. Volume fractions of starch and solute are 0.2 and about 0.1, respectively.

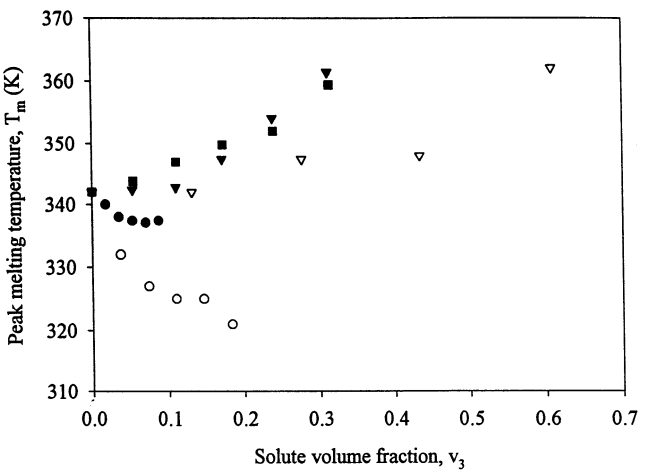

Fig. 3. The effect of solute concentration on the peak melting temperature of the B-type crystalline polymorph of starch. Volume fraction of starch, $v_2 = 0.2$. Solutes: urea, ●; guanidinium thiocyanate, ○; glycerol, ▽; D-glucitol, ■; glucose, ▼.

Table 3
Flory–Huggins parameters relevant to starch–solute–water mixtures[a]

Solute	g_{13}	χ_{32}
2.0 M urea	0.19	−1.58
4.1 M D-glucitol	0.74	−1.42
4.2 M glucose	0.74	−1.63

[a] χ_{32} was calculated from $(\partial\mu_2/\partial m_3)_{T,p,m_2}$ using value of g_{13} and assuming $\chi_{12} = 0.57$.

tropically and this effect alone would result in an increase in the melting temperature. Flory's estimate of the change due to the entropy of mixing depends solely on the relative sizes of the solute and solvent, $a_3 = V_3/V_1$, which for urea and water has the value 2.42. The other three terms which depend upon the interaction parameters g_{13}, χ_{12} and χ_{32} arise from the enthalpy of mixing [6] and, in this case, they are all negative. These terms outweigh the entropy term leading to an overall depression of the melting temperature. Term 5 is the largest enthalpic term, a result of the preferential starch–urea interactions.

Fig. 6 compares theory and experiment for the mixtures containing D-glucitol and glucose. The theoretical predictions are poor, whereas experimentally an elevation of the melting temperature was observed, the theory predicts a weak depression of the melting temperature. The bar chart (Fig. 7) shows the

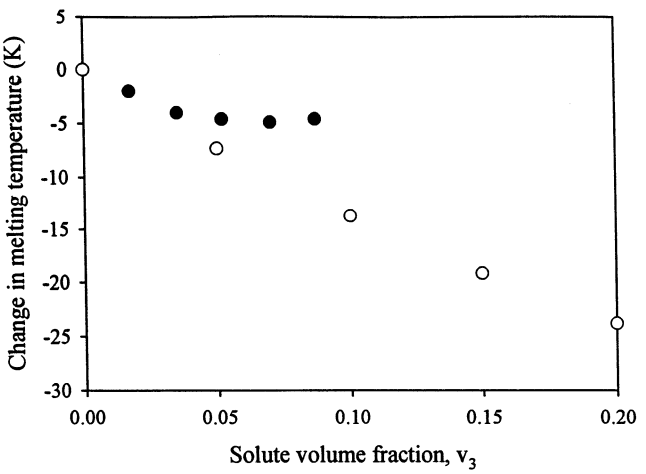

Fig. 4. A comparison of experiment and theoretical prediction for the effect of urea concentration on the peak melting temperature of the B-type crystalline polymorph of starch: experiment, ●; theory, ○. Volume fraction of starch, $v_2 = 0.2$.

