Colloids and Surfaces B: Biointerfaces 79 (2010) 377–383

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Interactions between whey protein isolate and gum Arabic

Miri Klein [1], Abraham Aserin, Paul Ben Ishai, Nissim Garti *

Casali Institute of Applied Chemistry, The Institute of Chemistry, The Hebrew University of Jerusalem, Edmond J. Safra Campus, Givat Ram, Jerusalem 91904, Israel

ARTICLE INFO

Article history:
Received 22 March 2010
Received in revised form 21 April 2010
Accepted 21 April 2010
Available online 29 April 2010

Keywords:
Gum Arabic
Whey protein isolate
DSC
Electrical conductivity
ζ-Potential
Surface tension

ABSTRACT

In this study we have attempted to understand the nature of "charge interactions" between two negatively charged biopolymers (whey protein isolate, WPI and gum Arabic, GA) and, consequently, why their mixture exhibits better interfacial activity.

Surface tension (γ_0) measurements indicated that at ca. 1 wt.% of the biopolymer mixture (3:1 wt. ratio) the air/water surface is saturated. At 5 wt.% the γ_0 of the mixture is lower than the calculated co-operative value.

The ζ-potential measurements revealed that the isoelectric point of the WPI:GA 3:1 wt. ratio mixture is 3.8. The ζ-potential values up to pH 6 are below those calculated. Similarly, the electrical conductivities of the mixture are lower than those calculated. All these measurements indicate: (1) partial charge neutralization in spite of the fact that both biopolymers are negative or (2) partial charge–charge interactions between the two biopolymers.

The thermal heating behavior of the frozen water in the aqueous mixture studied by DSC (heating cycle of the frozen sample) clearly indicates that the two biopolymers are interacting. We calculated the enthalpy, the free energy and the chemical potential of the interactions. We found that the interactions of the biopolymers are rather weak. They are likely derived from some local positively charged domains (pH 7) on the protein that neutralize some of the negatively charged GA. These interactions form weak charge adducts. These charge adducts are sufficient to improve its adsorption into the oil–water interface and enhance the emulsion stability.

1. Introduction

Proteins and hydrocolloids are natural biopolymers extensively used in many food colloids and emulsions [1–5]. The interactions between the proteins and hydrocolloids may play a significant role in the structure and stability of emulsions, double emulsions [6–11], drug release, microencapsulation, delivery systems, etc. [3,12,13].

Mixing oppositely charged proteins and hydrocolloids in aqueous solution was claimed to result in the formation of new electrostatically attracting moieties (complexes) with improved properties, such as lower sensitivity to environmental reactivity [2,5,11,12,14–16]. Calorimetric experiments have indeed shown that the complexation is mostly entropy driven [12,13]. The charge complex may "dissolve" in water to form a "molecular solution", or it may coacervate and precipitate into a two-phase system. Electrostatic interactions are dominated by the pH and the ionic strength and play a key role in the formation of complexes [4,12,16,17]. The soluble complex is formed at a pH above the isoelectric point (IEP) of the protein. We have suggested that at pH > IEP most of the side groups of the protein are negatively charged, but some moieties or fragments are still positively charged. The "peptide segments" are positively charged and may interact with the negatively charged hydrocolloid to form a weakly associated complex that does not precipitate [1,2,6,7,14,15,17–20]. If a complex does not form, a one-phase mixture of protein and hydrocolloid is macroscopically observed.

Benichou et al. [9,10] explored the use of mixtures of WPI (whey protein isolate) with xanthan gum (4:0.5, wt./wt.) to stabilize double emulsions. They found that the droplet sizes of the emulsions were smaller than 3 μm. Lutz et al. [18,21] examined emulsion stability and characteristics with a pre-prepared charge–charge complex of WPI and modified pectin (4:0.5, wt./wt.) at pH 6.

In our previous study, Klein et al. [8] demonstrated that an aqueous mixture of WPI and gum Arabic (3:1 wt. ratio) can stabilize oil-in-water (O/W) emulsions better than any of the biopolymers separately. Canola oil (10 wt.%) was emulsified with 10 wt.% of the aqueous mixture of the two biopolymers, forming a stable emulsion without creaming, aggregation, flocculation, and/or coalescence, even after 30 days of storage at 4 °C, 25 °C, and 32 °C.

WPI is considered an excellent amphiphilic biopolymer composed of a mixture of β-lactoglobulin (β-lg) (main component),

* Corresponding author. Tel.: +972 2 658 6574/5; fax: +972 2 652 0262.
E-mail address: garti@vms.huji.ac.il (N. Garti).

[1] The results presented in this manuscript will appear in the M.Sc. thesis of M.K. in partial fulfillment of the requirements for the M.Sc. degree in Applied Chemistry.

0927-7765/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.colsurfb.2010.04.021

α-lactalbumin (α-la), bovine serum albumin (BSA), and other proteins such as caseins [22]. The isoelectric point of β-lg is 5.3 at room temperature. This protein changes its structure as a function of pH [22,23]. The IEP of α-la is 4.1; therefore, at pH less than 4.1 the protein is always positively charged [22,24].

