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Glass transition temperature of protein/polysaccharide co-dried mixtures as affected by the extent and morphology of phase separation

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ABSTRACT

The glass transition temperature of whey proteins concentrate (WPC)/hydroxypropyl methylcellulose (HPMC) co-dried mixtures with different degrees of phase separation and morphologies were determined by differential scanning calorimetry. To this end the phase separation of aqueous mixtures of WPC (12 wt% or 20 wt%) and HPMC (2 wt% or 3 wt%) at pH 5 or 6, was arrested at different times before freeze-drying. Confocal microscopy allowed to characterize the morphology of phase separation.

Co-dried mixture from quenched phase-separated systems exhibited different numbers of Tgs, according to the degree of phase separation. Two Tgs were observed in the fully phase-separated systems. A single Tg was detected during the first stages of phase separation (i.e. below a 50% of phase separation). It is proposed to ascribe the observed single Tg to the predominance of the extremely large mixed protein/polysaccharide interface present, that would dominate the mobility of the whole system because acting as a network for the entanglement between the protein-rich and the polysaccharide-rich phases. WPC (12 wt%)/HPMC (2 wt%) co-dried mixture at pH 5, with a degree of phase separation above 50%, exhibited three Tgs which were related respectively to the mixed interface, protein-rich phase and polysaccharide-rich phase.

The non-phase-separated WPC (6 wt%)/HPMC (1 wt%) co-dried mixture also showed a single Tg with a reasonable agreement to the predicted value by a theoretical model.

1. Introduction

Drying is the most common method for stabilization of food ingredients and food products during processing and storage. Food materials are composed of multiple biopolymer molecules with different chemistry and properties. Proteins and polysaccharides are typical biopolymer components present in food and pharmaceutical products [1]. These components are often present in an amorphous state which is a non-equilibrium state and they may undergo time-dependent changes with increasing rate at increasing temperatures [1].

Amorphous protein and polysaccharide blends can be produced from mixed biopolymer solutions by spray drying or by rapid cooling and removal of water, i.e. freeze-drying. Mixed biopolymer solutions are usually not stable; after preparation, they generally lead to phase separation because of thermodynamic incompatibility. This phenomenon is the result of enthalpy and entropy barriers caused by the size and incompatible chemistry of different biopolymers [2]. Above the protein isoelectric point, thermodynamic incompatibility between proteins and polysaccharides generally occurs because of the repulsive electrostatic interactions and different affinities towards water. Then, above a critical concentration macroscopic phase separation occurs.

Moreover, the properties of phase-separated biopolymer mixtures are influenced by the morphology of the segregated domains and by the effect of surface energy and interfacial composition. Of all the properties of phase-separated aqueous polymer blends, the interface between adjacent phases is the least understood, because there are few techniques that allow to study it directly. However, there are several techniques available to characterize the microstructure of these blends, including some microscopy techniques. Tromp et al. [3] have demonstrated that confocal scanning light microscopy (CSLM) is a powerful technique to characterize the morphology of phase-separated blends of food biopolymers.

The glass transition is a critical parameter for amorphous food matrices that control their processability, properties and stability [4,5]. The glass transition temperature (Tg) occurs over a temperature range which is determined by the heterogeneity of the system [6,7]. Co-dried mixture heterogeneity is mainly determined by the physical properties of the polymers used as well as the interactions between them on the aqueous mixture, i.e. phase behaviour. Attempts to study immiscible biopolymer mixtures have often revealed two-Tg [8–11]. In contrast, perfectly compatible mixtures

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have a single Tg located between the Tgs of the individual components [12–15,2].

The understanding of the relationship between the phase behaviour of a protein/polysaccharide aqueous mixture and the thermal properties of the co-dried mixture produced from it, has a great importance in the development of food and biomaterials of desired properties and proper processing.

The current research aims to assess the relationship between the extent and morphology of phase separation of protein/polysaccharide aqueous mixtures and the thermal properties of the co-dried mixture obtained by freeze-drying. As a protein/polysaccharide mixture, we used a whey protein concentrate (WPC) and hydroxypropyl methylcellulose (HPMC) blend.

Phase-diagram of the WPC/HPMC mixed system at neutral pH was developed by Perez et al. [16]. The position of the binodal curve in this diagram indicated that the compatibility zone is relative small and phase separation takes place in a broad range of polymer concentrations.

Whey proteins have many technological applications. The main proteins present are β-lactoglobulin (β-lg), α-lactalbumin (α-lac) and bovine serum albumin (BSA) [17] and they account for by 70% of total whey proteins. These proteins are responsible for the hydration capacity, gelling, foaming and emulsifying properties of WPC.

Hydroxypropyl methylcellulose is used in the food industry, printing technology, and has pharmaceutical applications because is non-toxic and possesses good mechanical properties. The usefulness of HPMC is essentially based upon four key attributes: efficient thickening, surface activity, film forming ability, and the capacity to form thermal gels that melt upon cooling. These interesting properties are given by methyl substitutes along the cellulose backbone that constitute strong hydrophobic zones and hydroxypropyl groups that are more hydrophilic [16].

2. Experimental

2.1. Materials

HPMC E4M, food grade from The Dow Chemical Company was kindly supplied by Colorcon, Argentina. It was used without further purification. This cellulose derivative has 25% methyl groups, 10% hydroxypropyl groups, being the methyl/hydroxypropyl ratio 2.8%. Viscosity, measured on 2% (wt) aqueous solution (20 °C) determined by Ubbelohde viscometer, was 4995 cP and molecular weight 90,000 Da. Moisture content was 1.6%.

WPC powder was kindly given by Milka Frank, Santa Fe, Argentina. Its composition was: protein 78.9% (N × 6.25) [18]; lactose 5%; fat 6%; ash 4.3% and moisture 5.6%. SDS-PAGE-electrophoresis was made in a Mini-Protean II dual slab cell system (Bio-Rad Laboratories). Quantification of the protein bands was accomplished by means of Bio-Rad GS-670 imaging densitometer. Bio-Rad Molecular Analyst/PC Molecular Imager program allowed the analysis of molecular weight and band intensities under volumetric test option. WPC proteins composition was: β-lg 44%, α-lac 20%, BSA 8%. The remainder proteins were immunoglobulins and the proteose-petone fraction [19–21].

