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Effect of water partitioning on the glass-transition behaviour of phase separated amylopectin–gelatin mixtures

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Abstract

The glass transition behaviour of concentrated 1:1 and 3:1 amylopectin–gelatin mixtures (water contents between 18 and 36% dry basis) was studied for different compositions using FTIR microspectroscopy and DMTA. The thermograms of these mixtures exhibited 2 transitions indicating the existence of 2 phases. This was in agreement with the FTIR microspectroscopy results where 50–80 μm amylopectin-rich regions could be clearly identified in the continuous gelatin matrix. The assignment of the DMTA transitions was ascertained by varying the gelatin/amylopectin ratio. The prediction of the T_g for each of the 2 phases based on the existing values for the binary systems was best accomplished when the water partitioning between the polysaccharide and the protein was accounted for using the water vapour sorption/desorption isotherms of the individual biopolymer components of the blend. Finally, typical glass transition – "water partitioning" phase diagrams of phase separated amylopectin–gelatin mixtures were constructed.

Keywords: Glass transition; Starch; Amylopectin

1. Introduction

The role of water in modifying the stability and functional properties of biopolymers has been the subject of extensive studies due to its considerable scientific and commercial importance. Clearly, the hydration behaviour of biopolymers is of great relevance to various important processes such as the establishment of protein secondary structure as well as chemical and enzymatic reactivities. Although the hydration behaviour of individual biomolecules has been extensively studied [1,2], little has been reported regarding the distribution of water between the individual components during the hydration of mixed systems and the effects of selective hydration on the molecular and macroscopic properties [3].

A particularly interesting area is the plasticisation of biopolymers by water and the consequent effects on the molecular mobility, the mechanical properties and the amorphous–crystalline transition. Several workers ([4–8]) have successfully studied the plasticising effects of water and solutes on a range of biomolecules and used well established synthetic polymer models such as the Refs. [9,10] or Ref. [11] to describe the experimental results. However, attempts to study biopolymer mixtures have often revealed a two-transition behaviour. Indeed, the DSC and DMTA results reported by Kalichevsky and Blanshard [12] showed clearly two glass transitions for amylopectin/gluten mixtures at intermediate degrees of hydration e.g. samples stored at relative humidities (RH) of 54, 65 and 85%, but only a single transition at low (RH 12%) and high water contents (RH 97%) while for gluten/casein mixtures, two distinct transitions were observed even at high RH (97%). Although the authors rightly suggested that the system was phase separated, their suggestion that the observation of a single T_g in some hydration conditions could indicate a better miscibility is questionable.

In this work, a study of the behaviour of a typical concentrated non-homogenous mixed biopolymer system is reported. The aim of the work described in this paper is to compare the water distribution between the two biopolymers which would be predicted from the sorption isotherms with the actual distribution of water as inferred from mechanical measurements of the glass transition temperatures of the two biopolymers.

2. Material and methods

Amorphous amylopectin as pregelatinized waxy maize starch was supplied by National Starch and Chemical Corp. (Manchester, UK) Ref No 99AJD316. Commercial

