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XXIV Ciclo

Interface Rheology

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Relazione del Collegio dei Docenti del Dottorato di Ricerca in
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Nell'ambito del Corso di Dottorato di Ricerca in "Ambiente, Salute e Processi Ecosostenibili", l'ing. Lucia Seta ha svolto una ricerca riguardante diversi aspetti della caratterizzazione delle proprietà di interfaccia diretti all'uso alimentare, sotto la direzione del prof. Bruno de Cindio dal titolo "Interface Rheology".

Il lavoro sperimentale è stato realizzato in due fasi complementari tra di loro, la messa a punto della metodica e l'applicazione a sistemi specifici, che hanno richiesto notevoli dedizione e competenze scientifiche specifiche.

La ricerca è stata realizzata presso il, LAboratorio di Reologia ed Ingegneria Alimentare, del Dipartimento di Modellistica per l'Ingegneria, dove da tempo è stata avviata una ricerca di base indirizzata allo studio dei sistemi multifasici particolarmente per il settore alimentare.

Il lavoro svolto dall' ing. Lucia Seta ha presentato carattere di originalità e completezza, in quanto ha affrontato un argomento estremamente complesso usando le conoscenze di base della termodinamica e della reologia per caratterizzare superfici di separazione di fasi. Il lavoro ha affrontato inizialmente l'aspetto critico del classico approccio fenomenologico per introdurre una più solida teoria che definisce in maniera rigorosa il concetto di concentrazione in eccesso, nei casi di sistemi reologicamente complessi.

In questo contesto è da rimarcare la messa a punto di una nuova metodica di misura basata sulla deformazione dilatazionale che ha consentito di verificare l'applicabilità sulla superficie di una equazione costitutiva del tipo del gel critico, verificata per sistemi contenenti surfattanti proteici. Il lavoro risulta pioneristico sia sperimentalmente sia teoricamente, ed è esaustivo nello studio dello stato dell'arte dell'argomento eseguito con estrema competenza, I risultati generali ottenuti sinora sono direttamente calabili in realtà applicative per la predizione della stabilità di sistemi di interesse industriale.

L'intero lavoro di ricerca si è caratterizzato per l'assenza di specifici corsi universitari consolidati sull'argomento trattato, e per la necessità di ampliamento delle conoscenze di base per cui in questo senso è da apprezzare l'autonomia, la professionalità e la capacità di apprendere dell'ing. Lucia Seta, che si è dimostrata in grado di coordinare con successo i vari aspetti del lavoro, venendo in contatto con argomenti per larga parte nuovi.

L'ing. Lucia Seta partecipa a diversi progetti di ricerca attivi presso il laboratorio LaRIA anche in maniera autonoma, mettendo in evidenza le sue notevoli doti e capacità scientifiche. Si vogliono infine rimarcare le ammirevoli qualità relazionali messe in mostra in tutti questi anni di dottorato, che le hanno consentito una facile interazione con tutti i gruppi di ricerca non solo del laboratorio, come dimostrato dall'essere stata tra i promotori del progetto PePeCal selezionato come Spin Off per l'incubazione nel progetto Crescita e tarsformatosi in R&DCal con l'incorporazione di alcune società di servizio.

Il Collegio dei Docenti, visto l'impegno profuso e la qualità della sua attività, esprime un giudizio pienamente favorevole ai fini dell'ammissione dell'ing. Lucia Seta all'esame finale per il conseguimento del titolo di Dottore di Ricerca in "Ambiente Salute e Processi Ecosostenibili".

Addì, 28 novembre 2011

Il Coordinatore del Collegio
Prof. Bruno de Cindio

To Carlo

thanks for all the love and support

you've given me

Acknowledgements

I would like to express my gratitude to my academic supervisor, Prof. Bruno de Cindio who first believed in me and gave me all the tools for a successful career.
I can't thank you enough for giving me this tremendous opportunity. Working in your laboratory has been an extremely worthwhile experience, scientifically, professionally and personally.

Words are not enough to convey my sincere gratitude to Dr. Noemi Baldino, who gave a fundamental contribution to this work. She supported me in every step of PhD with great professionalism and effect. Thank you very much for your technical and human support.

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Table of contents

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Keywords: *food multiphase system, viscoelastic protein network, mechanical and kinetic aspects, model systems, proteins, emulsifiers, polysaccharides, synergic or competitive effects, dynamic interfacial tensions, interfacial dilational rheology, pendant drop method, case studies*

Introduction

In the present PhD thesis the functionalities of protein surfactants typically used as basic ingredients for the formation and stabilization of *food multhiphase systems* were investigated at the air-water (A/W) and oil-water (O/W) interfaces in order to predict their probable effect on the macroscopic properties of the final products.

The increasing importance of this subject is emphasized by the fact that the proteins are often the main surface active agents present in food emulsions and foams owing to their natural strong tendency to adsorb at the interfaces forming a network which is assumed to play a key role in the formation and stabilization of these systems. Emulsions are, in fact, inherently unstable owing to their large interfacial area, but they are made "kinetically stable" for a defined period of time by the inclusion of the surface active molecules. These molecules are able to adsorb at the interfaces between the dispersed and continuous phases and to reduce the interfacial tension, enhancing the dispersion of one phase into the other and forming a protective interfacial film layer around the bubbles/droplets, which prevents them from instability phenomena.

The aim of this thesis is to obtain systematic information on the importance of *mechanical and kinetic aspects* linked to the formation of a *viscoelastic protein network* at the O/W and A/W interfaces in a "*model systems*" (water-oil/air-surfactant) relevant for industrial applications and containing up to two types of surfactants. These simplified systems proved to be useful to evaluate the interactions between the single protein and surfactant at the interface and, at the same time, to study the evolution of *synergic or competitive effects* to be ascribed to the simultaneous presence in the system of other components (such as low molecular weight surfactants and polysaccharides) commonly used together with the proteins in emulsions and foams. *Proteins, emulsifiers and polysaccharides* are known to stabilize multiphase systems by very different mechanisms and they can interact both in the bulk and at the interface, strongly affecting the composition and the molecular

structures of the interfacial layer with important effects on the final properties of the system.

To understand the interfacial adsorption phenomena of different food surfactants and the effect of their interactions on the specific mechanical properties of the resulting viscoelastic layers, the adsorption rates and the interfacial structures have to be characterized.

To this purpose in this thesis *dynamic interfacial tensions* and interfacial rheological measurements were performed by using the pendant drop method, an experimental technique which is today widely employed both for tensiometric tests and for *dilational rheology* investigations owing to its always better applicability to both liquid-liquid and liquid-air interfaces. The rheological tests were carried out in the dilational regime, because it is a tool very sensitive to the kinetics of adsorption and desorption of the molecules at the interface and, then, suitable to monitor the evolution of the interfacial structures, since it implies a deformation in the interfacial area.

Different proteins (Ovalbumin, β-Casein, β-Lactoglobulin and milk whey proteins) varying in structure from flexible to rigid/globular and, as stated before, of interest for many industrial processes (involving mainly emulsion formation), were selected and their interfacial behavior, in the presence or not of other components, was investigated, separately, focusing mostly on the O/W interface characteristics. In fact, the interfacial adsorption and rheology of the protein Ovalbumin alone was evaluated at both A/W and O/W interfaces owing to its known foaming ability (chapter 3), whereas the other proteins, all derived from milk and typical ingredients for dairy emulsions, were characterized just at the O/W interface.

This approach allowed the development of different *case studies* concerning the investigation of the interactions between different surfactants mainly present in dairy emulsion interfaces (except for ovalbumin). The investigated case studies were structured as scientific papers and were reported, in the arrangement of the thesis, as separate chapters (3-7). In the preliminary chapters the state of the art on the nature of the interfacial region and the experimental techniques for characterizing its properties is presented; some key aspects of interfacial rheology under dilatational deformations (chapter 1) and the experimental technique known as *pendant drop method* (used in the present thesis) were widely described (chapter 2).

In the chapter 3 the competitive adsorption of ovalbumin protein and two different types of food emulsifiers, nonionic Tween 60 and anionic Admul Datem, respectively, was investigated at the air-water and sunflower oil-water interfaces.

Chapter 4 discusses the adsorption mechanism and the rheological behavior of milk proteins β-Casein and β-Lactoglobulin, both alone and in binary mixtures at the sunflower oil-water interface.

Chapters 5 and 6 deal with the kinetic and mechanical aspects investigated with a mixed β-Casein/ β-Lactoglobulin system (chosen among those studied in the previous case study, characterized by a bulk weight ratio between β-Casein and β-Lactoglobulin of 1:1) in the presence of two food emulsifiers (Tween 60 and Admul Datem) and of various polysaccharides (k-carrageenan , ι-carrageenan and guar gum) respectively.

Finally, in chapter 7, both bulk and interfacial rheological properties of biphasic systems, based on milk whey protein, iota-carrageenan and kappa-carrageenan biopolymers, were investigated in order to find a connection, between them, useful to analyze the short term stability of the investigated systems.

Chapter 1

General overview and state of the art

1.Introduction

The majority of manufactured processed foods are multiphase systems containing two or more immiscible phases (aqueous, oil or gas phases) in the form of foams and emulsions. These systems are inherently unstable owing to their large interfacial area, but they are made "kinetically stable" for a defined period of time by using surface active molecules able to adsorb at the surfaces/interfaces between the dispersed and continuous phases and to reduce the interfacial tension, thereby, enhancing the dispersion of one phase into the other and forming a protective interfacial film layer around the bubbles/droplets, which prevents the droplets/bubbles from coming close enough together to aggregate and, thereby, becoming unstable [McClements (1999)(a)].

Starting from this evidence it appears clearly that the bulk physicochemical properties of these systems, such as their ease of formation, stability, and texture, are governed by the nature of the interface, and thereby, by the composition of the interfacial region which strongly affects the bubble/drop interaction forces [McClements (1999)(a), Bos and van Vliet (2001), Murray (1998), Wilde (2000)]. The surface active molecules adsorbing at the interfaces need to be amphiphilic, and thus are attracted to the interfacial region so that their hydrophilic and hydrophobic components may associate with the respective polar and non-polar phases. There are two classes of surface-active molecules, which can stabilize foams and emulsions:

- ✓ Surfactants with low molecular weight (LMW) which include detergents, emulsifiers and lipids. They may be water or oil soluble, and usually form a compact adsorbed layer with a low interfacial tension.
- ✓ Polymers which are amphiphilic macromolecules, and the most commonly used are proteins. They typically form a visco-elastic, irreversibly adsorbed layer.

Surfactants stabilize foams and emulsions most effectively if they form a fluid adsorbed layer, which allows them to migrate to regions with a reduced surfactant concentration, due to perturbation during creation, mixing or transport processes. This is known as the Marangoni mechanism [McClements (1999)(a), Wilde (2000)]. In contrast, polymers are

most effective when they form a solid visco-elastic adsorbed layer, which is most commonly observed in proteins. They adsorb, partially unfold and form strong interactions, and this results in a visco-elastic adsorbed layer, the strength of which has been correlated with foam and emulsion stability [McClements (1999)(a), Wilde (2000)].