2.2. Dry systems preparation

From the phase-diagram of WPC/HPMC mixed system [16], different mixtures at pH 6, exhibiting phase separation or not, were selected to be studied: WPC (6 wt%)/HPMC (1 wt%), WPC (12 wt%)/HPMC (2 wt%) and WPC (20 wt%)/HPMC (3 wt%). To obtain the co-dried mixtures with different degrees of phase separation, each segregating mixture was quenched with liquid nitrogen to arrest phase separation. On mixtures WPC (12 wt%)/HPMC (2%) and WPC (20 wt%)/HPMC (3 wt%) the phase separation was arrested at three different times: (i) immediately after mixing the biopolymers solutions, (ii) 30 min after mixing them, and (iii) after centrifugation to fully separate the two phases. The WPC (12 wt%)/HPMC (2 wt%) mixture was also adjusted to pH 5 and 3 by HCl (0.1 M) addition.

Amorphous co-dried mixtures were obtained by freeze-drying the quenched samples for 48 h in Stokes freeze-dryer (operating at −40 °C condenser plate temperature and a chamber pressure of less than 100 μm Hg), and then placing them in a vacuum oven at 70 °C for 15 days in presence of $CaCl_2$ to eliminate residual water before measuring Tg.

Solutions of single WPC (15 wt%) or HPMC (3 wt%), were also subjected to the same procedure in order to measure the Tg of single dry components.

2.3. Kinetics of phase separation

On aqueous mixed systems WPC (20 wt%)/HPMC (3 wt%) at pH 6 and WPC (12 wt%)/HPMC (2 wt%) at pH 5 and 6, the kinetics of phase separation at room temperature, was determined by measuring the volume of bottom phase over time.

2.4. Differential scanning calorimetry

Thermal analysis was performed using a DSC 822 Mettler-Toledo calorimeter equipped with STARe 6.1 Thermal Analysis System software. The instrument was calibrated with indium and zinc. Each sample was heated at rate of 10 °C/min from 5 °C to 220 °C. Glass transition temperature was recorded as the midpoint of the discontinuities in the curves of heat-flow versus temperature from the second heating scan after cooling at 10 °C/min, in order to eliminate previous differences in the thermal treatment of samples. The onset and endset temperatures were recorded as temperatures at the very beginning and the end of these discontinuities in heat-flow over temperature, respectively.

Experiments were performed at least in duplicate in 40 μl punctured aluminium pans (Mettler-Toledo) containing 10–15 mg of dry sample. The samples were weighed on a Mettler-Toledo AG245 automatic electro-balance with an accuracy of ±0.01 mg. An empty pan was used as a reference.

2.5. Confocal scanning laser microscopy

Images of phase-separated WPC/HPMC aqueous mixtures were recorded with an Olympus FV300 CLSM, equipped with a vertical microscope (model Olympus BX61), used in the single photon mode with an Ar/HeNe visible light laser. The following Olympus objective lenses were used: UplanFl 10X/0.3NA/dry and UplanFl 10X/0.5NA/dry. Non-covalent labeling of protein was performed with few drops of rhodamine B 10 wt% solution (excitation wave length 560 nm; emission maximum 625 nm). Digital image files were acquired in multiple.tif format in 1024 × 1024 and 512 × 512 pixel resolutions.

3. Results and discussion

3.1. Phase separation of aqueous mixtures and glass transition temperature of quenched co-dried mixtures

3.1.1. Tg of individual dry biopolymers

The DSC curves for the individual dry biopolymers are shown in Fig. 1a and b. At the Tg, an endothermic step change in the heat-flow occurs, reflecting the transition from the glassy to the rubbery state.

The Tgs midpoints determined are shown in Table 1. Tg value for dry HPMC E4M was 167.7 °C which is consistent with a previously

