Carbohydrate Polymers 74 (2008) 358–365

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Rheological behavior of amylopectin and β-lactoglobulin phase-segregated aqueous system

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ARTICLE INFO

Article history:
Received 22 September 2007
Received in revised form 21 December 2007
Accepted 4 March 2008
Available online 20 March 2008

Keywords:
Phase segregation
Rheology
Takayanagi model
Amylopectin
β-Lactoglobulin

ABSTRACT

The rheological behavior of amylopectin–β-lactoglobulin–water system in the region of incompatibility was studied by performing constant shear rate and oscillatory measurements. Phase-segregated samples were prepared at concentrations that laid over the binodal and tie-lines.

The results were interpreted in terms of the isostress and isostrain blending laws. For this purpose the bases of Takayanagi model was used and a modified model considering the gradual formation of double emulsions close to the phase-inversion concentration that explain the transition was proposed.

Good agreement between observed and calculated values was obtained using the isostress and isostrain models.

Phase segregation and the phase-inversion control the rheological properties of the amylopectin–β-lactoglobulin mixture in aqueous media.

1. Introduction

The main components present in almost all foods are polysaccharides and proteins; therefore, to some extent they will determine the structure, chemical and physical properties of the whole system. The behavior of one of them is affected by the presence of the other and vice versa. In aqueous medium they can be compatible or incompatible. If they are thermodynamically incompatible they will segregate into two different phases, however, if they are thermodynamically compatible they will co-exist in a single-phase system or they will concentrate in one of the phases of the two-phase system (Tolstoguzov, 1986, chap 9).

Studies about polysaccharide–protein systems not only have scientific interest for a better understanding of the behavior of complex multicomponent systems but also for technological implications such as development of new food products (da Silva & Rao, 1992, chap 11).

We have been studying amylopectin and β-lactoglobulin in an aqueous system and used this system as a model for a multicomponent system, e.g. starch-based food systems. At high concentrations, amylopectin and β-lactoglobulin have been shown to phase segregate in one phase rich in polysaccharide and one phase rich in protein, when the system was not induced to phase segregate by heating, changes in pH or addition of salts. For samples with amylopectin:β-lactoglobulin ratios of around 1:1 an intermediate metastable phase was seen. The kinetics of phase segregation between amylopectin and β-lactoglobulin were controlled mainly by the polysaccharide because with time the branch of the binodal close to the polysaccharide concentration shifts to this axis. For this system, the critical concentration for phase segregation was quite high, above 20% (Quiroga & Bergenståhl, 2008); this critical concentration was dropped to lower concentrations on heat treatment above 60 °C (Quiroga & Bergenståhl, submitted for publication).

Since the physical properties of phase-segregated mixtures depend on the composition of each phase and its volume fraction and distribution, and the extent of interaction between phases (Manson & Sperling, 1976, chap 2), we have decided to continue studying this system to get more information about its rheological properties. Therefore, the aims of this work were: (i) to study the rheological properties of amylopectin–β-lactoglobulin aqueous mixtures in the region of incompatibility and (ii) to explain the rheological results according to an approach similar to Takayanagi's model.

The Takayanagi's model was the first and simplest model to explain the mechanical behavior of a binary polymer system, where both polymers have different moduli (Takayanagi & Harima, 1963). Later on, this model was modified and applied for describing the mechanical properties of ternary aqueous systems, where the solvent was partitioned into both polymer phases (Clark, Richardson, Ross-Murphy, & Stubbs, 1983; Lopes da Silva & Rao, 1999, chap 6; Sperling, 1986, chap 8).

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doi:10.1016/j.carbpol.2008.03.011

Amylopectin is one of the two components of starch. It is a highly branched polymer of glucose units linked in a linear way with α-D-(1 → 4) bonds. Branching takes place with α-D-(1 → 6) bonds occurring every 24–30 glucose units. The molecular weight values range from 2 to 700 10^6 (Bertoft, 2004, chap 2; Zobel, 1988).

β-Lactoglobulin is the major whey protein of cow's milk (~3 g/l). It is a relatively small protein of 162 residues, with an 18.4 kDa molecular weight. In physiological conditions it is predominantly dimeric (Kontopidis, Holt, & Sawyer, 2004; Sawyer, 1992, chap 7).

2. Materials and methods

2.1. Materials

Amylopectin (molecular weight 20 MDa) from unmodified waxy maize starch (extracted from hybrid corn grain) was bought from Sigma (product number A-7788). β-Lactoglobulin was kindly supplied by Arla Foods (PSDI 2400).

