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Rheology of water-in-water emulsions: caseinate-pectin and caseinate-alginate systems

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Rheology of water-in-water emulsions: caseinate-pectin and caseinate-alginate systems

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Abstract

Conventional models developed for oil-water emulsions do not fit viscosity of caseinate-pectin and caseinate-alginate water-in-water emulsions, which is always lower than predicted, except for high viscosities of disperse phase. These models do not consider strong deformations, prevented by the high interfacial tension of oil-water interphases. The ultra-low interfacial tension of water-in-water emulsions facilitates the creation of interphase and very elongated droplets. Capron model considers interfacial tension, fitting results when the dispersed phase is the most viscous, but, for other cases, lower experimental values are obtained related to the shear-induced stratification. Even values below the stratification model are observed for some samples, related to the influence of the interphase in the viscosity of the emulsion. A model that takes into account the presence of a relatively thick interphase poor in both polymers is proposed. Intermediate structures between highly elongated and stratified fluids, with influence of interphase viscosity could explain results.

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1. Introduction

Emulsions, i.e. mixtures of two immiscible liquids where droplets of one liquid are dispersed in a continuous medium, are extensively present in our daily life. One can think, for example, in a high variety of household, agrochemicals, cosmetics, and pharmaceutical products. Water-in-oil (W/O) and oil-in-water (O/W) single emulsions have been widely studied, regarding their formation, droplet diameter, destabilization mechanisms and rheological characterization (Becher, 1986; Delmas et al., 2011; Derkach, 2009; Gutiérrez et al., 2008; Weiss & Muschiolik, 2007). Multiple emulsions such as oil-in-water-in-oil (O/W/O) or water-in-oil-in-water (W/O/W) have also been studied although their formation and behavior are more difficult to control as they are more complex systems (Florence & Whitehill, 1981; Pal, 2011).

Water-in-water emulsions (W/W) are emulsions where both, disperse and continuous phase, are aqueous. Other notations used in literature for these systems are Aqueous-Two-Phase-Systems (ATPS) (Atefi, Fyffe, Kaylan, & Tavana, 2016) or Aqueous Biphasic Systems (Bridges, Gutowski, & Rogers, 2007; Onghena, Opsomer, & Binnemans, 2015). Although in appearance these systems resemble water-in-oil or oil-in-water systems, their dynamic behavior is far more complex (Sagis, 2008). They are very interesting colloidal dispersions that can result from mixtures of two hydrophilic polymers, a hydrophilic polymer with a polyelectrolyte or a surfactant, or just surfactant solutions, leading to a complex phase behavior (Esquena, 2016). Particularly, biopolymer mixtures are very common in the food industry. Some of these polymers (or biopolymers), although being water-soluble, result in two thermodynamically incompatible aqueous phases when mixed, depending on composition, temperature and chemical environment. Two different interactions between polymers can lead to phase separation: segregative, when repulsion of polymers occurs, and associative, when attraction takes place (Matalanis, Jones, & McClements, 2011; Piculell & Lindman, 1992). When segregation occurs, at low polymer concentrations the mixture remains in a single phase, with both components in solution. However, above some minimum concentrations, the two components separate into two immiscible aqueous phases in thermodynamic equilibrium, each phase enriched with one of the hydrophilic components. The phase separation is caused by thermodynamic incompatibility of the two components, because the small entropy of mixing is overruled by the repulsive interaction between the polymers, in certain conditions where the two molecules have no opposite charges (Piculell & Lindman , 1992). That is, when the solvent-biopolymer interactions are favored compared to biopolymer-biopolymer interactions (Corredig, Sharafbafi, & Kristo, 2011). Therefore, repulsive interactions between polymer molecules produce segregative phase separation (at intermediate macromolecule concentrations) or co-solubilization (at low concentrations). The second mechanism, associative phase separation, is based on attractive interaction of the hydrophilic components. This phenomenon is quite different because the mixtures of the two components and water separate in a solid-like precipitate, or coacervate, which contains high concentrations of both polymers, and a supernatant with just a residual amount of the two polymers. Associative phase separation takes place usually by electrostatic complexation of the oppositely charged polymers (Esquena, 2016; Schmitt & Turgeon, 2011).

W/W emulsions have been described for polymer mixtures like poly(ethylene glycol) (PEG) and poly(acrylates) (Johansson, Feitosa, & Pessoa, 2011; Lu & Weiss, 1995), or just PEG with salts. Such aqueous liquid-liquid two-phase systems have been proposed to be useful in the extractive separation of metals (Karmakar & Sen, 2019; Onghena et al., 2015) and some biomolecules such as proteins and enzymes. Separation can take place in milder conditions thanks to the low interfacial tension between the phases that, in proper conditions, allows small droplet size and a large interphase with gentle stirring and an ulterior rapid partition (Willauer, Huddleston, & Rogers, 2002).

Other promising application of W/W emulsions is their use as microreactors, with the advantages of maintaining reaction internal conditions and allowing exchange of reactants and products with surroundings under mild conditions (Cacace, Rowland, Stapleton, Dewey, & Keating, 2015). They could also be used as templates for the preparation of size-controlled microgels, where hydrophilic monomers and initiators could be used in a mild aqueous medium, as an alternative to the emulsion polymerization in O/W emulsions (Kasapis, Morris, Norton, & Clark, 1993).

Regarding biopolymers, mixtures of proteins and polysaccharides in aqueous solutions are commonly found in daily life, in food, pharmaceutical and cosmetic fields, and they are the most used biopolymers to form W/W emulsions. Interactions between proteins and polysaccharides have been reported for a long time ago. W/W emulsions were reported and deeply studied in mixtures like gelatin-hydrolyzed starch, agar-gelatin (Esquena, 2016), dextran-gelatin (Tromp & de Hoog, 2008), locust bean gum-caseinate (Norton & Frith, 2001), bovine serum albumin-dextran (Antonov, Wolf, & Moldenaers, 2015), Na-caseinate-Na-alginate (Antonov & Moldenaers, 2011; Capron, Costeux, & Djabourov, 2001; Simeone, Alfani, & Guido, 2004; Simeone, Molè, & Guido, 2002), pectin-caseinate (Rediguieri, de Freitas, Lettinga, & Tuinier, 2007) as examples. A review listed more than 100 polysaccharide-protein combinations that resulted in W/W emulsion formation (Grinberg & Tolstoguzov, 1997). Water-in-water emulsions prepared with biopolymers can be used for encapsulation and controlled release applications in pharmaceutical, food, or cosmetic products (Dinsmore et al., 2002; Gibbs, Kermasha, Alli, & Mulligan, 1999; Norton, Espinosa, Watson, Spyropoulos, & Norton, 2015). W/W emulsion droplets could be very appropriate in food formulations and functional food for the design of novel encapsulation and delivery systems for labile molecules (Sagis,2008). If W/W emulsions are prepared using food-grade components, the biocompatibility can be ensured; moreover, these delivery systems can be formulated by using simple processing operations.

Water-protein-polysaccharide W/W emulsions present interesting uses as thickeners, rheology and texture modifiers in the food industry, and manufacture of innovative products in cosmetic and pharmaceutical industry (Rediguieri et al., 2007). As an example, they could be used in foods as thickeners and textural-mimics for low or zero fat products (Capron et al., 2001; Simeone et al., 2004). Casein-polysaccharide mixtures are in fact present in many dairy products (Jensen, Rolin, & Ipsen, 2010). Other possible uses are related to the shear of these systems as a tool for obtaining structures with certain sensory functionality, for example, in manufacture of meat replacement products (van der Goot, Peighambardoust, Akkermans, & van Oosten-Manski, 2008). Due to the low interfacial tension of W/W emulsions, single shear can easily elongate droplets and a fibrous structure similar to that of meat could then form through gelation, for example (van de Velde, de Hoog, Oosterveld, & Tromp, 2015). Shear banding observed in some conditions offers an additional method to obtain certain shear-induced structures for W/W emulsions, where ultra-low interfacial tension favors extremely high elongation of droplets (Tromp & de Hoog, 2008).

