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Thermochimica Acta 328 (1999) 143–153

thermochimica acta

Thermodynamic behavior of mixed biopolymers in solution and in gel phase

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Accepted 6 October 1998

Abstract

The thermodynamic properties of mixtures of two biopolymers, namely maltodextrin and gelatin, have been studied from the gelation as well as from the solution properties points of view. Differential scanning calorimetry has been used to monitor the changes in enthalpy due to the melting of the gel and to evaluate the cooperativity parameter of the gelatin in dilute and semi-dilute concentration. Heats of dilution of the single biopolymers and heats of dilution of the mixed biopolymer solutions have been used to evaluate the Flory interaction parameters within the framework of a new experimental procedure. These data are useful in the description of the thermodynamics of mixed biopolymers and complement other data in progress on this or similar systems.

Keywords: Gelatin; Maltodextrin; Gelation; Cooperativity; Phase separation; Flory interaction parameter

1. Introduction

Mixtures of food biopolymer systems exhibit a variety of behaviours which are much more complex than those occurring in the synthetic polymer field. This is mainly due to the fact that, in addition to the several phase transitions and possible phase separation, they are often characterised by a local order–disorder equilibrium, not very common in other polymers. Compatibility regions have to be, therefore, identified in the phase diagrams with the additional specification about the molecular conformational features which may also be responsible for some of the phenomenological features [1,2].

The mixture of two or more biopolymers with water is an almost constant occurrence in all food preparations, and the interest in the ordered–disordered conformational states in food biopolymers is now being recognised with a thorough characterisation of the structural and thermodynamic features. However, concerning industrially important biopolymers, such as gelatin, either reliable enthalpy data in dilute and semi-dilute aqueous solutions are not available or low sensitivity calorimeters are used (≈0.03 mW, or lower). For example, the only direct calorimetric study on the melting enthalpy of gelatin in a wide range of concentrations (≈5–100%) appears to be that of Tserately and Smirnova [3], using a Setaram DSC-111. Furthermore, the fundamental approach to the thermodynamics of polymeric solutions seems to have been completely neglected, even if there is a large amount of work on the thermodynamic properties of polymer solutions and polymer blends. Thermodynamic theories, based on the use of the Flory–Huggins lattice model [4], or its generalisation thereof [5], are

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0040-6031/99/$ – see front matter
PII: S0040-6031(98)00635-2

broadly accepted for the interpretation of experimental data. However, in the field of binary and ternary systems, a search of the literature reveals that the actual splitting of the excess Gibbs free-energy term (i.e. the parameter χ) in the entropy and enthalpy contributions (χ^H and χ^S) has been almost elusive. This is due exclusively to the fact that direct calorimetric measurements on mixed systems are scarce. As a result, even the formal definition of the experimental quantities involved in the evaluation, say of χ^H, is somewhat unsatisfactory.

The current research in our laboratory deals with the study of the energetics of the interaction between biopolymer chains in solution and in gel phases [6–8]. The final aim is the understanding of the structure–property relationships in these organised macromolecular systems (physical gels) and, eventually, the prediction of the behaviour of mixed polymer systems. Within the framework of a larger project [9], we present the results of two stages of investigation on the aqueous system of a gelatin/maltodextrin mixture. The first part, given the evidence of gelation phenomena of the biopolymers, deals with the melting process of the two individual systems and with their behaviour in the mixtures. It is understood that biopolymer gelation occurs through an association mechanism of helical, ordered segments of chains. The second part is concentrated on the determination of the enthalpy contributions in the isothermal mixing of gelatin with maltodextrin under conditions of soluble macromolecular components. The theoretical framework is the Flory–Huggins approach to the thermodynamics of random coil polymer solutions. The results are expected to help in an understanding of the relevance of the enthalpy changes to the phase stability with temperature and composition.

2. Experimental

2.1. Materials

The gelatin sample (LH1e) is a first extract alkaline-process sample obtained from lime hide, very kindly provided by Systems Bio-Industries (SBI). The pI-value of the biopolymer is ca. 4.5. The solid sample, used without further purification, contains ca. 12 wt% of water and an amount of associated salts which was accurately determined by the producer. All ionic species, however, were $<10^{-7}$ mol/g of dry power, except that Na^+ and NH_4^+ were 1.3×10^{-4} and 1.1×10^{-4} mol g^{-1}, respectively. Gelatin dispersion was solubilised by heating up to 65°C, under stirring, for 45 min.

The maltodextrin sample (Paselli SA2, produced by AVEBE, and provided by Unilever Research, UK) is a potato starch enzymatic hydrolysate. The original amylose/amylopectin ratio was 21/79 with molecular weight (Mw) of 5×10^5 and 3.4×10^8, respectively, for the linear and the branched fraction. The sample (SA2) is characterised by a DE (dextrose equivalent) of ca. 2–3, which gives an approximate Mw of 10^4. However, the molecular-weight distribution curve, determined by DAWN size-exclusion chromatography coupled with LALLS at Unilever, gives evidence of a broad bimodal distribution with peak values centred at ca. Mw of 8×10^5 and 7×10^3. The sample is assumed to contain polydisperse linear, and branched, fractions of poly(α-D-glucose). Maltodextrin solutions were prepared by dispersing the powder in water and the dispersion was heated and held at 90°C for half-an-hour. Solutions were prepared by weight.