Gum Arabic (GA) has been continuously studied for over 50 years and in spite of all the efforts, its exact structure is still not entirely resolved [25–28]. What is known is that GA consists of galactose, arabinose, rhamnose and glucuronic acid and that it has a highly branched structure, with a small proportion of proteinaceous material [29–32]. The average molecular weight of GA is considered to be about ~300–800 kDa [30,33,34]. The main thrust of the previous research has been to understand arabinogalactan-protein (AGP), a component of GA that represents approximately 10% of the total mass and contains about 10% protein. AGP has a high molecular weight of about $(1–2) \times 10^3$ kDa, and its polysaccharide moieties are bound covalently to protein [35]. This component is surface active and tends to be adsorbed into oil droplets and can stabilize O/W emulsions. Additionally the arabinogalactan component (AG) exists separately from AGP and has a molecular mass of $\sim 2.5 \times 10^2$ kDa, representing ~90% of the gum and contains <1% protein [35]. The third component is a glycoprotein with molecular mass of $\sim 2 \times 10^2$ kDa, representing only ~1% of the total mass with up to 50% protein [35].

Much effort has been made to understand the structure of AGP. It is believed to have a 'wattle blossom' type structure, with 5–6 large carbohydrate blocks linked to a polypeptide chain [36]. However, recent studies have demonstrated a somewhat different organization of the 'wattle blossom' structure [35], but the overall surface activity remains similar. In addition, as it is known that the GP component is poor in glucuronic acid, it is reasonable to believe that it exists in abundance in the AG component [37].

In order to explain the unique stabilization of the O/W emulsions by the WPI:GA 3:1 wt. ratio [8], we assumed that the two negatively charged biopolymers form charge–charge interactions. At pH 4 formation of coacervates was clearly observed and documented (both by eye and microscopically) [22]. At $5 < pH < 7$ the aqueous mixture is almost transparent (or somewhat colloidal with weak turbidity) and without precipitation. In this pH range, the two biopolymers (3:1 wt. ratio) formed the most stable O/W emulsion [8]. We speculated that some positively charged domains of the WPI are interacting at these pHs with the negatively charged GA, without causing detectable coacervation.

The aim of our present work is to explore and gain a deeper understanding of the interactions between the two biopolymers. To this end we employed a number of different analytical techniques such as surface tension, ζ-potential, electrical conductivity, differential scanning calorimetry, and confocal microscopy.

2. Materials and methods

2.1. Materials

Whey protein isolate (WPI) (BiPRO, Davisco, Le Sueur, MN, USA), consisted of 97.8 wt.% protein with lactose content less than 1 wt.%. Gum Arabic (food grade) was provided by Colloides Naturels International Co. (Rouen, France). Water was double distilled and contained 0.01 wt.% sodium azide (BDH, Poole, England) to prevent bacterial contamination. Rhodamine B was purchased from Sigma–Aldrich Co. (St. Louis, MO, USA).

2.2. Sample preparation

Gum Arabic (GA) and WPI were dispersed in water and mixed at room temperature until practically clear solutions were formed. The resulting aqueous biopolymer mixtures were aged at room temperature for an additional 24 h to insure complete solubility equilibrium.

2.3. Characterization

Surface tension was measured with a DCAT 21 tensiometer (DataPhysics Instruments, GmbH, Germany) equipped with an platinum–iridium plate using the Wilhelmy plate technique. A surface tension value of 72.8 ± 0.2 mN m^{-1} was measured for distilled water (HPLC grade, J.T. Baker, Mallinckrodt Baker, Deventer, Netherlands) and was used as a reference. All the measurements were carried out for 5 h until a constant value was obtained.

Electrophoretic mobility expressed as ζ-potential values was obtained from the perturbations of Brownian diffusivity under a pulsating electrical field. ζ-Potential has been used previously by several authors for biopolymers, emulsions, etc. [7,10,38–41]. The surface charge of a biopolymer arises primarily from the ionization of its surface polar groups. This ionization is pH-dependent. Measurements of electrophoretic mobility were carried out in aqueous solutions over a wide range of pH values using a Zatasizer. A NICOMP 380 SLZ (Particle Sizing Systems, PSS•Nicomp, Santa Barbara, CA, USA) was used to determine the ζ-potential values of the aqueous solutions composed of WPI or GA or WPI:GA at different pHs. Each test was repeated three times on three different samples of the same composition at 25 °C.

Electrical conductivity (Mettler Toledo S30, Greifensee, Switzerland) was measured directly by inserting the electrode into the biopolymer solutions; electrical conductivity was performed in triplicate at 25 °C. The result is an average of the three measurements.

For the confocal laser scanning microscope (CLSM) observations, WPI:GA 3:1 wt. ratio solutions were prepared using a fluorescent dye, rhodamine B (0.32 mL of 0.01% rhodamine B solution/20 mL biopolymer solution), to stain the protein. The combined solutions were stored 24 h. Samples were scanned using the Olympus FV-1000 confocal microscope (Tokyo, Japan), equipped with an IX80 inverted microscope. A 40×/1.3 oil immersion objective was used. Rhodamine B fluorescence was collected, using an excitation line of 543 nm and 560–620 nm filter for the emission.

Differential scanning calorimetry (DSC) measurements were performed with a Mettler Toledo DSC822 (Greifensee, Switzerland) calorimeter. The measurements were carried out as follows: 10–20 mg solution was weighed, using a Mettler M3 microbalance, in standard 40 μL aluminum pans, and immediately sealed by a press. The samples were rapidly cooled in liquid nitrogen from 25 °C to −30 °C, at a rate of 3 °C min^{-1}. The samples remained at this temperature for 10 min and then were heated at 3 °C min^{-1} to 25 °C. The instrument determined the fusion temperatures of the frozen components and the total heat transferred in any of the observed thermal processes. The enthalpy change associated with each thermal transition was obtained by integrating the area of the relevant DSC peak. DSC temperatures reported were reproducible to ±0.1 °C.