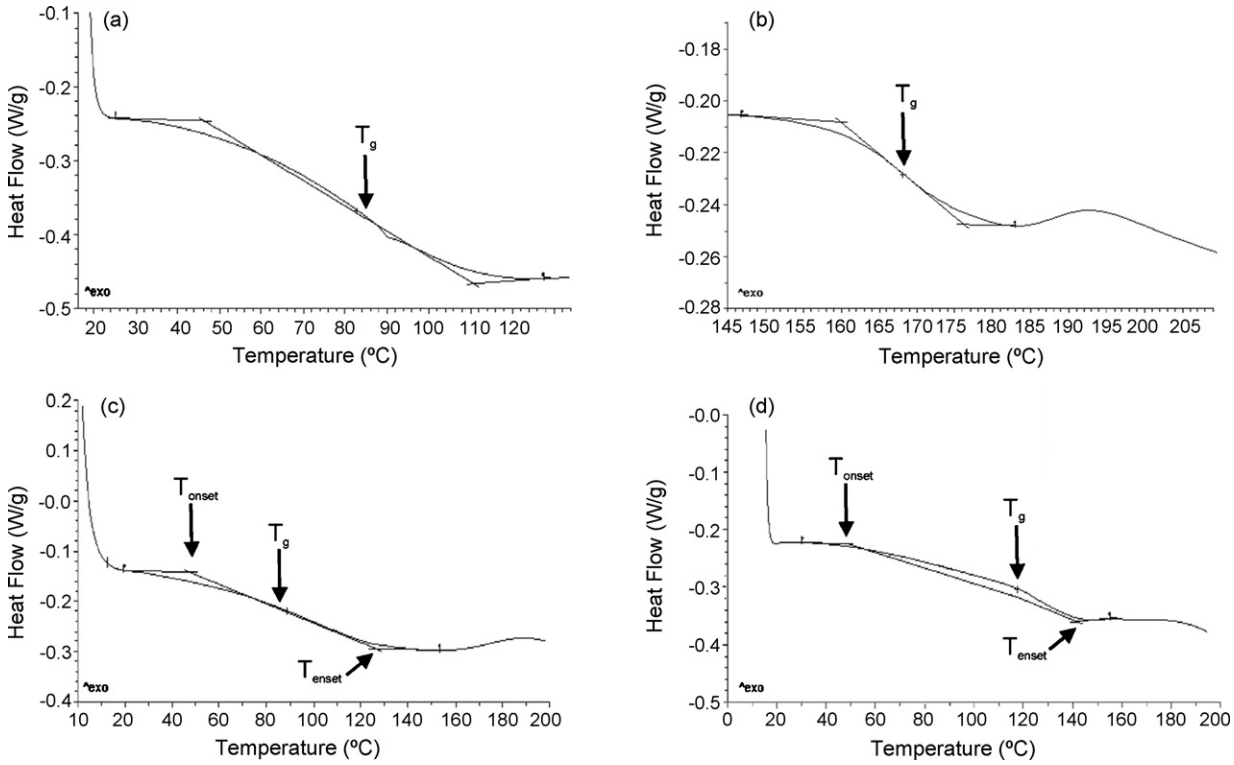

Fig. 1. DSC thermograms of individual dry biopolymers WPC (a) and HPMC (b), and non-phase-separated co-dried mixtures: WPC (6 wt%)/HPMC (1 wt%) at pH 6 (c) and WPC (12 wt%)/HPMC (2 wt%) at pH 3 (d).

reported value of 167.2 °C for anhydrous HPMC E4M using modulated temperature differential scanning calorimetry [22].

On the other hand, Tg value for dry WPC was 84.8 °C. Burin et al. [23] determined Tg values between 80 °C and 85 °C for dehydrated modified whey powders. Jouppila and Roos [24] and Karmas et al. [25] reported Tg values for dehydrated skim milk and lactose-based model systems, respectively, that were also within the same range. The similarity of Tg values of anhydrous whey systems determined by DSC to that of lactose [23] suggests that either lactose governs the Tg values of the whey systems or lactose and proteins may exist in different phases, with only the lactose-rich phase being detectable by DSC [24].

3.1.2. Tg of co-dried mixtures from non-phase-separated aqueous mixtures

Fig. 1c and d shows DSC thermograms corresponding to co-dried mixtures, WPC (6 wt%)/HPMC (1 wt%) at pH 6 and WPC (12 wt%)/HPMC (2 wt%) at pH 3, respectively. Both co-dried mixtures exhibited a single Tg, which is consistent with the fact that aqueous mixtures of these systems did not show any macroscopic phase separation after mixing as can be seen in Fig. 2a and b.

Several equations have been developed to relate the dependence of the Tg of a compatible polymer blend to its composition. One of the most used relations is the Couchman–Karasz equation:

$$\mathrm{Tg} = \frac{W_1 \mathrm{Tg}_1 + (\Delta Cp_2/\Delta Cp_1) W_2 \mathrm{Tg}_2}{W_1 + (\Delta Cp_2/\Delta Cp_1) W_2} \quad (1)$$

where Tg is the glass transition temperature of the blend, Tg_1 and Tg_2 are the glass transition temperatures of the individual polymers,

Table 1
Parameters of glass transition in the investigated individual dry biopolymers and their non-phase-separated co-dried mixtures.

System	$\mathrm{Tg_e}$ (°C)	ΔCp (J/g °K)	$\mathrm{Tg_{C-K}}$ (°C)
Dry HPMC	167.7 ± 0.7	0.25 ± 0.01	
Dry WPC	84.8 ± 1.5	1.02 ± 0.02	
WPC/HPMC 6/1 pH 6	82.4 ± 1.5		87.5
WPC/HPMC 12/2 pH 3	117.3 ± 1.2		87.5

$\mathrm{Tg_e}$ and $\mathrm{Tg_{C-K}}$ are the experimental and the predicted by Couchman–Karasz equation Tg values.

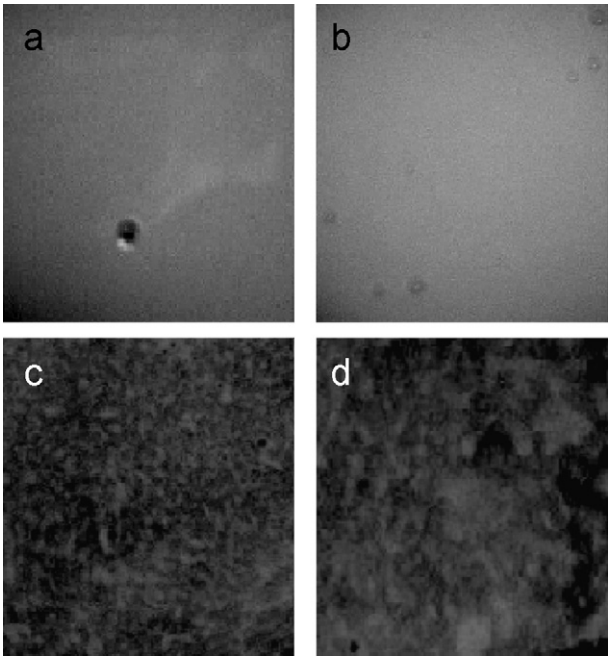

Fig. 2. WPC/HPMC aqueous blends immediately after mixing the biopolymers. (a) WPC (6 wt%)/HPMC (1 wt%) at pH 6 and (b–d) WPC (12 wt%)/HPMC (2 wt%) at pH 3, 5 and 6, respectively. For the two last cases macroscopic phase separation is evident (dark areas: HPMC rich domains; grey areas: WPC rich domains).

Table 2
δTg deviation for non-phase-separated co-dried mixtures.

System	ΔTg_e (°C)	ΔTg_p (°C)	δTg (°C)
WPC/HPMC 6/1 pH 6	75	74	1
WPC/HPMC 12/2 pH 3	93	74	19

ΔTg_e is the difference between the experimental values of onset and endset temperatures. ΔTg_p and δTg are parameters of miscibility calculated using equations development by Song et al. [27].

W_1 and W_2 are the weight fractions of the individual polymers, and ΔCp_1 and ΔCp_2 are the change in heat capacity at the glass transition, for individual polymers [26].

The ΔCp values for each individual component, the experimental and predicted Tg values for the co-dried mixtures are summarized in Table 1.