2.2. Scanning calorimetry

The thermal behaviour of binary and ternary systems was studied by means of differential scanning calorimetry with Setaram Micro DSC-I and DSC-III. In order to obtain the actual temperature of the sample, calibrations were made to correlate the nominal temperature (or the block temperature) with the measured temperature in the sample cell at the different scan rates used. All the DSC data refer, therefore, to this 'real' temperature.

The ternary systems were prepared and treated according to the following steps: (1) the solutions of maltodextrin and gelatin (prepared as described above) were mixed at 60°C and NaCl was added in order to reach the desired ionic strength; (2) the calorimetric cells were filled with the ternary systems (sample weight ranging from 0.6 to 0.8 g); (3) a heating scan from 25° to 95°C with a scanning rate of 2 K min^{-1} was run to cancel the previous thermal history; and (4) the sample were then treated according to a standard procedure, namely cooling at 1 K min^{-1} from 95° to 10°C curing for 16 h and

subsequent heating with a scanning rate of 0.5 K min^{-1}.

2.3. Mixing calorimetry

The measurements of the heats of dilution and of mixing were carried out with an LKB 10700 batch-type microcalorimeter, equipped with gold cells. The electric circuit of the control unit of the calorimeter was modified to allow it to operate at 45°C. Electrical calibration of the heat effects was performed (averaged over many independent data points). Many repeated experiments proved to be irreproducible if the solutions were left to equilibrate for a long time, given the instability (though slow) of the maltodextrin solutions at high concentration. Therefore, operation times were standardised in order to optimise the equilibrium time of the calorimeter for a suitable registration of the power–time curve. Experiments carried out with the standard procedure were occasionally affected by long-term drift and/or curvature of the signal after mixing. The areas were evaluated by constantly neglecting these undesirable heat effects, when they occurred. No such problems were encountered with the gelatin solution experiments.

3. Theory

Phase diagrams on the composition triangle for ternary systems consisting of two chemically different polymers and a pure solvent vary with the compatibility of the polymer components as well as the polymer/solvent affinity. The main thermodynamic factor leading to incompatibility of a three-component mixture made by two polymers in an otherwise good solvent is the poor attractive interactions between the polymer components. In other cases (very seldom reported in the literature), the polymers may also undergo induced conformational transitions as functions of the composition and/or the temperature. These conformational transitions are *in themselves* conceptually analysed as phase transitions, since the polymer state is characterised by a difference in the structural and thermodynamic properties. We shall shortly summarise the analyses of the experimental results which can be obtained by differential scanning calorimetry on the helix → coil (in this case, gel → sol) conformational transition in linear biopolymer chains. Thereafter, a derivation of the relevant equations for the evaluation of the interaction parameter in binary and ternary systems will be summarised.

3.1. Thermodynamics of helix–coil and gel–sol transition

Let us briefly recall some concepts underlying the helix–coil 'phase' transition in biopolymers [10]. In the case of globular proteins, it is worth mentioning that the Gibbs free energy of the native species is often ca. 40–60 kJ/mol of protein lower than the denatured random coil form. On account of this low free energy difference, the temperature of transition between the two species is higher than the ambient temperature (i.e. $T_m > 25°C$) only if the whole macromolecule can be thermodynamically considered a single domain. The hypothesis was, therefore, made that the denaturation is a 'cooperative' process between two distinct, thermodynamically defined states, in equilibrium with each other at the transition temperature. The confirmation of the validity of this hypothesis, by means of DSC, has been one of the most significant milestones in the thermodynamics of biopolymer systems [10].

Whenever biopolymers have a regular sequence of units, which does not give a globular folding, helical segments are formed, as the stability of ordered structures is also a function of the chain length with a critical value above which the helix is interrupted [11]. This concept was introduced, before the above findings for globular proteins, by the Zimm–Bragg theory [11] through the cooperativity parameter σ. This parameter essentially defines the excess free energy of formation of an isolated helical conformation with respect to the same process occurring as a neighbour of a helical sequence, for which the associated free-energy change is described by the parameter s. Terms like initiation and propagation of a cooperative helical transition were then connoted. The σ parameter is related to the sharpness of the change in any property measured as a function of a variable inducing helix–coil transition. Without going into the details of the theoretical treatment [12], the prediction is that the cooperativity of the transition depends on the chain length, n, and on the parameter σ, whilst the average transition temperature depends on n and mainly on the value of s.

From the calorimetric point of view, the heat of transition evaluated by DSC experiments differs from that evaluated by using the van't Hoff isochore for the apparent equilibrium constant. This discrepancy is a direct consequence of, and theoretically related to, the existence of 'molecular blocks of monomer units' which undergo a phase transition, with a change of enthalpy that is larger than the unitary change (i.e. per residue) by a factor of $N^0 \approx \sigma^{-1/2}$. In this approach, N^0 is defined as the length of the cooperative unit. Calorimetric measurements directly provide the value of N^0 as the ratio of the apparent van't Hoff and the calorimetric heat of transition.

Here, we do not discuss whether a statistical mechanical analysis can be made on a biopolymer which, in addition to the helix–coil transition, exhibits further changes due to association of helical segments in larger aggregates and/or supramolecular structures. Theoretical works on some of these additional processes have been recently published ([12–14]). At this stage, only the stability and the size of the thermodynamic domains are defined through the DSC experiments.