3. Results and discussion

3.1. Surface tension

Surface tension of 5 wt.% aqueous solution of GA was 51.7 ± 0.2 mN m^{-1} (Fig. 1). Greater concentrations did not show any further reduction in γ_0 (not shown). The WPI, at a similar concentration (5 wt.%), reduced the surface tension of water to 47.8 ± 0.2 mN m^{-1}. The mixture of the two biopolymers (WPI:GA, 5 wt.%) at different weight ratios (1:1–4:1) reduced the surface tension to 49.7, 48.6, 48.3, and 48.1, respectively (Fig. 1). We calculated

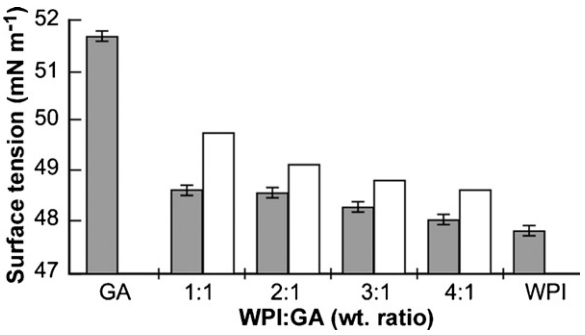

Fig. 1. Surface tension measurements (gray – measured) and co-operative calculated surface tension (white – calculated) values, as a function of WPI:GA wt. ratio of aqueous solutions composed of each of the biopolymers alone and the corresponding mixture at various ratios. The total concentration of the mixtures of WPI:GA was kept constant at 5 wt.% (measurements recorded after a plateau is reached after 5 h).

the co-operative (additive) expected surface tension (Fig. 1) of the two biopolymers as if there was no interaction between them (co-operative behavior). It can be seen that the experimental values of surface tension are always less than the calculated values. Yet, the surface tension of the mixtures was never less than that of WPI alone, even after prolonged equilibration (by dipping the plate for 5 h to reach equilibrium). To the best of our interpretation, the results may indicate that the protein moieties in the WPI:GA mixture are located in the vicinity of the air/water interface but in part are replaced by some domain of the hydrocolloid.

Similar measurements were carried out with 0.01–5.0 wt.% WPI, GA, and a mixture of 3:1 WPI:GA (Fig. 2). It can be seen that with increases in the biopolymer concentration the surface tension reduction of GA is more moderate than that of WPI, suggesting again that the surface is occupied primarily by the WPI. At ca. 1 wt.% of the mixture, the surface is saturated with the biopolymers and the surface tension (γ_0) reaches a plateau ($\sim$48 mN m^{-1}). The main effect of the mixture is detected at the low mixture concentrations (0.01–1.0 wt.%), reflecting a situation in which the air–water surface is not yet saturated.

3.2. ζ-Potential

ζ-Potential is an analytical tool that should point to the existence of strong charge–charge interactions between the two biopolymers. ζ-Potential values (Fig. 3) of GA are negative at all pH (from −7 to ca. −30 mV) and they level off at ca. pH 4. On the other hand,

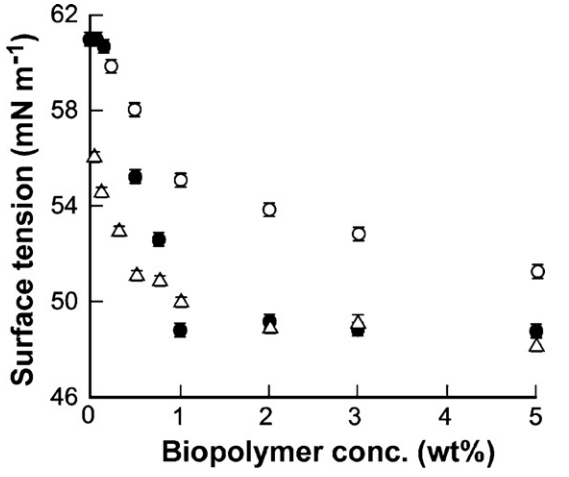

Fig. 2. Surface tension measurements of aqueous solutions composed of WPI (△), GA (○), and WPI:GA (3:1 wt. ratio) (●) as a function of the biopolymer concentration.

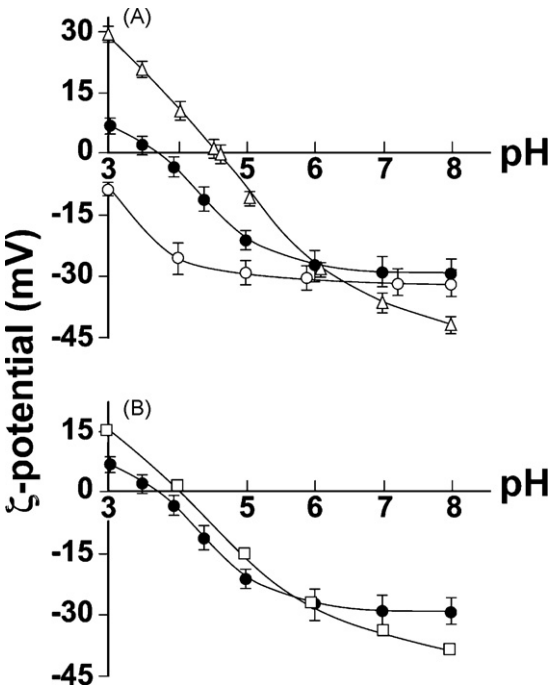

Fig. 3. (A) ζ-potential measurements of the aqueous solutions of WPI (△), GA (○), and WPI:GA (3:1 wt. ratio) (●) against pH, and (B) the ζ-potential values of the aqueous solutions of WPI:GA 3:1 wt. ratio (●) against pH in comparison to the calculated co-operative values (□). The connecting lines are for ease of visualization.