But a problem that occurs particularly in food foams and emulsions is that they are stabilized by including a mixture of different types of surface-active components (e.g., proteins, polysaccharides, phospholipids, and surfactants), rather than a single chemically pure type, which can compete for the interface causing often also a drastic loss of stability [McClements (1999)(b), Maldonado-Valderrama and Rodrìguez-Patino (2010)]. Among them, proteins and low molecular weight (LMW) surfactants play a crucial role in defining both the structure and behavior of the interfacial layer which in turn control functionality of the bulk material whereas generally polysaccharides imparted a sufficiently thick continuous phase that inhibited the tendency of the dispersed phases to aggregate [McClements (1999)(b), Bos and van Vliet (2001)].

The main aspects to consider about the formation and stabilization of emulsions and foams can be divided in those related to the physical properties of the fluids and those related to the presence of surface-active species in the interface between the fluids, which are the focus of this thesis.

Important fluid properties are the viscosity of the continuous phase η_c and of the dispersed phase η_d, their densities, the purity and the polarity of these phases and the pH and ionic strength of the continuous phase [McClements (1999)(b), Bos and van Vliet (2001)].

Important aspects regarding potential interfacial surface active molecules are their capability to lower the interfacial tension and the rate of lowering; the adsorbed amount; their ability to desorb; the possibility to change their conformation during and after adsorption; the thickness of the adsorbed layer; the interaction between the adsorbed molecules; and their lateral mobility [Bos and van Vliet (2001)].

There are several methods and techniques which can give information about these latter properties, e.g. ellipsometry to determine the adsorbed amount and layer thickness, and spectroscopic methods to determine lateral mobility and conformational changes, tensiometry to establish the mechanisms and the rate of the adsorption of the molecules at the interfaces and the interfacial rheology techniques to study the response of the interfacial film to a specific deformation [McClements (1999)(a), Bos and van Vliet (2001)]. Both the latter two techniques often used in a synergic way, proved to be particular important to characterize and to discretize the interfacial properties of many

surface active molecules adsorbed at different interfaces air/water (A/W) or oil/water (O/W), giving useful information to control and predict the mechanisms underlying their functionality in the multiphase systems.

Although the surface activity of surfactant molecules is certainly an important aspect to investigate so as to determine the mechanical work necessary to form the multiphase system, the lowering of interfacial tension does not by itself explain the stability of surfactant-based emulsions/foams. The essential stabilizing function of surfactants is that they enable the fluid interface to resist tangential stresses and area changes from the adjoin flowing liquids preventing the droplets/bubbles from coming close enough together to aggregate and becoming unstable [Dickinson (1998), Murray (1998), Wilde (2000)]. This function can be investigated by interfacial rheological measurements, which are defined typically for both compressional deformation (dilational rheology) and shearing motion of the interface (shear rheology).

Dilational rheology is determined by measuring the change in interfacial tension, due to a specific change in interfacial area, maintaining a constant shape and it is a measure of the resistance to compression and expansion of the adsorbed layer, whereas shear rheology accounts for changes in shape at constant area, giving a direct measure of the mechanical strength of the adsorbed layer subject to a shear strain [McClements (1999)(a), Bos and van Vliet (2001), Maldonado-Valderrama and Rodrìguez-Patino (2010)].

These two methods are complementary and focus on different aspects of the interfacial layer, and their combination is useful to better interpret the composition, interactions and mechanical behavior of a deformed film. But while shear viscosity may contribute appreciably to the long-term stability of dispersions, dilatational rheology plays an important role in the short-term stability [Bos and van Vliet (2001), Benjamins and Lucassen-Reynders (1998)]. Specifically, interfacial dilatational rheology is a very sensitive technique to monitor the interfacial structure and concentration of single emulsifiers at the interface [Rodrìguez-Patino et al.(2007), Rodrìguez-Patino et al.(2003), Wilde et al. (2004)] or the relative concentration, the competitive adsorption, and the magnitude of interactions between different emulsifiers at the interface [Rodrìguez-Patino et al.(2008)].

Since the rheological interfacial dilational properties of proteins, emulsifiers and polysaccharides of food use adsorbed at the O/W and A/W interfaces, were object of study of this thesis, this chapter will describe the investigations and importance of these properties and, then, on the results obtained in this study area

Specifically, in this chapter an overview will be given of the nature of the interfacial region, experimental techniques for characterizing its properties, and the role that it plays in determining the bulk physicochemical properties of multiphase systems. The nature of the interface will be described by using both a phenomenological, thermodynamic and rheological approach.

A brief summary of some key aspects of interfacial rheology follows with an emphasis on interfacial rheology of adsorbed proteins and the protein-surfactant mixtures under dilatational deformations.

Finally, the relation between interfacial dilational rheology and the stability of emulsions and foams will be addressed.

2. Molecular basis of interfacial properties: phenomenological approach

2.1 Interfaces between two pure phases

The interface that separates the oil and water phases is often assumed to be a planar surface of infinitesimal thickness (figure 1(a)). This assumption is convenient for many purposes, but it ignores the highly dynamic nature of the interfacial region, as well as the structure and organization of the various types of molecules involved (figure 1(b)). The composition of the system therefore varies smoothly across the interfacial region, rather than changing abruptly. The thickness and dynamics of the interfacial region depend on the relative magnitude of the interactions between the molecules involved (oil–oil, water-water, and water–oil).

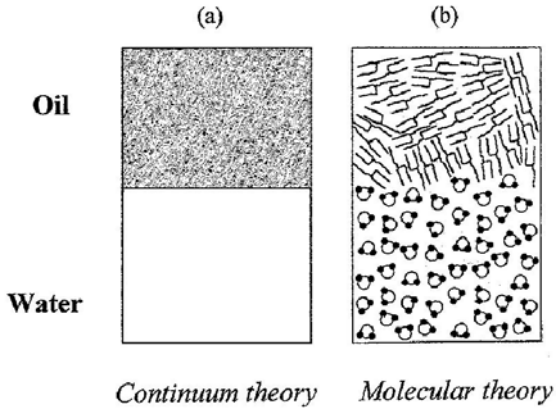


Figure 1. Scheme of Oil/Water interface according to the Continuum and Molecular theories, taken from McClements (1999)(a)

Oil molecules are incapable of forming hydrogen bonds with water molecules, and so the mixing of oil and water is strongly unfavorable because of the hydrophobic effect

[McClements (1999)(c)]. It is therefore necessary to supply energy to the system in order to increase the contact area between oil and water molecules. The amount of energy which must be supplied is proportional to the increase in contact area between the oil and water molecules [McClements (1999)(a)]:

$$\Delta G = \gamma \Delta A \qquad (1)$$

where ΔG is the free energy required to increase the contact area between the two immiscible liquids by ΔA (at constant temperature and pressure), and γ is a constant of proportionality called the in*terfacial tension*, which can be replaced by the *surface tension* if one of the phases is a gas.

The interfacial tension is a physical characteristic of a system which is determined by the imbalance of molecular forces across an interface: the greater the interfacial tension, the greater the imbalance of forces.

2.2 Interfaces with adsorbed surfactants

2.2.1 Surface Activity and the Reduction of Interfacial Tension

The surface activity of a molecule is a measure of its ability to accumulate at an interface. A molecule tends to accumulate at an interface when the free energy of the adsorbed state is significantly lower than that of the unadsorbed state [McClements (1999)(a)]. The difference in free energy between the adsorbed and unadsorbed states (ΔG_{ads}) is determined by changes in the interaction energies of the molecules involved, as well as by various entropy effects.

The change in the interaction energies which occurs as a result of adsorption comes from two sources, one associated with the interface and the other with the surfactant molecule itself. First, by adsorbing to an oil–water interface, a surfactant molecule is able to “shield” the oil molecules from the water molecules. The direct contact between oil and water molecules is replaced by contacts between the nonpolar segments of the surfactant and oil molecules and between the polar segments of the emulsifier and water molecules. These interactions are less energetically unfavorable than the direct interactions between oil and water molecules. Second, surfactant molecules usually have both polar and nonpolar segments, and when they are dispersed in bulk water, some of the nonpolar segments come into contact with water, which is energetically unfavorable because of the hydrophobic effect. By adsorbing to an interface, they are able to maximize the number of energetically favorable interactions between the polar segments and water while minimizing the number of unfavorable interactions between the nonpolar segments and water (figure 2).

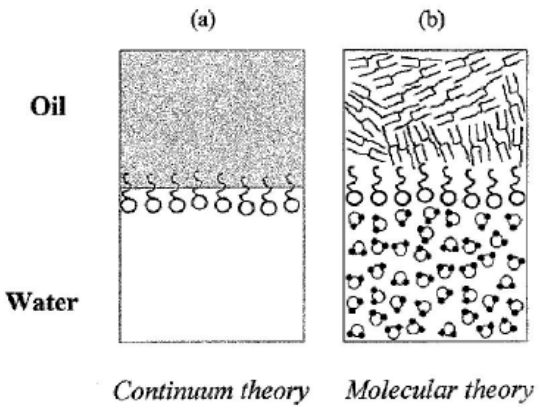


Figure 2. Scheme of Oil/Water interface with adsorbed emulsifiers, according to the Continuum and Molecular theories, taken from McClements (1999)(a)

The major driving force favoring the adsorption of an amphiphilic molecule at an interface is therefore the hydrophobic effect. Nevertheless, various other types of interaction may also contribute to the surface activity, which may either favor or oppose adsorption (e.g., hydration repulsion, electrostatic interactions, steric interactions, and hydrogen bonding).

The entropy effects associated with adsorption are mainly due to the fact that when a molecule adsorbs to an interface, it is confined to a region which is considerably smaller than the volume it would occupy in a bulk liquid and that its molecular rotation is restricted. Both of these effects are entropically unfavorable, and so a molecule will only adsorb to an interface if the energy gained by optimizing the interaction energies is sufficiently large to offset the entropy lost.

When the adsorption energy is large compared to the thermal energy (i.e., $-\Delta G_{ads} >> kT$), a molecule strongly "binds" to the surface and has a high surface activity. When the adsorption energy is small compared to the thermal energy (i.e., $-\Delta G_{ads} << kT$), a molecule tends to be located mainly in the bulk liquid and has a low surface activity.

The decrease in the free energy of a system which occurs when a surface-active molecule adsorbs to an interface manifests itself as a decrease in the interfacial tension (i.e., less energy is required to increase the surface area between the oil and water phases). The extent of this decrease depends on the effectiveness of the molecule at "shielding" the direct interactions between the oil and water molecules, as well as on the strength of the interactions between the hydrophilic segments and water, and between the hydrophobic segments and oil.