Details on the derivation of the thermodynamic parameters for a triple helix-to-coil transition from DSC experiments are given elsewhere (Sist et al., in preparation). The transition can be analysed within the framework of the polysteric model [13] for conformational transitions, as it has already been done for the polysaccharide succinoglycan [7]. The most simple approach gives the length of the cooperative unit in terms of the specific excess heat capacity of the system at the transition mid-point, T_m, and of the specific enthalpy change for the transition Δh:

$$\frac{\Delta H^{v_H}}{\Delta H^{cal}} = \frac{4RT_m^2 \Delta c_p^{T_m}}{\Delta h^2}$$

where ΔH^{v_H} is the van't Hoff enthalpy of the 'equilibrium process', defined in terms of the partition function Q:

$$\Delta H^{v_H} = RT^2 \frac{d\ln Q}{dT}$$

3.2. Thermodynamics of binary and ternary systems

Within the logical premises earlier formalised by Flory [4,15,16], the total excess Gibbs free energy of mixing (*per mole of lattice sites*) of a multicomponent solution containing k components is, in the most general form:

$$\frac{\Delta G}{RT} = \sum_{i=1}^{k} \frac{\phi_i}{N_i} \ln \phi_i + \sum_{i \neq j}^{k} \sum_{j=1}^{k} \chi_{ij} \phi_t \phi_j \qquad (1)$$

where χ is the interaction parameter (total) and ϕ the volume fraction. ΔG must be negative for the mixing of k components to give a compatible system, although the stability of the system is determined by the condition that the second derivatives of ΔG or, in the general case, the matrix determined of the terms $\{\Delta G_{ij}\}$ is zero.

The interaction parameters have formally been split into the two contributions, enthalpic and entropic, according to the definition $\chi = \chi^H + \chi^S$. In this paper, however, we deal specifically with the direct measurement of the heat involved in a change of state (concentration) and, therefore, the experimental data by definition are related exclusively to the enthalpic contributions of the *excess* free-energy change of the system. To the best of our knowledge, a clear derivation from the experimental data of the enthalpic component of the Flory parameter, χ^H, is lacking. Most of the literature data refer either to the determination of the free-energy parameter, χ, from chemical potential relations or to the evaluation of the enthalpic component of χ from the temperature dependence of χ itself. The full derivation and the limitations of the equations reported here are given elsewhere (Cesàro et al., in preparation); only a short summary is given in the following.

In a binary (polymer-1/solvent-0) system, the enthalpy of mixing $\Delta_m H$ of a pure solvent with a pure polymer gives:

$$\Delta_m H = kT\chi_{01}^H N_0 \phi_1 \qquad (2)$$

where ϕ_1 is the polymer volume fraction and N_0 the number of solvent molecules.

The enthalpy of dilution is defined as $\Delta_{dil} H_{c'' \leftarrow c'} = \Delta_m H_{c''} - \Delta_m H_{c'}$, where c' and c'' are the initial and final concentrations for the dilution process. It is easy to verify that the concentration-dependent Flory enthalpic parameter, χ^H, can be extracted by a simple evaluation of the heat of dilution as a function of the polymer concentration:

$$\chi_{01}^H = -\frac{Q_{dil}}{RT\Delta(\phi_1 n_0)} \qquad (3)$$

where the term $\Delta(\phi_1 n_0)$ is the change in the volume fraction of the polymer times the number of moles of the solvent.

Although the evaluation of χ^H is done here by using Eq. (3), a comment is necessary on other approximated equations which can be derived, for example, with the assumption that the dilution process is infinitesimal; such approximate equations have been used even without an infinitesimal dilution process being made [17,18]. In general, the validity of the approximations relies on the condition that χ^H does not depend on the concentration and, furthermore, that an infinitesimal dilution experiment is made.

For a ternary system (polymer-1, polymer-2, solvent-0) some methods have been suggested based on thermodynamic cycles involving the dissolution of the solid polymer into the solvent. These cycles always contain an algebraic summation of several steps of relatively large heat measurements, leading to a relatively large error accumulation. Alternatively, the interaction parameter between two polymeric solutes can be extracted from the equations already derived for the evaluation of cross-interaction coefficient h_{ij}, from the *excess* enthalpy of a solution containing two low-molecular weight solutes [19]. A straightforward experimental procedure is obtained by diluting the ternary systems at a constant polymer-1/polymer-2 ratio by the addition of a solvent, such as that already applied for low-molecular weight solutes within the framework of the McMillan–Mayer theory of solutions [20,21].