From the last paragraph it seems clear that rheological properties of these emulsions play an important role in their possible uses. Rheology of conventional emulsions, where oil-water interphases are involved, has been extensively studied and comprehensive reviews have been published for simple and multiple emulsions (Derkach, 2009; Pal, 2011). Derkach (2009) reviews equations to predict apparent viscosity of dispersions starting from the simplest one, the Einstein law, developed for the viscosity of very diluted dispersions of non-deformable particles or droplets with no hydrodynamic interactions between them (Eq. 1),

$$\eta_E = \eta_c\left(1+2.5\phi\right) \qquad (1)$$

where η_E is the apparent viscosity of the dispersion, η_c is the viscosity of the continuous phase and ϕ is the volume fraction of the disperse phase. Taylor equation extended the prediction to very diluted emulsions with small deformation of droplets, introducing the viscosity of disperse phase, η_d, where $\lambda=\eta_d/\eta_c$, according to Eq. (2):

$$\eta_T = \eta_c\left(1+\frac{1+2.5\lambda}{1+\lambda}\phi\right) \qquad (2)$$

Further modifications of these equations consider the surface shear viscosity, the dilatational viscosity, or the interactions between droplets in intermediate and concentrated emulsions (Derkach, 2009). For example, hydrodynamic interactions can be taken into account by the modified Taylor model in Eq. (3):

$$\tilde{\eta}_T = \eta_c \frac{5+3(\eta_T/\eta_c-1)}{5-2(\eta_T/\eta_c-1)} \tag{3}$$

However, these models always predict an increase of apparent emulsion viscosity with respect to the viscosity of the continuous phase, as only moderate deformation of droplets is allowed, and usually work well when oil-water interphases are involved. Pal (2011) published another review where models for multiple emulsions appeared, in which a part of the continuous phase is entrapped into the disperse one and increases its effective volume fraction. The use of equations mentioned previously is limited to emulsions where deformation of droplets is weak and do not include influence of interfacial tension, droplet size and shear rate. On the other hand, some models (Frankel & Acrivos, 1970) added a crucial parameter for the measurement of drop deformation, the Capillary dimensionless number (Eq. 4):

$$Ca = \frac{\eta_c R \dot{\gamma}}{\sigma} \tag{4}$$

Where R is the radius of the droplets, $\dot{\gamma}$ is the shear rate and σ is the interfacial tension. Capillary number measures the balance between the force favoring deformation, i.e., the applied shear stress, $\eta_c\dot{\gamma}$, and the force opposing deformation or creation of new surface, the interfacial tension. At very low interfacial tensions, Ca number can be very high, predicting strong deformations. For W/W emulsions interfacial tensions can be of the order of 10^{-3} to 10^{-5} mN/m, in contrast to 1-5 mN/m for a conventional emulsion. Oldroyd (1953) had before presented a similar model where a characteristic relaxation time was used, τ_1, defined as the time required for drops to recover their spherical shape once stress is removed, and dependent on the Ca number, given by Eq. (5):

$$\tau_1 = \frac{\eta_c R}{\sigma} \frac{(2\lambda+3)(19\lambda+16)}{40(\lambda+1)} \tag{5}$$

Based on the Oldroyd's work, Capron et al. (2001) published a model in which, again, Ca number was relevant. They observed, depending on λ and shear rate applied and according to other authors (Suchkov, Grinberg, & Tolstogusov, 1981), a decrease in emulsion viscosity compared to the viscosity of the continuous phase and reported, in some cases, values below the average viscosity of both phases. Their model was proposed to fit results, for no hydrodynamic interactions between droplets:

$$\eta_{Cap} = \frac{\eta_c}{1+(\tau_1\dot{\gamma})^2}\left[1+\phi\,\frac{5\lambda+2}{2\lambda+2}+\left(1+\phi\,\frac{5(\lambda-1)}{2\lambda+3}\right)(\tau_1\dot{\gamma})^2\right] \tag{6}$$

Note that if the term $(\tau_1\dot{\gamma}) \rightarrow 0$ this model transforms into the Taylor's equation. When interfacial tension is high, the relaxation time is very small because droplets tend to quickly recover the spherical shape once stress disappears, so the influence of interfacial tension can be neglected for conventional emulsions; for W/W emulsions this could not be done due to their low interfacial tension.

If hydrodynamic interactions are considered, Eq.(5) and (6) transform into Eq. (7) and (8) (Capron et al., 2001):

$$\tilde{\tau}_1 = \frac{\eta_c R}{\sigma}\frac{(2\lambda + 3 - 2\phi(\lambda - 1))(19\lambda + 16)}{40(\lambda + 1) - 8\phi(5\lambda + 2)} \tag{7}$$

$$\tilde{\eta}_{Cap} = \frac{\eta_c}{1 + (\tilde{\tau}_1\dot{\gamma})^2}\left[\frac{2\lambda + 2 + 3\phi\left(\lambda + \frac{2}{5}\right)}{2\lambda + 2 - 2\phi\left(\lambda + \frac{2}{5}\right)} + \frac{2\lambda + 3 + 3\phi(\lambda - 1)}{2\lambda + 3 - 2\phi(\lambda - 1)}(\tilde{\tau}_1\dot{\gamma})^2\right] \tag{8}$$

However, the authors talk about deviations from Eq.(8) when drops become extremely elongated. Note again that in the limit $(\tilde{\tau}_1\dot{\gamma}) \rightarrow 0$, related to weak deformations (low shear rates and/or high interfacial tension), Eq.(8) becomes Eq.(3), the modified Taylor model.

Suchkov et al. (1981), when working with caseinate-alginate-water systems, proposed, depending on the composition and shear rate, the appearance of a third low-viscosity thin film in the interphase that would act as a lubricant, resulting in a lower than predicted viscosity.

As it can be seen from this introduction, there are several rheological models, which try to fit and explain rheological behavior; however, this representation is extremely difficult due to different reasons. Moreover, biopolymers, of natural origin, and even synthetic polymers, do not have a defined composition, which makes the reproducibility of experiments difficult. On the other hand, if representation in all the range of volumetric fractions of the phases is intended, capillary numbers are changing because of the exchange of the disperse and continuous phases, even assuming that the radius of the drops does not change with the volumetric fraction. Besides, as emulsions are non-equilibrium systems, their properties will depend on their preparation procedure and not only on the composition and temperature; finally, stability and results of rheological measurements are difficultly reproducible.

All these reasons make that only a qualitative representation of rheological behavior can be obtained through mathematical models proposed; however, this qualitative representation can be a useful tool to understand the variety of behaviors that can be observed in such complex systems. In the present work, these models are discussed and compared with experimental results. A variant of one of these models is also presented. Caseinate-pectin-water system is used as a model system. Phase diagram is determined and emulsions are prepared along several tie-lines. High and low shear viscosity of obtained emulsions are compared with predictions of some of the cited models, and adequacy of the models as a function of the properties of the phases is discussed. We propose that the capillary number and the disperse/continuous viscosity ratio are relevant for the structure of water-in-water emulsions, and the viscosity of the water-water interphase affects the viscosity of the emulsion. Experimental results for other systems such as caseinate-alginate-water system will be used in discussion for stablishing conclusions.

2. Experimental

2.1. Materials

Sodium caseinate (Cas) from bovine milk, around 0.72% sulfur and 0.70% phosphorus, and pectin (poly-D-galacturonic acid), with ≥74% galacturonic acid, ~80% esterified with methyl and with $M_w \approx 90000$ g/mol were purchased from Sigma-Aldrich. Sodium alginate with a ratio β-D-mannuronic acid/α-L-guluronic acid = 58.85/41.15, $M_n \approx 668000$ and $M_w \approx 1750000$ was supplied by Panreac. Sodium hydroxide required for pH adjustment was purchased from Sigma-Aldrich. In order to slow down microbial growth processes, solutions of biopolymers were prepared with a 0.04% w/w of sodium azide supplied by Sigma-Aldrich. All products were used as received. MilliQ water was used to prepare solutions.

2.2. Preparation of mother solutions

Aqueous mother solutions of polymers were prepared, 3.5% w/w for Alg; 8% w/w for Pec and 15% w/w for Cas. Sodium azide 0.04% was added to all mother solutions as antibacterial agent. In order to avoid lump formation during dissolution, powder was added gradually to MilliQ water while stirring. High speed Ultra-Turrax was used to dissolve alginate, due to the high viscosity of the solution. The required NaOH was added to reach pH = 8. Mixing was maintained overnight at room temperature and solutions were subsequently stored at 4^{o}C for 24 h before use to assure complete hydration.

2.3. Determination of the phase diagram

Binodal and tie-lines were determined in separated steps, as described below.

2.3.1. Binodal line determination

Binodal line was determined by titration for the systems Cas-Pec-Water and Cas-Alg-Water at 25^{o}C; a solution of a single polymer with a known concentration was located in a tube and the solution of the other polymer was dropwise added while mixing until two phases were observed. As solutions alone presented slight turbidity, centrifugation after each addition was required for an accurate determination of the single-two phase boundary. After centrifugation at 25ºC, no separation was observed in the single-phase region and an interphase was clearly distinguished in the two-phases region. This procedure was carried out for several concentrations of each polymer, adding the coupled one. For the limits of low concentrations, the binodal line was determined starting from a mixture in the biphasic zone and adding water until a single phase was observed after centrifugation.

2.3.2. Tie-lines determination

Once the binodal line was determined, tie-lines were obtained as follows. For each system, mixtures of both polymers at different concentrations in the two-phases region were prepared and gently mixed at 25ºC to ensure that equilibrium of phases is reached. Phase separation was allowed and accelerated by centrifugation. Then, 5 g of each phase was weighed in a petri dish and dried at 120ºC until constant weight. As a result, the %w/w of total polymers for each phase was known. As binodal line was already drawn, and for each point of binodal line the %w/w of total polymers was known, it allowed situating each equilibrium phase composition on it. For both phases of each tie-line, it was searched on the binodal line the point where the sum of %w/w of both polymers was the total %w/w experimentally obtained. Therefore, tie-lines were obtained. The fact that the global composition and the composition of the two phases in equilibrium must be on the same tie-line, was used as a proof of the goodness of the determination.