2.2. Sample preparation

Two stock solutions were prepared: one solution of AP (~18%) and one solution of βlg (~40%).The first solution was prepared by dissolving the polysaccharide in water at 120 °C for 1 h and the second solution by mixing the protein with water at room temperature. For removing the impurities and insoluble material from the solutions, they were centrifuged at 20,000 rpm for 1 h. The concentration of the solution was recalculated by gravimetry, i.e. by weighing an specific amount of sample and leaving at 105 °C until the weight was constant.

AP–βlg mixtures were prepared by mixing the stock solutions at different ratios and by adding some amount of water if needed to the mixture. In order to prevent bacterial growth in the samples during equilibration (1 week), sodium azide (0.01 wt%) was added. All samples were calculated on a weight percentage basis (wt%).

Two sets of samples were prepared in the region of incompatibility (along the tie-lines S1–S5 and S6–S9), and one sample (S10) in the region of compatibility, see the phase diagram in Fig. 1. The volume of each sample was 20 mL.

2.3. Rheology

For the study of the rheological behavior of the mixtures, three different programs were used: constant rate, oscillation stress sweep and oscillation strain control; and the measurements were performed in a Viscotech rheometer (ReoLogica Instruments AB, Lund, Sweden) with a concentric cylinder (23 mm internal diameter, 27 mm external diameter and 37.5 mm height).

Before the measurements the set-up of the equipment was done according to the rheometer manufacturer specifications, and all the parameters were set for the different programs.

To ensure that the measurements were done properly we gave the samples enough time to reach an equilibrium flow before the sampling of the data, (delay time). In addition, the values presented in this paper were the result of the calculations of the measurements recorded during a period of time (integration time).

2.3.1. Constant rate

The measurements were made in the range of 0.01 and 100 s^{-1}, although the graphs show the shear rate range between 1 and 100 s^{-1}, with a delay time of 10 s and integration time of 30 s.

2.3.2. Oscillation stress sweep

The measurements were performed in order to find the linear viscoelastic region. The stress sweep was in the range of 0.07146 and 10 Pa divided in 20 logarithmic steps, the frequency was set to 1 Hz, the delay time was 10 s and the integration period 30 s. All further oscillation measurements were carried out at strains inside this region.

2.3.3. Oscillation strain control

Frequency was varied between 0.02 and 100 Hz in 60 steps, though the graphs display the frequency range between 0.02 and 10 Hz. The delay time was 10 s. The integration period for the first 20 steps was 60 s, for the next 20 steps 30 s and for the last 20 steps 10 s. The integral period was decreased with increasing the frequency to shorten the experimental time but still keeping sufficient time for completing at least one oscillation cycle. The tolerance limit of the strain was 1%.

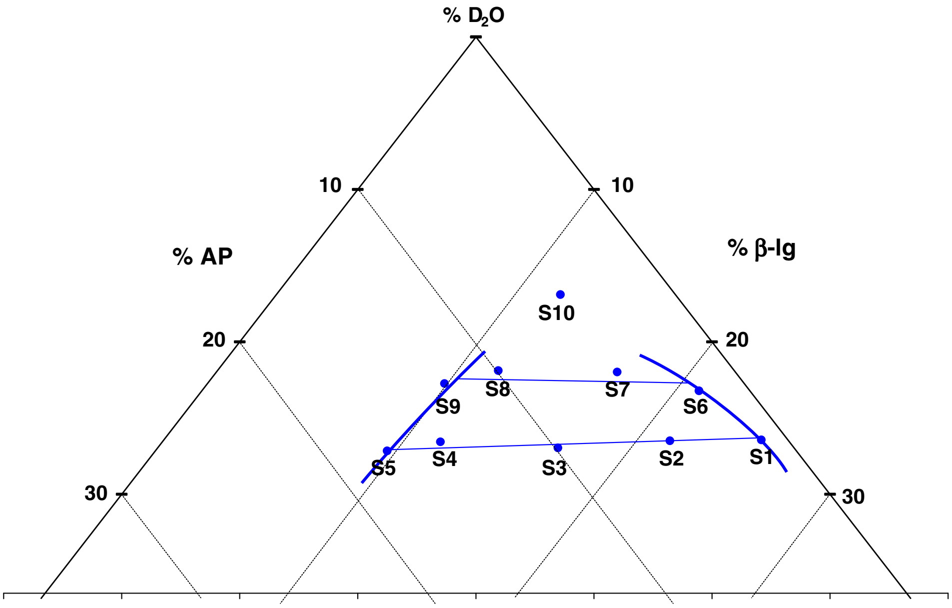

Fig. 1. AP–βlg–D_2O phase diagram, 1 week equilibration time: (●) AP and βlg samples used for the rheology studies, (—) binodal and tie-lines. Note that the AP-rich phase has a metastable nature and does not represent the true equilibrium condition. Figure modified from "Effect of Heat Treatment on the Phase Segregation of Amylopectin and β-lactoglobulin Aqueous System" (Quiroga and Bergenståhl, submitted for publication).