In this way, the χ^H_{12} for the ternary system can be derived, once the parameters for the binary systems are known in addition to the operational quantity χ^* which defines the *excess* property of the ternary system.

$$\chi^H_{12} = \frac{\Psi_1\chi^H_{01} + \Psi_2\chi^H_{02} - \chi^*}{\Psi_1\Psi_2} \tag{4}$$

and

$$\chi^* = -\frac{Q_{dil}}{RT\Delta(\phi_{tot}n_0)} \equiv \Psi_1\chi^H_{01} + \Psi_2\chi^H_{02} + \Psi_1\Psi_2\chi^H_{12} \tag{5}$$

where $\Delta(\phi_{tot}n_0)$ is the change in the total volume fraction of polymer-1 plus polymer-2 in the mixture times the number of moles of the solvent, and Ψ_1 and Ψ_2, respectively, the volume fractions of polymer 1 and polymer 2 in the total volume of polymer 1 plus polymer 2. When no formal distinction is made between polymer-1 and polymer-2, the dilution of a ternary systems gives, in analogy with the premises, Eq. (3). Dilution experiments can therefore be made on ternary systems of polymer 1/polymer-2/solvent by adding the pure solvent and, thus, maintaining the concentration ratios of the two polymers constant.

4. Results

4.1. Thermal behaviour by scanning calorimetry

4.1.1. Binary systems: Gelatin H1e–0.1 M NaCl and Maltodextrin SA2–0.1 M NaCl

The influence of the cooling rate on the gelation and on the following melting was studied by DSC for the binary system, LH1e–0.1 M NaCl. A decrease in the gelation temperature of LH1e with increasing cooling rates was observed in agreement with the mechanism of non-isothermal crystallisation. After a curing of 16 h at 10°C, during the subsequent heating the values of T_m and the enthalpy of melting ΔH_m of LH1e are not influenced by the cooling rate (i.e. the curing of 16 h at 10°C cancels any difference in the properties of the gel formed during the cooling step). As for SA2, no influence of the cooling rate could be detected on the broad melting curve, also because the gelation occurs during the cooling with a much slower kinetics (therefore, not detected in the cooling mode).

Curing at temperatures >10°C gives a slower kinetics of gelation for gelatin, while the same cannot be said for maltodextrin, which does not show detectable signals. Melting temperatures increase slightly with the curing temperature for both the components and melting profiles do not change appreciably for curing times >12 h. Because a heating up to 90°C during the first preparation of the samples had to be carried out in order to solubilise all the material in the mixed systems, a check on the influence of the upper temperature limit of the scan on the stability of the LH1e seemed necessary. Repeated thermal cycles on the same specimen show that both, the area and temperature of LH1e peaks shift towards lower values with the successive heating scans, if the upper temperature is >60°C. A cyclic heating–cooling scan (to 60°C) without curing (Fig. 1) shows the effect of the

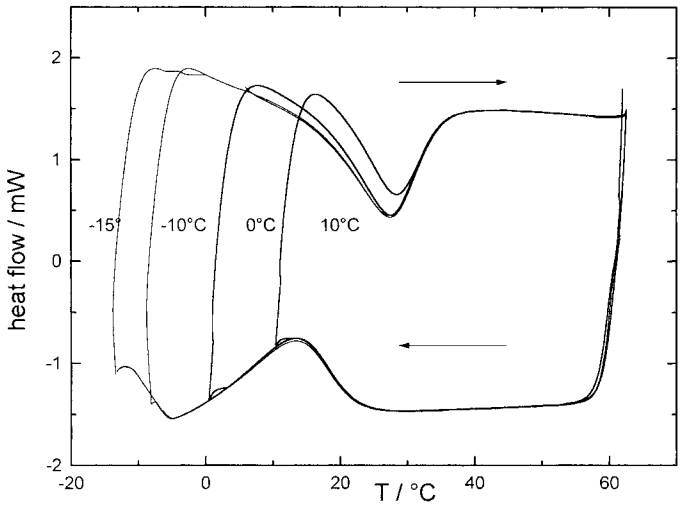

Fig. 1. Cyclic heating (→) and cooling (←) scan of gelatin LH1e (up to 60°C) showing the effect of the lower temperature limit of the scan (10°, 0°, −10° and −15°C, respectively) on the melting peak (4% in 0.1 M NaCl).

lower temperature limit of the scan (10°, 0°, −10° and −15°C, respectively) on the melting behaviour; apparently, a low temperature, e.g. ≈−10°C) over a short time (≈5 min) provide gelation conditions which give a gel structure similar to that reached by curing at the higher temperature of 10°C for a long time (16 h).

Melting thermograms of gelatin have been analysed in the theoretical framework of the helix–coil transition (as mentioned above). The hypothesis was made of a two-state equilibrium process

$$(H_3)_n \leftrightarrow 3(C)_n$$

between a triple-helical segment of n residues per chain and the random coil chains. The model assumes that the cooperativity parameter $N^0 = 3n$ gives the number of residues contained in the cooperative unit.

Table 1 shows the results of analysis of the experimental data of gelatin at two different ionic strengths and at several gelatin concentrations. The melting temperature of gelatin at 0.025 M ($T_m = 27.6 \pm 0.1$) appears slightly higher than that measured at a high ionic strength ($T_m = 27.3 \pm 0.1$). The cooperativity parameter N^0 (of the order of ca. 200) is a function of the gelatin concentration and decreases by about 10%, going from dilute solution to 12 wt% LH1e concentration, while no difference can be detected between low and high ionic strengths.