WPI is positive at low pHs and its IEP is 4.6. Above this pH the WPI is negatively charged (Fig. 3A). The IEP of 3:1 WPI:GA mixture was 3.8 and the ζ-potential values at pHs 4–6 are between the two biopolymers. Above pH 6 the ζ-potential of the mixture is higher than that of each of the biopolymers alone (Fig. 3A). These measurements demonstrate charge neutralization that is enhanced by the pH increase.

From Fig. 3B it can be seen that at pH < 6 the ζ-potential values are smaller than the calculated values. In contrast, at pH > 6 the situation is inverted and the measured ζ-potential values are higher than those calculated. These results indicate that the biopolymer charge–charge moiety consists of some domains that are partially neutralized. These domains might change their conformation with the pH, and subsequently change their exposure to the aqueous phase with the pH fluctuations.

3.3. Electrical conductivity

Since both biopolymers (WPI and GA) are charged, the electrical conductivity of the mixture might reflect possible interactions between them. It was found (Fig. 4), as expected, that GA is much more negatively charged than the WPI in all of the tested concentrations (1–10 wt.%), and the difference becomes very significant at high concentrations (10 wt.%). The 4:1 wt. ratio of WPI:GA shows only minor electrical conductivity differences over the WPI alone, but as the WPI content within the WPI:GA mixture decreases, the electrical conductivity deviates from the electrical conductivity of WPI (Fig. 4).

Since the differences between the aqueous solution conductivities are most significant at 10 wt.% biopolymer concentration, we plot the electrical conductivity values (10 wt.%) of the aqueous solution mixtures at different ratios and compare them to the calculated value (Fig. 5).

At 10 wt.% biopolymers, surprisingly, the electrical conductivity of all of the mixtures is lower than the calculated values and very similar to that of the WPI (Fig. 5). It seems that GA contri-

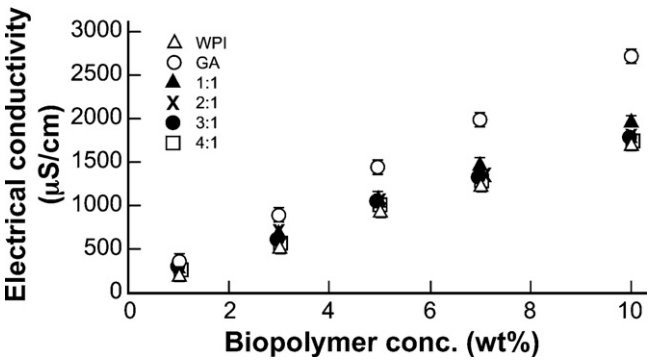

Fig. 4. The electrical conductivity of aqueous solutions of WPI, GA, and WPI:GA at different ratios as a function of biopolymer concentration.

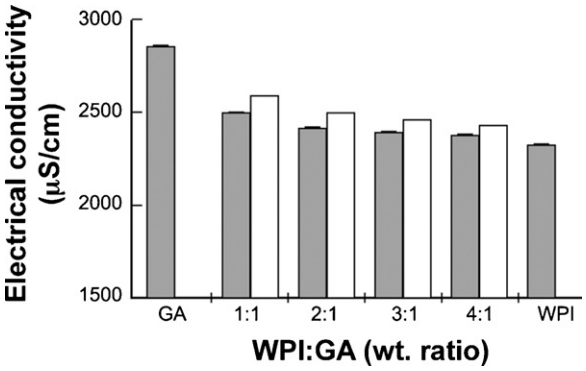

Fig. 5. Electrical conductivity measurements (gray) in comparison to calculated (white) values of aqueous solutions composed of 10 wt.% WPI, GA, and WPI:GA at different wt. ratios.

bution to the mixture is minor in spite of the fact that it is so much more negatively charged. The measured electrical conductivity values might be a result of the low GA ratio in the mixture but more likely are derived from the mutual charge neutralization between the two biopolymers neutralizing the charge of the GA. These results indicate the existence of partial charge neutralization (or charge–charge interactions) between the two biopolymers that reduces the electrical conductivity of the mixture.

3.4. Confocal microscope

While the surface tension, electrical conductivity, and ζ-potential are measured events occurring in the aqueous solution, the CLSM observations reflect the nature of the precipitates. The WPI of the mixture of the two biopolymers at pH 3–5 (near the IEP) has a strong tendency to charge neutralization and coacervation followed by precipitation (Fig. 6A). The coacervate can be seen even by the naked eye. A typical protein precipitate is observed under CLSM by using the dye rhodamine B (which binds only to the protein and not to the GA) [6].

At 3 > pH > 5 the aqueous mixture remains clear and no fluorescence or precipitation was detected (Fig. 6B). The lack of fluorescence is not clear evidence for such complex formation, since it might hint that at this pH range there are only very weak interaction incompatibilities (no interaction) between the biopolymers.