2.3.3. Preparation of emulsions

To study the effect of disperse phase fraction, ϕ, on rheology, without changes in composition of phases nor redistribution of components while mixing, emulsions were always prepared along a tie-line through the mixture of the corresponding equilibrium phases previously obtained. First of all, a large amount of mixture of a known composition inside the biphasic region was prepared and allowed to equilibrate. Afterwards, the equilibrium phases were separated. Then, the required quantities of these phases were mixed in the desired proportions in order to obtain the different W/W emulsions along the chosen tie-line, with the fixed volume fractions of the equilibrium phases to be studied. For this, density of the equilibrium phases was determined and used for having the precise volume of each phase by weighting. The phases were weighted in a vessel until 8 g of total mixture was obtained and stirred with a magnetic mixer for 10 min at 500 rpm. All the emulsions were prepared in the same way in order to minimize the influence of preparation variables in non-equilibrium systems. This procedure was repeated for all the tie-lines investigated. For the system casein-pectin-water, emulsions of the following volume fractions of pectin rich phase were prepared: 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and 95% along each of the three tie-lines studied.

2.3.4. Rheological measurements

The rheological properties of the emulsions were studied using a HAAKE MARS III (Modular Advanced Rheometer System) rheometer at a controlled temperature of 25°C ± 0.1°C. A coaxial double gap cylinder sensor was used. After loading, samples were held for 5 min at a constant 5 s^{-1} shear rate before the formal test to allow a proper distribution of the sample, temperature and stress equilibration and remove bubbles.

Stationary flow, oscillatory stress sweep and oscillatory frequency sweep tests were carried out.

The conditions of each essay are listed below:

- *Stationary flow (steady-state viscosity vs. shear rate)*: The viscosity of the samples was measured for a range of shear rates 1-2000 s^{-1}. Rheometer was programmed to fix a shear rate and measure viscosity *vs*. time, in order to record the viscosity value only when it reached a constant value (i.e. the equilibrium or steady state viscosity). This viscosity was then registered and a new shear rate was subsequently stablished until new steady state for all the range of shear rates stablished. Regularity in the variation of the viscosity with the gradient was observed, indicating that there were no distortions due to turbulence.
- *Oscillatory stress sweep test*: It was a preliminary test in order to determine the linear viscoelastic range, LVR, for the ulterior frequency sweep test. Frequency of 1 Hz and 25°C were fixed and shear stress amplitude of oscillation was changed from 0.01 to 10 Pa. The complex modulus G* was measured. From these results, stress amplitude for frequency sweep test was chosen in the range where complex modulus was constant and independent of stress.
- *Frequency sweep test*: Storage (G’) and loss (G”) moduli viscoelastic functions were measured through a frequency sweep test. The frequency range was 0.01 to 100 Hz, corresponding to 0.063 - 63 s^{-1}. The shear stress amplitude was 1 Pa, chosen through the preliminary oscillatory stress sweep test, in order to work in the LVR.

3. Results and discussion

3.1. Phase diagram

The phase diagrams were determined at 25°C and are shown in Figures 1 and 2.

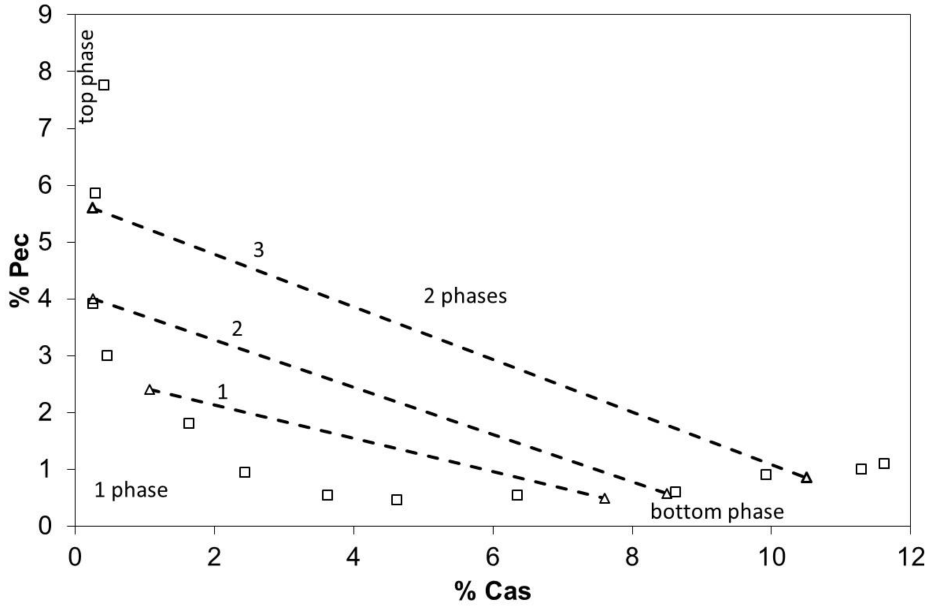

Figure 1. Phase diagram of caseinate-pectin-water (Cas-Pec-Water) mixtures at 25°C, pH = 8. Squares are the experimentally determined points of binodal line. Triangles are equilibrium phases, joined by their tie-lines (dotted lines).

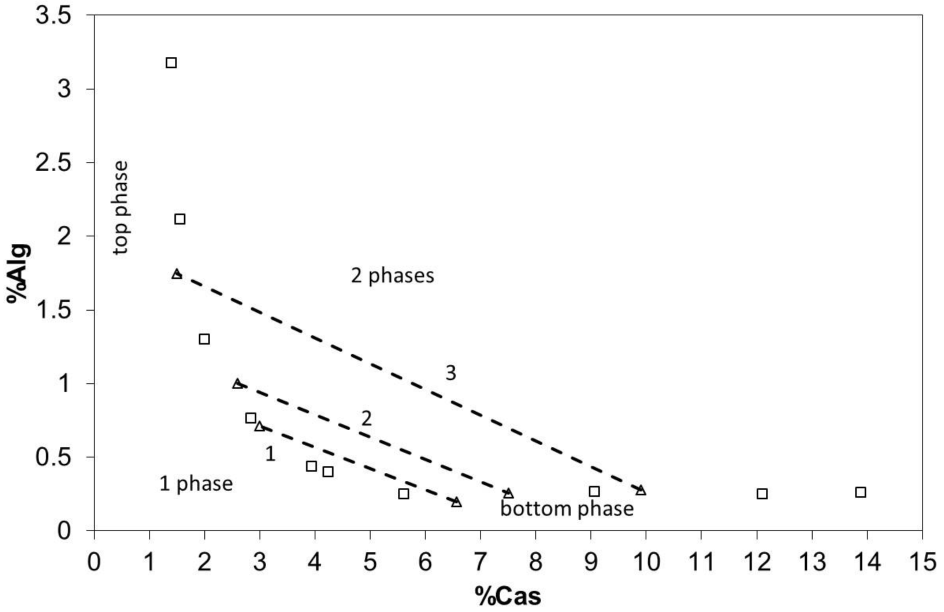

Figure 2. Phase diagram of caseinate-alginate-water mixtures (Cas-Alg-Water) at 25°C, pH = 8. Squares are the experimentally determined points of binodal line. Triangles are equilibrium phases, joined by their tie-lines (dotted lines).

Figures 1 and 2 show that both systems behaved in a segregative way, so that when the two phases were present, each phase was enriched in one biopolymer, and at low concentrations of polymers a single phase appeared. For the Cas-Pec-Water system, the top, lighter phase was rich in pectin and the bottom phase was rich in caseinate. For the Cas-Alg-Water system, the top phase was rich in alginate and the bottom one was enriched in caseinate. Densities of both phases were similar, especially, as expected, for the more diluted tie-lines, located near the critical point. For example, for the Cas-Alg-Water and the most diluted tie-line tested (tie-line 1 on Fig. 2), densities of top and bottom equilibrium phases were 1.054 and 1.095 g/mL, respectively. It indicated that, once emulsion was formed, destabilization was not produced by sedimentation, at least in the beginning, but, due to the low interfacial tension, coalescence took place instead. Only when droplets big enough formed through coalescence, sedimentation and subsequent macroscopic separation appeared. For the most diluted tie-line of the Cas-Pec-Water system (tie-line 1 on Fig. 1), more similar densities were yet obtained, 1.028 and 1.032 g/mL for top and bottom phases.

3.2. Rheology of emulsions

In order to investigate the influence of volume fraction of phases in the rheology of water-in-water emulsions, composition and volume fraction of phases must be known and well stablished. If solutions of single polymers are mixed, components redistribute between the phases and both composition and volume fraction change. To avoid this, emulsions were prepared mixing equilibrium phases. So, whatever proportion of phases was used, the system just moved along a tie-line and these variables were unaltered. Linear viscoelasticity and steady state viscosity were measured in the W/W emulsions along the tie-lines drawn in Figures 1 and 2. The rheological results are presented and discussed to be used in proposing and eventually validating behavioral models of these emulsions.