About 15 g of sample was placed in the cup, allowed to equilibrate at 20 °C for 5 min and measured.

For each sample the measurements were done twice with an interval of 200 s between the measurements.

The experiments were performed before and after equilibration. The time of equilibration was 1 week.

3. Results

The composition of the samples is described in Table 1. The chosen compositions lay over the tie-lines of the co-existent phases and over the binodal of the phase diagram of the phase-segregated AP–βlg–D_2O system. Samples S1 and S6 were rich in protein with low polysaccharide content, samples S5 and S9 were rich in polysaccharide with important protein content. The composition of the other samples was between those that were described. Sample S10 was not in the region of incompatibility; it was co-soluble. Fig. 1 shows the position of each sample in the phase diagram.

The total macromolecular concentration of the samples was quite high; for the phase-segregated samples it was between 21% and 27%.

Table 1
Composition of samples and the distribution between the AP-rich and βlg-rich phase in the phase-segregated region

Sample code	% AP	% Blg	$\phi_{\text{AP rich phase}}$[a]	$\phi_{\beta\text{lg rich phase}}$[a]
S1	1.13	25.31	0.00	1.00
S2	5.03	21.47	0.23	0.77
S3	10.00	16.96	0.54	0.46
S4	14.78	11.79	0.84	0.16
S5	17.33	9.82	1.00	0.00
S6	2.16	21.06	0.00	1.00
S7	5.00	16.98	0.24	0.76
S8	9.99	11.89	0.79	0.21
S9	12.69	10.04	1.00	0.00
S10[b]	4.87	12.04	–	–

[a] Estimated from Fig. 1.
[b] Non-phase-segregated sample.

3.1. Flow behavior

All the samples before and after phase segregation exhibited a “power law” relationship between shear rate and shear stress. The samples with high polysaccharide content exhibited a shear-thinning behavior, however, the samples with high protein content showed Newtonian behavior.

The results of the different rheological parameters of the samples were different before and after 1 week of equilibration, though the differences between them were small, e.g. for the measurements shear stress versus shear strain the differences in the slope were less than 5%.

For the samples rich in polysaccharide the apparent viscosity decreases with increasing the shear rate; however, for the samples rich in proteins the apparent viscosity was more or less the same at low and high shear rates (see Figs. 2 and 3). This agrees with the flow curve, i.e. samples rich in polysaccharide behaved as shear-thinning fluids and samples rich in protein as Newtonian fluids.

Oscillatory data give a fingerprint of the state of the microstructure (Barnes, 2000, chap 13); for this kind of measurements a sinusoidal varying stress is applied and the equally varying strain and the shift between stress and strain are measured (Ferry, 1970, chap 5). However, great care has to be taken to ensure that the experiment takes place in the linear region where the storage modulus (G') and loss modulus (G'') are independent of the maximum amplitude of stress of the oscillation.

3.2. Viscoelastic region

The moduli, storage modulus (G') and loss modulus (G''), were independent of stress over a range between 0.07 and approximately 3 Pa. The tendency of the curves looked quite similar before and after equilibration; therefore only the graph after equilibration is presented (see Fig. 4). The viscous samples showed that G' decreased slowly with increasing stress; however, the less viscous samples showed that G' increased drastically with increasing stress.

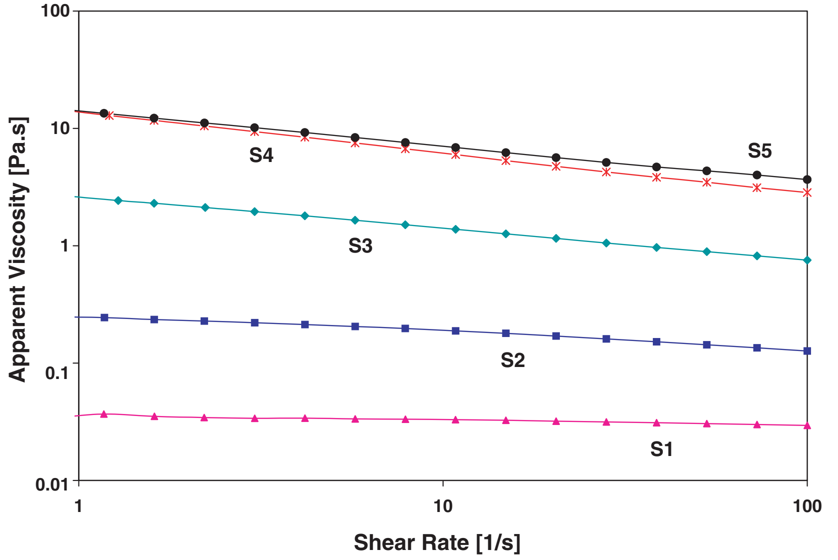

Fig. 2. Viscosity curve, shear rate versus viscosity; the composition of the samples are described in Table 1: S1(▲), S2(■), S3(◆), S4(✱) and S5(●) rheological measurements before equilibration.