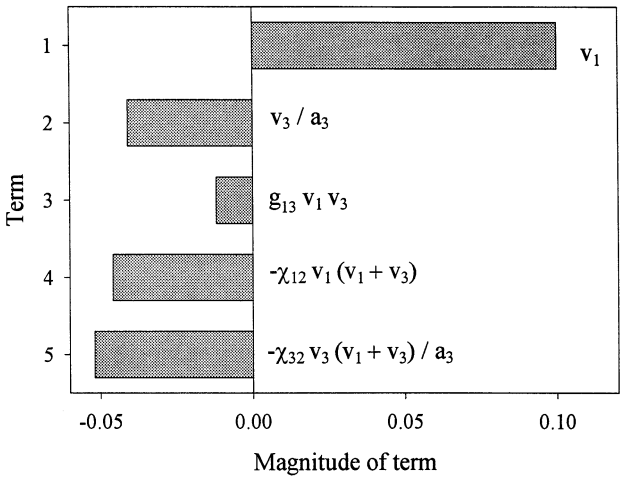

Fig. 5. A comparison of the contributions to the melting temperature reduction in a 20% v/v starch–water mixture due to replacement of 10% v/v water by urea as predicted by Flory's theory of melting (see Eq. (9)).

change in magnitude of the various terms when 0.4 of the volume fraction of water is replaced by D-glucitol. As with the urea mixtures, the enthalpic terms (terms 3–5) are all negative and outweigh the entropic terms (terms 1 and 2) leading to a depression of the melting temperature. D-Glucitol is larger than urea and so the entropy of mixing term (term 2) is correspondingly smaller. Term 5 is the smallest enthalpic term, a result of the preferential hydration of the starch. The Flory modelling of preferential interactions and melting are both related to variations of the chemical potential of the polymer with solvent composition hence the similarity between Eqs. (5)

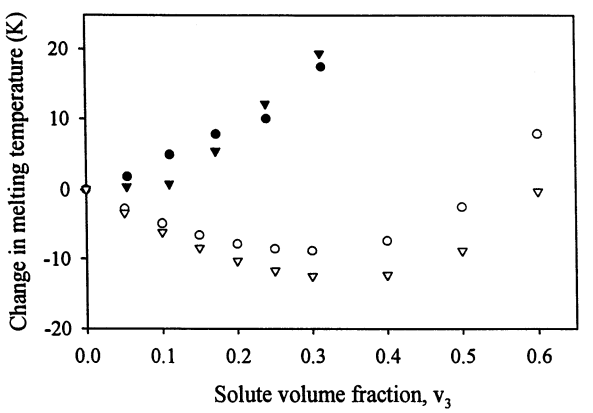

Fig. 6. A comparison of experiment and theoretical prediction for the effect of D-glucitol and glucose concentration on the peak melting temperature of the B-type crystalline polymorph of starch: D-glucitol experiment, ●; D-glucitol theory, ○; glucose experiment, ▼; glucose theory, ▽.

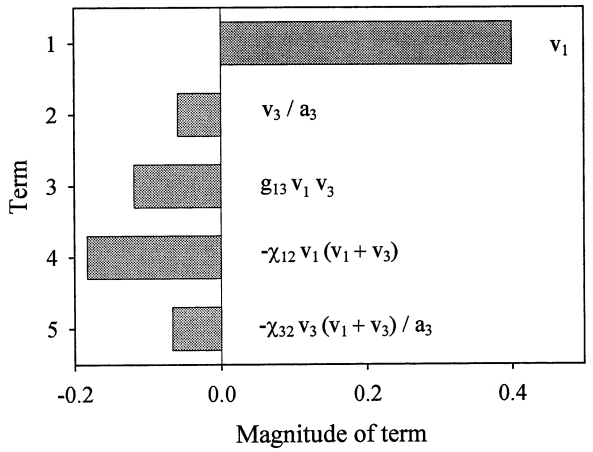

Fig. 7. A comparison of the contributions to the melting temperature reduction in a 20% v/v starch–water mixture due to replacement of 40% v/v water by D-glucitol as predicted by Flory's theory of melting (see Eq. (9)).