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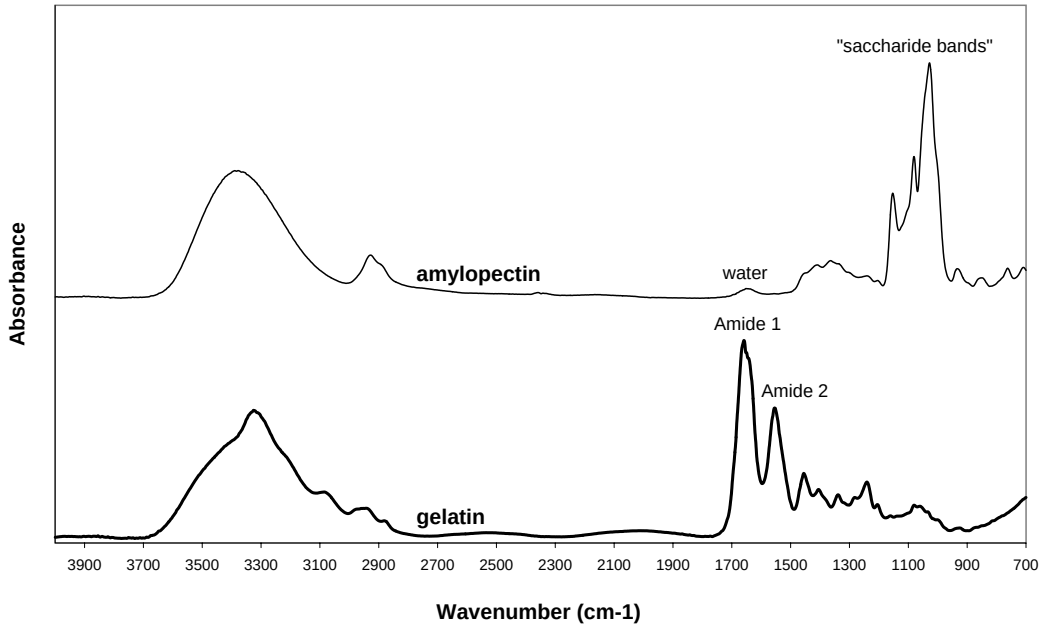

Fig. 1. Transmission FTIR of thin films of amylopectin (top) and gelatin (bottom) extrudates.

grade limed hide gelatin (Bloom 225) was purchased from Chemcolloids Ltd (UK).

2.1. Sample preparation

A given amount of amylopectin and gelatin was mixed in a Kenwood Peerless mixer for 90 min prior to extrusion. Fine gelatin powder (average particle size ≈ 200 μm) was used in order to insure a satisfactory mixing. The mix was extruded through a 1 mm × 30 mm slit die using a Clextral BC-21 co-rotating, intermeshing, twin screw extruder. The 40 cm long extruder barrel was divided into four temperature zones (40, 90, 110 and 80°C). A screw speed of 200 rpm and a solids feed rate of 5 kg/h were used. Distilled water was introduced into the barrel to achieve water contents of approximately 25% w.b in the final extruded ribbons. The moisture contents were determined gravimetrically by vacuum drying at 70°C until for 24 h. 75:25 and 50:50 amylopectin–gelatin blends were produced. Additional water contents were obtained by storing a fraction of the samples at 57% RH (5°C) for 10 days. Water vapour sorption/desorption isotherms were calculated from the weight

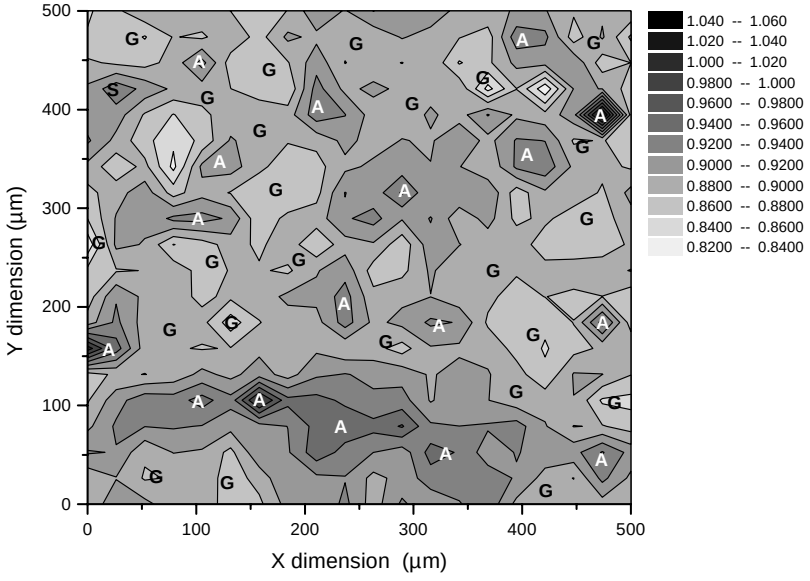

Fig. 2. Contour plot of the ratio of the area of the saccharide bands (1180–953 cm^{-1}) divided by that of the combined amide 1 and 2 bands (1750–1483 cm^{-1}). The letter G and A depicts gelatin rich and amylopectin rich areas respectively.