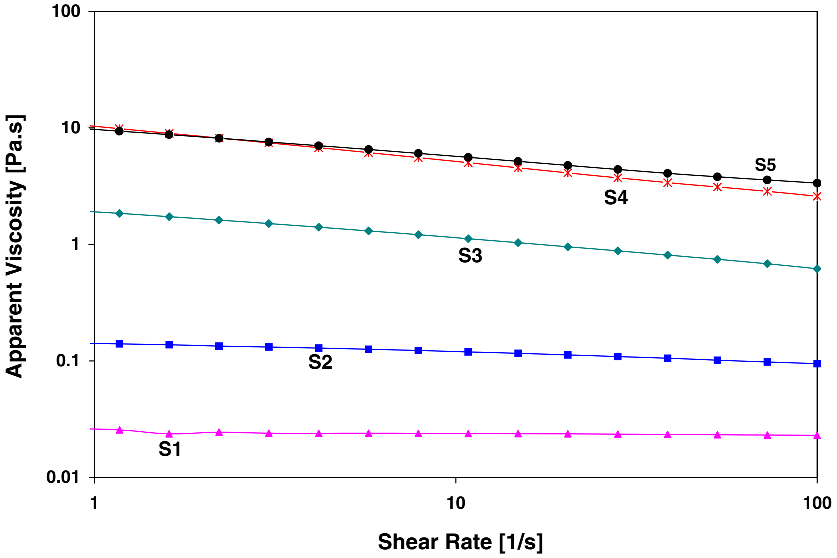

Fig. 3. Viscosity curve, shear rate versus viscosity; the composition of the samples are described in Table 1: S1(▲), S2(■), S3(◆), S4(✱) and S5(●); rheological measurements after 1 week of equilibration.

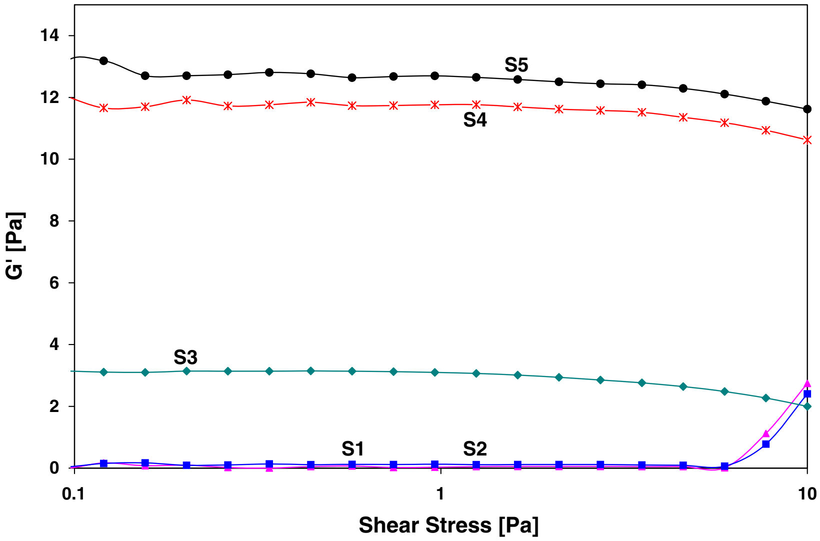

Fig. 4. Linear viscoelastic region, shear stress versus storage modulus recorded at 1 Hz frequency; the composition of the samples are described in Table 1: S1(▲), S2(■), S3(◆), S4(✱) and S5(●); rheological measurements after 1 week of equilibration.

3.3. *Mechanical spectra*

G'' values were higher than G' values, the loss modulus values range was between 0.15 and 26.70 Pa and the storage modulus values range was between 0.05 and 15.90 Pa, at least in the range between 0.2 and 10 Hz, indicating that the viscous component had a more important role in the samples than the elastic, i.e. the samples were liquid-like (see Fig. 5).

The difference between G' and G'' was larger for the samples rich in polysaccharide than the samples rich in protein; e.g. for S5 the difference between the moduli was around 13 Pa.

G' and G'' values are larger for the samples rich in AP than those rich in βlg. For all the samples, G' and G'' displayed marked frequency dependence, the moduli values increased with increasing frequency. At low G' and G'', the moduli of the samples converged with increasing frequency (above 2 Hz), thereby displaying a viscoelastic liquid characteristic. The noisiness in these samples at increasing frequency might be because the measurements were performed close to the operational limits of the equipment.