3.5. DSC measurements

Solutions of 1 wt.%, 5 wt.%, and 10 wt.% WPI, GA, and 3:1 WPI:GA were prepared. These were cooled from room temperature to −30 °C (at 3 °C min^{-1}), aged for 10 min at −30 °C and reheated (3 °C min^{-1}) back to room temperature (25 °C). From the record of the heating cycle, we deduced the endothermic melting point of water along with its melting enthalpy (ΔH_m of water). In Fig. 7 typical thermograms of the endothermic events are presented.

Table 1 shows that as the concentration of the biopolymers increases (1–10 wt.%), the melting point of the water decreases from −0.1 °C, 0.0 °C and −0.1 °C for WPI, GA, and WPI:GA mixture (3:1 wt. ratio), respectively, to −1.0 °C, −1.5 °C, and −1.8 °C, respectively.

The ΔH_m values show a similar trend. With an increase in the biopolymer content the absolute values of ΔH_m decrease (−284; −283; −270 J/g for WPI, GA, and WPI:GA (3:1 wt. ratio) to −250; −226; and −187 J/g, respectively).

The melting events of the biopolymers of 1 wt.% are almost like that of water (ca. 0 °C). However, from 5 wt.% to 10 wt.% of the biopolymer in solution, the water thawed at a temperature slightly below 0 °C, indicating a weak binding of the water to the biopolymer.

If we compare the mixture containing 1 wt.% of the two biopolymers to solutions of the single components alone, one can see that

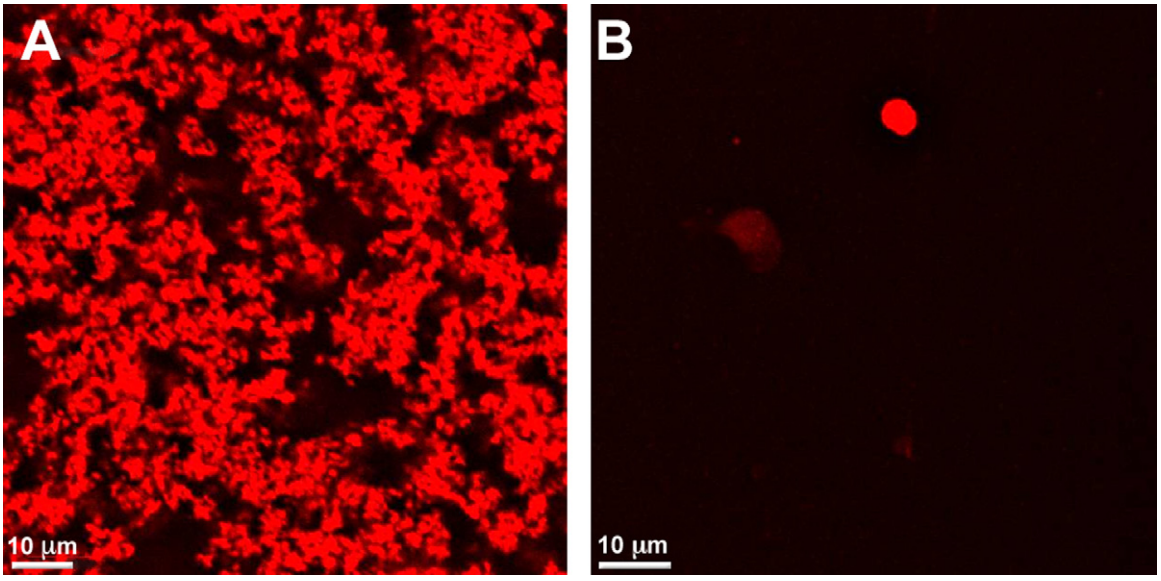

Fig. 6. CLSM images of 10 wt.% WPI:GA 3:1 wt. ratio at (A) pH 4 and (B) pH 7.

Table 1
Melting temperatures (water peak) and its enthalpies (ΔH_m) of 1 wt.%, 5 wt.%, and 10 wt.% WPI, GA, and WPI:GA (3:1 wt. ratio) aqueous solutions.

Biopolymer concentration (wt.%)	WPI		GA		WPI:GA (3:1 wt. ratio)	
	T_{melt} (°C)[a]	ΔH_m (J/g)	T_{melt} (°C)[a]	ΔH_m (J/g)	T_{melt} (°C)[a]	ΔH_m (J/g)
1	−0.1	−284.12 ± 1.62	0.0	−283.02 ± 3.48	−0.1	−269.73 ± 0.67
5	−0.2	−267.56 ± 1.53	−0.9	−259.58 ± 3.19	−0.5	−267.24 ± 0.67
10	−1.0	−249.68 ± 1.42	−1.5	−225.90 ± 2.78	−1.8	−186.66 ± 0.47

[a] Temperatures reported were reproducible to ±0.1 °C.

the enthalpy of each of the biopolymers is ca. −280 J/g, while the mixture is slightly lower, −270 J/g; but once the concentration was increased to 10 wt.% the differences are obvious. The enthalpy of water of 10 wt.% of the mixture is significantly reduced to −187 J/g in comparison to −250 and −226 for WPI and GA, respectively. The decrease of the temperature of the melting events and the reduction in the enthalpy of the water clearly indicates that in the mixture the ratio of bound to free water increases, as opposed to the single component solutions where it is almost negligible (at these concentrations). Subsequently we deduce that the biopolymers, via a selective exposure of the hydrophilic groups, significantly interact with the aqueous phase and bind the water molecules more efficiently than each of biopolymers alone.