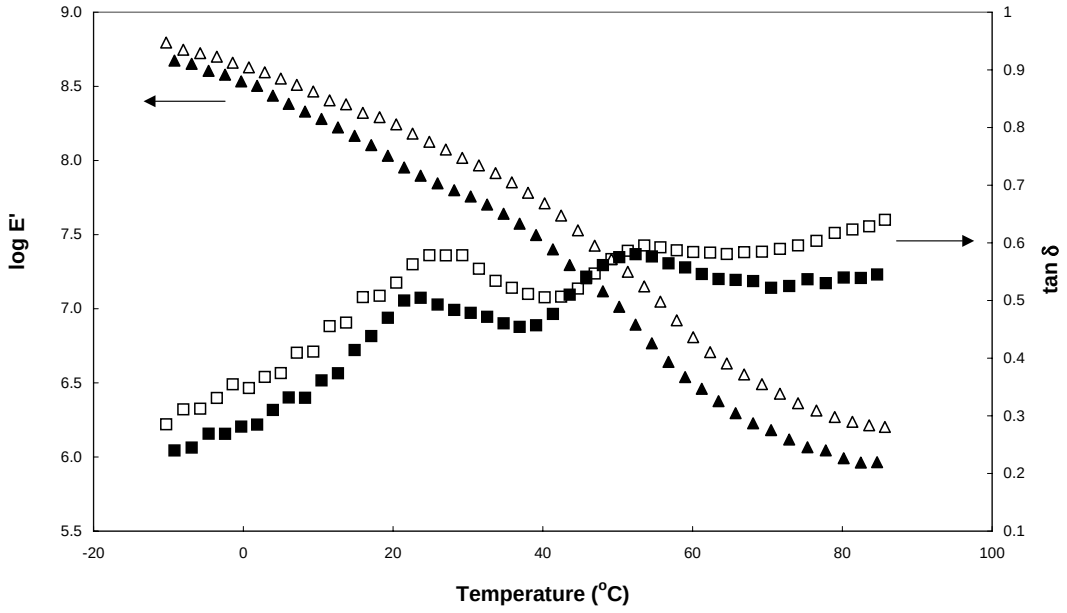

Fig. 3. DMTA thermograms of a gelatin/amylopectin/water 50:50:32 system acquired at frequencies of 1 Hz (solid symbols) and 5 Hz (open symbols).

change of extruded starch and gelatin samples stored at 5°C for a period of 10 days in sealed containers over saturated salt solutions providing a range of RH values.

2.2. FTIR microspectroscopy

Thin films (5–10 μm thick) were obtained by cryo-microtoming the extruded samples and were placed on ZnSe windows for transmission FTIR measurements. A Bruker IFS 48 (Bruker, UK) mid-infrared spectrometer equipped with Bruker's IRscope-1 microspectroscopy attachement and a liquid N_2 cooled MCT detector was used. The computer controlled motorised stage was programmed to acquire 500 μm × 500 μm 2D-maps consisting of 20 spectra in each dimension with a 20 μm apperature. A spectral resolution of 4 cm^{-1} was used and 128 scans were acquired for each spectrum. Air was humidified by bubbling in distilled water and was circulated to avoid the drying of the samples which contained approximately 25% water (w.b).

2.3. DMTA measurements

A Rheometrics Scientific (Mark III) dynamic mechanical thermal analyser operating in bending mode was used. Rectangular (length = 5, width = 14 and thickness = 1 mm) strips were cut from the extruded ribbons and clamped in single cantilever geometry. DMTA thermograms were acquired between −50 and +100°C at two frequencies (1 and 5 Hz) and a heating rate of 2°C/min. Samples were covered with silicone oil in order to alleviate the problem of water loss at high temperatures.

3. Results and discussion

3.1. FTIR microscpectroscopy and DMTA characterisation of gelatin/amylopectin blends

Typical mid-infrared transmission spectra of extruded gelatin, pregelatinised amylopectin and amylopectin–gelatin blend acquired using the FTIR microspectroscopy equipment are shown in Fig. 1. It is clear that the amide 1 and 2 bands centered at approximately 1650 and 1540 cm^{-1} could be used to probe the protein in the blend while the so-called saccharide bands, a series of overlapping peaks in the region 1180–950 cm^{-1} provide information on the distribution of the polysaccharide in the blend.