The Cox–Merz rule was applied to the system in order to check the relationship between the rheological parameters determined from oscillatory test such as complex viscosity on one hand and the apparent viscosity, from shear stress and shear rate, on the other hand (Fig. 6). The less viscous samples showed almost a superimposition of the shear rate dependence of steady shear viscosity and of the frequency dependence of the complex viscosity at equal values of frequency (below about 5 Hz) and shear rate. For the viscous samples, no superimposition was seen, that might mean that these samples were less homogeneous than the others.

4. Discussion

In this study we have characterized the rheological properties in the two-phase region of a phase-segregating system. The results

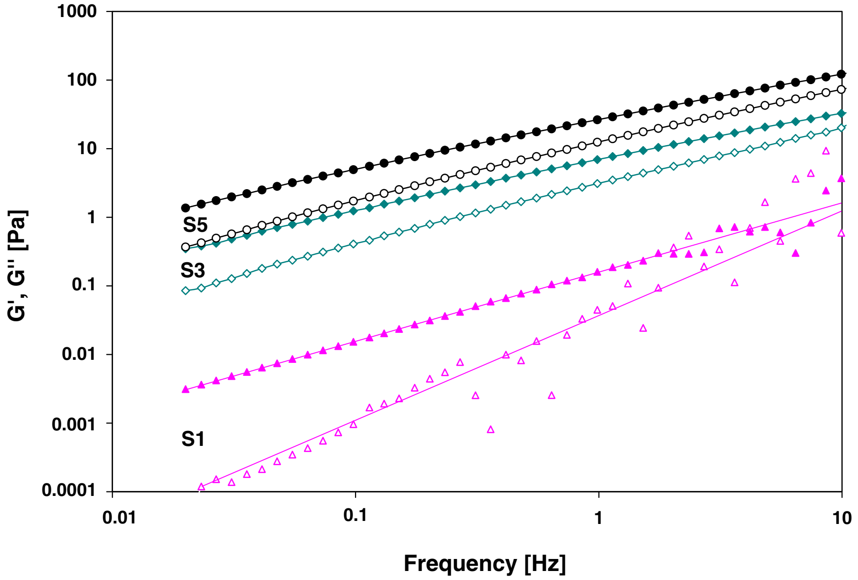

Fig. 5. Storage and loss moduli versus frequency. The composition of the samples are described in Table 1: S1(▲), S3(◆) and S5(●); filled symbols correspond to loss modulus (G'') and non-filled symbols correspond to storage modulus (G'); rheological measurements after 1 week of equilibration.

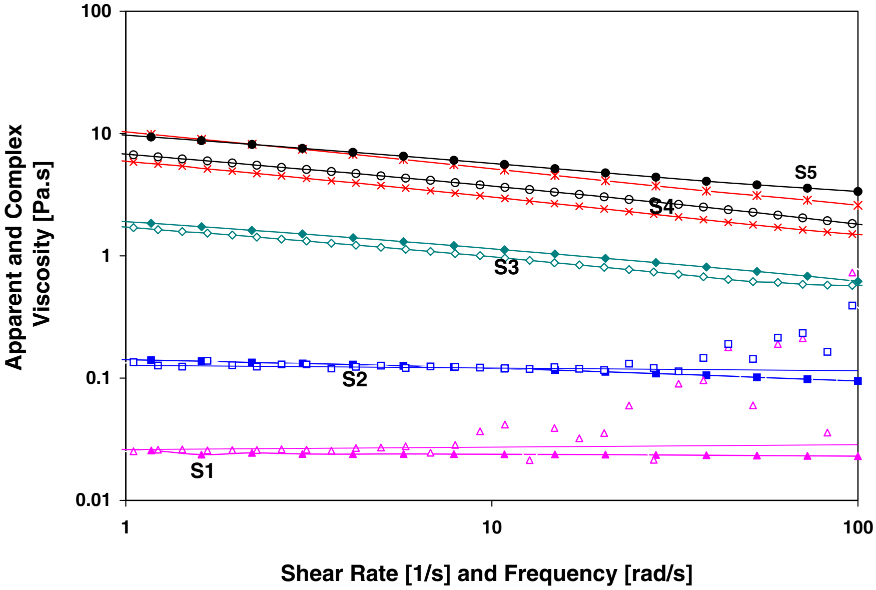

Fig. 6. Cox–Merz rule for AP–βlg–H_2O system; the composition of the samples are described in Table 1: S1(▲), S2(■), S3(◆), S4(✱) and S5(●); filled symbols correspond to apparent viscosity versus shear rate and non-filled symbols correspond to complex viscosity versus frequency; rheological measurements after 1 week of equilibration.

show that the highly viscous AP-rich phase is in equilibrium with a low-viscous βlg-rich phase. The ratio between the low-viscous and the high-viscous phase is about 400 for viscosity (flow curves), about 100 for G' and about 100 also for G''. In the mixed region the dominating phase controls the properties and when the volume fractions become about equal, we can observe a dramatic change in the properties (Fig. 7).