3.2.1. Viscoelasticity

Linear viscoelasticity was measured for separated single equilibrium phases and their mixtures (emulsions) in several volume fractions. Dynamic spectra, storage and loss moduli, are presented in Figure 3(a) for some compositions along the tie-line 1 of the Cas-Pec-Water system. For the sake of clarity, only 4 experiments are shown, corresponding to bottom phase (rich in caseinate), 30% top phase, 70% top phase and 100% top phase (rich in pectin). Both moduli presented low values with a strong predominance of viscous behavior, as G” was clearly higher than G’ in nearly all the range of frequencies, with high crossover frequencies, between 10 and 100 rad/s, indicating short relaxation times. The bottom phase was the least viscous one and, when the rich-in-pectin, top phase was added, the loss modulus G”, related to viscous behavior, progressively increased up to that of pure top phase. G’ was practically unaltered with volume fraction of phases. Although not shown, intermediate volume fractions 5, 10, 20, 30, 40, 60, 70, 80, 90 and 95% were tested and all of them were ordered in the same way, with a gradual increase on G” and no significant changes in G’, that presented low values in all cases. This allowed to stablish that the continuous phase was always the one present in major proportion since, otherwise, stronger viscoelasticity should be observed in one side of the range tested, related to interaction of droplets in concentrated and highly concentrated emulsions. Therefore, it was assumed that below 40% of top phase the continuous one was the bottom phase, and above 60%, the continuous phase was the top phase. Around 50% uncertainties occurred. When tie-lines 2 and 3 were tested, G” curve raised to higher values, ordered in the same way as tie-line 1, from the less viscous, bottom phase, to the most viscous, top phase, as the proportion of top phase was increased, obtaining higher values for the tie-line 3, the most concentrated one tested, as observed in Figure 3(b). Again, low values of G’ are obtained, with no significant changes with composition.

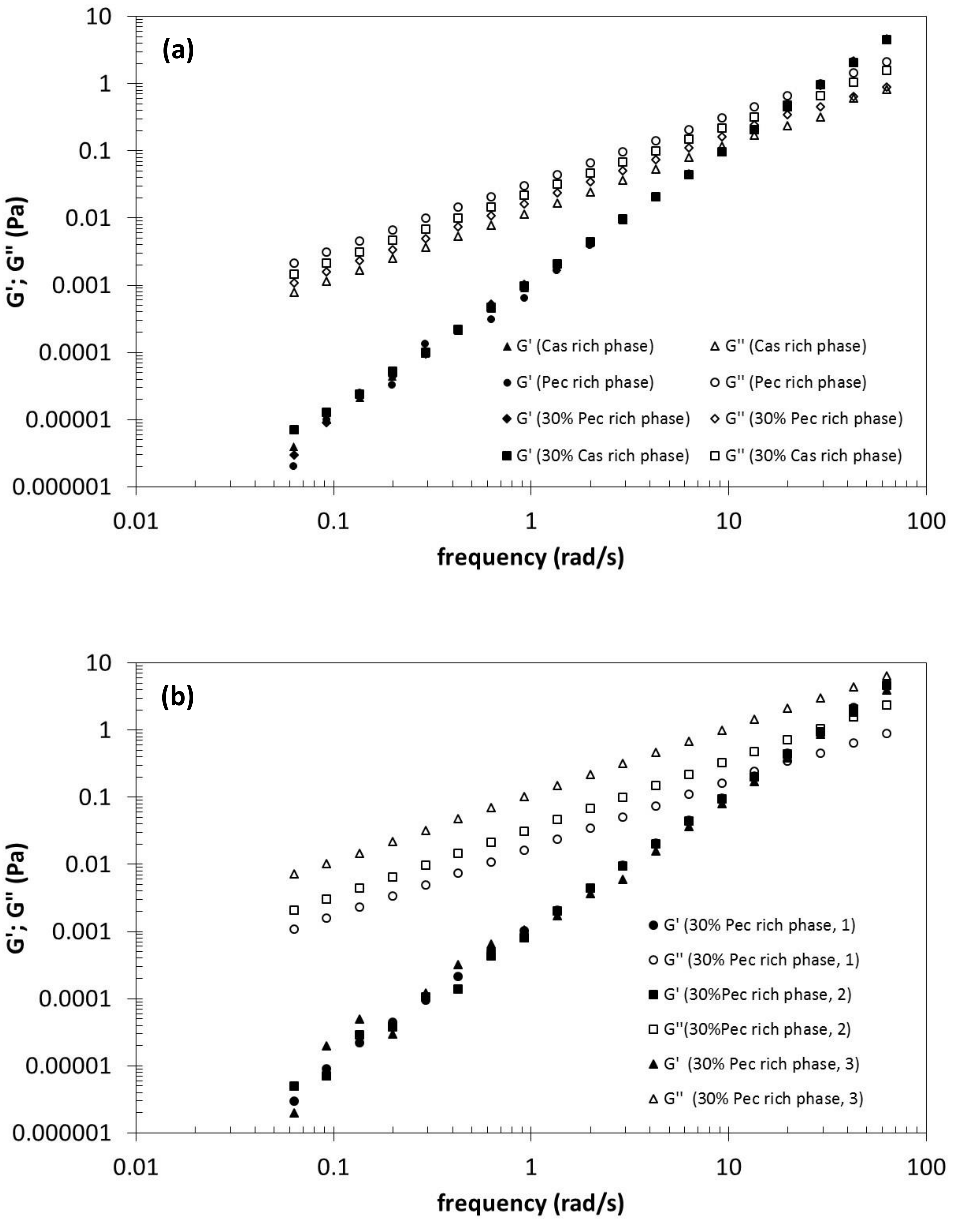

Figure 3. Dynamic shear moduli of caseinate-pectin-water system (Cas-Pec-Water), (a) for equilibrium phases, 30% pectin rich and 30% caseinate rich phases, for tie-line 1 on Figure 1. (b) for 30% equilibrium top phase (Pec rich) for tie-lines 1, 2 and 3 of Figure 1.

Elastic modulus did not change with volumetric fraction of dispersed phase while loss modulus increased; therefore, crossover frequency increased, and the related relaxation time decreased. This can be seen in Figure 4 for tie-line 1.

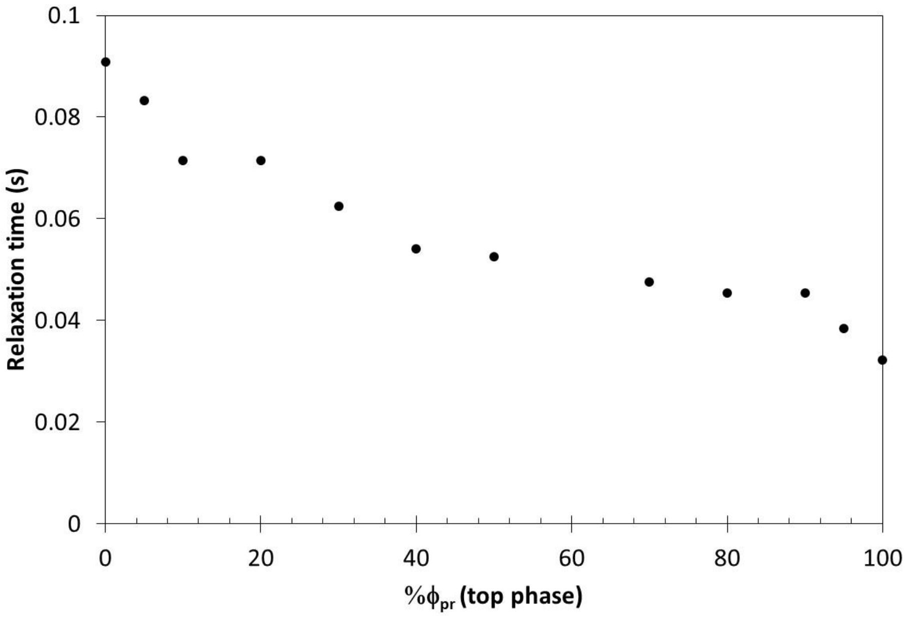

Figure 4. Relaxation time *vs*. shear rate for single phases and emulsions along tie-line 1 of Figure 1 (Cas-Pec-water system).

Relaxation times of these emulsions were clearly related with relaxation time of continuous phase, as it can be seen from the tendencies when volumetric fraction of dispersed phase approaches to zero, i.e., approaching to zero of pectin rich phase or approaching to 100% of pectin rich phase. Near these limits the continuous phase was the majority one. On the contrary, a high elasticity should be observed, related to the presence of concentrated or highly concentrated emulsions (Cohen-Addad and Höler, 2014). For intermediate volumetric fractions the tendencies were not so clear. This fact could indicate that at these volumetric it was not well stablished which was the continuous phase and bicontinuous structures could occur, as it is generally admitted (see for example Norton and Frith, 2001).