Tg values of WPC (6 wt%)/HPMC (1 wt%) co-dried mixture at pH 6 and WPC (12 wt%)/HPMC (2 wt%) co-dried mixture at pH 3 are 82.4 °C and 117.3 °C, respectively, while predicted Tg for both systems is 87.5 °C. For the WPC (6 wt%)/HPMC (1 wt%) co-dried mixture at pH 6, Tg value predicted by Couchman–Karasz equation shows a reasonably good agreement to the experimental data, but in the case of WPC (12 wt%)/HPMC (2 wt%) co-dried mixture at pH 3 there is a strong deviation.

For a polymer mixed system, the breadth of glass transition region, ΔTg, which is the difference between the onset and endset temperatures, is proportional to the polymers compatibility. Song et al. [27] suggested the following approximate relationship:

$$Tg \approx \frac{W_1\,\Delta Tg_1 + W_2}{\Delta Tg_2} \quad (2)$$

where W_1 and W_2 are the weight fractions of polymer 1 and polymer 2, respectively, and ΔTg_1 and ΔTg_2 are the widths of the glass transition regions for polymers 1 and 2, respectively. These authors also proposed the following quantification of the deviation:

$$\delta Tg = \Delta Tg_e - \Delta Tg_p \quad (3)$$

where ΔTg_e and ΔTg_p are the experimental and the predicted by Eq. (2) values of widths of the glass transition for a mixed system. A fully compatible polymers pair has a very small δTg value, close to zero, while a partial compatibility causes a larger δT value, i.e. 10–40 °C approximately [27]. Table 2 shows δTg values calculated for WPC (6 wt%)/HPMC (1 wt%) and WPC (12 wt%)/HPMC (2 wt%) co-dried mixtures at pH 6 and 3, respectively. Clearly, on the first one the biopolymers are compatible, while in the second one there is a partial compatibility between them. Blends of poly(epichlorohydrin) with poly(methyl methacrylate) are known to be non-compatible, although manipulation of kinetic of phase separation may result in a temporarily locked homogeneous phase structure [28]. Moreover, partial compatible poly(methyl methacrylate)/polystyrene blends contain sub-micron size heterogeneous domains, resulting in the material properties, i.e. glass transition, sensitively depending on this microscopic phase separation [29].

This partial compatibility could correspond to a microscopic phase separation, resulting in a temporarily locked homogeneous phase showing an apparent single Tg, but deviated from theoretical value.

3.1.3. Tg of co-dried mixtures from phase-separated aqueous mixtures

Phase separation of WPC (12 wt%)/HPMC (2 wt%) aqueous blend at pH 6 was arrested at three different times: immediately after mixing the biopolymers solutions, 30 min after mixing them, and finally after centrifugation to fully separate the two phases. The DSC thermograms of these quenched dry systems are shown in Fig. 3a–c, respectively and Table 3 shows the corresponding Tg values.

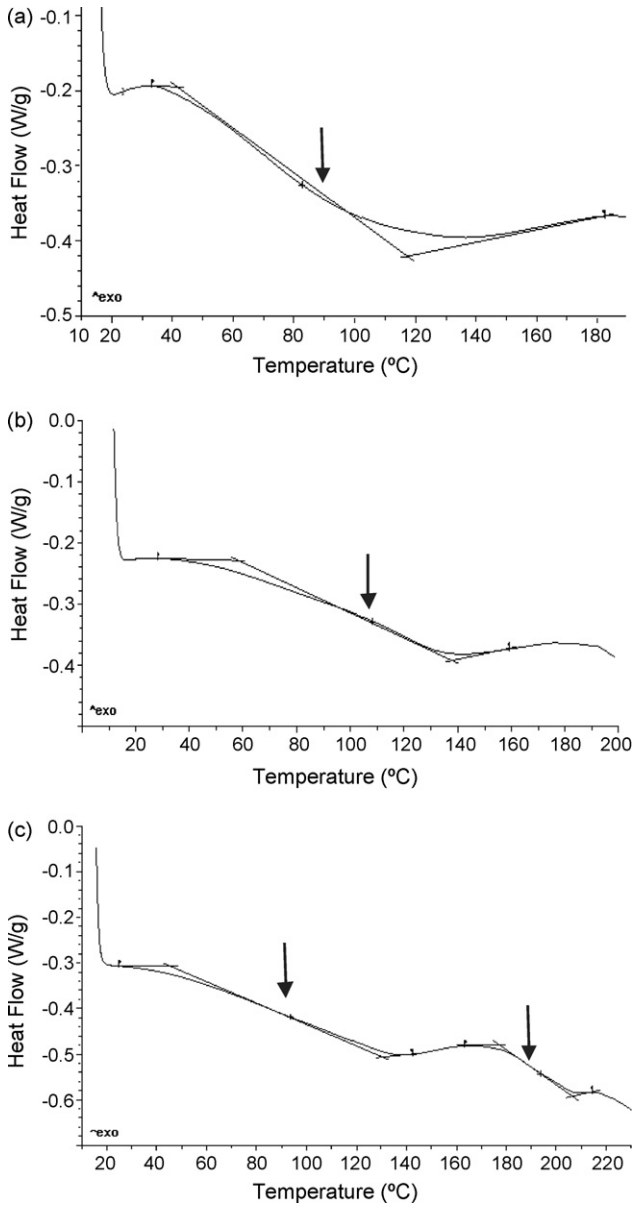

Fig. 3. DSC thermograms of WPC (12 wt%)/HPMC (2 wt%) co-dried mixture at pH 6, when phase separation of the corresponding aqueous mixture was arrested, immediately after mixing the biopolymers (a), 30 min after mixing them (b), and after centrifugation to fully separate the two phases (c). Arrows indicate the glass transition temperature.

This aqueous mixture was found to be visually phase separated immediately after mixing (Fig. 1d). However, when it was dried, it exhibited a single Tg value of 91.4 °C which slightly deviated from the predicted value by Couchman–Karasz equation (87.5 °C).

When the phase separation of this aqueous blend was arrested 30 min after mixing the biopolymer solutions, the corresponding quenched dried system showed a single Tg of 113.6 °C.

Two Tg values of 94.7 °C and 193.2 °C were only observed in the fully phase-separated co-dried mixture, but they resulted higher than those corresponding to single dry WPC or HPMC.