It is based on these clear differences in the mid-infrared spectra of gelatin and amylopectin that maping the composition of extruded amylopectin–gelatin blends was performed. Fig. 2 depicts the variation of the ratio of the area of the saccharide bands divided by the combined areas of the amide 1 and 2 bands. Band ratios rather absolute area values were used in order to normalise for possible variations in the film thickness. Despite the limited resolution of the technique (20 μm), the inhomogeneity of the blend is evident. Furthermore, gelatin rich domains constitute the continuous phase of the blend.

The DMTA thermogram of all the gelatin/amylopectin blends investigated in this study showed two glass transition events. A typical DMTA result, e.g. that of a 50:50:32 gelatin/amylopectin /water system is shown in Fig. 3 where two glass transitions as characterised by the drop in the elastic component of the modulus, *E*' and the peak in the loss tangent, tan δ(could be clearly seen. The existence of two transitions suggesting the existence of two phases in the

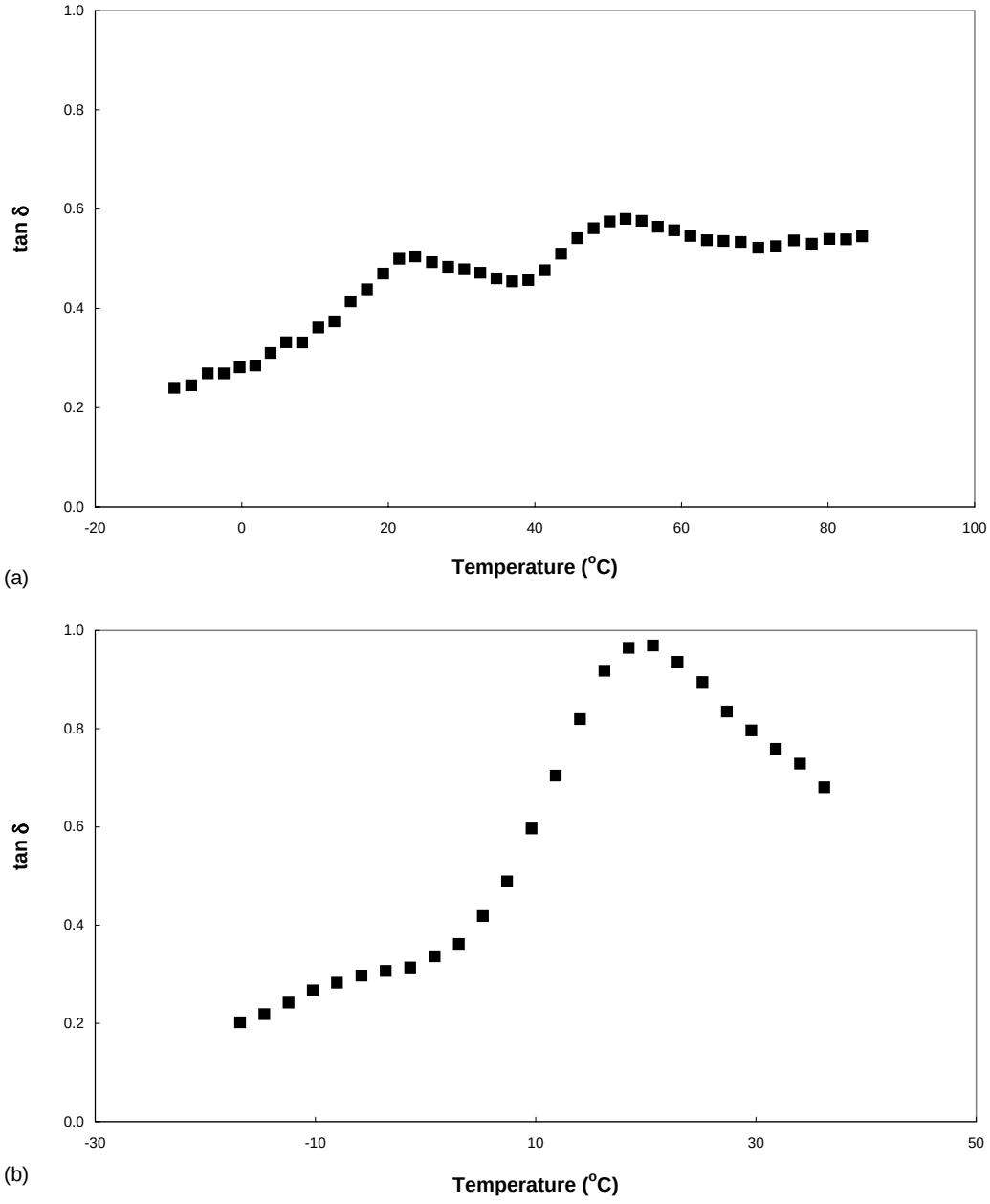

Fig. 4. tan δ results (1 Hz) obtained for 50:50:32 (a) and 25:75:36 (b) gelatin/amylopectin extrudates.

system is in agreement with FTIR microspectroscopy results.