3.2.2. Steady state viscosity

Steady state viscosity *vs*. applied shear rate was measured along tie-lines. Figure 5 shows results obtained along tie-line 3 for the Cas-Pec-Water system. Shear-thinning behavior was observed for all samples, including those of single phases. It could be related to shear-induced disentanglement and alignment of polymer chains or small aggregates in single phases, together with stretching of droplets in the direction of the flow for emulsions. Smoother shear-thinning behavior was observed for tie-lines 2 and 1, related to more diluted solutions, indicating that polymer entanglements, more important at higher concentrations, and their disentanglement with shear, have a strong influence in flow curves. Results for tie-line 1 are shown in Figure 6. In agreement with dynamic results, the Cas-rich phase presented the lowest viscosity, and it increased gradually as the % Pec-rich phase was increased. Relatively small values of viscosity were obtained in all cases. It indicated, as discussed for dynamic results, that highly concentrated emulsions were not formed in any case and, therefore, it could be assumed that the continuous phase was the one present in major ratio. No discontinuity was observed around 50% and surroundings. Some bicontinuous and/or multiple emulsion zones could exist, as a transition from caseinate-rich continuous phase to pectin-rich continuous phase was produced at intermediate volumetric fractions. The extremely low interfacial tension of water-in-water emulsions could favor the presence of these bicontinuous zones. It would only be speculation if it had to be based on these data alone; however, there are numerous references (such as Norton and Frith, 2001, or Corredig et al., 2011) with graphic evidence that bicontinuous emulsions are common in these systems.

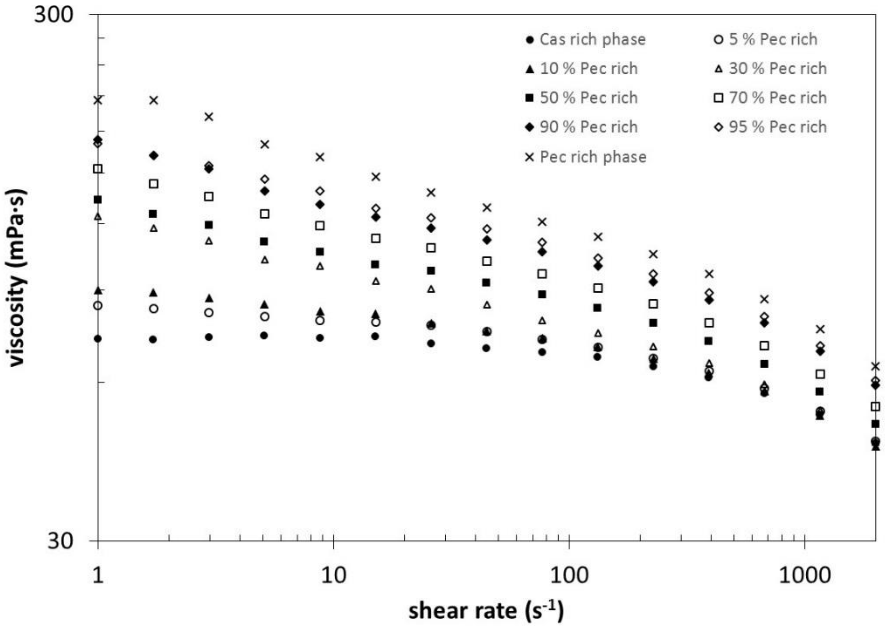

Figure 5. Steady state viscosity *vs*. shear rate for single phases and emulsions along tie-line 3 of Figure 1 (Cas-Pec-water system).

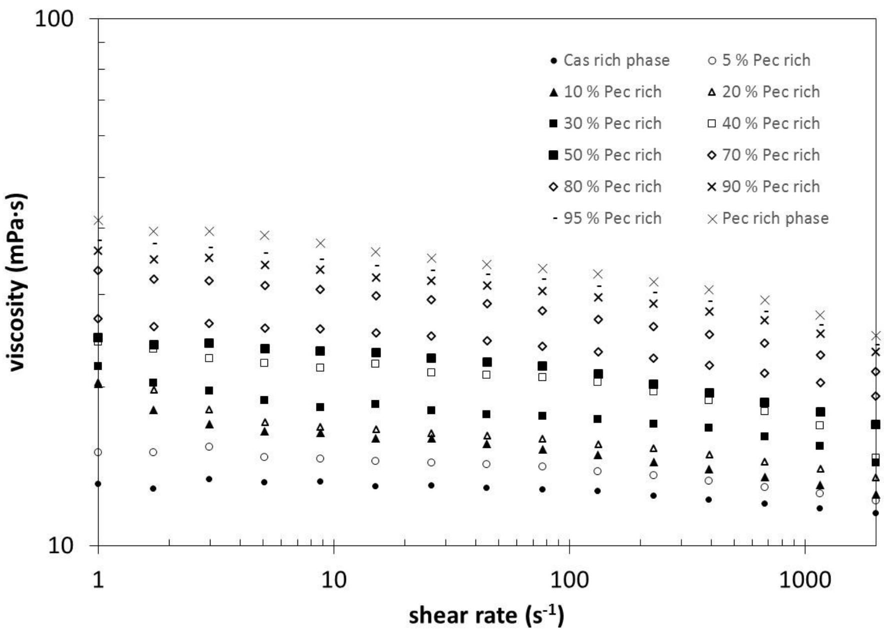

Figure 6. Steady state viscosity vs. shear rate for single phases and emulsions along tie-line 1 of Figure 1 (Cas-Pec-water system).

Figures 5 and 6 show that for samples with casein rich single-phase viscosity remained constant for a wide range of shear rates and began to decrease at a shear rate ~100 s^{-1}. However, for emulsions with pectin rich phase, shear thinning appeared at lower shear rates, and viscosity began to decrease around 1 s^{-1}.

In order to compare flow results with dynamic ones, and according to Cox-Merz rule, steady state viscosity was plotted *vs*. shear rate together with dynamic viscosity, $\eta'(\omega)=\frac{G''}{\omega}$, *vs*. ω, where ω is oscillation frequency in rad/s. Figure 7 shows that Cox-Merz rule was quite well fulfilled, with some deviations at the lowest shear rates measured in flow curves, which could be explained as for low shear rates steady state experiments were situated in the limit of the range of valid measurement for the geometry used in the rheometer, although these deviations only occurred for samples with pectin while single casein sample and casein-alginate emulsion showed good agreement with Cox-Merz rule. Therefore, using Cox-Merz superposition, flow curves could be extended to very low shear rates, where a Newtonian zone appeared and the samples could be considered undisturbed, while for pectin-containing samples it might be that single shear could cause some kind of interactions provoking the deviations.

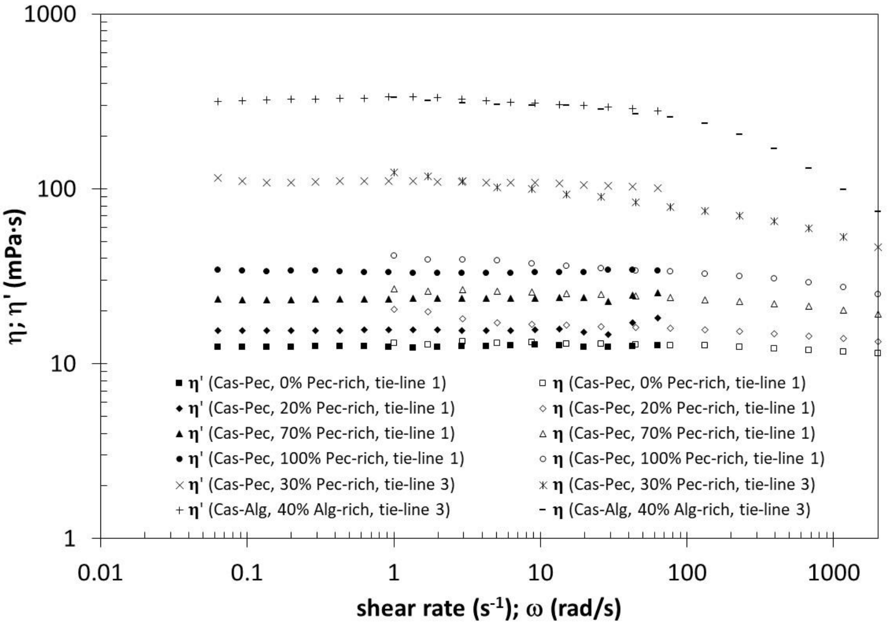

Figure 7. Cox-Merz superposition (η vs. shear rate and η' vs. ω) for several samples along some of the tie-lines studied, for Cas-Pec-Water and Cas-Alg-Water systems. T=25ºC.

3.2.3. Influence of volume fraction on the viscosity of emulsions

Viscosity of emulsions depends on viscosity of separated phases, volume fraction, interaction and elongation/orientation of droplets, which is be governed by capillary number, Ca. The Grace curve (Grace, 1982) shows the critical Capillary number, Ca_c, which must be reached for shear-induced break-up of droplets. Ca_c is dependent on the disperse/continuous viscosity ratio, λ. Ca_c decreases gradually until λ around 1, and then abruptly increases. A strong deformation is predicted at values near Ca_c, especially at low λ ratios, where the high viscosity of the continuous phase implies a high applied shear stress for a fixed shear rate, $\tau = \eta_c\dot{\gamma}$, and the viscosity of droplets is low and does not oppose resistance to deformation (Bazhlekov, Anderson, & Meijer, 2006). At high λ, the high viscosity of fluid in droplets makes them behave like a solid, and low deformation and no break-up occur, as Ca_c is high. At λ around 0.1-1 a minimum in Ca_c appears. Capillary number is inversely proportional to interfacial tension, so, for typical ultra-low interfacial tensions of W/W emulsions, about 10^{-5} N/m (Norton and Frith, 2001), even at low shear rates there will be high Ca numbers causing strong deformations.