According to the phase-diagram of WPC/HPMC mixed system [16], it can be observed that the protein-rich phase of the fully phase-separated WPC (12 wt%)/HPMC (2 wt%) aqueous blend contains 0.62 wt% of HPMC. Thus, the increase in the Tg of the protein-rich phase of the co-dried mixture, can be accounted for by this remainder amount of HPMC.

Table 3
Glass transition temperature (Tg) of phase-separated co-dried mixtures at different times/degrees of phase separation.

System	Time (min)	Tg (°C)	Tg_{C-K} (°C)	$V_{Lower\ phase}/V_{max}$ (%)
WPC/HPMC, 12/2 pH 6	0	91.4 ± 1.8	87.5	0
	30	113.6 ± 0.9		44
	After centrifugation	94.7 ± 2.3 193.2 ± 1.0		100
WPC/HPMC, 12/2 pH 5	0	107.0 ± 0.7	87.5	0
	30	82.0 ± 0.2 142.3 ± 0.3 187.4 ± 2.2		82
	After centrifugation	79.0 ± 1.7 153.3 ± 2.2		100
WPC/HPMC, 20/3 pH 6	0	149.5 ± 2.0	88	0
	30	160.0 ± 0.5		7
	After centrifugation	80.1 ± 2.3 152.0 ± 2.1		100

Tg_{C-K} is the Tg value predicted by Couchman–Karasz equation. $V_{Lower\ phase}/V_{max}$ is a measure of the degree of phase separation.

Regarding the HPMC-rich phase of the co-dried mixture, one might hypothesize that a molar mass fractionation of HPMC could take place during phase separation because of its high polydispersity. Often biopolymers, like cellwall biopolymers, used in food products are polydisperse in their molar mass. Moreover, all METHOCEL cellulose ethers consist of a distribution of chain lengths because they are manufactured in a heterogeneous process in which a statistical distribution of substitution and sequence isomers is created [30].

Due to this polydispersity, fractionation in molar mass takes place during phase separation, resulting in different molar mass distributions in the coexisting phases. This phenomenon has been observed for maltodextrin/agarose mixtures by Loret et al. [31], who showed that the phase separation resulted in fractionation in molar mass of maltodextrin due to its high polydispersity.

Thus, different HPMC molecular weight fractions would not show the same degree of compatibility with WPC. The higher molecular weight fractions of HPMC would concentrate in the HPMC-rich phase of the co-dried mixture, rising its Tg value, while the lower molecular weight fractions would segregate in the protein-rich phase.

Fig. 4 shows the kinetics of phase separation of the aqueous mixed systems WPC (12 wt%)/HPMC (2 wt%) at pH 5 and 6. In both cases rapid phase separation occurred, but at pH 5 the phases segregated faster.

The kinetics of phase separation was fitted using the following equation:

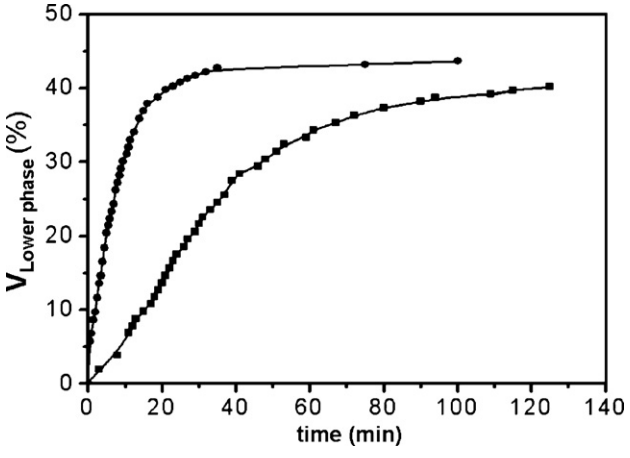

Fig. 4. Kinetics of phase separation for WPC (12 wt%)/HPMC (2 wt%) aqueous blend at pH 5 (●) and 6 (■).

$$V_{Lower\ phase} = \frac{V_{max}t}{B+t} \quad (4)$$

where $V_{Lower\ phase}$ is the volume of bottom phase, t is the time, B is a constant and V_{max} is the maximum separated volume of bottom phase. A measurement of the degree of phase separation is the ratio between $V_{Lower\ phase}$ over time and V_{max}.

Thirty minutes after mixing the biopolymers solutions, this ratio was 44% at pH 6, but it was almost two fold higher at pH 5 (Table 3).

The thermograms corresponding to the dry quenched system at pH 5 are shown in Fig. 5a–c, and Tg values in Table 3. Again, the aqueous mixture was found to be visually phase separated immediately after mixing (Fig. 1c) but the corresponding co-dried mixture showed a single Tg at 107 °C. As soon as the biopolymers segregation was almost completed, 30 min after mixing them (82% degree of phase separation), the co-dried mixture exhibited three Tg values of 82.0 °C, 142.3 °C and 187.4 °C. The first one resulted slightly lower than the Tg value of dry WPC, the last one was higher than Tg of dry HPMC, while the second one was within those two values.

As expected, two Tg values were observed in the co-dried mixture when phase separation was completed, but the Tg of each dry phase was lower than Tg of single dry biopolymers (Table 1).

For WPC (20 wt%)/HPMC (3 wt%) aqueous blend at pH 6 the phase separation was also arrested, immediately after mixing of the biopolymers, 30 min after mixing them, and after centrifugation to fully separate the two phases. Table 3 shows the Tg values of co-dried mixtures and DSC curves are shown in Fig. 6a–c.

Immediately after mixing the biopolymers and 30 min after mixing them, the co-dried mixture exhibited a single Tg value of 149.5 °C and 160 °C, respectively. In both cases the experimental Tg value presented a strong deviation from the predicted value by Couchman–Karasz equation (88 °C). As can be seen in Table 3, the rate of phase separation of this aqueous mixture was extremely low. In fact, after 30 min this blend separated only by 7% in comparison to 44% and 82% of phase separation of the blend WPC (12 wt%)/HPMC (2 wt%) at pH 6 or 5, respectively.

In the fully phase separated co-dried mixture two Tgs were found, but again the Tg values of each phase were lower than Tg of single biopolymers.