Takayanagi assumed that when the high modulus phase forms the continuous structure, the deformation is controlled by the outer structure and results in an equal deformation of both inner and outer structure elements (so-called isostrain). The equal deformation of all structure elements corresponds to a mechanical model of parallel elements. When the low modulus phase forms the continuous structure the deformation of the outer material will be more extensive than the deformation of the inner structures although the tension can be assumed to be evenly distributed (so-called isostress). Equal stress on all structure elements corresponds to a mechanical model where the elements are arranged in series.

The isostrain model gives that the modulus of the composite is obtained as a volumetric average of the modulus of the both phases:

$$G_{\mathrm{C,strain}} = \phi_{\mathrm{IP}} \cdot G_{\mathrm{IP}} + \phi_{\mathrm{OP}} \cdot G_{\mathrm{OP}} \qquad G_{\mathrm{OP}} >> G_{\mathrm{IP}} \tag{1}$$

where G_{C} is the modulus of the composite, G_{OP} and G_{IP} are the moduli of the outer and inner phases and ϕ refers to the volume fraction of the phases.

The isostress model gives reciprocal volumetric average:

$$G_{\mathrm{C,stress}} = \frac{1}{\frac{\phi_{\mathrm{IP}}}{G_{\mathrm{IP}}} + \frac{\phi_{\mathrm{OP}}}{G_{\mathrm{OP}}}} \qquad G_{\mathrm{OP}} << G_{\mathrm{IP}} \tag{2}$$

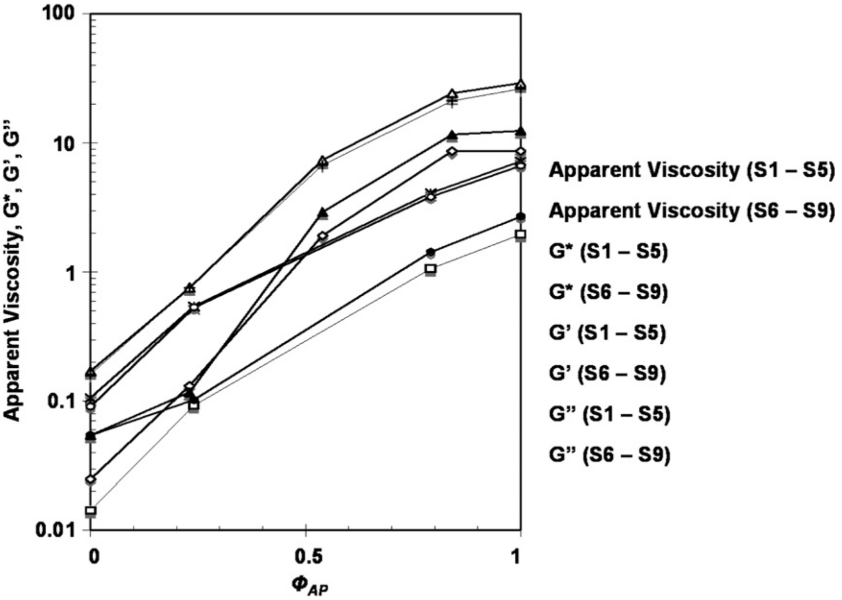

Fig. 7. Apparent viscosity, complex modulus (G^*), storage modulus (G') and loss modulus (G'') versus the volume fraction of AP-rich phase along the tie-lines S1–S5 and S6–S9 in Fig. 1. Data were recorded at 1 Hz frequency and 2 Pa shear stress.

The Takaynagi model demands certain conditions to provide a decent description of the mechanical properties of a fluid with a composite character. Both phases need to form an isotropic dispersion. The deformation would be small if the dispersion remained isotropic. The mechanical properties of the domains are described by only considering the bulk modulus, e.g. no interfacial tension effects are considered. No hydrodynamic interactions are considered between the domains, which also limit the model to small-deformation rheology.

The AP–βlg system can be described using the Takayanagi model using the moduli obtained at the phase boundaries (S1 and S5) as measures of the moduli of the pure phases. By estimating the volume fractions of the respective phases (Table 1) from the phase diagram (Fig. 1) using the isostrain model, Eq. (1) and the isostress model, Eq. (2), the G^*, G' and G'' (obtained at 1 Hz and a shear stress of 2 Pa) as well as the viscosity from the flow curves were compared.

The results from the viscometric measurements (not shown) deviated somewhat, most likely because the results were influenced by hydrodynamic interactions between domains (particularly in the low-viscous region). Similar deviations from the Takayanagi theory were also observed for G'' and G^*.