Steady state viscosity was plotted *vs*. volume fraction of top phase at a constant low shear rate (1 s^{-1}) and high shear rate (675 s^{-1}) along a tie-line in order to study the influence of ϕ in the behavior of the water-in-water emulsions studied. Figures 8a and 8b show results for tie-line 2 of Figure 1, Cas-Pec-Water system. Predictions of Einstein, Taylor, and modified Taylor are also plotted. Capron and modified Capron models take into account the Capillary number, which is not known since R under flow is difficult to measure. However, as said above, their limits for weak deformations (low Ca) are Taylor and modified Taylor models. At very low interfacial tensions τ_1 is very high, so Eqs.(6) and (8) become nearly insensitive to shear rate (Capron, 2001). Then, Eqs.(6) and (8) can be truncated as follows:

$$\eta_{Cap} = \eta_c \left[1 + \phi \, \frac{5(\lambda-1)}{2\lambda+3}\right] \tag{9}$$

$$\tilde{\eta}_{Cap} = \eta_c \left[\frac{2\lambda+3+3\phi(\lambda-1)}{2\lambda+3-2\phi(\lambda-1)}\right] \tag{10}$$

These limits are also represented in Figures 8 labeled as Capron-low and Modif. Capron-low. Therefore, models of Eq.(6) and (8) move in the range between Eq.(2) – Eq.(9) and Eq.(3) – Eq.(10), respectively, depending on deformation of droplets. It could be assumed that interactions between droplets cannot be neglected for finite concentrations of disperse phase, so Eqs.(3) and (10) are probably more suitable. Remember that it is assumed that the disperse phase is the minority one, due to the low values of G'. Therefore, in the left hand of Figure 8 the disperse phase is the top phase, the most viscous, pectin-rich, and in the right hand, the disperse phase is the bottom one, casein-rich, less viscous. Figure 8a shows that for low shear rates the low limit of modified Capron model fits results reasonably well, especially when the disperse phase is the least viscous (ϕ_{pr}>0.5, λ<1), consistent with a strong elongation of droplets that would correspond to high Ca (because of the low interfacial tension) and low λ, in agreement to that predicted by Bazhlekov et al. (2006), for λ<1. When the disperse phase is the most viscous (λ>1, below 50% of top phase) a slight positive deviation of viscosity can be observed, related to the fact that the viscosity of droplets opposes deformation. But results are yet in the range between Eqs.(3) and (10), as predicted by the Modif. Capron model, and very near to its low limit, consistent yet with strong deformations. On the other hand, when high shear rate viscosity is plotted (Figure 8b) a negative deviation of the Modif. Capron-low model is observed, as viscosity is lower than predicted, especially when the disperse phase is the least viscous, λ<1 and strong elongations are expected. In this case, results are below the range of modified Capron model. Tromp and De Hoog (2008), working with a dextran-gelatin-Water system, demonstrated that in some cases, for systems with a low interfacial tension, shear could induce coalescence of very elongated droplets, forming wormlike structures that eventually developed into doughnut-shaped bands. In these cases, it could be considered that the phases are stratified, and the effect of edges of droplets could be neglected. Then, the resistance to momentum transfer, $1/\eta_s$, could be calculated as the sum of consecutive resistances related to the resistance of each phase in the direction of transfer and proportional to the volume fraction of each one, according to Eq.(11), also plotted in Figure 8, labeled as stratified fluids:

$$\eta_s = \left[\frac{\phi}{\eta_d} + \frac{1-\phi}{\eta_c}\right]^{-1} \tag{11}$$

where η_s is the viscosity predicted by the stratification model.

The stratified phases model fits results obtained with high shear rate surprisingly well.

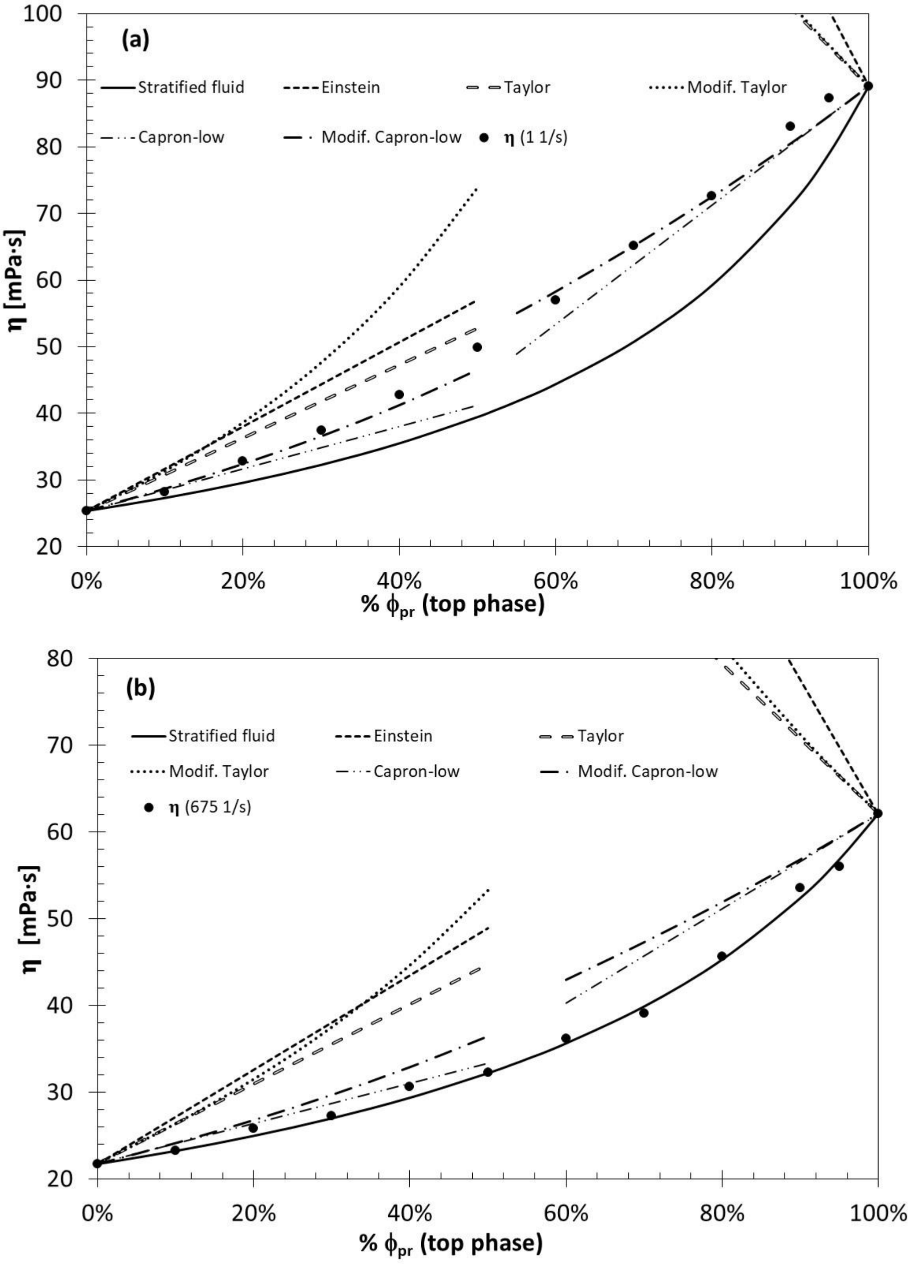

Figure 8. Stationary viscosity vs. volume fraction of pectin rich phase, ϕ_{pr}, for emulsions at 1 s^{-1} (a) and 675 s^{-1} (b), along tie-line 2 of Figure 1 (Cas-Pec-Water system).

Figure 9 shows steady state viscosity as a function of top phase volume fraction, %ϕ_{pr}, for tie-line 3 of Figure 1. As for tie-line 2, at low shear rate the system behaves into the Capron modified model, near to the low range, when the dispersed phase is the pectin rich one (λ>1, left hand of Figure 9a). However, when λ<1 (right hand), viscosities registered are below the Capron range, being even lower than those estimated by the stratification model. Here, very high elongations are expected according to the Grace curve (Grace, 1982) and Bazhlekov et al. (2006). This behavior is extended to the whole range of ϕ when a high shear rate is used (Figure 9b), when Ca is higher. To explain values below the model of two stratified phases, the contribution of a third, low-viscosity domain should be considered.