3.2. Relationship between the glass transition temperature of co-dried mixtures and the morphology of phase separation

The separation of aqueous biopolymer mixtures into two phases can proceed throughout two main ways, spinodal decomposition (SD) and nucleation and growth (NG) [32].

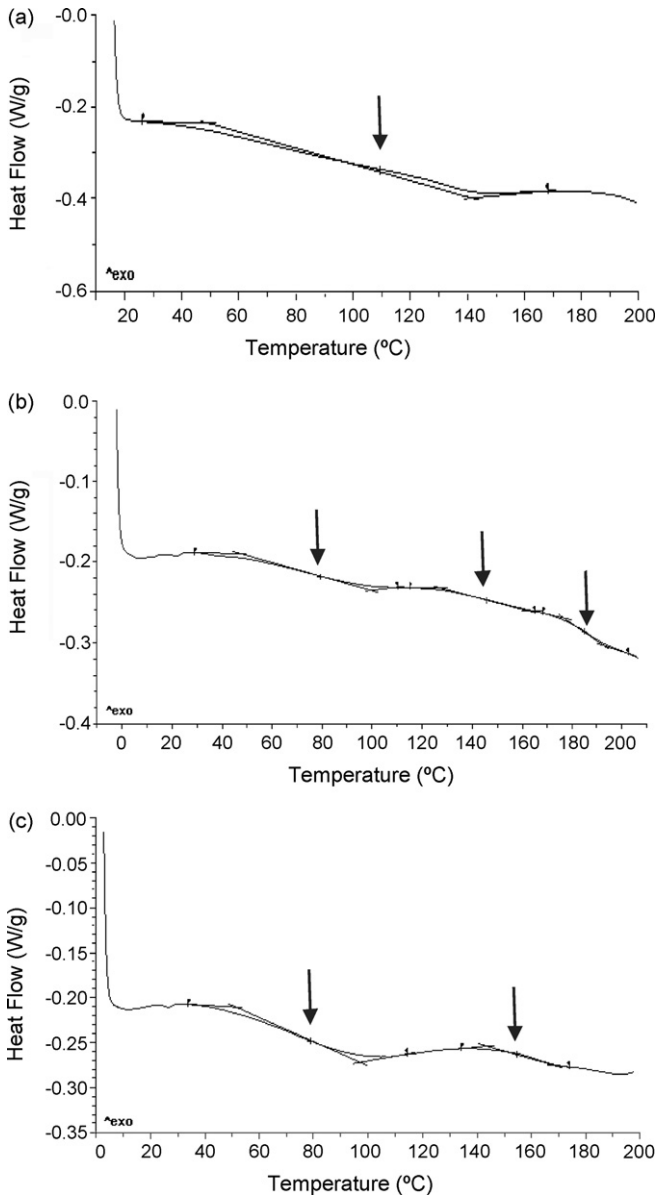

Fig. 5. DSC thermograms of WPC (12 wt%)/HPMC (2 wt%) co-dried mixture at pH 5, when the phase separation of the corresponding aqueous mixture was arrested, immediately after mixing the biopolymers (a), 30 min after mixing them (b), and after centrifugation to fully separate the two phases (c). Arrows indicate the glass transition temperature.

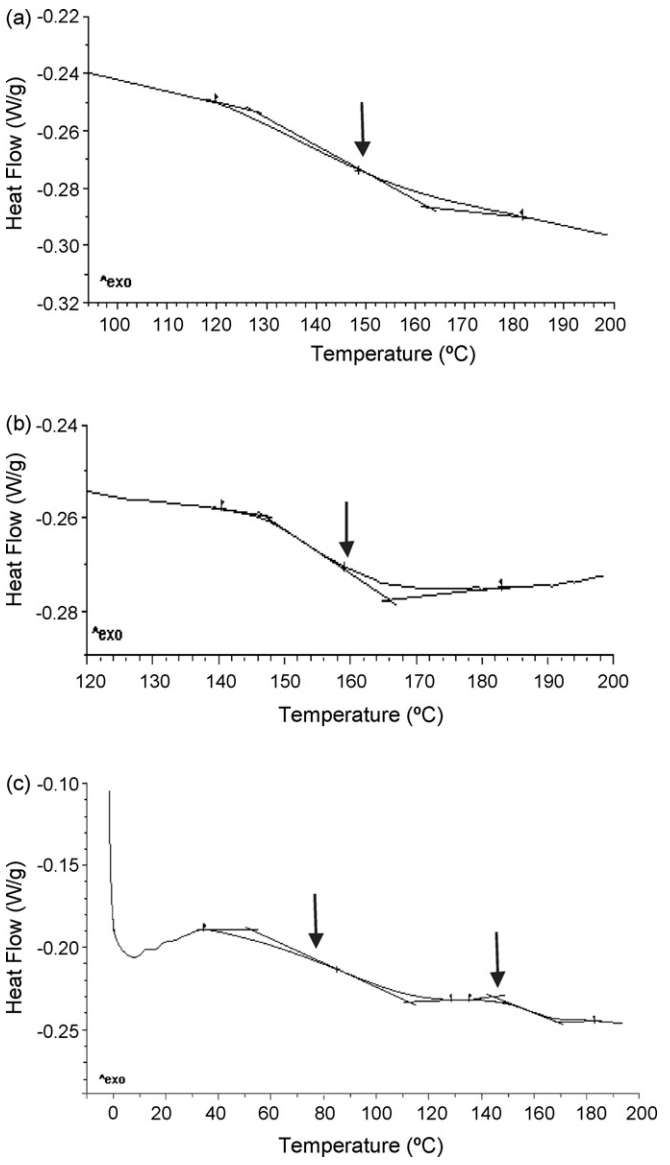

Fig. 6. DSC curves of WPC (20 wt%)/HPMC (3 wt%) co-dried mixture at pH 6, when phase separation of the corresponding aqueous mixture was arrested, immediately after mixing the biopolymers (a), 30 min after mixing them (b), and after centrifugation to fully separate the two phases (c). Arrows indicate the glass transition temperature.