In order to assign the observed transitions to the components present in the system, two samples with different gelatin/amylopectin ratios and comparable water contents were produced and examined by DMTA. The results in Fig. 4 clearly demonstrate that gelatin yields the low temperature transition while amylopectin is associated with the higher temperature tan δ peak. The experimental results were successfully fitted using a bigaussian model.

3.2. Effect of water content

As expected, the position of the tan δ peaks shifted to higher temperatures when the water content of the samples was reduced by storage over 57% RH and 5°C but the two transitions remained distinct. It is interesting to note that the relative positions of the DMTA transition of gelatin and amylopectin changed at low water contents for both gelatin/amylopectin ratios. Indeed, while at high water contents (in the 50:50:32 and 25:75:36 systems) the transition assigned to gelatin was found at lower temperature than that of amylopectin, at lower water contents (for the 25:75:19 and 50:50:19 systems) the T_g of gelatin was higher than that of amylopectin. This is clearly displayed in Fig. 5 where the transitions could be easily identified from their relative height of the tan δ peaks.

At this point, results of glass transition temperatures for

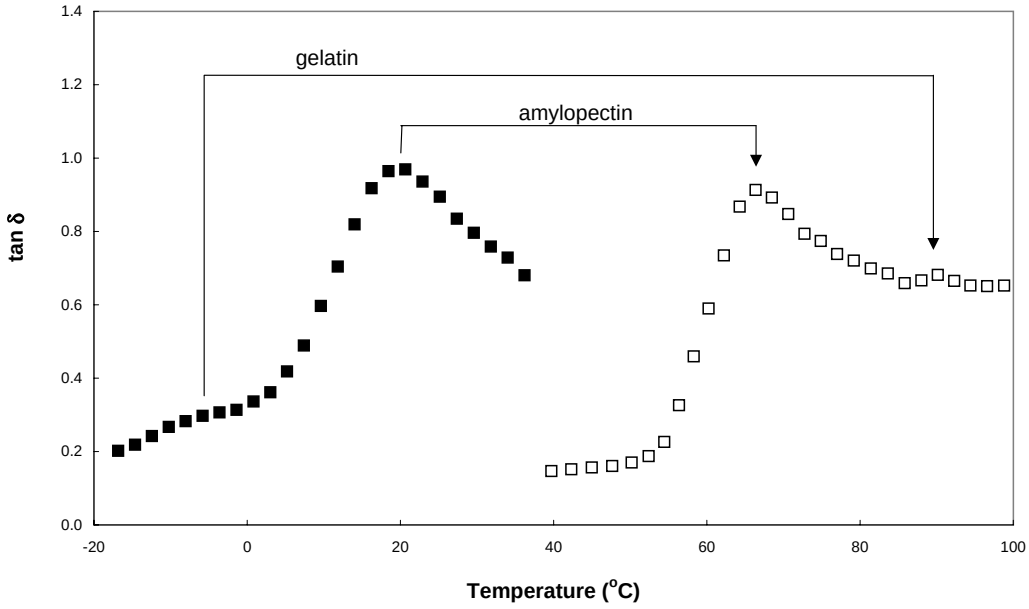

Fig. 5. DMTA tan δ results for the gelatin/amylopectin 25/75 extruded containing 35.75% water (d.s.b) (■) and stored subsequently at RH ≈ 57% and 5°C to reach an equilibrium water content of 19.4% (□).