and (9). Experimentally, whilst the preferential interactions are measured at low starch compositions and extrapolated to zero concentration, the melting study samples were at volume fractions of 20%. However, the balance between the terms in the melting calculations (Fig. 7) and the fitting of χ_{32} using Eq. (5) to obtain the experimental value of $(\partial\mu_{2u}/\partial m_3)_{T,\,p,\,m_2}$ are similar. In the Flory fit of the preferential hydration data, it is the large entropy of mixing term (term 1) which leads to the negative value of χ_{32}. This, in turn, leads to the prediction that D-glucitol is a very good solvent for starch whilst water is a poor solvent. The melting data (Fig. 3) clearly contradict this, underlining the poor performance of the Flory model in this instance.

4. Discussion

The addition of a low-molecular-weight solute to an aqueous polysaccharide solution can influence several aspects of behaviour including gelation, crystallisation [24] and dissolution [4,7], and liquid–liquid phase separation [22,23]. Approaches for predicting the behaviour of comparable synthetic polymer systems are well-developed and there is a corresponding body of literature on experimental studies. In this study we have employed an approach developed to examine interactions in ternary aqueous protein solutions to aqueous starch mixtures. A relatively weak interaction between starch and the hydroxy compounds glycerol, D-glucitol and D-glucose was observed at room temperature. In contrast, urea and guanidinium thiocyanate showed a preferred interaction with the starch chain.

In the present work the link between preferential interactions and melting has been made using Flory's statistical thermodynamic models. However, the correlation between the two experimental measurements and the similarities between the statistical theories indicates that it may be possible to derive a purely thermodynamic relationship between these two properties. The difference in polymer concentration between the two measurements would need to be incorporated into this relationship.

While the agreement between experiment and theory for the melting of starch–urea–water mixtures is encouraging, the lack of agreement for the starch–hydroxy compound–water mixtures shows that, at worst, this may have been fortuitous. More thermodynamic data is required for model building and testing. With the present state of knowledge the Flory model provides a useful framework on which to build. The small molecular size of urea and its strong preferential interaction with starch lead to a relatively small change in the entropy of mixing and a dominant starch–urea contribution to the enthalpy of mixing (Fig. 5). In contrast, D-glucitol is larger and is weakly excluded from the domain of starch which leads to a relatively large change in the entropy of mixing and the enthalpy of mixing is dominated by the interactions of starch and D-glucitol with water (Fig. 7). Flory [6] states that contributions to the entropy from specific interactions are not accounted for in the entropy of mixing term, this is solely due to configurational entropy. Instead it is recognised that these contributions are, to some extent, accounted for in the enthalpy of mixing term, e.g., χ_{12} for starch–water. This explains the poor performance of Flory theory in situations in which non-configurational contributions to the entropy of mixing are important and identifies this as the area in which progress needs to be made.

The role of water of hydration in melting of starch crystals remains to be clarified. Here the Flory model (Eq. (8)) was applied to starch–water melting data and so values of T_m^0, ΔH_u and χ_{12} are appropriate to B-type crystals which contain significant amounts of water of hydration. Strictly speaking Flory's theory of melting is derived for crystals containing no solvent, i.e., an anhydrous polymorph, and, in the crystal, only the chemical potential of the polymer is considered. By fitting this model to melting data of hydrated crystals the effects of water activity on melting have only partially been taken into account.

Despite its shortcomings, the Flory model illustrates the features of a thermodynamically correct model, e.g., the interplay between the various entropic and enthalpic terms. These features should figure in any thermodynamically valid explanation of equilibrium behaviour of polysaccharides in solvent mixtures. The model also predicts an effect of molecular size on the entropy of mixing, a prediction which requires further study.

If agreement between experiment and theory is to be obtained, further experimentation using spherulites is required to ensure equilibrium melting has been achieved. Finally, we believe that by developing this approach, a deeper understanding of the thermodynamic aspects of polysaccharide functionality in mixtures will be obtained.

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