The morphology of phase separated WPC (12 wt%)/HPMC (2 wt%) aqueous blend at pH 6 system is shown in Fig. 7. According to its microstructure, phase separation proceeds via SD exhibiting a characteristic three-dimensional interconnected network [33]. Similar results were found for this aqueous blend at pH 5 (Fig. 8).

Contrarily, phase-separated WPC (20 wt%)/HPMC (3 wt%) aqueous blend at pH 6 showed a different morphology with spherical droplets of HPMC dispersed in a continuous phase of WPC (Fig. 9) which is characteristic of NG [32].

The results exposed in the previous sections showed that a single Tg was detected by DSC in some co-dried mixtures of macroscopically phase-separated aqueous mixtures, i.e. WPC (20 wt%)/HPMC (3 wt%) at pH 6 and WPC (12 wt%)/HPMC (2 wt%) at pH 6.

On the other hand, the co-dried mixture of WPC (12 wt%)/HPMC (2 wt%) aqueous blend at pH 5 with an advanced degree of phase separation (i.e. 30 min after mixing the biopolymers), presented three Tg which no one coincided with the Tg of single dry biopolymers.

One possible hypothesis to explain these results is that the DSC mainly detects, up to a certain degree of phase separation, the Tg of the water–water interface between the protein-rich and polysaccharide-rich phases of the co-dried mixture. This interface constitutes an homogeneous third phase formed by absorbed HPMC and WPC. As shown in Fig. 7, the morphology of this aqueous system was formed by bicontinuous structures with both phases completely interconnected by a very large interface. Moreover, when magnification was increased a secondary phase-in-phase morphology was observed, with small dark HPMC domains trapped within a network of aggregated protein particles (Fig. 7). Therefore, on co-dried mixture the DSC would detect a single Tg because of the magnitude of the interfacial domain, that would dominate the mobility of the whole system.

Nevertheless, the detection of a single Tg does not preclude the existence of other Tgs associated to the protein and polysaccharide-

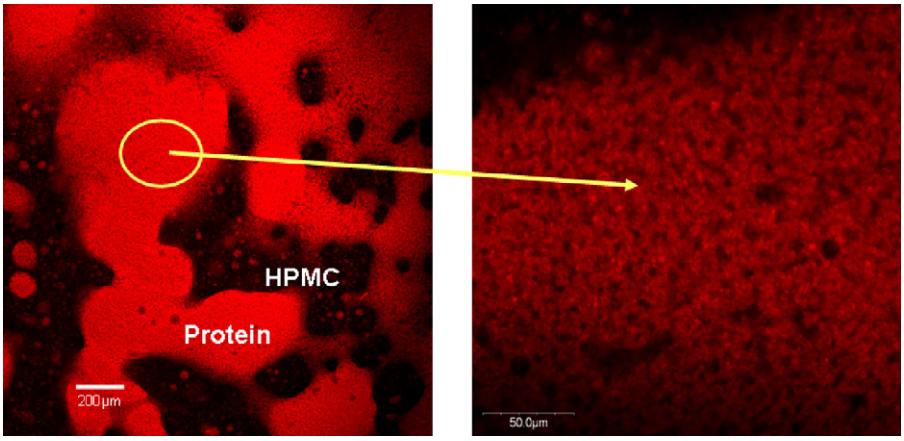

Fig. 7. Confocal image of WPC (12 wt%)/HPMC (2 wt%) aqueous blend at pH 6 (images widths 200 μm and 50 μm).

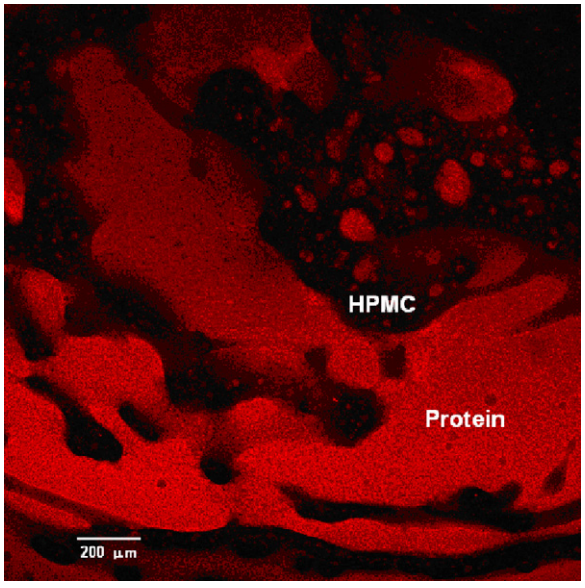

Fig. 8. Confocal image of WPC (12 wt%)/HPMC (2 wt%) aqueous blend at pH 5 (image width 200 μm).

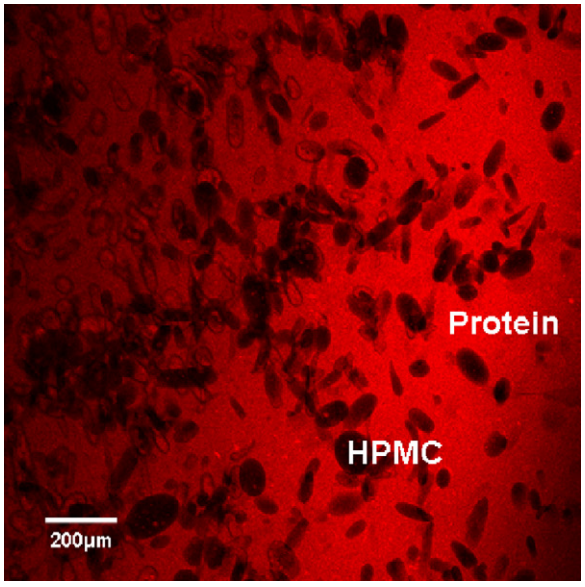

Fig. 9. Confocal image of WPC (20 wt%)/HPMC (3 wt%) aqueous blend at pH 6 (image width 200 μm).

rich phases that could be detected with a more sensible technique (i.e. mechanical spectroscopy). What the results point out certainly, is the predominance of a single Tg associated to the existence of a very large interface upon segregation of protein/polysaccharide mixtures.