The ability of surfactant molecules to shield direct interactions between two immiscible liquids is governed by their optimum packing at an interface, which depends on their

molecular geometry [McClements (1999)(c)]. When the curvature of an interface is equal to the optimum curvature of a surfactant monolayer (i.e., optimum packing is possible), the interfacial tension is ultralow because direct interactions between the oil and water molecules are effectively eliminated. On the other hand, when the curvature of an interface is not at its optimum, the interfacial tension increases because some of the oil molecules are exposed to the polar regions of the surfactant or some of the water molecules come into contact with the hydrophobic part of the surfactant. Surfactants can usually screen the interactions between the oil and water phases more efficiently than biopolymers, which means they are more effective at reducing the interfacial tension. This is because biopolymers cannot pack as efficiently at the interface and because they often have some nonpolar regions on their surface exposed to the water phase and some polar regions exposed to the oil phase [McClements (1999)(a)].

The reduction of the interfacial tension by the presence of a surfactant is referred to as the surface pressure defined by the equation (2):

$$\pi = \gamma_0 - \gamma \qquad (2)$$

where γ_0 is the interfacial tension of a pure oil–water (or gas/water) interface and γ is the interfacial tension in the presence of the emulsifier.

2.2.2 Conformation of Surfactants at Interfaces

The conformation and orientation of molecules at an interface are governed by their attempt to reduce the free energy of the system. Amphiphilic molecules arrange themselves so that the maximum number of nonpolar groups are in contact with the oil phase, while the maximum number of polar groups are in contact with the aqueous phase (figure 3). For this reason, small-molecule surfactants tend to have their polar head groups protruding into the aqueous phase and their hydrocarbon tails protruding into the oil phase (Myers 1988). Similarly, biopolymer molecules adsorb so that predominantly nonpolar segments are located within the oil phase, whereas predominantly polar segments are located within the water phase. Biopolymer molecules often undergo structural rearrangements after adsorption to an interface in order to maximize the number of favorable interactions. In aqueous solution, globular proteins adopt a three-dimensional conformation in which the nonpolar amino acids are predominantly located in the hydrophobic interior of the molecule so that they can be away from the water. When they adsorb to an oil–water interface, they are no longer completely surrounded by water, and so the protein can reduce

its free energy by altering its conformation so that more of the hydrophobic amino acids are located in the oil phase and more of the polar amino acids are located in the water phase [McClements (1999)(a)].

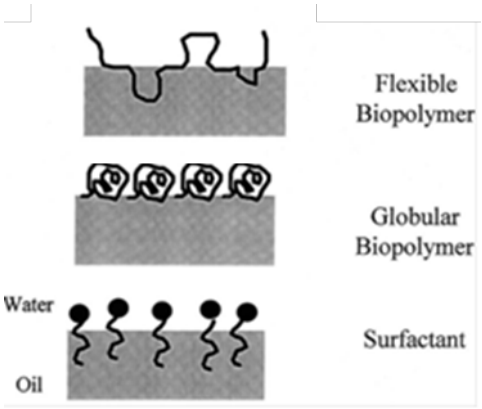


Figure 3. Scheme of the orientation and conformation of molecules at an interface are determined by their tendency to reduce the free energy of the system, taken from McClements (1999)(a)

The rate at which the conformation of a biopolymer changes at an oil–water interface depends on its molecular structure [Dickinson and Tanai (1992)]. Flexible random-coil molecules can rapidly alter their conformation, whereas rigid globular molecules change more slowly because of various kinetic constraints. Immediately after adsorption to an interface, a globular protein has a conformation that is similar to that in the bulk aqueous phase. With time, it alters its conformation so that it can optimize the number of favorable interactions between the nonpolar amino acids and the oil molecules. An intermediate stage in this unfolding process is the exposure of some of the nonpolar amino acids to water, which is energetically unfavorable because of the hydrophobic effect, and so there is an energy barrier which must be overcome before unfolding can occur. In this case, the rate of any conformational changes will depend on the height of the energy barriers compared to the thermal energy. The configuration of emulsifier molecules at an interface can have an important influence on the bulk physicochemical properties of food emulsions. The coalescence stability of many oil-in-water emulsions depends on the unfolding and interaction of protein molecules at the droplet surface. When globular proteins unfold, they expose reactive amino acids that are capable of forming hydrophobic and disulfide bonds with their neighbors, thus generating a highly viscoelastic membrane that is resistant to coalescence.

3. Interfacial properties: thermodynamic approach

3.1 Interfaces between two pure phases

To define the surface/interfacial tension with a systematic approach it is possible to consider as reference system an infinitesimal rectangular curved surface separating two immiscible fluids and having centre in the point P (figure 4).

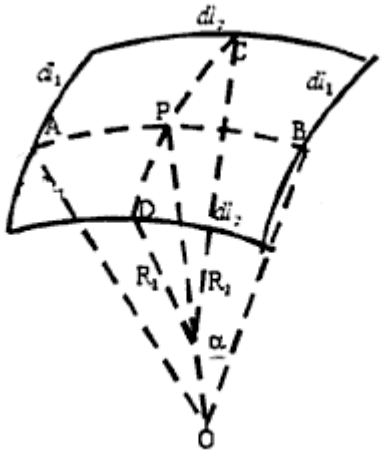


Figure 4. Schematic of an infinitesimal rectangular element of a curved surface [Paul Joos (1999)]

The lengths of this surface element are dl_1 and dl_2. At the point A, the surface tension γ, stretches the surface over a distance dl_2, giving rise to a force

$$dF_1=\gamma dl_1 \tag{3}$$

The same holds for the point B. Drawing at the points A and B lines normal to these forces, which meet each other in the point O, the α can be individuated as the angle between these lines with the line PO, representing the uniform deformation of the surface. These forces can decomposed into two normal forces (dF_{1N}, dF_{2N}) and two tangential forces ((dF_{1T}, dF_{2T}):

$$\begin{aligned} dF_{1N} &= \gamma dl_1 \sin\alpha \approx \gamma dl_1\,\alpha \\ dF_{2N} &= \gamma dl_2 \sin\alpha \approx \gamma dl_2\,\alpha \\ dF_{1T} &= \gamma dl_1 \cos\alpha \\ dF_{2T} &= \gamma dl_2 \cos\alpha \end{aligned} \tag{4}$$

where $\sin\alpha\approx\alpha$ due to the small curvature of the infinitesimal surface. The two tangential forces cancel each other due to the equilibrium to rotation, whereas the resulting total normal force is directed inside the curved surface.

Since $\alpha = dl_1/2R_1 = dl_2/2R_2$ and $dA = dl_1\, dl_2$ is the area of the surface element, we obtain for the total normal force dF_N:

$$dF_N = 2dF_{1N} + 2dF_{2N} = \gamma dA(1/R_1 + 1/R_2) \tag{5}$$

This normal force is balanced by the pressure difference inside and outside the curved surface (Laplace equation).

Then, in general and similarly to the case of the internal stress, the surface/interfacial tension can be defined as the stress, expressed as an force per unit length, which has to be applied to the surface to allow it to resist to an external force. This stress is caused by the cohesion of similar molecules, and is responsible for many of the behaviors of liquids.

Thus, considering a free surface which separates two phases the deformation power can be written:

$$W = \gamma \dot{\tilde{a}} \tag{6}$$

So the first and second thermodynamic laws for the system object of analysis can be written as:

$$\begin{aligned} \dot{\tilde{U}} &= q + \gamma \dot{\tilde{a}} \\ \dot{\tilde{S}} &\geq \frac{q}{T} \end{aligned} \tag{7}$$

And then

$$\dot{\tilde{U}} \leq T\dot{\tilde{S}} + \gamma\dot{\tilde{a}} \tag{8}$$

The Helmotz free energy in this case proves a continuous and differentiable function of the three state variable T, $\tilde{V}$, $\tilde{a}$ ($\tilde{A} = \tilde{A}(T, \tilde{a})$).

Realizing the time derivate it is obtained:

$$\dot{\tilde{A}} = \left.\frac{\delta\tilde{A}}{\delta T}\right|_{\tilde{V},\tilde{a}} \dot{T} + \left.\frac{\delta\tilde{A}}{\delta\tilde{a}}\right|_{T,\tilde{V}} \dot{\tilde{a}} \tag{9}$$

And considering the Helmotz free energy definition:

$$\dot{\tilde{U}} = \dot{\tilde{A}} + T\dot{\tilde{S}} + \tilde{S}\dot{T} \tag{10}$$

By using the equation 9, the equation 8 becomes

$$\left.\frac{\delta\tilde{A}}{\delta T}\right|_{\tilde{V},\tilde{a}} \dot{T} + \left.\frac{\delta\tilde{A}}{\delta\tilde{a}}\right|_{T,\tilde{V}} \dot{\tilde{a}} + T\dot{\tilde{S}} + S\dot{T} \leq T\dot{\tilde{S}} + \gamma\dot{\tilde{a}} \tag{11}$$

$$\left(\left.\frac{\delta\tilde{A}}{\delta T}\right|_{\tilde{V},\tilde{a}} + \tilde{S}\right)\dot{T} + \left(\left.\frac{\delta\tilde{A}}{\delta\tilde{a}}\right|_{T,\tilde{V}} - \gamma\right)\dot{\tilde{a}} \leq 0 \tag{12}$$

Because the relationship 12 must hold for any given transformation, it results:

$$\tilde{S} = -\left.\frac{\delta\tilde{A}}{\delta T}\right|_{\tilde{V},\tilde{a}} \quad p = -\left.\frac{\delta\tilde{A}}{\delta\tilde{V}}\right|_{T,\tilde{a}} \quad \gamma = \left.\frac{\delta\tilde{A}}{\delta\tilde{a}}\right|_{T,\tilde{V}} \tag{13}$$

An the following four Maxwell equations are obtained:

$$\begin{aligned}
\dot{\tilde{U}} &= T\dot{\tilde{S}} + \gamma\dot{\tilde{a}} \\
\dot{\tilde{H}} &= T\dot{\tilde{S}} + \gamma\dot{\tilde{a}} \\
\dot{\tilde{A}} &= -\tilde{S}\dot{T} + \gamma\dot{\tilde{a}} \\
\dot{\tilde{G}} &= -\tilde{S}\dot{T} + \gamma\dot{\tilde{a}}
\end{aligned} \tag{14}$$

3.2 Interfaces with adsorbed surfactants

When an interface is characterized by the presence of an adsorbed surfactant monolayer, the variation of the surface tension with the surfactant composition has to be considered and this causes some differences in the thermodynamic treatment of the interfacial behavior from that concerning the interface between two pure phases.

The complexity of this treatment is owing to the fact that the surfactant concentration has to be defined at the interface where the continuity condition is not valid. Then, it is necessary to consider the surface excess properties and to give a new definition and location of the interface. One should consider the fact that the interface is not a two-dimensional surface in the mathematical sense but a very thin layer of extremely small volume but finite mass. There are, however, distinct advantages in regarding it as a surface, and the thermodynamic theory is developed by substituting for the real system with an "ideal" model system consisting of two bulk phases and a surface-two dimensional with finite mass in the analysis. This allows the definition of the surface excess concentration.