From the thermograms one can extract the thermodynamic values related to these interactions. From Eqs. (1) and (2) we extracted the entropy (ΔS) of the system, plotted against the heating temperature.

$$C_v = \frac{dQ}{dT} = \frac{dH}{dT} \quad (P = \text{const}) \tag{1}$$

$$\Delta S = \int C_v (\ln\ T)\, d\ \ln T \tag{2}$$

The ΔS values of 10 wt.% WPI, GA, and the mixture against temperature are shown in Fig. 8, and the change in ΔS values within the melting event are then calculated (Table 2).

The molecular weight of the WPI is 18.3 kDa while that of the GA is 300–800 kDa. In order to extract the chemical potential of the system, related to the ratio of bound to free water, we calculated the number of molecules, N_i, involved in the process for two extreme molecular weights of the GA and the average molecular weight (300, 800, and 550 kDa). The chemical potentials are extracted from Eq. (3)

$$\mu_i = \left(\frac{\partial G}{\partial N_i}\right)_{T,P,N_{Ij}} \approx \frac{\Delta S}{N_i} \tag{3}$$

and are summarized in Table 3

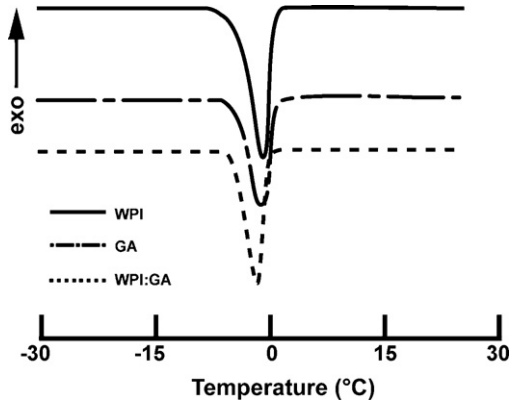

Fig. 7. Typical thermograms of the endothermic events of 10 wt.% WPI, GA, and 3:1 WPI:GA mixture.

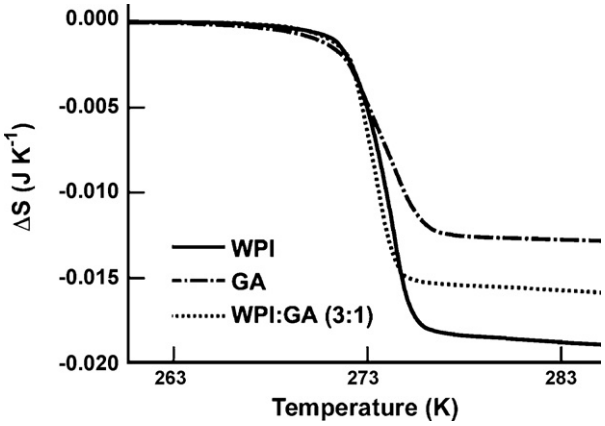

Fig. 8. ΔS values of 10 wt.% solutions of WPI, GA, and WPI:GA mixture (3:1 wt. ratio) against temperature.

It can be seen that in all concentrations of the 3:1 WPI:GA in use and regardless of the molecular weight of the GA, experimental chemical potentials are smaller than those that were calculated if the GA and WPI were not interacting. Regardless of whether the molecular weight of GA was 300, 800, or averaged 550 kDa, the obtained values of the mixture were always one-fourth the size than that when no interactions existed.

The calculations indeed confirm that the two biopolymers form a charge interaction. The low chemical potential values indicate that the new adduct formed between the two biopolymers is larger in size than each of them separately.

The charge interactions are sufficient to cause a molecular adduct migration (diffusion) of the two biopolymers together to the air–water or the oil–water interface. We assume that the AGP component is not involved in such interaction, since the glucuronic acid content in this component is limited and the polysaccharide moiety is linked covalently to other polysaccharide chains. As a result there is practically no negative charge on the AGP component and therefore it is less susceptible to interactions with the WPI. However, the AG component practically does not have proteins attached to it. Furthermore, the disordered polysaccharide has a relatively high concentration of glucuronic acid that imparts negative charges to the AG component. Consequently, AG has a higher feasibility to

Table 2
The change in ΔS values within the melting event of 1 wt.%, 5 wt.%, and 10 wt.% solutions of WPI, GA, and WPI:GA (3:1 wt. ratio).

Biopolymer concentration (wt.%)	Biopolymer type	Entropy ΔS (JK^{-1}) ($\times 10^{-2}$)
1	WPI	1.74 ± 0.02
	GA	1.75 ± 0.02
	WPI:GA (3:1 wt. ratio)	1.75 ± 0.01
5	WPI	2.13 ± 0.03
	GA	1.79 ± 0.02
	WPI:GA (3:1 wt. ratio)	2.32 ± 0.03
10	WPI	1.84 ± 0.02
	GA	1.26 ± 0.02
	WPI:GA (3:1 wt. ratio)	1.55 ± 0.01

Table 3
Chemical potential of the WPI:GA aqueous solutions as obtained from the DSC measurements in comparison to the calculated values.