The comparison shows that the Takayanagi model describes the elastic modulus quite well close to the phase boundary. This suggests that the deformation is sufficiently small to leave the structure unaltered and that the contribution of interfacial energies is very limited. As expected, G' is less sensitive to hydrodynamic phenomena and the results in the low-viscous region fit with the model as well as the results obtained in the high-viscous region.

However, in the middle of the range there appears to be a transition region where the system changes from the isostress to the isostrain behavior. This transition is not described by the Takayanagi model directly.

The microstructure of phase-segregating AP–βlg mixtures in dried films was investigated in a previous study (Quiroga & Bergenståhl, 2007) using atomic force microscope (AFM). In that work, clear emulsion structures were observed close to the phase boundaries, continuous AP close to the AP-rich phase and continuous βlg close to the βlg-rich phase. In the intermediate range of the phase-segregated region many more undefined structures were observed. The microstructure displayed a spinodal character with an unclear continuity. Close to this phase-inversion region it appeared that the emulsions obtained a gradual tendency to form double emulsions. Based on these observations we suggest the following tentative explanation of the mechanical properties of the system.

4.1. The Takayanagi model applied on a phase-inverting system

We are assuming that the phase inversion is a gradual process (along the compositional line). Thus we can imagine that double emulsion domains are formed in the vicinity of the phase-inversion concentration. A formation of double emulsion can be assumed to make the effective internal phase volume much higher than the internal phase volume if no double emulsion would be formed and would approach one at the phase-inversion concentration (Fig. 8). This concept can be introduced into the simple Takayanagi model.

Let's define a critical inversion concentration ϕ_{IPinv} of the internal phase and let's assume that a double emulsion is formed when AP is added to a βlg-rich phase: βlg-rich phase/AP-rich phase/βlg-rich phase emulsion; and that this process starts above a critical concentration of the internal phase $\phi_{\mathrm{IP^*}}$. Similarly the double emul-

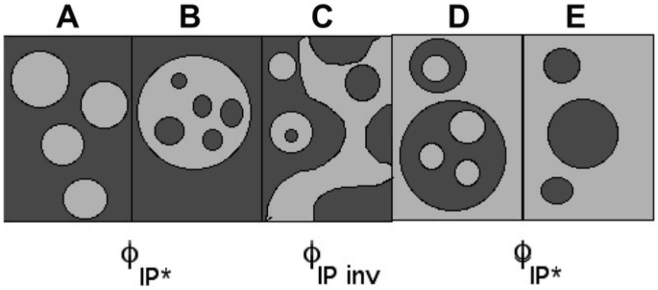

Fig. 8. This illustration shows how the phase-segregating polymer emulsion (A and E) passes through a double emulsion state (B and D) above a critical concentration, $\phi_{\mathrm{IP^*}}$, with an increased effective internal phase volume ($\hat{\phi}_{\mathrm{IP}}$), close to the phase-inversion concentration, ϕ_{IPinv} (C).

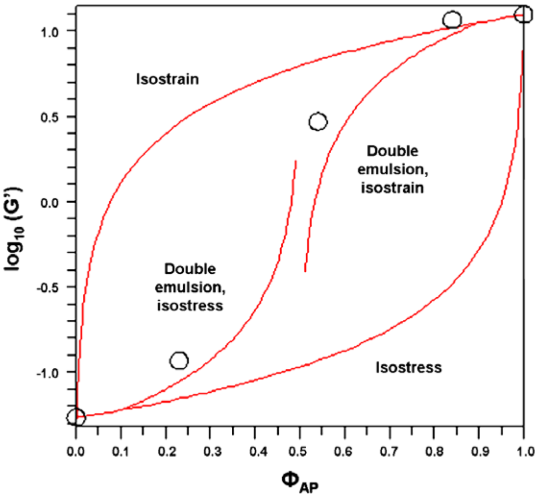

Fig. 9. The modified Takayanagi isostrain/isostress models considering the gradual formation of double emulsion when the system approaches the phase-inversion concentration (Eq. (6)). The model is compared with experimental G' data along the tie-line S1–S5 in Fig. 1. ϕ_{AP} at the X-axis refers to the volume fraction of the AP-rich phase along the phase-segregated tie-line (S1–S5). Data were recorded at 1 Hz frequency and 2 Pa shear stress.