four different compositions are available (gelatin/amylopectin/water 50:50:32, 50:50:19, 25:75:36 and 25:75:19). The next step was to rationalise these data in relation to the behaviour of the binary amylopectin–water and gelatin–water systems. To achieve this, (i) the phase diagram i.e. the relationship between T_g and the water content for the binary systems was established (Fig. 6) and, (ii) the amount of water associated with each component was estimated from the water vapour sorption/desorption isotherms acquired on extruded amylopectin and extruded gelatin samples (Fig. 7).

The degree of hydration of each component in the mixture was obtained as follows:

$$X_{mix} = w_a X_a + w_g X_g \quad (1)$$

where w_a and w_g are the dry weight fractions of amylopectin and gelatin, respectively, and X_a and X_g the water contents (w/w d.s.b) as given by the sorption/desorption isotherms while the water content of the mixed system X_{mix} was determined gravimetrically on the investigated samples.

The phase diagram of the binary systems was constructed from the results of tan δ peak temperature published by [4] for pregelatinised waxy maize starch and those obtained on gelatin extrudates (this study). The data were subsequently modelled using the Gordon–Taylor [9] equation:

$$T_g = \frac{w_1 T_{g1} + k w_2 T_{g2}}{w_1 + k w_2} \quad (2)$$

where w_1 and w_2 are the weight fractions and T_{g1} and T_{g2} the glass transition temperatures of the pure components for water and the biopolymer respectively and k is a constant. A value of $T_{g1} = 134$ K [13] was used while T_{g2} and k were obtained by χ^2-minimisation. T_{g2} values of 495 and 502 K, and k values of 0.25 and 0.34 were obtained for waxy maize starch and gelatin respectively (Fig. 6). These values are in agreement with the DSC results of Orford et al. [13] where the T_g of amylopectin was estimated at 500 ± 10 K.

The tan δ glass transition temperatures were estimated from the phase diagrams in Fig. 8 for all four samples assuming (i) equal partitioning of water between amylopectin and gelatin and then (ii) accounting for the unequal distribution of water. The calculated values were compared to the experimentally measured T_g values (Table 1). The glass transition temperatures for the mixtures were calculated by extending Eq. (2) to three components assuming total homogeneity.

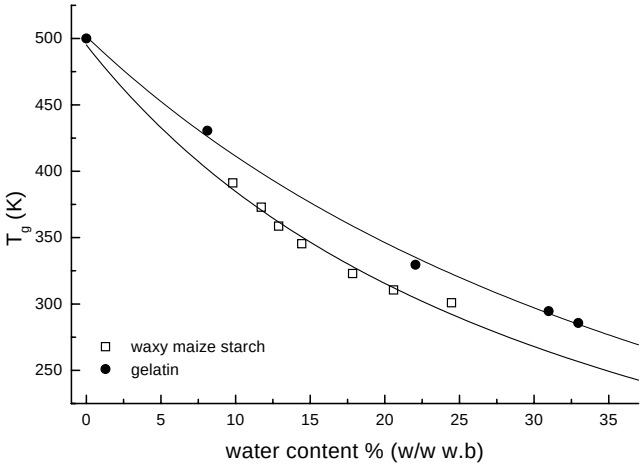

Fig. 6. The glass transition temperatures of pregelatinised waxy maize starch (adapted from [4]) and gelatin (this study) obtained from the DMTA tan δ peak. The lines represent the best fit to the experimental data using Eq. (2).