4.1.2. Ternary systems: Gelatin LH1e–Maltodextrin SA2–0.1 M NaCl

The ternary system was studied using the standard protocol reported in Section 2; each experiment was

Table 1
Calorimetric and van't Hoff enthalpy of transition and cooperativity parameter, N^0, for the helix–coil transition of LH1e

C_{LH1e}/(wt%)	C_{salt}/M	T_m/°C	ΔH^{cal}/(J g^{-1})	ΔH^{vH}/(J g^{-1})	N^0
2	0.1	27.4	2.78×10^3	6.22×10^5	222
2	0.025	27.7	2.67×10^3	6.13×10^5	228
3	0.1	27.3	2.78×10^3	6.22×10^5	222
4	0.025	27.6	2.89×10^3	5.52×10^5	192
6	0.1	27.4	2.82×10^3	5.50×10^5	195
6	0.025	27.6	2.79×10^3	5.38×10^5	192
8	0.1	27.2	2.70×10^3	5.32×10^5	198
12	0.1	27.3	2.63×10^3	4.82×10^5	186

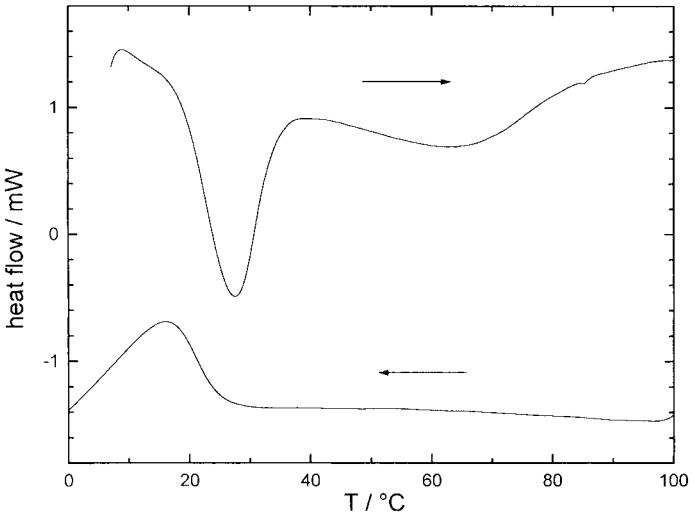

Fig. 2. Heating (→) thermogram for the ternary system (LH1e 4 wt% + SA2 12.5 wt%) after curing at 10°C for 16 h. In the following cooling (←), the gelation of gelatin is shown.

made with a freshly prepared solution. In addition to the composition, several experimental conditions were changed (like the time and temperature of curing, the heating and cooling scanning rates) to study their influence (if any) on the separation and/or gelation process. Fig. 2 shows the features of a heating thermogram for the ternary system after the thermal pretreatment and after curing at 10°C for 16 h. The first peak is due to the melting of gelatin while the second, broader peak is due to the melting of maltodextrin. Upon cooling, only the gelation of gelatin can be detected.

Melting temperatures, T_m, of gelatin LH1e in the mixed systems vary, with few exceptions, within 1°C in the covered range of composition while enthalpies of melting, ΔH_m, for gelatin decrease slightly with increasing maltodextrin content (Fig. 3). As for maltodextrin, the values of ΔH_m slightly increase and those of T_m decrease for the systems with higher maltodextrin concentrations. The values of ΔH_m for gelatin are lower (by a few J g^{-1}) in the mixed systems than those of gelatin alone (see below). All these small changes should be interpreted primarily in terms of gelatin/maltodextrin interaction. However, according to other experiments, microscopic and macroscopic phase separation has been eventually observed and tie lines determined [9]. Therefore, changes in the enthalpy of melting in a separated phase are actually expected, because both T_m and ΔH_m depend on the polymer concentration. The data reported here for the ternary systems do not take into account the fact that the effective concentration of gelatin in the mixture becomes higher because of the phase separation. For example, if the two phases are separated in two equal volumes, the concentration is doubled. As a marginal fact but relevant for the phase separation process, visible, concentric phase separation has been observed in the cylindrical calorimetric cell (ca. 1 ml of volume) if repeated heating–cooling cycles are run. The phenomenon must be seen as a freeze-thawing process which facilitates phase extraction of the micro-segregated drops.

4.2. *Thermodynamics of mixed solution by isothermal calorimetry*

Heats of mixing have been measured at 45°C, where the gelatin does not form gel, and the gelation of maltodextrin is slow enough with respect to the calorimetric experiments to attempt at meaningful results. The heats of dilution have been measured by mixing a volume of polymer solution (2 ml) with an equal volume of the solvent, in order to ensure a measurable although small heat effect in the calorimeter (up to ca. −5 mJ and 25 mJ for SA2 and LH1e, respectively). The heats of dilution can be used, in principle, to

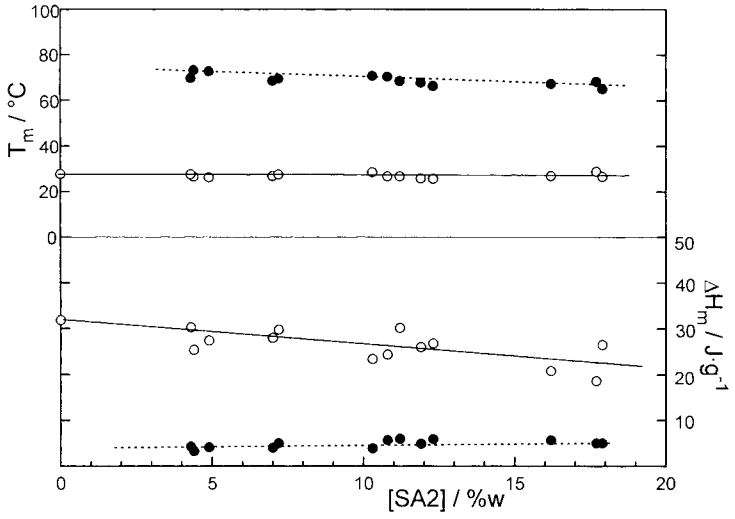

Fig. 3. Temperatures of melting, T_m, and enthalpies of melting, ΔH_m, of LH1e (○) and SA2 (●) as a function of SA2 concentration (LH1e = 4 wt%).