Biopolymer concentration (wt.%)	μ (300 kDa)		μ (550 kDa)		μ (800 kDa)	
	Calculated ($\times 10^{-17}$)	Measured ($\times 10^{-17}$)	Calculated ($\times 10^{-17}$)	Measured ($\times 10^{-17}$)	Calculated ($\times 10^{-17}$)	Measured ($\times 10^{-17}$)
1	6.85	1.99 ± 0.02	12.24	3.39 ± 0.03	17.60	4.79 ± 0.04
5	6.82	1.72 ± 0.01	12.19	2.94 ± 0.02	17.60	4.15 ± 0.03
10	5.39	1.54 ± 0.01	9.55	2.59 ± 0.01	13.70	3.67 ± 0.03

Note: Since GA is a complex mixture with average molecular weight of 550 kDa, but with some fractions of 300 and others of 800 kDa, we calculated the chemical potential of the three possible cases.

interact with the positively charged domains of the WPI and can form a charge–charge adduct.

We also know that the molecular weight of the WPI is about 18.3 kDa and the AG molecular mass is about 2.5×10^2 kDa. Probably each AG polymer can bind ca. 13 molecules of WPI. However, since the glucuronic acid is ca. 6–15 wt.% of the AG polymer we assume that there are ca. 120 glucuronic acid sites on the AG that can bind to the WPI.

It is clear that if WPI only binds to the AG the performance of GA as an emulsifier in O/W emulsions, is enhanced by the presence of the WPI:AG complex in the mixture. It is reasonable to believe that the extra surface activity of the GA is mostly derived from the WPI:AG adduct, which is more surface active than each of the components alone. We think that the existence of two different surface active components (the WPI:AG and AGP) better stabilize the interface between the water and the oil. Their activity is complementary to that of the AGP but not competitive in the pH range where the protein is positively charged and the GA is negatively charged. Padala et al. [42] studied GA with egg white protein and found that at pH 7.5 they do not interact. The egg white protein is adsorbed preferentially due to its greater surface activity. But in conditions where the interactions occur (pH 3.5) the electrostatic complexes are adsorbed at the O/W interface leading to a more stable emulsion.

Nevertheless we cannot completely rule out the possibility that the WPI is co-adsorbed on the AGP component, complementary to the covalently bound protein. Fig. 9 is an schematic illustration of one possible scenario of charge–charge interaction between AG and WPI.

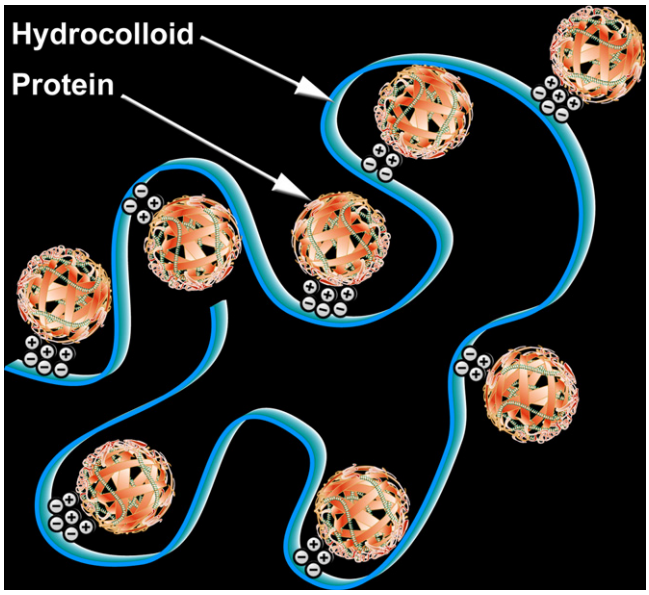

Fig. 9. Schematic illustration of a possible scenario of charge–charge interaction between arabinogalactan (AG) component and WPI at aqueous solution.

4. Conclusions

The analytical techniques performed on the solutions of WPI, GA, and their mixture indicates that within pH 5–7 there are weak charge interactions between the biopolymers.

The surface tension of the mixtures is lower than their cooperative calculated values. ζ-Potential values up to pH 6 are less than the calculated ones. Yet, at pH > 6 the values are greater than those calculated. The electrical conductivity measurements support the surface tension results. The DSC measurements and the calculated chemical potentials of the interactions clearly indicate that the two biopolymers are weakly interacting.

The charge interactions occur only when the protein is not totally negative or totally positive. In this range of pHs, the protein consists of some positive domains of amino peptides that are not fully neutralized. These positively charged domains interact with the negatively charged GA to form weak complexes that do not form precipitating coacervates but rather soluble or colloidal adducts that behave like a large molecular entity with amphiphilic and interfacial characteristics. The charge interactions are sufficient to cause a molecular adduct migration of the two biopolymers together to the air–water interface.

Acknowledgments

We would like to thank Miss. C. Pemberton for her careful experimental work, using SD-NMR and Mrs. I. Svitov for her constructive assistance.