Thus, following on the formation of an oriented surfactant monolayer, a fundamental associated physical quantity is the surface excess, which is defined as the concentration of surfactant molecules in a surface plane, relative to that at a similar plane in the bulk. A common thermodynamic treatment of the variation of surface tension with composition has been derived by Gibbs. An important approximation associated with this Gibbs adsorption equation is the 'exact' location of the interface.

Consider a surfactant aqueous phase α in equilibrium with vapour β. The interface is a region of indeterminate thickness τ across which the properties of the system vary from values specific to phase α to those characteristic of β [Eastoe (2010)]. Since properties within this real interface cannot be well defined, a convenient assumption is to consider a mathematical plane, with zero thickness, so that the properties of α and β apply right up to that dividing plane positioned at some specific value X', and a physical volume σ across the two phases α and β. Figure 5 illustrates this idealized system.

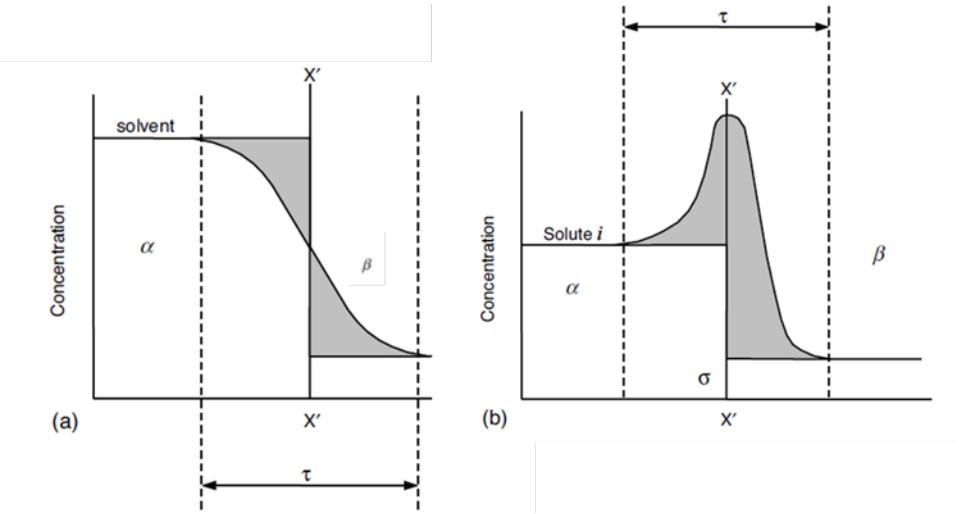


Figure 5. Idealized system for the definition of the Gibbs dividing surface [Eastoe (2010)]

In the definition of the Gibbs dividing surface X'X' is arbitrarily chosen so that the surface excess adsorption of the solvent is zero. Then, the surface excess concentration of component *i* is given by:

$$\Gamma_i^{\sigma} = \frac{n_i^{\sigma}}{A} \tag{15}$$

where A is the interfacial area. The term n_i^{σ} is the amount of component *i* in the surface phase σ . Γ_i^{σ} may be positive or negative, and its magnitude clearly depends on the location of XX'.

According to Gibbs multicomponent system theory, the internal energy U of the total system is the sum of the energy of each phase and assuming valid Maxwell equations:

$$\begin{aligned} U &= U^{\alpha} + U^{\beta} + U^{\sigma} \\ U^{\alpha} &= TS^{\alpha} - PV^{\alpha} + \sum_i \mu_i n_i^{\alpha} \\ U^{\beta} &= TS^{\beta} - PV^{\beta} + \sum_i \mu_i n_i^{\beta} \end{aligned} \tag{16}$$

The corresponding expression for the internal energy of the interfacial region σ is

$$U^{\sigma} = TS^{\sigma} + \gamma A + \sum_i \mu_i n_i^{\sigma} \tag{17}$$

Where the last term of the second side takes into account the presence of different components. In the equation 17 the pressure term, is the surface tension and the sign has to change as it is a tension instead of a pressure. The phase volume is replaced by the area of the surface. For any infinitesimal change in *T*, *S*, *A*, μ, *n*, the total derivate of U^{σ} gives

$$\dot{U}^{\sigma} = T\dot{S}^{\sigma} + S^{\sigma}\dot{T} + \gamma\dot{A} + A\dot{\gamma} + \sum_i \mu_i \dot{n}_i^{\sigma} + \sum_i n_i \dot{\mu}_i^{\sigma} \tag{18}$$

For a small, isobaric, isothermal, reversible change the differential total internal energy in any bulk phase is

$$\dot{U} = T\dot{S} - P\dot{V} + \sum_i \mu_i \dot{n}_i \tag{19}$$

Similarly, for the differential internal energy in the interfacial region

$$\dot{U}^{\sigma} = T\dot{S}^{\sigma} + \gamma\dot{A} + \sum_i \mu_i \dot{n}_i^{\sigma} \tag{20}$$

Subtracting equation (20) from equation (18) leads to

$$S^{\sigma}\dot{T} + A\dot{\gamma} + \sum_i n_i \dot{\mu}_i^{\sigma} = 0 \tag{21}$$

Then at constant temperature, with the surface excess of component *i*, Γ_i^{σ} , as defined in equation (15), the general form of the Gibbs equation (Gibbs-Duhem equation for the surface) is

$$d\gamma = -\sum_i \Gamma_i^{\sigma} d\mu_i \tag{22}$$

For a simple system consisting of a solvent and a solute, denoted by the subscripts 1 and 2 respectively, then equation (22) reduces to

$$d\gamma = -\Gamma_1^{\sigma} d\mu_1 - \Gamma_2^{\sigma} d\mu_2 \tag{23}$$

Considering the choice of the Gibbs dividing surface position, i.e. so that $\Gamma_1^{\sigma} = 0$, then equation (23) simplifies to

$$d\gamma = -\Gamma_2^{\sigma} d\mu_2 \tag{24}$$

Where Γ_2^{σ} is the solute surface excess constration.

The chemical potential is given by

$$\mu_1 = \mu_i^0 + RT \ln a_i \tag{25}$$

where μ_i^0 is the reference chemical potential of component *I* (p=1atm, and T of the system). So at constant temperature

$$d\mu_i = RTd \ln a_i \tag{26}$$

Therefore applying to equation (24) gives the common form of the Gibbs equation for non-dissociating materials (e.g. non-ionic surfactants)

$$\begin{aligned} d\gamma &= -\Gamma_2^\sigma RTd \ln a_2 \\ \Gamma_2^\sigma &= -\frac{1}{RT}\frac{d\gamma}{d \ln a_2} \end{aligned} \tag{27}$$

For dissociating solutes, such as ionic surfactants of the form R^-M^+ and assuming ideal behavior below the CMC, equation (15) becomes

$$d\gamma = -\Gamma_R^\sigma d\mu_R - \Gamma_M^\sigma d\mu_M \tag{28}$$

If no electrolyte is added, electroneutrality of the interface requires that $\Gamma_R^\sigma = \Gamma_M^\sigma$. Using the mean ionic activities so that $a_2 = (a_R a_M)^{1/2}$ and substituting in equation (28) gives the Gibbs equation for 1 : 1 dissociating compounds:

$$\Gamma_2^\sigma = -\frac{1}{2RT}\frac{d\gamma}{d \ln a_2} \tag{29}$$

The surface excess concentration of a surfactant can be determined from measurements of the variation in the surface tension of an air–liquid interface as the surfactant concentration in the bulk liquid is increased (figure 6).

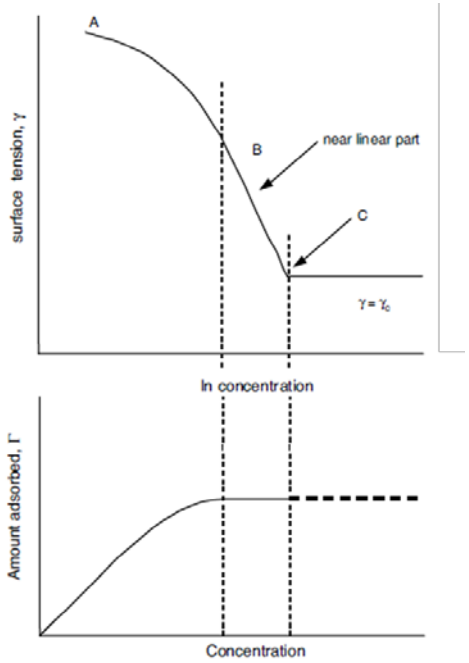


Figure 6. Dependence of the surface tension on the concentration of surfactant, taken from [Eastoe (2010)]

Knowledge of the surface excess concentration is important for formulating food emulsions because it determines the minimum amount of emulsifier which can be used to create an emulsion with a given size distribution. The smaller the value of Γ, the greater the area of the interface which can be covered per gram of surfactant, and therefore the smaller the size of droplets which can be effectively stabilized by the emulsifier. Plots of surface tension versus surfactant concentration are also useful because they indicate the maximum surface pressure (π_{max}) which can be achieved when the surface is saturated by an emulsifier, which has important consequences for the formation and stability of food emulsions and foams.

4. Interfacial rheology

In the previous section the interfacial tension has been considered as a scalar similar tio the pressure for the bulk. From a general point of view, the interfacial tension should be considered as a 2D tensor, and should be linked to specific deformation tensors. Interfacial rheology is the study of the relationship between interfacial stress and the deformation and it has long been thought important to the understanding of foams and emulsions; hence the scientific literature devoted to this subject is quite extensive [Murray (1998), Bos and van Vliet (2001)].

While the most apparent outcome of surfactant adsorption is the reduction in interfacial tension, the response of a surfactant covered interface to deformation is more relevant to understanding emulsion/foam functional properties than equilibrium interfacial tension values [Murray (1998), Bos and van Vliet (2001)], because it allow the individuation of constitutive equation valid for the interface.

In the interfacial rheology developed recently, the continuum is considered to be two-dimensional, the contact forces are proportional to the contact line length and the field forces are proportional to a unit area.

The two-dimensional rheology of an interfacial layer is very different than three-dimensional rheology. This is primarily determined by the fact that the monolayers of surfactants are characterized by an enormous (per unit of mass) contact area with adjacent phases. It is especially true for the uniform compressibility-related deformations [Krotov et al.(2009)].

It is well known that the compressibility of condensed insoluble monolayer is immeasurably higher than the compressibility of the three-dimensional condensed bodies, and soluble monolayers have no direct analogues in the three-dimensional case.