calculate the apparent molar relative enthalpy, ϕL_2, and, therefore, converted to a definite thermodynamic quantity which can be compared with other literature results. Very regrettably, a scrutiny of the literature reveals that the heat-of-dilution data for biopolymers are very scarce and none of them reported as ϕL_2. Some comparison could only be made, therefore, with the solution properties of monomeric analogues [22,23] which are, however, not very useful.

Evaluation of the concentration in volume fraction ϕ has been made by converting the weight concentration through the relation: $\phi = \mathrm{w\%}\rho_{sol}/100\rho_{pol}$, where ρ_{pol} and ρ_{sol} are the inverse of the specific volumes of the solution and that of the polymer. The values of 1.44 and 1.62 g ml^{-1} have been used for ρ from the literature values of the specific volume of gelatin [24] and maltodextrin [23], respectively, in aqueous solutions.

Since the purpose of these data was primarily to calculate the enthalpy part of the interaction parameter χ^H_{12} for the components 1 and 2 of the mixture, the values of χ^H_{01} and χ^H_{02} for the binary systems have been extracted from the heats of dilution (Fig. 4(a) and Fig. 5(a)) according to Eq. (3). In addition, to facilitate an interpretation of the trend of the interaction parameters vs. polymer concentration, it was decided that the polynomial equations which could be used to fit the heats of dilution also had to describe correctly the concentration dependence of χ^H_{01} and χ^H_{02}. It turns out that the heat-of-dilution data of Fig. 4(a) and Fig. 5(a) are expressed by polynomials of the following type:

$$Q_{dil} - n_2\Delta_{dil}H = a_2x^2 + a_3x^3 + \ldots$$

with the first two terms a_0 and a_1 equalling zero. Non-zero values of these two terms would give incorrect values of ϕL_2 and an unrealistic limit as $\chi^H_{01}, \chi^H_{02} \to \infty$ for the concentration approaching zero.

With these conditions, and by using Eq. (3), the fitting polynomials have been used to evaluate the continuous functions of χ^H_{01} and χ^H_{02} of Fig. 4(b) and Fig. 5(b), where the data point of the individual experimental heats of dilution are also reported. The slight but measurable concentration dependence of both, χ^H_{01} and χ^H_{02} supports the current belief that χ^H cannot be, in general, extracted by measurements at a single concentration with the assumption of its independence from the polymer concentration. The value of ca. -0.02 ± 0.01 can be extracted for χ^H_{01} (of LH1e) at infinite dilution (in 0.1 M NaCl), while a smooth negative slope is observed with increasing concentrations. The curve of LH1e at low ionic strength diverges from this trend at low polymer concentrations. This deviation can clearly be ascribed to the slight polyelectrolytic character of the polymer, which undergoes coil expansion upon dilution. Let us simply define the interaction parameter of LH1e at low