Table 2
Estimations of the parameters describing the microstructure and the mechanical properties using the gradual phase-inversion model

Fraction βlg-rich phase	$\hat{\phi}_{IP}$[a]	$\hat{\phi}_{OP/IP}$[b]	$\hat{G}_{IP}$[c]	$\hat{G}_{C\ phase\ inv}$[d]
0.00	0.00	–	–	0.10
0.05	0.05	0.00	10.00	0.10
0.10	0.10	0.00	10.00	0.11
0.15	0.21	0.29	7.09	0.13
0.20	0.32	0.38	6.19	0.15
0.25	0.44	0.43	5.76	0.18
0.30	0.55	0.46	5.50	0.22
0.35	0.66	0.47	5.33	0.29
0.40	0.78	0.48	5.21	0.42
0.45	0.89	0.49	5.12	0.77
0.50	1.00	0.50	–	–
0.55	0.89	0.49	0.20	1.30
0.60	0.78	0.48	0.19	2.40
0.65	0.66	0.47	0.19	3.50
0.70	0.55	0.46	0.18	4.60
0.75	0.44	0.43	0.17	5.70
0.80	0.32	0.38	0.16	6.80
0.85	0.21	0.29	0.14	7.90
0.90	0.10	0.00	0.10	9.01
0.95	0.05	0.00	0.10	9.50
1.00	0.00	–	–	10.00

The phase-inversion concentration, ϕ_{IPinv}, is set to 0.5, the critical concentration when the double emulsion starts to form, ϕ_{IP^*}, is set to 0.1. The modulus of the βlg-rich phase is set to 0.1 and the modulus of the AP-rich phase is set to 10.

[a] Effective volume fraction internal phase (Eq. (3)).

[b] The volume fraction of the continuous phase in the effective internal phase (Eq. (4)).

[c] The modulus of the effective internal phase (Eq. (5)).

[d] The modulus of the phase-inverting polymer system (Eq. (6)).

sion, AP-rich phase/βlg-rich phase/AP-rich phase emulsion, can be assumed to be formed above a volume fraction ϕ_{IP^*} when the βlg content in the system is increased. A simple linear model describing the formation of double emulsion from 0 to 1 between ϕ_{IP^*} and ϕ_{IPinv} can be assumed to illustrate the consequences of the gradual inversion process. An effective volume fraction internal phase ($\hat{\phi}_{IP}$) can thus be shown as:

$$\hat{\phi}_{IP} = \phi_{IP^*} + (1 - \phi_{IP^*}) \cdot \left(\frac{\phi_{IP} - \phi_{IP^*}}{\phi_{IPinv} - \phi_{IP^*}}\right) \tag{3}$$

The effective internal phase consists of a continuous emulsion in the original internal phase and with the original outer phase as internal phase. The volume fraction dispersed phase in the effective internal phase ($\hat{\phi}_{OP/IP}$) is shown as:

$$\hat{\phi}_{OP/IP} = \frac{\hat{\phi}_{IP} - \phi_{IP}}{\hat{\phi}_{IP}} \tag{4}$$

Using the composition given by Eq. (4) the mechanical properties of the effective inner phase can be estimated:

$$\hat{G}_{IP} = f(\hat{\phi}_{OP/IP}, G_{OP}, (1 - \hat{\phi}_{OP/IP}), G_{IP}) \tag{5}$$

where f refers as a function of (1), (2) depending on the relative strength of the moduli.

The result is the modulus of a gradually phase inversing polymer system:

$$\hat{G}_{C\,\mathrm{phaseinv}} = f(\hat{\phi}_{IP}, \hat{G}_{IP}, (1 - \hat{\phi}_{IP}), G_{OP}) \tag{6}$$

where f refers to a function of (1), (2) depending on the relative strength of the moduli.

The model can be applied from the low modulus side (in our case with the βlg-rich phase as continuous phase) or from the high modulus side (in our case with the AP-rich phase as the continuous phase).

Equation [6] has been solved using $\phi_{IPinv} = 0.5$, $\phi_{IP^*} = 0.1$. The obtained G' from S1 and S5 was used as $G'_{\beta lg\text{-rich phase}}$ and $G'_{AP\text{-rich phase}}$. The result is shown in Fig. 9 and the derivation of the curves plotted in this figure is shown in Table 2.

The nature of the rapid change in the mechanical properties as the system passes through the region of phase inversion is clearly obtained. Thus this result shows that the gradual character of the phase-inversion process and the formation of double emulsions close to the phase-inversion concentration result in the dramatic change in the modulus that is observed experimentally. These results suggest that the internal microstructure in a segregating polymer system has a drastic influence on its mechanical properties.

5. Conclusions

Phase segregation and phase-inversion control the rheological properties of phase-segregated amylopectin–β-lactoglobulin system. The phase-inversion leads to a gradual change in the elastic modulus that cannot be described with the classical Takayanagi model. A modified Takanayagi model considering the gradual formation of double emulsions close to the phase-inversion concentration explains the transition.

Acknowledgement

This work was financially supported by the Swedish International Development Agency (Sida/SAREC).

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