In the interfacial rheology, there are two primary types of interfacial deformation (figure 7):

- ✓ shear
- ✓ dilatation

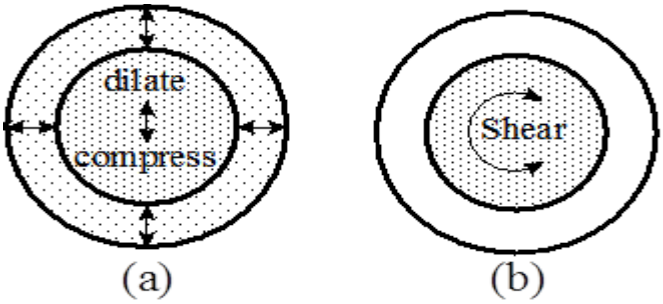


Figure 7. Example of a two-dimensional shear and dilational deformation of an interface

Shear involves perturbing a 2-dimensional interface in any direction parallel to the interfacial plane. On the contrary, in the dilatational rheology the interfacial area is changed while and the interfacial tension, γ, which is the surface stress and tends to oppose any increase in the area is measured [Murray (2002), Murray (1998), Bos and van Vliet (2001)]. While shear viscosity may contribute appreciably to the long-term stability of dispersions, dilatational rheology plays an important role in short-term stability.

Interfacial shear rheology is most useful for protein and mixed protein surfactant adsorption layers and insoluble monolayers and gives access to interaction forces in two dimensional layers [Maldonado-Valderrama (2010)]. On the other hand, interfacial dilatational rheology is a very sensitive technique to monitor the interfacial structure and concentration of single emulsifiers at the interface [Ravera et al. (2010)] or the relative concentration, the competitive adsorption, and the magnitude of interactions between different emulsifiers at the interface. However, these two contributions are usually combined in real process conditions, and therefore the composition, interactions and mechanical behavior of a deformed film is better interpreted through a synergy of dilatational and shear techniques.

The interfacial rheology has the aim to write a constitutive equation linking the surface stress to deformation and in the following two examples are considered: a purely elastic and purely viscous two-dimensional surface equations. They can be used as good approximations for monolayers of surfactant when the viscosity of the two fluid phases is sufficiently small. The generalization of a two-dimensional starting from a three-dimensional one implies a formal replacement of 3 by 2 in the expressions.

4.1 Purely elastic two-dimensional continuum

Considering a monolayer of surfactant at the interface and *x* and *y* as the Cartesian coordinates located in the monolayer interface, this monolayer can be characterized by for values of stress, expressed in a matrix form:

$$\hat{\gamma} = \begin{vmatrix} \gamma_{xx} & \gamma_{xy} \\ \gamma_{yx} & \gamma_{yy} \end{vmatrix} \tag{30}$$

Where the component γ_{xy} determine the forces in the x direction per unit length of the contact line perpendicular to the y direction.

We will consider only small perturbations, because only for this case simplest linear rules can be derived and the superposition principle applies. Therefore, the 2D tensor $\hat{\gamma}$ is represented as the sum of the non-perturbed (31)and perturbed contribution (32) [Krotov et al.(2009)]:

$$\begin{vmatrix} \gamma_0 & 0 \\ 0 & \gamma_0 \end{vmatrix} = \gamma_0 \cdot \hat{1} \tag{31}$$

$$\Delta\hat{\gamma} = \begin{vmatrix} \gamma_{xx} - \gamma_0 & \gamma_{xy} \\ \gamma_{yx} & \gamma_{yy} - \gamma_0 \end{vmatrix} \tag{32}$$

In turn, the perturbed contribution $\Delta\hat{\gamma}$ of the tension tensor can be decomposed as:

$$\Delta\hat{\gamma} = \Delta\hat{\gamma}_i + \Delta\hat{\gamma}_d = \begin{vmatrix} \frac{\Delta\gamma_{xx} + \Delta\gamma_{yy}}{2} & 0 \\ 0 & \frac{\Delta\gamma_{xx} + \Delta\gamma_{yy}}{2} \end{vmatrix} + \begin{vmatrix} \frac{\Delta\gamma_{xx} + \Delta\gamma_{yy}}{2} & \gamma_{xy} \\ \gamma_{yx} & \frac{\Delta\gamma_{xx} + \Delta\gamma_{yy}}{2} \end{vmatrix} \tag{33}$$

Where $\Delta\hat{\gamma}_i$ is responsible for the isotropic part of the tensor $\Delta\hat{\gamma}$, while $\Delta\hat{\gamma}_d$ is the deviatoric tensor, corresponding to the non-isotropic residual of the tensor $\Delta\hat{\gamma}$.

As the rotation of the coordinate system within the interface is determined by a single parameter, and $\gamma_{xy} = \gamma_{yx}$, thus one can apply the rotation to let the two non-diagonal components of the tensor in $\Delta\hat{\gamma}_d$ vanish, and, therefore, the corresponding components of interfacial tension tensor $\hat{\gamma}$ [Krotov et al.(2009)].

In a similar way, considering that the displacements of the points at a plane interface are determined by the displacement vector $\vec{u}(x, y)$, for the deformation tensor one has:

$$\hat{e} = \hat{e}_i + \hat{e}_d$$

$$\hat{e} = \begin{vmatrix} \left(\frac{\delta u_x}{\delta x} + \frac{\delta u_y}{\delta y}\right)/2 & 0 \\ 0 & \left(\frac{\delta u_x}{\delta x} + \frac{\delta u_y}{\delta y}\right)/2 \end{vmatrix} + \begin{vmatrix} \left(\frac{\delta u_x}{\delta x} - \frac{\delta u_y}{\delta y}\right)/2 & \left(\frac{\delta u_x}{\delta y} + \frac{\delta u_y}{\delta x}\right)/2 \\ \left(\frac{\delta u_x}{\delta y} + \frac{\delta u_y}{\delta x}\right)/2 & \left(\frac{\delta u_y}{\delta y} - \frac{\delta u_x}{\delta x}\right)/2 \end{vmatrix} \quad (34)$$

Where $\hat{e}_i$ and $\hat{e}_d$ are the isotropic and deviatoric contribution of the deformation tensor, respectively [Krotov et al.(2009)].

For purely elastic surfaces a linear constitutive equation gives:

$$\Delta\hat{\gamma}_i = k_i \hat{e}_i \quad (35)$$

$$\Delta\hat{\gamma}_d = k_d \hat{e}_d \quad (36)$$

where k_i and k_d are two independent constants which characterize the elastic properties of a two-dimensional continuum subject to small perturbations.

In the case of dilational and shear kinematics, it is possible to relate them to a surface elastic modulus E and shear modulus G:

$$k_i = 2E \quad (37)$$

$$k_d = 2G \quad (38)$$

One obtains from equations 35 and 36:

$$\Delta\hat{\gamma}_i = 2E\hat{e}_i \quad (39)$$

$$\Delta\hat{\gamma}_d = 2G\hat{e}_d \quad (40)$$

4.2 Purely viscous two-dimensional continuum

We turn now to the tensor formulation of rheological equations for two-dimensional viscous liquid. The simplest case, the purely viscous liquid, is defined as the continuum in which the arising forces depend only on the velocities of perturbations. In two-dimensional case the velocities are defined by the components v_x and v_y , and the tensor of deformation velocity can obtained as:

$$\hat{\dot{e}} = \begin{vmatrix} \dfrac{\delta v_x}{\delta x} & \dfrac{\dfrac{\delta v_x}{\delta y} + \dfrac{\delta v_y}{\delta x}}{2} \\ \dfrac{\dfrac{\delta v_x}{\delta y} + \dfrac{\delta v_y}{\delta x}}{2} & \dfrac{\delta v_y}{\delta y} \end{vmatrix} \tag{41}$$

Similarly with the previous discussion presented above, the tensor (41) shoud be decomposed in two tensor:

$$\hat{\dot{e}} = \hat{\dot{e}}_i + \hat{\dot{e}}_d \tag{42}$$

Then, combining the equation (42) with the equation (33) it can be found[Krotov et al.(2009)]:

$$\Delta\hat{\gamma}_i = 2\eta_d \hat{e}_i \tag{43}$$

$$\Delta\hat{\gamma}_d = 2\eta \hat{e}_d \tag{44}$$

where η is the interfacial shear viscosity and η_d the interfacial dilational viscosity. The liquid which obeys the rheological equations (43) and (44) is called Newtonian liquid, on the contrary, for the three-dimensional case, no physical continuum is known to obey simultaneously these equations.

4.3 Viscoelastic behavior

It is known from experiments that with respect to dilational and shear deformation, three-dimensional and two-dimensional continua exhibit both elastic (reversible processes) and viscous (irreversible processes) properties [Krotov et al.(2009)]. This fact is obvious from the point of view of general thermodynamics, because each process which takes place with finite velocity is accompanied by a dissipation of energy. The perturbation of the tension of the continuum can be caused by two factors acting simultaneously: the existence of deformations of different types (39,40) and the existence of deformation rates of different types (43, 44). As the perturbations are assumed to be small and, therefore, the

superposition principle for the deformation should be valid, the resulting stress is described by the tensors:

$$\Delta\hat{\gamma}_i = 2E\hat{e}_i + 2\eta_d\hat{\dot{e}}_i \quad (45)$$

$$\Delta\hat{\gamma}_d = 2G\hat{e}_d + 2\eta\ \hat{\dot{e}}_d \quad (46)$$

All components of the tensors $\Delta\hat{\gamma}_i(t)$ and $\Delta\hat{\gamma}_d(t)$ are determined by the equations 45 and 46, if the $\hat{e}_i(t)$ and $\hat{e}_d(t)$ are defined by the experimental conditions.

The integrations of the 43 and 44 equations leads to obtain:

$$\Delta\hat{\dot{\gamma}}_i + (E/\eta_d)\Delta\hat{\gamma}_i = 2E\hat{\dot{e}}_i \quad (47)$$

$$\Delta\hat{\dot{\gamma}}_d + (G/\eta)\Delta\hat{\gamma}_d = 2\ G\hat{\dot{e}}_d \quad (48)$$

The two dimensional continuum which can be described by the equations 45-46 and 47-48 are called the Kelvin-Voigt solid body and Maxwellian liquid, respectively [Krotov et al.(2009)].

Starting from the same initial expressions for the isotropic tensors and deviators, which characterize the elastic and viscous properties of the continuum, one can develop different constitutive equations for possible rheological properties.

4.4 Measurement

The measurement of interfacial rheology can take one of two approaches, either dilational or shear. The choice of approach will depend on its suitability to particular applications. The most accurate and reproducible results tends to come from methods which utilise small, reversible applied stresses or strains, thus minimising any disruption or damage to the interfacial layer (region of linear viscoelasticity). They will be described briefly below.

4.4.1 Interfacial dilational rheology

Dilational rheology, as the name suggests, deals with the expansion and compression of the interface. Simply, a mechanical system is constructed, which allows the interface to be expanded and contracted, usually in a sinusoidal manner, while the interfacial tension is simultaneously monitored.