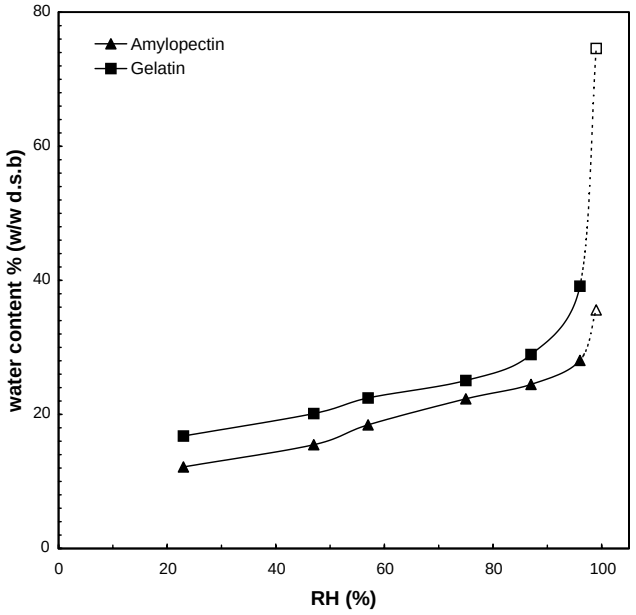

Fig. 7. The water vapour sorption/desorption isotherms of pregelatinised waxy maize starch and gelatin extrudates (5°C). The data points depicted by open symbols were obtained on sorption while the remaining points were obtained on desorption.

Table 1 demonstrates clearly that the values calculated on the basis of equal partitioning predicted wrongly the experimental results. Furthermore, for all the formulations investigated, the calculation of T_g based on the equal hydration hypothesis anticipated values for gelatin that are higher than those of amylopectin. This was found to contradict the experimental results obtained on the sample with high water contents where there was little doubt regarding the assignment of the low temperature transition to gelatin and the high temperature transition to amylopectin. Furthermore, the calculation based on unequal water partitioning predicted that at low water contents i.e. for the 25:75:19 and 50:50:19 gelatin/amylopectin/water samples the T_g of gelatin would be higher than that of amylopectin.

On the basis of the phase diagrams of the binary systems described in Fig. 6, and by using the extended form of Eq. (2) to calculated the T_g line for the hypothetically homogeneous ternary mixtures the complete glass transition, water partitioning diagrams of biphasic 75:25 and 50:50 amylopectin/gelatin extrudates were constructed (Fig. 8). The plots demonstrate clearly the satisfactory agreement between the experimental values and those calculated assuming the unequal water partitioning.

4. Conclusion

This study of the glass transition behaviour of non-homogenous extruded pregelatinised waxy maize starch–gelatin mixtures has demonstrated the need for considering the non-equal partitioning of water between the components of a phase separated system in order to predict the T_g values for the individual phases. The water vapour sorption/desorption isotherms of the single components provided a useful mean to estimate the amount of water associated with each component when coexisting in a phase separated/non-miscible system for different conditions of RH.

Consequently, it is not unreasonable to envisage conditions where the individual T_g values in multiphase systems could converge and become unresolved by DMTA, DSC, etc. Such observations may be wrongly interpreted as that obtained from a one phase homogeneous system.

Acknowledgements

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Table 1
The predicted and measured glass transition temperatures for the various gelatin/amylopectin/water blends investigated

		Homogeneous Mixture	Heterogeneous Equal partitioning		Mixture = two phases Unequal partitioning	
			Starch	Gelatin	Starch	Gelatin
Wms/gelatin 50:50	Water content % d.s.b	32.25	32.25	32.25	23.5	41.0
	Calc. T_g (°C)	35.8	19.5	49.9	48.0	27.8
	Meas. T_g (°C)				52	24
	Water content % d.s.b	19.0	19.0	19.0	17.0	21.0
	Calc. T_g (°C)	83.4	67.0	97.1	76.8	88.5
	Meas. T_g (°C)				55	80
Wms/gelatin 75:25	Water content % d.s.b	35.75	35.75	35.75	29.0	56.0
	Calc. T_g (°C)	18.7	10.4	40.4	29.0	0.0
	Meas. T_g (°C)				19	− 5
	Water content % d.s.b	19.4	19.4	19.4	18.0	23.6
	Calc. T_g (°C)	73.8	65.2	95.3	71.8	78.2
	Meas. T_g (°C)				66	87