Suchkov et al. (1981) observed a similar behavior when working with the casein-sodium alginate-water system. They suggested that the emulsion could behave like a three-phase system with the protein-rich phase, the casein-rich phase, and the interphase layer, where, as polymers interact in a segregative way, it could be assumed that the concentration of polymers is much lower than those in the phases where each polymer is dominant. Therefore, the viscosity of this interphase would be considerably lower than the viscosities of the both, disperse and continuous phases. Then, the interphase could act as a lubricant and produce slippage, decreasing the observed apparent viscosity. In fact, it has been reported that interphases of W/W emulsions are usually thicker than oil-in-water interphases. Water-water interphases have length scales larger than the correlation length of the polymer solutions (Esquena, 2016; Nguyen, Wang, Saunders, Benyahia, & Nicolai, 2015).

If the thickness of the interphase is not negligible and its viscosity is considerably lower than the viscosity of continuous and disperse phases, the contribution of the interphase on the apparent viscosity of the emulsion could be relevant. A new model is proposed where the droplets could be completely elongated and, due to shear-induced coalescence, form doughnut-shaped bands, consistent with Tromp and Hoog (2008), surrounded by a relatively thick interphase, consistent with Nguyen et al. (2015) and Suchkov et al. (1981). Now the resistance to momentum transfer for three stratified phases, 1/η_{3s}, could be calculated as the sum of three consecutive resistances corresponding to the resistances of the continuous and disperse phases and the resistance of the interphase. For this configuration, the effective volume fraction of each phase must be redefined in such a way that:

$$y_{pr} + y_{cr} + y_i = 1 \qquad (12)$$

where y_{pr} is the effective volume fraction of pectin-rich phase, y_{cr} is the effective volume fraction of casein-rich phase, regardless of which is the disperse one, and y_i is the effective volume fraction of interphase.

The model proposed would result in:

$$\frac{1}{\eta_{3s}} = \frac{y_{pr}}{\eta_{pr}} + \frac{y_{cr}}{\eta_{cr}} + \frac{y_i}{\eta_i} \tag{13}$$

Assuming, as a first approximation, that the thickness of the interphase is constant and the size and shape of droplets do not change along the tie-line, y_i would be proportional to the volume fraction of disperse phase, according to:

$$y_i = a \cdot \phi = a \cdot \phi_{pr} \quad \text{if} \quad \phi_{pr} < 0.5 \tag{14}$$

$$y_i = a \cdot \phi = a \cdot \left(1 - \phi_{pr}\right) \quad \text{if} \quad \phi_{pr} > 0.5 \tag{15}$$

Eqs.(14) and (15) could approximately be described by the continuous function:

$$y_i = a \cdot \phi_{pr} \cdot \left(1 - \phi_{pr}\right) \tag{16}$$

Accepting that both ϕ_{pr} and ϕ_{cr} contribute in the same proportion to the formation of the interfacial phase and combining Eq.(12) and (16):

$$y_{pr} = \phi_{pr} - 0.5 \cdot a \cdot \phi_{pr} \cdot (1 - \phi_{pr}) \tag{17}$$

$$y_{cr} = \left(1 - \phi_{pr}\right) - 0.5 \cdot a \cdot \phi_{pr} \cdot \left(1 - \phi_{pr}\right) \tag{18}$$

And Eq.(13) can be rewritten as:

$$\eta_{3s} = \left[\frac{\phi_{pr} - 0.5 \cdot a \cdot \phi_{pr} \cdot (1 - \phi_{pr})}{\eta_{pr}} + \frac{1 - \phi_{pr} - 0.5 \cdot a \cdot \phi_{pr} \cdot \left(1 - \phi_{pr}\right)}{\eta_{cr}} + \frac{a \cdot \phi_{pr} \cdot (1 - \phi_{pr})}{\eta_i}\right]^{-1} \tag{19}$$

The viscosity of the interphase has been roughly assumed to be the viscosity of pure water at 25°C. Therefore, the only fitting parameter is *a*, that is calculated to be around 0.0037 for the best fitting results to the whole range of ϕ_{pr} (Figure 9b). It can be observed that a good correspondence between results and prediction is obtained. It means that a promising fitting is obtained assuming a percentage of interphase thickness below the 0.4% of the disperse phase, which could be acceptable. Of course, viscosity of the interphase is not known and could be slightly higher than the one of water, but this would just produce a slight increase of *a*, maintaining it in a reasonably low percentage of disperse phase, which can be physically possible for highly elongated droplets. On the other hand, it has been assumed that the shape of droplets is constant along the tie-line. This fact does not need to be true, firstly because interactions, break-up and coalescence between droplets can change if ϕ changes, but especially because when disperse and continuous phases are inverted, Ca number changes, since the viscosity of continuous phase changes its value. Therefore, a better correspondence could be obtained with different *a* parameter for ϕ_{pr} above and below 50 %, and even fit results for $\phi_{pr}>0.5$ for low shear rates (right hand on Figure 9a). However, the aim of this work is not to obtain a perfect fitting, just to point out a physical phenomenon (stratification and influence of an interphase with low viscosity), and propose a mathematic model consistent with this physical behavior. This physical phenomenon could be considered to understand the actual structure of water-in-water emulsions under shear. Too many unknown factors affect results, due to the fact that emulsions are non-equilibrium systems, to pretend to perfectly predict viscosity.

In fact, results seem to indicate that several phenomena can appear simultaneously. Tromp and De Hoog (2008) reported the coexistence of coalesced doughnut-shaped, stratified droplets, with just elongated ones. So, intermediate behaviors between Capron and stratified model could appear and the effect of the low viscosity interphase could be masked, depending on the actual structure, that would be influenced by viscosity of disperse and continuous phase, preparation variables, especially Ca, the Ca number during measurement and its relation with Ca_c, interfacial tension, λ, ϕ… However, the point of view of this work could be useful in order to deep on structures present in W/W emulsions under shear, which could be helpful, for example, in their use as texture makers especially in food field. For example, gelation of disperse and/or continuous phase at different shears could conduct to structures with different influence of this third, interfacial phase, that could provide lubrication to the mixture to mimic some textures, like that of fat, to partially substitute it.

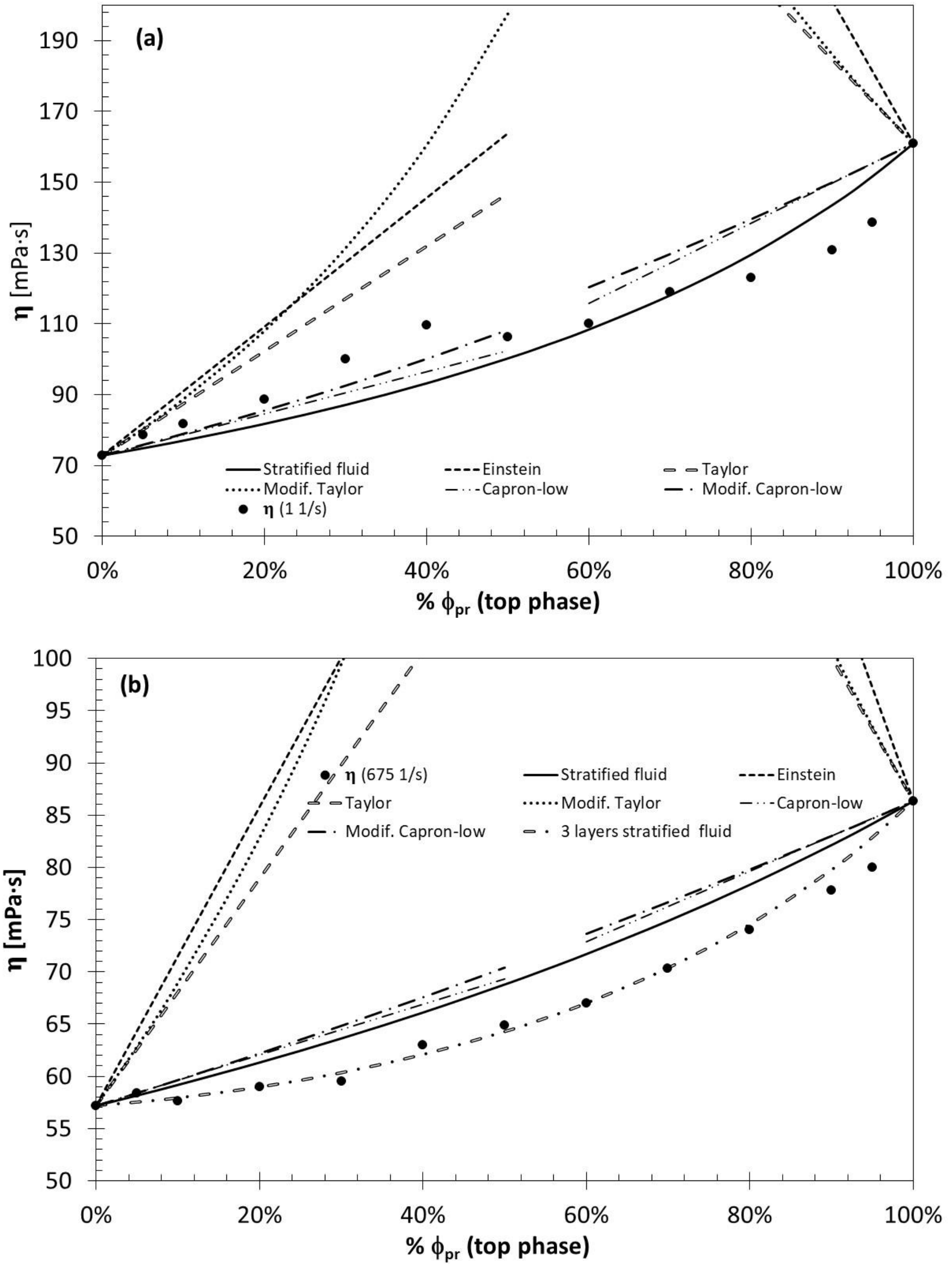

Figure 9. Stationary viscosity vs. volume fraction of pectin rich phase, ϕ_{pr}, for emulsions at 1 s^{-1} (a) and 675 s^{-1} (b) along tie-line 3 of Figure 1 (Cas-Pec-Water system).