The original method used a standard Langmuir trough, as shown in figure 8. Normally, the barriers are used to gradually compress or expand the interface to control the surface concentration of insoluble monolayers. A small modification to this method allows the barriers to be oscillated sinusoidally, producing small changes in the surface area.

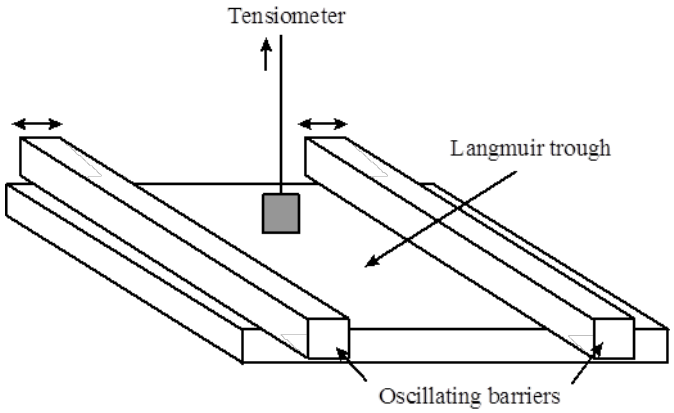


Figure 8. Use of a Langmuir trough fitted with oscillating barriers to change the surface area *A*, whilst simultaneously monitoring surface tension.

Assuming no exchange of surfactant between the surface and the bulk during the compression/expansion cycle, there will be a change in the surface tension. As the surface is compressed, the effective surface concentration increases, and the interfacial tension will go down. Conversely, expanding the surface will result in an increase in the surface tension. The relationship between surface area and surface tension is shown in figure 9.

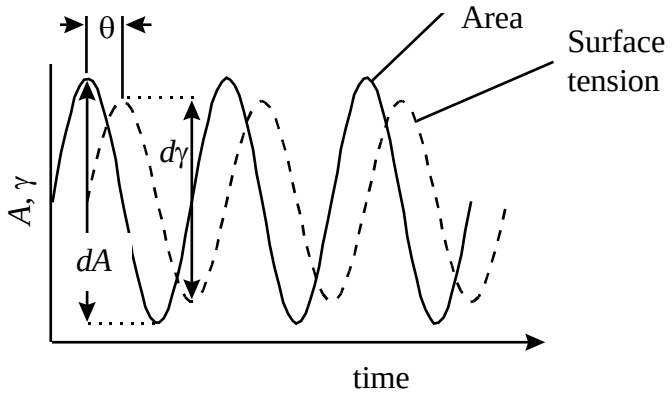


Figure 9. Time dependent relationship between area and surface tension during a typical dilational rheology experiment

The surface dilational modulus (|E|) is given as :

$$|E| = A.\frac{d\gamma}{dA} \tag{49}$$

This is then split into the elastic (E') and viscous (E'') components. If the surface is purely elastic, then the phase lag (θ) will be zero, if it is viscous then θ=90°. In practice, the

behaviour is usually intermediate between the two extremes, and the two components can be calculated as follows:

$$E'=|E|\cos\theta \qquad E''=|E|\sin\theta \tag{50}$$

Normally, experiments should be conducted in the linear region, but the length of the linear region does give information about the resistance of the surface to compression and collapse.

One limitation of the Langmuir trough technique, is that sometimes their maybe leakage of surfac active material below or around the edge of the barriers, leading to anomalies in the surface tension. Another approach is to use a ringtrough according the method of Kokelaar et al. (1991). The setup is shown in figure 10.

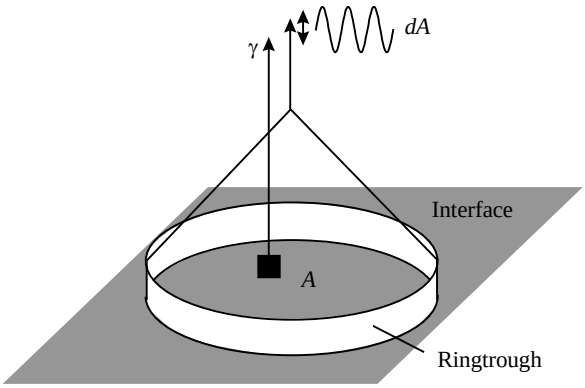


Figure 10. Schematic setup for the Ringtrough method. The ring is oscillated vertically, while the surface tension is monitored within the ring

The area *A* is located within a ring, held in the interface. The ring is oscillated up and down through the interface, effectively stretching and compressing the area within the ring. The area within the ring is totally sealed, so leakage effects are eliminated. The surface tension is measured within the ring, as close to the centre as possible, this ensures that the expansion/compression is purely dilatory, rather than having a shear component, which is the case in the Langmuir trough approach. A dilational method which has attracted much attention over recent years is the use of the pendant drop method (see chapter 2). This is particularly useful for looking at the oil-water interfacial rheology. The interfacial tension is calculated by measuring the size and shape of a liquid drop suspended from a capillary, in a less dense fluid. The interfacial area is changed by increasing or decreasing the size of the drop by controlling the liquid flow through the capillary. Changes in the interfacial area, and interfacial tension can be measured simultaneously from the dimensions of the

drop, and hence the interfacial dilational modulus can be calculated. This is a very useful technique for looking at small sample volumes, and it avoids the hydrodynamic problems encountered when trying to expand/compress oil/water interfaces.

4.4.2 Interfacial shear rheology

In contrast to the dilational technique, the surface shear methods are direct determinations of the mechanical properties of an interface. The simplest approach is a two-dimensional adaptation of standard three-dimensional viscoelastic measurements performed on a standard rheometer. The only difference is the sensitivity and the geometry. Figure 11 shows the geometries commonly used for the air-water and oil-water interfaces.

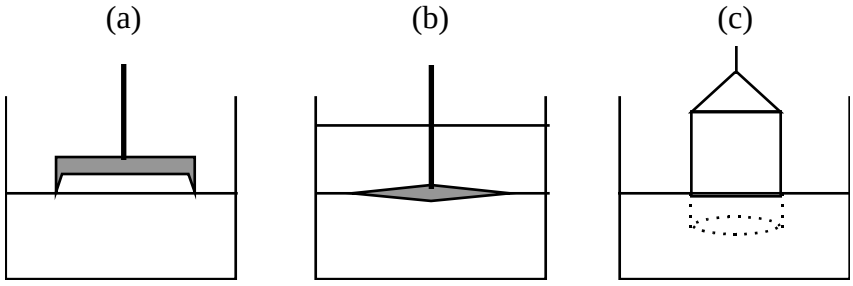


Figure 11. Typical geometries for measuring interfacial shear geometry (a) knifedge for air/water interface. (b) bicone for oil-water interface. (c) Du Nouy ring for sensitive measurements

For the air water interface, a circular knife-edge geometry is commonly used, as the component from the gas phase can be neglected, and the knife edge does not penetrate into the aqueous phase, thus maximising the response from the interface itself. For fluid interfaces such as the oil-water interface, a very shallow biconical geometry is often used. As contact has to be made with both phases, the components from those phases need to be subtracted to reveal the contribution from the interface. The final geometry is a Du Nouy ring, commonly used for measuring interfacial tension, but here it has been specifically designed to measure the surface shear viscoelasticity using the method developed by Sherriff and Warburton (1974). The light construction of this geometry makes it very sensitive to interfaces with very low rheological properties.

In these techniques, an oscillatory motion (strain), which, if small enough, should not breakdown any structures formed at the interface, is applied and the resultant oscillating stress is measured (figure 12).

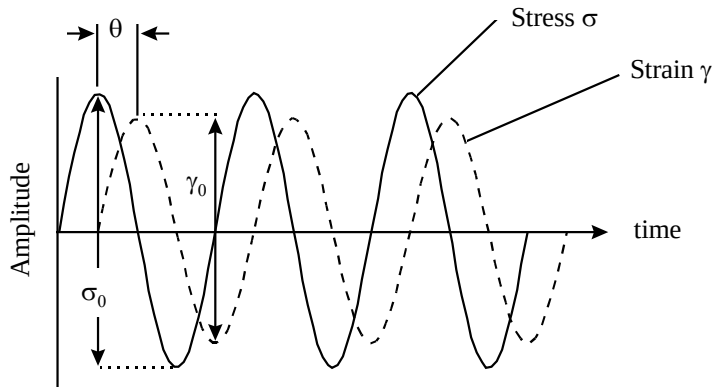


Figure 12. Stress - strain relationship for a typical oscillatory surface shear viscoelasticity measurement.

The total viscoelastic modulus G^* is given as :

$$G^* = \frac{\sigma_0}{\gamma_0} \tag{51}$$

σ_0 and γ_0 are the amplitudes of the stress and the strain respectively. The stresses and strains are the effective two dimensional equivalents of the three dimensional standard viscosity measurements. So the stress here is the applied force per unit distance, and the strain is the distance moved relative to the gap between the geometry and the outer vessel. Similar to the dilational method, if the phase lag θ is 0 or 90° , then G^* is either totally elastic or viscous respectively. The elastic (G') and viscous (G'') moduli can be calculated as follows.

$$G' = G^* \cos\theta \quad G'' = G^* \sin\theta \tag{52}$$

5. Interfacial properties of proteins and LMW surfactants

Food emulsions and foams are stabilized by surface active agents that largely fall into two main categories: surfactants and proteins. These two species strongly differ in their molecular structure and hence in the mechanisms of adsorption, exhibiting surface properties different and conferring stability via dissimilar and incompatible mechanisms [McClements (1999)(a), Bos and va Vliet (2001), Mackie and Wilde (2005)].

Proteins are biopolymers, in which the monomer units are amino acids, joined head-to-tail via peptide bonds. There are 20 amino acids which are directly expressed in the genetic code. Their polypeptide chains fold into a 3-dimensional structure, with four levels of protein structure:

- ✓ *Primary structure:* the linear amino acid sequence forming the protein
- ✓ *Secondary structure:* structures stabilized by hydrogen bonds between the C=O and N-H groups of different peptide bonds
- ✓ *Tertiary structure:* structures stabilized by interactions between the amino acid side chains within a single protein
- ✓ *Quaternary structure:* the association of multiple protein subunits to form a functional protein complex.

The functional properties of proteins are determined by their structure, and then, modifications of ambient conditions like changes of pH, temperature, ionic strength, pressure, etc., may lead to rearrangement of the protein conformation (secondary, tertiary, and quaternary structure) and thus alter its functional properties.

Proteins are surface active, comparable with LMW surfactants, resulting in a lowering of the interfacial tension of fluid interfaces. Depending on the type of oil used, the interfacial tension varies between 8 and 22 mN/m, whereas the surface excess Γ of most proteins is found to be approximately 2-3 mg/m^2 , depending on the pH and ionic strength of the solution, tending to be higher at a solution pH close to the isoelectric point of the protein. Some typical values were reported in table (1).