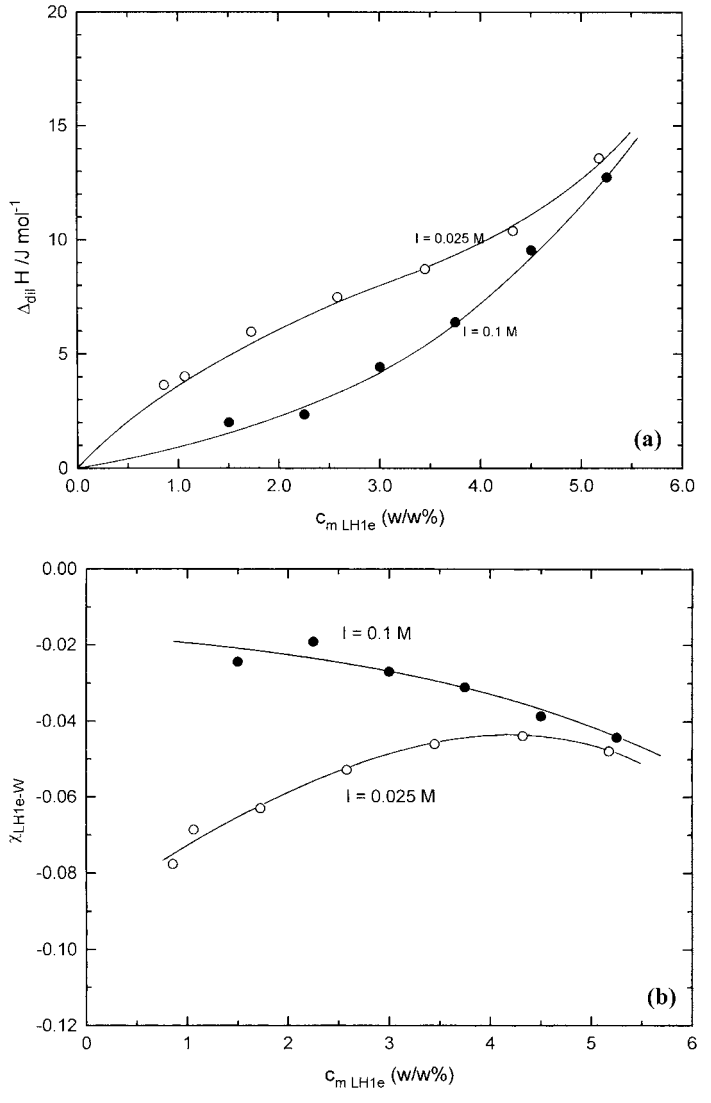

Fig. 4. $\Delta_{dil}H$ (a) and χ^{H}_{01} (b) for the LH1e in aqueous solution (ionic strengths 0.025 and 0.1 M).

ionic strength as an *apparent* quantity. Whether the naif Flory–Huggins lattice model could be used to correctly evaluate the interaction parameter for weak polyelectrolytes in surely questionable, and deserves more comment. This is, however, beyond the scope of the present article.

The values of the interaction parameter χ^{H}_{12} for the ternary system have been obtained from the heats of dilution of the ternary system, as outlined in Section 3. The plot of Fig. 6 shows schematically the values of χ^{H}_{12} in the portion of the ternary diagram investigated in this work. These data for the ternary system clearly demonstrate the enthalpic incompatibility of the two biopolymers in aqueous solution at 45°C, despite the experimental errors and the experimental difficulties. The reported values can be usefully introduced into the free-energy equations to simulate several features (e.g. spinodal decomposition) and to understand the fundamental behaviour of the ternary systems, without the common practice of using 'assumed interaction parameters'. While the entropic part is more amenable to modelling with some appropriate solution theory,

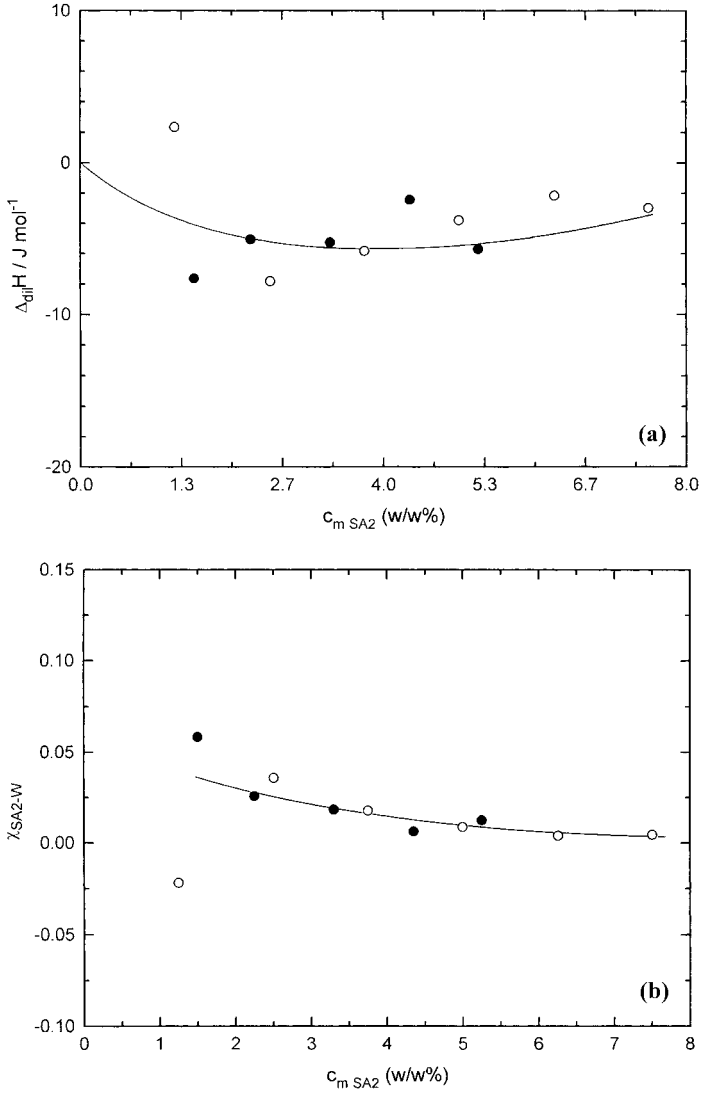

Fig. 5. $\Delta_{dil}H$ (a) and χ_{02}^{H}(b) for the SA2 in aqueous solution (ionic strengths 0.025 and 0.1 M).

the direct calorimetric determination of the enthalpic component to the Flory interaction parameters must be appreciated [25].