For comparison purposes, results for the tie-line 3 on Figure 2 (Cas-Alg-Water system) have been plotted together with discussed models in Figure 10. It can be observed that the behavior is completely different if the disperse phase is the alginate rich (ϕ_{ar}<0.5) or the caseinate rich phase (ϕ_{ar}>0.5), due to the strong difference between the viscosities of alginate-rich and caseinate-rich phases. Because of these differences, all the models run quite close for ϕ_{ar}<0.5. In order to separate curves, viscosity was plotted in a semi-logarithmic scale.

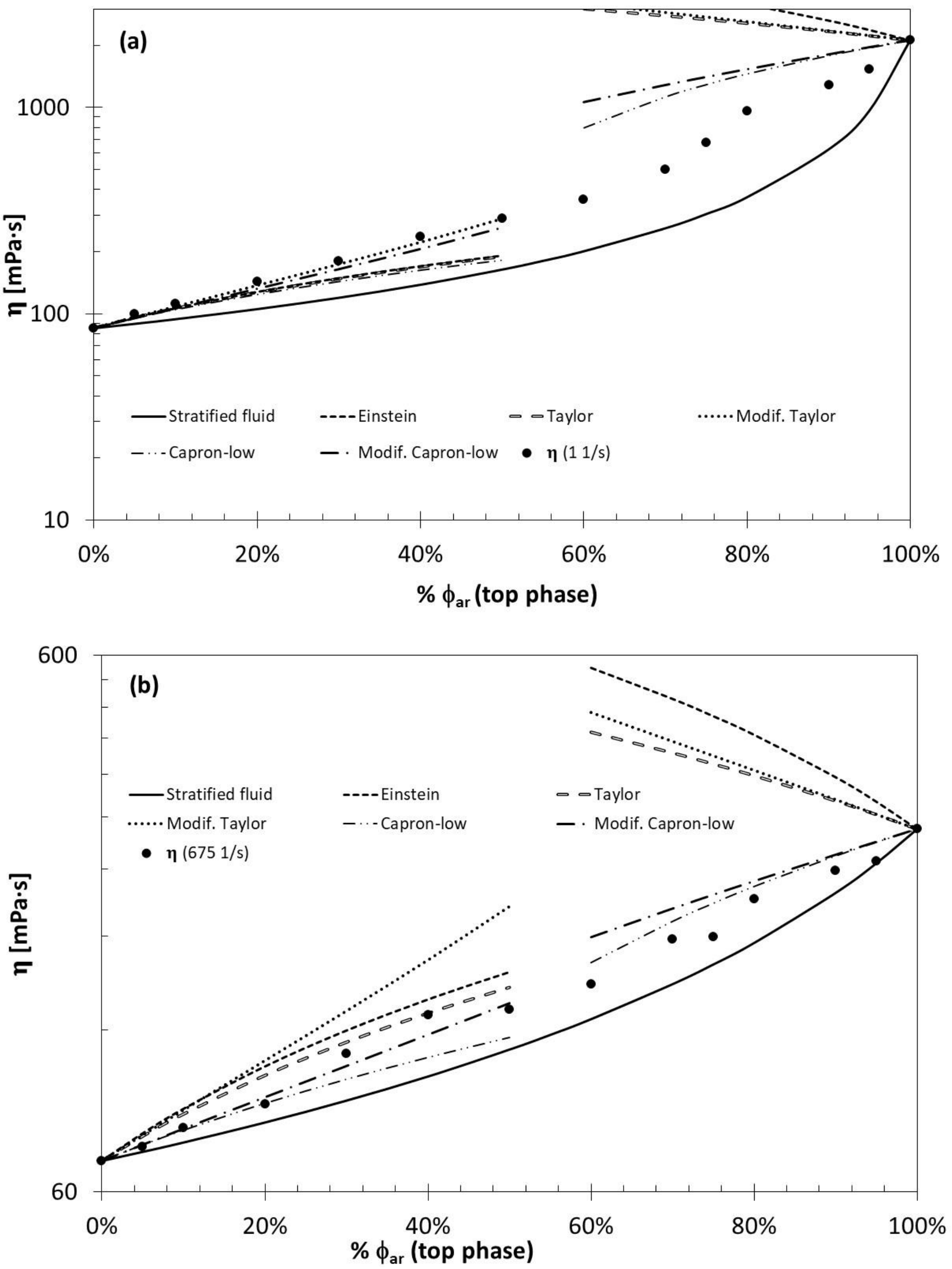

Figure 10. Semi-logarithmic representation of stationary viscosity vs. volume fraction of alginate rich phase, ϕ_{ar}, for emulsions at 1 s^{-1} (a) and 675 s^{-1} (b) along tie-line 3 of Figure 2 (Cas-Alg-Water system).

Figure 10a shows that when the disperse phase is the alginate-rich one (ϕ_{ar}<0.5) and a low shear rate is applied, results can be fitted quite well by the modified Taylor model, that takes into account hydrodynamic interactions between droplets and just small deformations.

These results are in agreement with the existing high $\lambda \approx 25$. According to the Grace curve (Grace, 1982), very poor deformation is expected when the disperse phase is much more viscous than the continuous one, as the high viscosity of droplets fights against deformation and they behave nearly as solid particles. When a high shear is applied to the same emulsions (Figure 10b, left hand) results are located in the range of modified Capron model, near the low range, indicating a more important deformation of droplets, as λ decreases to lower values, around 4.2, due to the high shear-thinning behavior of the alginate-rich phase. However, this decrease is not enough to observe stratification, as results do not cross the low limit, due to the yet high value of λ. On the other hand, when the disperse phase is the caseinate-rich one ($\phi_{ar}>0.5$), apparent viscosity values of emulsions are located below the Capron model range but they do not reach that of stratified model whatever the shear rate, indicating an intermediate situation where, as observed by Tromp and De Hoog (2008), coalesced stratified droplets could coexist with elongated individual ones. These high deformations are in agreement with the low λ values for these cases, around 0.04 for low shear rates and around 0.24 for high shear rates. As experimental viscosities do not reach the stratification model, it has no sense to apply the 3 phase stratification model. This fact does not exclude the presence of a thick interphase, predicted for the cas-pec-water model and discussed by Nguyen et al. (2015) and Suchkov et al. (1981). If stratification is not strong enough, the influence of the interphase on the apparent viscosity may not be relevant or be masked, as it will affect only the stratified zones. Grace (1982) reported that for λ below a critical value break-up of droplets can take place, especially when the viscosity of the continuous phase is very high, as then the Ca applied is high and can reach the critical Ca_c. Grace states that prediction of emulsion viscosity is very difficult in this regime, where arbitrary strong drop deformations or break-up can appear. Therefore, a single model may not be able to fit results, as a complex structure is expected involving elongated and stratified droplets, with a relatively thick interphase. However, a qualitative comparison of results with involved models can help to understand the shear-induced microstructure.

Although the structures of emulsions under shear proposed are not validated by direct observation in this work, they are supported by literature (Nguyen et al., 2015; Suchkov et al. 1981; Tromp & De Hoog, 2008), and equations developed here following the models explain extraordinarily well the experimental results for viscosity obtained in this work.

4. Conclusions

Viscoelastic behavior of W/W emulsions of the casein-pectin-water system show much higher loss than elastic moduli with crossovers at high frequencies, which increase with pectin rich phase volumetric phase.

Regarding steady state viscosity, models typically used to predict viscosity of oil-in-water or water-in-oil emulsions do not fit the apparent viscosity of the water-in-water emulsions studied, neither for low nor for high shear rates. Experimental viscosity was lower than that predicted by these models, except when the viscosity of the disperse phase was much higher than the one of the continuous phase. In this last case, modified Taylor model was able to fit results as very low deformation of droplets was expected. When higher droplet deformation can be expected, models assuming two stratified phases or even the presence of a third phase, as a thick interphase between the two phases of the emulsions, are needed for explaining the viscosity obtained for the W/W emulsions. In fact, for high shear rates a very good fitting of experimental results is achieved with these models. At intermediate shear rates, intermediate behaviors among the different proposed models can explain the experimental results.

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