Protein	Condition Conc. (g l^{-1})	pH	Surface pressure (mN m^{-1})	Adsorbed amount (mg m^{-2})
BSA	0.1	6.7	17.8	2.4
	0.01	7.1	16.7	1.95
β-casein	0.01	6.7	19.8	2.95
	0.1	7.1	22	4.2
Na Caseinate	0.3	6.7	25	3.3
Ovalbumin	0.1	6.7	16.3	1.52

Table 1. Some typical values for the adsorbed amount and surface pressures obtained for some proteins under various conditions [Bos and van Vliet (2001)]

Once the proteins are adsorbed, their conformation may change considerably at hydrophobic surfaces; there may be a difference in the extent of conformational changes between an oil/water and an air/water interface, and in the former case it may depend on the oil used [Bos and van Vliet (2001)]. Such conformational changes can be seen as a form of interfacial denaturation of the protein. However, thermal or pressure denaturation of proteins before adsorption at interfaces may lead to completely different surface and emulsification properties [Galazka et al. (1996)].

Protein adsorption may often be considered as being irreversible. Related to protein desorption is its displacement by surfactants or other proteins adsorbing at interfaces. Due to their markedly different propensity to lower the surface tension, one protein may displace the other from the interface. β-casein and αs_1-casein displace each other, forming a composite layer with a constant ratio between both proteins [Galazka et al. (1996)].

The mutual displacement of proteins is related to the difference in the ability of proteins to change their conformation on adsorption. Flexible proteins, also called “soft” proteins, change their conformation more easily than “hard” proteins (often called globular proteins) [Arai and Norde (1990)(a), Arai and Norde (1990)(b)]. They often displace the more globular proteins from the interface. This difference in surface behaviour is also reflected in the surface shearviscosity, ηs, which is in the order of 10^{-3} N.s.m^{-1} for flexible proteins, and for ‘hard’ or globular proteins in the order of 1 N.s.m^{-1} as for lysozyme [Bos and van Vliet (2001)].

Unlike proteins, surfactants are in general relatively simple amphiphilic molecules, with low molecular weight (LMW). They can be classified into two groups according to the charge of the head group (table 2); non-ionic or uncharged surfactants and ionic surfactants with a either a negative anionic or positive charge (cationic). A classification of LMW surfactants can also be based on their ability to dissolve in an oil or water phase,

respectively. A measure of this is the so-called hydrophilic lipophilic balance (the HLB value). The HLB number can be calculated using the relation: $HLB = 7 + \sum hydrophilic\ \ group\ \ numbers - \sum lipophilic\ \ group\ \ numbers$. HLB values vary between 0 and 20, and in table 3 a classification based on the application of the surfactants and their accompanying HLB values is given.

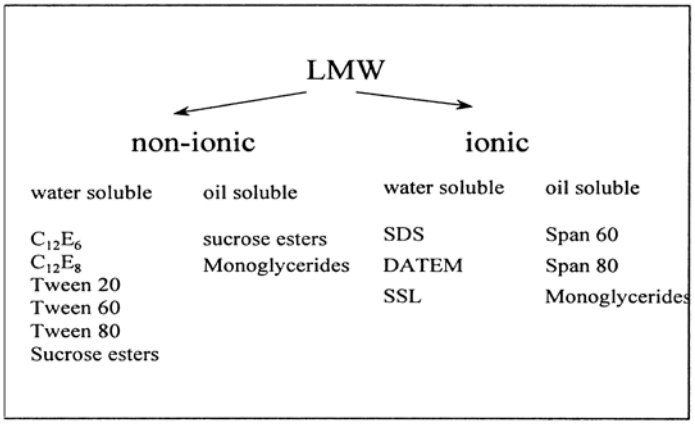


Table 2. Overview of the main LMW surfactants used in the literature to study the properties of mixed protein_surfactant interfacial layers. Abbreviations: SDS, sodium dodecyl sulphate; SSL, sodium stearoyl-2-lactylate; DATEM, diacetyl tartaric acid ester of monoglycerides; C12E6 , hexaoxyethylene *n*-dodecyl ether; C12E8, octaoxyethylene *n*-dodecyl ether. Span 60, sorbitan monostearate; Span 80, sorbitan monooleate; Tweens, polyoxyethylene sorbitan esters of monoglycerides [Bos and van Vliet (2001)]

HLB value	Application
3–6	Emulsifiers of w/o emulsions
7–9	Wetting agents
8–18	Emulsifiers of o/w emulsions
13–15	Detergents
15–18	Solubilisers

Table 3. Ranges of applications of surfactants with given HLB values [Bos and van Vliet (2001)]

The most frequently used LMW surfactants in foods are: phospholipids (lecithin), mono- and diglycerides (glycerol monostearate), polysorbates (Tweens); sorbitan monostearate; sorbitan monooleate (Span 80), polyoxyethylene sorbitan monostearate (Tween 20) and sucrose esters. They lower the interfacial tension to a greater extent than high molecular weight surfactants such as proteins.

LMW surfactants have higher adsorption energies per m^2 than proteins, but the latter can adsorb at the interface with several segments. Moreover, changes in their conformation and in their orientation allow more segments to adsorb.

Although LMW surfactants are more effective than proteins in reducing interfacial tension, foams and emulsions formed by such surfactants are mostly less stable against coalescence. This is because steric repulsion between the protein-covered oil droplets is very effective in opposing aggregation [McClements (1999)(a), Bos and va Vliet (2001), Mackie and Wilde (2005), Gunning et al. (2004)].

Furthermore, proteins can form a visco-elastic network around the oil droplets or air cells via non-covalent intermolecular interactions, sometimes referred to as a two-dimensional gel and via covalent intermolecular disulphide cross-linking [Bos and va Vliet (2001), Mackie and Wilde (2005), Gunning et al. (2004)], whereas the LMW surfactants cannot and rely on a high degree of surface mobility to counter deformation, via the Gibbs-Marangoni effect, developed to describe the role of mobile surfactants in stabilizing the film present in the emulsions and foams [Gunning et al. (2004)].

In many food systems LMW surfactants are used together with proteins in order to improve the long term stability of these systems than those formed with the single surfactant. In multicomponent systems the surface active species can compete for the interface. In fact, in emulsions and foams containing proteins and LMW surfactants the displacement of proteins by LMW surfactants, or the interaction between them, either in solution or in the interface, is responsible for system specific properties. The displacement of protein from liquid surfaces by various LMW surfactants can be described by two extreme mechanisms [Bos and va Vliet (2001)]:

- ✓ *Solubilisation*: the water-soluble surfactant binds to the protein to form a soluble protein-surfactant complex. The surfactant does not have to adsorb at the interface, but it must strongly interacts with the protein.
- ✓ *Replacement*: the surfactant adsorbs at the interface and displaces the protein because the Gibbs interfacial energy (interfacial tension) for the surfactant is lower than that for the protein (or protein-surfactant complex). The surfactant does not have to interact with the protein, but it has to bind at the surface.

In practice, the actual mechanism will often be in between both extremes, with competitive adsorption involving ionic surfactants mainly proceeding via the solubilisation mechanism,

and that involving non-ionic surfactants mainly proceeding via replacement [Bos and va Vliet (2001)].

Then, it is important to emphasize that in the multicomponent systems a wide variety of factors can influence interfacial composition, such as the emulsifier concentration, emulsifier type, solution conditions, temperature, and time [McClements (1999)(a)]. In this regard, of particular importance is the affinity of an emulsifier molecule for an interface, which can be described by its *adsorption efficiency* and its *surface activity* [Dickinson (1992)]. The adsorption efficiency is a measure of the minimum amount of emulsifier required to saturate an interface, whereas the surface activity is a measure of the maximum decrease in interfacial tension achievable when an interface is completely saturated. Adsorption efficiencies and surface activities depend on the molecular structure of emulsifiers, as well as the prevailing environmental conditions. Amphiphilic biopolymers, such as proteins, tend to have higher adsorption efficiencies, but lower surface activities, than small-molecule surfactants (figure 6).

A small molecule tends to have one fairly strong binding site (its hydrophobic tail), whereas biopolymer molecules tend to have a large number of relatively weak binding sites (nonpolar amino acid side groups). The overall binding energy of a biopolymer molecule tends to be greater than that of a small-molecule surfactant, and therefore it binds more efficiently (i.e., less emulsifier must be added to the bulk aqueous phase before the interface becomes completely saturated). For the same reason, small-molecule surfactants tend to rapidly exchange between the adsorbed and unadsorbed states, whereas biopolymer molecules tend to remain at an interface for extended periods after adsorption.

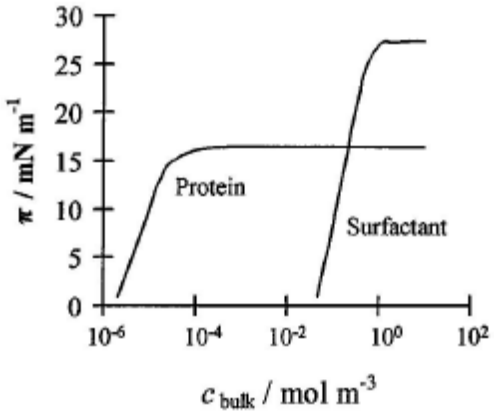


Figure 13. Comparison of the affinity of amphiphilic biopolymers and small-molecule surfactants for an oil–water interface [McClements (1999)(a)]

Thus, at low emulsifier concentrations, biopolymers have a greater affinity for an interface than small-molecule surfactants. On the other hand, small-molecule surfactants tend to decrease the interfacial tension by a greater amount than biopolymer molecules at concentrations where the interface is completely saturated, because they pack more efficiently and therefore screen the unfavorable interactions between the oil and water molecules more effectively. Thus, at high emulsifier concentrations, small-molecule surfactants have a greater affinity for an interface than biopolymers and will tend to displace them. This accounts for the ability of relatively high concentrations of surfactant molecules (e.g., 1% Tween 20) to displace proteins from the surface of oil droplets [McClements (1999)(a)].

6. Adsorption kinetics

The rate at which a surfactant adsorbs to an interface is one of the most important factors determining its efficacy as a food ingredient. The adsorption rate depends on many factors such as the molecular characteristics of the surfactant (e.g., size, conformation, and interactions), the nature of the bulk liquid (e.g., viscosity), and the prevailing environmental conditions (e.g., temperature and mechanical agitation) [McClements (1999)(a)].

In regard to the first factor it is important to emphasize that the kinetics of surfactant adsorption at gas/liquid interface are different for LMW surfactants and biopolymers or proteins [Murray (1998)], and specifically the protein adsorption tends to be considerably slower than that of LMW surfactants owing to the higher molecular weight and the co-operative nature of their adsorption

Then, the most notable difference in their adsorption is that proteins can undergo internal rearrangements at phase boundaries, further exposing their hydrophobic groups to the more hydrophobic phase (air in foams), whereas the LMW surfactants are typically much simpler structures and hence undergo little to any rearrangements at the interface [Murray (1998), W*ü*stneck et al. (1996), Beverung et al. (1999), Miller et al. (2000)].