As mentioned earlier, phase separation of this aqueous blend at pH 5 proceeds more quickly. Again, at the beginning of phase separation, bicontinuous structures appeared and showed both phases completely interconnected by a very large interface. Thus, on the corresponding co-dried mixture the DSC detects a single Tg because of the magnitude of this interfacial domain. As phase separation of aqueous mixture proceeds three phases turned up, two separated phases enriched in one of the components linked by an interface; at this point the DSC starts to detect three Tgs on the co-dried mixture, corresponding to these three domains. Finally, WPC and HPMC-rich phases increase at the expense of the continuous decrease of the interfacial domain. Thus, when the phase separation in the aqueous mixtures was completed (centrifuged mixtures) the corresponding co-dried mixture showed two Tgs attributable to WPC and HPMC-rich phases. In this case the interfacial domain is negligible.

On the other hand, WPC (20 wt%)/HPMC (3 wt%) co-dried mixture at pH 6 exhibited a single Tg even 30 min after mixing of biopolymers because of the very low degree of phase separation, 7% (Table 3). So, it can be considered that co-dried mixture of this system is almost completely constituted by a very large interface and the DSC only detects a single Tg. At the end of phase separation, the co-dried mixture of this system showed two Tgs, close to 80 °C and 150 °C attributable to WPC and HPMC-rich phases, respectively. Tg value of protein-rich phase shows a reasonably good agreement with Tg of dry WPC. On the other hand, as shown by Perez et al. [16] the HPMC-rich phase of segregated WPC (20 wt%)/HPMC (3 wt%) aqueous blend, contains approximately 0.81 wt% of WPC. This can explain why the Tg of the polysaccharide-rich phase on the co-dried mixture, was lower than the Tg corresponding to dry HPMC (167.7 °C).

The interfacial domain of an aqueous WPC/HPMC blend may be described as a miscible blend of absorbed HPMC and WPC, in an unknown proportion. Previous studies on the dynamics of adsorption at the air–water interface of the WPC/HPMC mixed systems [34] have shown that HPMC adsorbs in competence with WPC, being HPMC more surface-active than WPC. Therefore, HPMC dominates the interfacial properties at long adsorption times. From these previous results, it can be extrapolated that the composition and structure of the water–water interface between coexisting protein-rich and polysaccharide-rich phases can change over time, being the interfacial behaviour dominated by the HPMC because of its

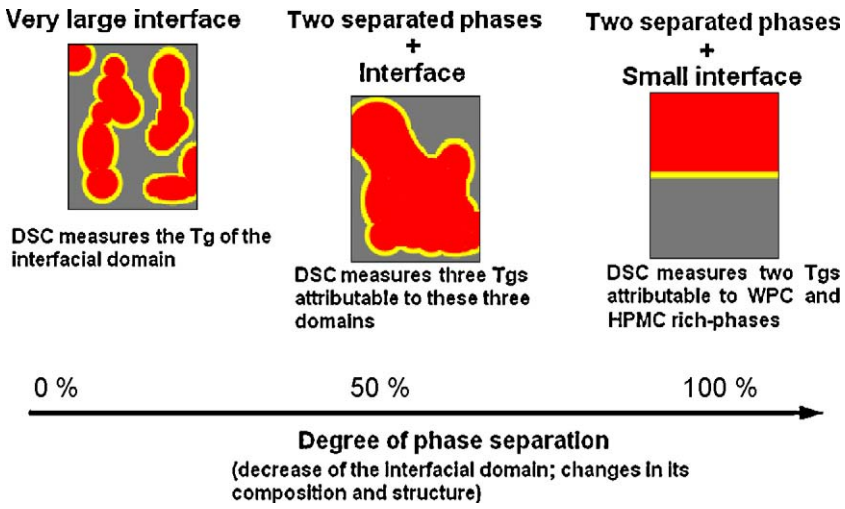

Fig. 10. Schematic representation of phase separation in aqueous WPC–HPMC mixtures and its relationship with measured Tg on the corresponding co-dried mixture blend.

higher surface activity. Thus, the observed single Tg in co-dried mixtures from dried-quenched segregated aqueous mixed systems can be attributed to HPMC/WPC miscible mixture forming the interfacial domain. This Tg value located within the Tg of single dry components, could change with the advance of phase separation, towards the value of HPMC because of changes in composition of the interfacial domain with time (Table 3).

Fig. 10 shows a graphical picture explaining the relationship between the morphology of the phase separation of aqueous mixture and the detected Tg on the corresponding co-dried mixture. It is proposed to ascribe the observed single Tg to the interface formed of absorbed HPMC and WPC, whose volume would be predominant up to advanced degrees of macroscopic phase separation (i.e. 50%). The interface would dominate the mobility of the whole system because it would act as a network for the entanglement between the protein-rich and the polysaccharide-rich phases. At advanced degrees of phase separation (i.e. >50%) three Tgs can be found, which could be attributed to the interface, protein-rich and polysaccharide-rich phases, being the values of these Tgs related to the interfacial structures arising from different morphologies and composition of the coexisting phases. Finally, two Tgs are observed for very advanced degrees of phase separation (i.e. the fully phase separated co-dried mixture) because the interfacial domain reaches a negligible volume.

4. Conclusions

Through thermal analysis and microscopy, the dependence of glass transition temperature of a WPC/HPMC co-dried mixture on the phase behaviour of the corresponding aqueous mixture was determined.

For non-phase-separated co-dried mixtures, WPC (6 wt%)/HPMC (1 wt%) at pH 6 and WPC (12 wt%)/HPMC (2 wt%) at pH 3, glass transition temperature had a reasonably good fit to traditional models used to calculate the Tg of polymeric mixtures.

In the case of partially phase-separated co-dried mixtures, WPC (20 wt%)/HPMC (3 wt%) and WPC (12 wt%)/HPMC (2 wt%) at pH 6, glass transition temperature depends mainly on the morphology and degree of phase separation of aqueous mixture (Fig. 10).

The knowledge of the relationship between the extent and morphology of phase separation of protein/polysaccharide aqueous mixtures and the thermal properties of co-dried mixtures produced from them, can be used as a tool to design food and biomaterials of desired or improved properties.

In the present study CLSM and DSC were used as complementary techniques to further the understanding on properties of co-dried mixtures from protein/polysaccharide aqueous mixtures.

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