References

[1] A. Benichou, A. Aserin, N. Garti, Polym. Adv. Technol. 13 (2002) 1019–1031.
[2] D.J. McClements, Biotechnol. Adv. 24 (2006) 621–625.
[3] O.G. Jones, U. Lesmes, P. Dubin, D.J. McClements, Food Hydrocolloids 24 (2010) 374–383.
[4] S.H. Liu, N.H. Low, M.T. Nickerson, J. Agric. Food Chem. 57 (2009) 1521–1526.
[5] K. Xu, P. Yao, Langmuir 25 (2009) 9714–9720.
[6] R. Lutz, A. Aserin, Y. Portnoy, M. Gottlieb, N. Garti, Colloids Surf. B 69 (2009) 43–50.
[7] A. Benichou, A. Aserin, R. Lutz, N. Garti, Food Hydrocolloids 21 (2007) 379–391.
[8] M. Klein, A. Aserin, I. Svitov, N. Garti, Colloids Surf. B 77 (2010) 75–81.
[9] A. Benichou, A. Aserin, N. Garti, Colloids Surf. A 297 (2007) 211–220.
[10] A. Benichou, A. Aserin, N. Garti, Colloids Surf. A 294 (2007) 20–32.
[11] R. Jimenez-Alvarado, C.I. Beristain, L. Medina-Torres, A. Roman-Guerrero, E.J. Vernon-Carter, Food Hydrocolloids 23 (2009) 2425–2433.
[12] I. Schmidt, F. Cousin, C. Huchon, F. Boue, M.A.V. Axelos, Biomacromolecules 10 (2009) 1346–1357.
[13] E. Dickinson, Soft Matter 4 (2008) 932–942.
[14] A. Benichou, A. Aserin, N. Garti, Adv. Colloid Interface Sci. 108–109 (2004) 29–41.
[15] A. Benichou, A. Aserin, N. Garti, J. Dispersion Sci. Technol. 23 (2002) 93–123.
[16] S.L. Turgeon, C. Schmitt, C. Sanchez, Current Opin. Colloid Interface Sci. 12 (2007) 166–178.
[17] T.V. Burova, N.V. Grinberg, V.Y. Grinberg, A.I. Usov, V.B. Tolstoguzov, C.G. de, Kruif, Biomacromolecules 8 (2007) 368–375.
[18] R. Lutz, A. Aserin, L. Wicker, N. Garti, Colloids Surf. B 74 (2009) 178–185.
[19] E. Dickinson, Food Hydrocolloids 17 (2003) 25–39.
[20] V. Tolstoguzov, Food Hydrocolloids 17 (2003) 1–23.
[21] R. Lutz, A. Aserin, L. Wicker, N. Garti, Colloids Surf. B 72 (2009) 121–127.
[22] K. de Kruif, F. Weinbreck, R. de, Vries, Complex Coacervation of Proteins and Anionic Polysaccharides, University of Wageningen Press, Utrecht, Netherlands, 2004, pp. 6–7.

[23] M. Gottdchalk, H. Nilsson, H. Roos, B. Halle, Protein Sci. 12 (2003) 2404–2411.
[24] F. Weinbreck, R. de Vries, P. Schrooyen, C.G. de, Kruif, Biomacromolecules 4 (2003) 293–303.
[25] Y. Akiyama, S. Eda, K. Kato, Agric. Biol. Chem. 48 (1984) 235–237.
[26] R.C. Randall, G.O. Phillips, P.A. Williams, Food Hydrocolloids 2 (1988) 131–140.
[27] R.C. Randall, G.O. Phillips, P.A. Williams, Food Hydrocolloids 3 (1989) 65–75.
[28] M.E. Osman, A.R. Menzies, P.A. Williams, G.O. Phillips, T.C. Baldwin, Carbohydr. Res. 1246 (1993) 303–318.
[29] D.M.W. Anderson, E. Hirst, J.F.J. Stoddart, Chem. Soc. C (1966) 1959–1966.
[30] S.A. Assaf, G.O. Phillips, P.A. Williams, Food Hydrocolloids 19 (2005) 647–660.
[31] M.J. Kieliszewski, L.J. Goodrum, A. Patel, J.F. Leykam, Phytochemistry 54 (2000) 99–106.
[32] C. Sanchez, C. Schmitt, E. Kolodziejczyk, A. Lapp, C. Gaillard, D. Renard, Biophys. J. 94 (2008) 629–639.
[33] C. Flindt, M.E. Osman, S.A. Assaf, G.O. Phillips, P.A. Williams, Food Hydrocolloids 19 (2005) 687–702.
[34] E.A. Hassan, S.A. Assaf, G.O. Phillips, P.A. Williams, Food Hydrocolloids 19 (2005) 669–678.
[35] T. Mahendran, P.A. Williams, G.O. Phillips, S.A. Assaf, T.C. Baldwin, J. Agric. Food Chem. 56 (2008) 9269–9276.
[36] S. Connolly, J.C. Fenyo, M.C. Vandevelde, Carbohydr. Res. 8 (1988) 23–32.
[37] P.A. Williams, G.O. Phillips, A.M. Stephen, Food Hydrocolloids 4 (1990) 305.
[38] R. Lutz, A. Aserin, L. Wicker, N. Garti, Food Hydrocolloids 23 (2009) 786–794.
[39] K. Duan, X. Zhang, X. Tang, J. Yu, S. Liu, D. Wang, Y. Li, J. Huang, Colloids Surf. B 76 (2010) 475–482.
[40] J.F.P.F. Gomes, S. Rocha, M. do Carmo Pereira, I. Peres, S. Moreni, L. Toca-Herrera, M.A.N. Coelho, Colloids Surf. B 76 (2010) 449–455.
[41] S. Ogawa, E.A. Decker, D.J. McClements, J. Agric. Food Chem 52 (2004) 3595–3600.
[42] S.R. Padala, P.A. Williams, G.O. Phillips, J. Agric. Food Chem. 57 (2009) 4964–4973.