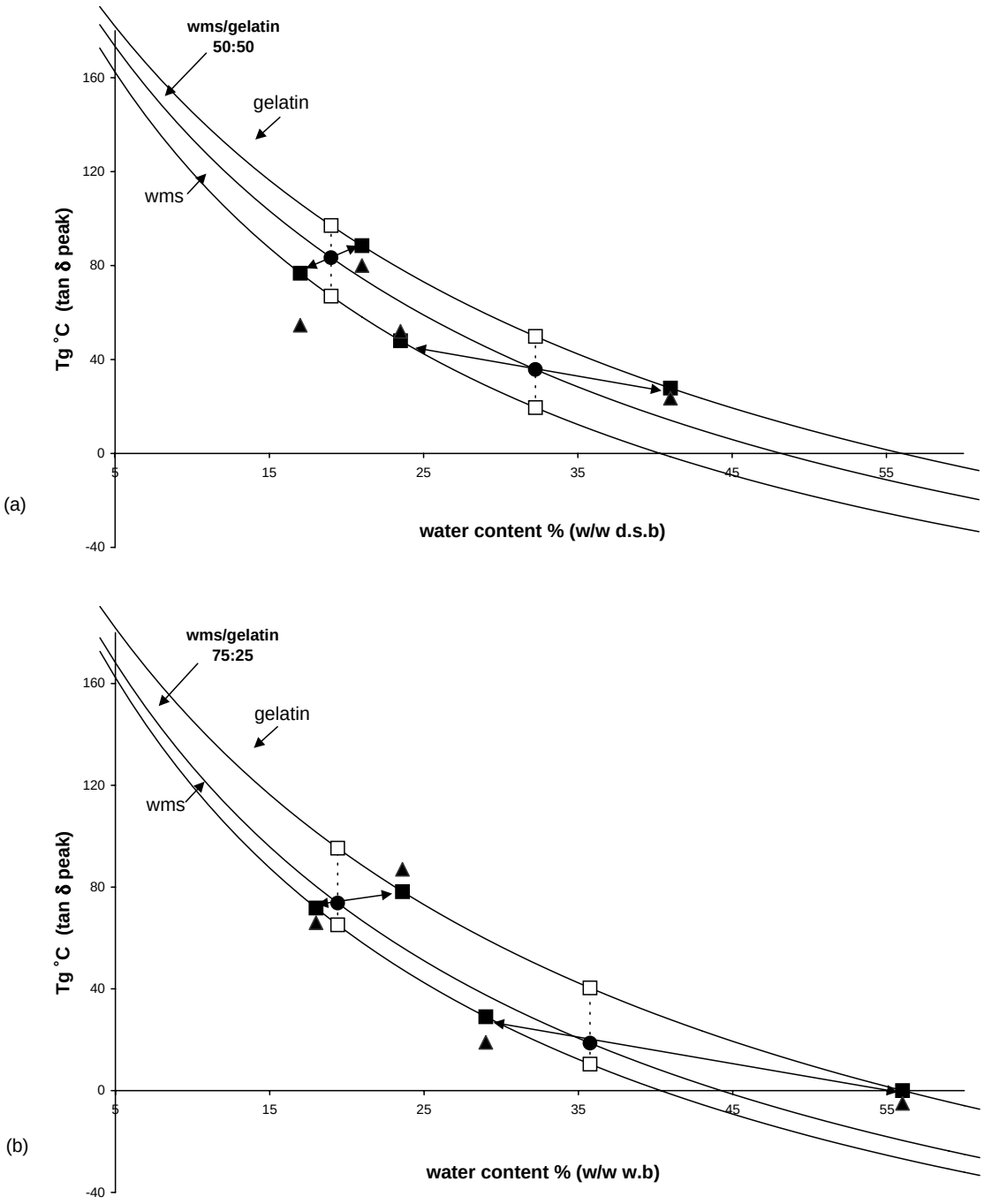

Fig. 8. The glass transition temperature-water partitioning phase diagram for the two-phase gelatin/amylopectin systems investigated: (a) 50:50; and (b) 25/75. The solid lines represent the best Gordon–Taylor fit (Fig. 6) for the experimental data of the binary biopolymer–water mixtures. The symbols are as follows: predicted T_g for the homogeneous ternary mixtures (●); predicted T_g assuming equal partitioning of water (□); predicted T_g accounting for unequal partitioning of water (■); experimental results (▲).

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