5. Conclusions

The main result of this work has been the determination of the enthalpic part of thermodynamic interaction parameter(s) in the phase diagram of the ternary system of gelatin/maltodextrin/water at 45°C. These enthalpic terms can be used together with the parallel free-energy terms to evaluate the entropic part which is usually theoretically calculated with appropriate solution models. No assumptions have been made in the treatment of the experimental data and a simple procedure is suggested which can be used to study ternary systems of two polymers with a third solvent component. In addition, a characterisation of the thermal stability of the biopolymers, gelatin and maltodextrin, is given. These data are used to control the conditions for reliable experiments and to study the

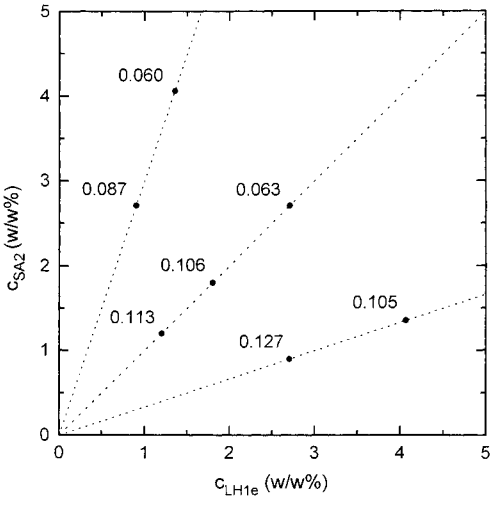

Fig. 6. Interaction parameter χ_{12}^{H} for the ternary gelatin/maltodextrin/water system in the portion of the ternary diagram investigated in this work.

effect of phase separation on the thermodynamics of the gelation and melting processes.

Acknowledgements

This work was carried out within the Project Fair CT96-1015 and through funding of the University of Trieste (Grants for Thermal Equipments). F. Cuppo, D. Fabri and F. Sussich are recipient of fellowships within the Project. Thanks are due to the other participants of the Project for providing data which helped the understanding of the results and to F. Delben and R. Urbani for helpful discussions.

References

[1] V.Ya. Griberg, V.B. Tolstoguzof, Food Hydrocoll. 11 (1997) 145–158.
[2] (a) S. Kasapis, E.R. Morris, I.T. Norton, A.H. Clark, Carbohydr. Polymers 21 (1993) 243–248; (b) S. Kasapis, E.R. Morris, I.T. Norton, M.J. Gidley, Carbohydr. Polymers 21 (1993) 249–259; (c) S. Kasapis, E.R. Morris, I.T. Norton, C.R.T. Brown, Carbohydr. Polymers 21 (1993) 261–268; (d) S. Kasapis, E.R. Morris, I.T. Norton, A.H. Clark, Carbohydr. Polymers 21 (1993) 269–276.
[3] G.I. Tseretely, O.I. Smirnova, J. Thermal Anal. 38 (1992) 189–1201.
[4] P.J. Flory, Principles of Polymer Chemistry, Chap. 12, Cornell University Press, Ithaca, 1953.
[5] see for example, M. Doi, S.F. Edwards, The Theory of Polymer Dynamics, Oxford Science Publ., Oxford, 1986.
[6] T.V. Burova, I.A. Golubeva, N.V. Grinberg, A.Ya. Mashkevich, V.Ya. Grinberg, A.I. Usov, L. Navarini, A. Cesàro, Biopolymers 39 (1996) 517–529.
[7] R. Geciova, A. Flaibani, F. Delben, G. Liut, R. Urbani, A. Cesàro, Macromol. Chem. Phys. 1196 (1995) 2891–2903.
[8] A. Cesàro, Pure Appl. Chem. 67 (1995) 561–568.
[9] Internal Reports, FAIR CT96 1015 1997–1998.
[10] P.L. Privalov, S.A. Potekhin, Methods Enzymology 131 (1986) 4–51.
[11] B.H. Zimm, J.K. Bragg, J. Chem. Phys. 31 (1959) 526–535.
[12] D. Poland, Cooperative Equilibria in Physical Biochemistry, Oxford University Press, Oxford, 1978.
[13] S.-I. Kidokoro, A. Wada, Biopolym. 26 (1987) 213–229.
[14] C.H. Robert, A. Colosimo, S.J. Gill, Biopolym. 28 (1989) 1705–1729.
[15] P.J. Flory, Discuss. Faraday Soc. 20 (1970) 7–29.
[16] M. Kurata, Thermodynamics of Polymer Solutions, Chap. 2, Harwood Academic Publ., Chur, Switzerland, 1982, p. 128.
[17] A. Kagemoto, S. Murakami, R. Fujishiro, Makromol. Chem. 105 (1967) 154–163.
[18] A.M. Basedow, K.H. Ebert, W. Geigenbutz, Makromol. Chem. 181 (1980) 1071–1080.
[19] W.G. McMillan, J.E. Mayer, J. Chem. Phys. 13 (1945) 276–306.
[20] G. Barone, P. Cacace, V. Elia, A. Cesàro, J. Chem. Soc., Faraday Trans. I 80 (1984) 2073–2086.
[21] A. Cesàro, Thermochim. Acta 96 (1985) 333–348.
[22] R.N. Goldberg, Y.B. Tewari, J. Phys. Chem. Ref. Data 18 (1989) 809–882.
[23] A. Cesàro, in: H-J Hinz (Ed.), Thermodynamic Data for Biochemistry and Biotechnology, Springer, New York, 1986, p. 17.
[24] H.B. Bohidar, S.S. Jena, J. Chem. Phys. 100 (1994) 6888–6895.
[25] C. Vinches, A. Parker, W.F. Reed, Biopolym. 41 (1997) 607–622.