Moreover, the interfacial area occupied by the adsorbed protein molecule is large compared to that of a small surfactant and it cannot be assumed constant, the number of

configurations of adsorbed protein molecules exceeds that of proteins in the bulk solution, protein adsorption and subsequent changes within the adsorbed layer take place on a time scale several orders of magnitude higher than for small molecular weight surfactants [Wilde (2000)] and protein adsorption can be considered irreversible, differently from low molecular weight surfactants, which can leave the interface and penetrate into the bulk phase [Van Hunsel et al. (1986), Demeter-Vodnàr et al. (1996), Rosen (2004)].

In general, the degree of interfacial protein rearrangement depends in turn on numerous factors, including the intrinsic flexibility of the molecules, solutions conditions, and the surface pressure (π) of the interface [Murray (1998), Wüstneck et al. (1996), Davis (2005)]. If the surface pressure is high, i.e. there are many surfactants already at the interface, a newly arriving protein should unfold to a lesser degree than a protein that adsorbs at an empty interface.

Nevertheless, the main features of the adsorption kinetics of proteins and LMW surfactants can include [Pérez et al. (2009), Pérez et al. (2010), Camino et al. (2009), Van Hunsel et al. (1986), Demeter-Vodnàr et al. (1996), Rosen (2004), Davis (2005)]:

- ✓ the diffusion of the molecules from the bulk onto the interface;
- ✓ the adsorption and penetration of molecules at the interface;
- ✓ the interfacial aggregation and rearrangement of molecules adsorbed within the interfacial layer, which is very important in the case of proteins.

The surfactant adsorption at the interface is accompanied by a kinetic gradient in the interfacial tension. As the molecules adsorb at the interface, the interfacial tension will dynamically decrease from the pure solvent value, until some lower equilibrium value is attained (figure 14). The dynamic interfacial tension can be characterized by up to three general kinetic regimes prior to attainment of its equilibrium value. The first regime is the induction time, which is recorder only for the adsorption phenomena of protein at relatively small bulk concentration, during which the interfacial tension remains nearly equal to that of the pure solvent's value, with little or no apparent decrease [Tripp et al. (1995)]. The solution interfacial tension decreases from the pure solvent's value only after a "substantial" amount of protein has adsorbed to the interface. The second kinetic interfacial tension regime is characterized by a rapid decrease in interfacial tension.

The third kinetic regime is the mesoequilibrium interfacial tension (MIT) regime. Attainment of the MIT regime is indicated by a large decrease in the magnitude of the dynamic interfacial tension slope at the end of regime 2.

The slow rate of decrease in interfacial tension during the MIT regime is thought to be a result of molecular reorientation and conformational change in the adsorbed protein molecules. Attainment of steady–state interfacial tension during the MIT regime indicates that the adsorbed protein molecules have achieved their equilibrium conformation and surface concentration [Tripp et al. (1995)].

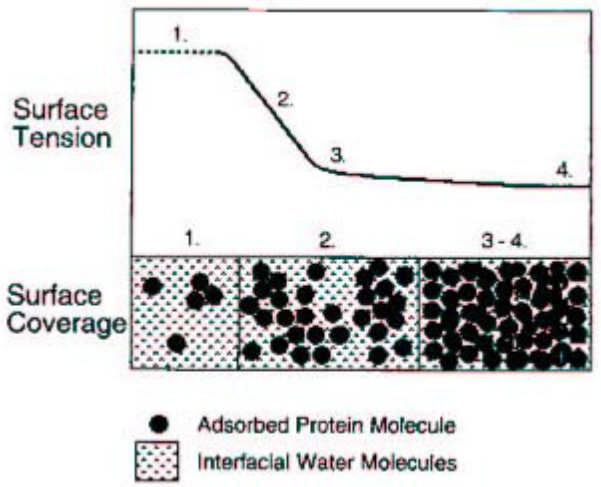


Figure 14. Idealized diagram of dynamic interfacial tension – surface coverage relationship, illustrating the three possible dynamic interfacial tension kinetic regimes: induction time (1), rapid interfacial tension decrease (2), mesoequilibrium interfacial tension (3), resulting in the steady – state interfacial tension (4) [Tripp et al. (1995)]

6.1 Macroscopic description

According to the Gibbs equation for ideal systems, the adsorption of a single classical surface-active agent at a fluid interface decreases the interfacial tension (γ) according the equation (5):

$$d\gamma = -\Gamma RTd \ln c \qquad (53)$$

Where Γ is the surface excess concentration, R is a constant, T is a temperature and c is the bulk concentration of surfactant.

If diffusion of the surface active molecule to the interface is the rate limiting process, then the 1-D dimensional diffusion equation can be employed:

$$\delta c(x,t)/\delta t = D\delta^2 c(x,t)/\delta x^2 \tag{54}$$

where D is the diffusivity of the surface active molecule in the solvent, x is the spatial coordinate from the interface and t is the adsorption time.

The boundary condition for conservation of mass at the surface (x = 0) is given by an equivalent form of Fick's first law:

$$d\Gamma(t)/dt = D \cdot \frac{\delta c(x,t)}{\delta x} / x = 0 \tag{55}$$

and the boundary condition far away from the surface:

$$x \to \infty; c = c_0 \tag{56}$$

where c_0 is the bulk concentration. The initial conditions are:

$$c(x,0) = c_0; \Gamma(0) = \Gamma_0 \tag{57}$$

Ward and Tordai (1946) integrated this diffusional problem using Green's functions, resulting in Ward and Tordai's equation:

$$\Gamma(t) = \Gamma_0 + (D/\pi)^{1/2}\left[c_0 t - \int_0^{\sqrt{t}} c_s(t-\lambda)d\ln\lambda\right] \tag{58}$$

where λ is a relaxation time and c_s is the subsurface concentration. If Γ_0 is assumed to be zero, then at early times during the diffusion controlled process $c_s \approx 0$ and the convolution integral is neglected, resulting in a "short-time" approximation [Ward and Torday (1946)]:

$$\Gamma(t) = 2c_0(D\ t/3.14)^{\frac{1}{2}} \tag{59}$$

The change in Γ(t) with time is given by:

$$d\Gamma(t)/dt = c_0(D\ /3.14 \cdot t)^{\frac{1}{2}} \tag{60}$$

In terms of surface pressure, π, this can be rearranged to give equation (26):

$$\pi(t) = \gamma_0 - \gamma(t) = \Gamma(t)RT \tag{61}$$

$$\pi(t) = RTc_0(Dt/3.14)^{1/2} \tag{62}$$

The measured dynamic surface pressure, Π(t), should therefore increase linearly with t in the early times of adsorption. For a long-time approximation the Ward and Tordai equation is reformed as:

$$\Gamma(t) = -2(D/\pi)^{1/2}\left[\int_0^{\sqrt{t}} \Delta c_s(t-\lambda)d\ln\lambda\right] \tag{63}$$

where $\Delta c_s = c_s - c_0$. Over the interval at large times, Δ Cs changes little and the equation (63) becomes:

$$\Gamma(t) = \left(4\mathrm{Dt}/3.14\right)^{1/2}\left(c_0 - c_s\right) \tag{64}$$

If (c_0 - c_s) is linearized with respect to interfacial tension, γ(t):

$$c_0 - c_s = dc / d\gamma \cdot (\gamma_\infty - \gamma) \tag{65}$$

Equation (64) now becomes

$$\gamma(t) = \gamma_\infty - d\gamma / dc \cdot \Gamma(t)\,(\pi / 4Dt)^{1/2} \tag{66}$$

and using the Gibbs equation, equation 54 becomes the "long-time" approximation to Ward and Tordai [Ward and Torday (1946)]:

$$(\gamma(t) - \gamma_\infty) = RT\Gamma(t)^2 / c_0 \cdot (\pi / 4Dt)^{1/2} \tag{67}$$

After adsorption to an interface has started the adsorbed molecules may relax, in that they reorientate themselves in the interfacial layer. At the interface the following reorientation reaction takes place: $\Gamma_1 \leftrightarrow \Gamma_2$, where Γ_1 is the adsorption at t = 0, and Γ_2 is the subsequent adsorption of the reoriented molecules. At equilibrium (*e*) there is a total adsorption of $\Gamma_1^e + \Gamma_2^e$ [Führling (2004)]. According to Lucassen-Reynders [1966], only 1 is directly exchangeable with the subsurface, and 2 is exchangeable only via modification 1 (figure 15).

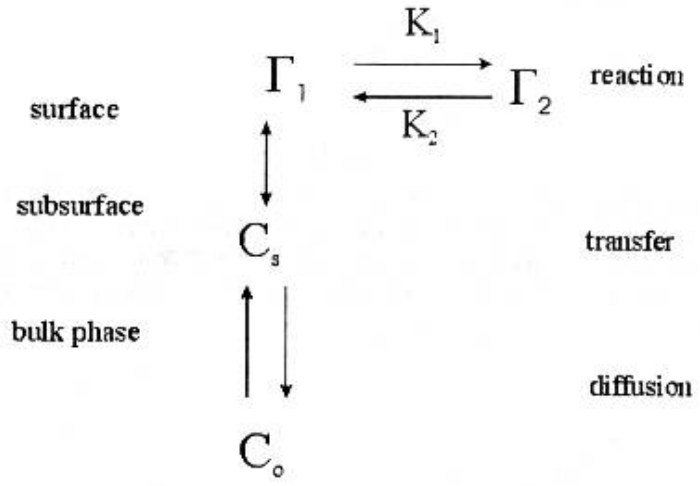


Figure 15. Schematic of the diffusional and surface reorientation reactions close to the interface [Führling (2004)]

Following this scheme, the mass balances at the surface are given by:

$$d\Gamma(t)_1 / dt = D(\delta c / \delta x)_{x=0} - k_1(\Gamma(t)_1 - \Gamma_{1e}) + k_2(\Gamma(t)_2 - \Gamma_{2e}) \tag{68}$$

$$d\Gamma(t)_2 / dt = k_1(\Gamma(t)_1 - \Gamma_{1e}) - k_2(\Gamma(t)_2 - \Gamma_{2e}) \tag{69}$$

The concentration gradient, $D\,(\delta c / \delta x)_{x=0}$, is given by the diffusion equation. Joos (1999) gives for this model the approximate solution:

$$\Delta\gamma = \Delta\gamma_0 \left[\alpha_1 \exp - (4t / \pi\tau D)^{1/2} + \alpha_2 \right] e^{-kt} \quad (70)$$

where $\Delta\gamma$ is the dynamic change in interfacial tension, α_1 is a factor describing diffusion equilibration, α_2 a factor describing equilibration by the reorientation reaction, and k = k1+k2. This equation describes the interfacial tension as a function of time quite well, especially for protein solutions. The exponential-type relation was already discovered empirically for protein solutions by Graham and Phillips [1979], which proposed the following equation in terms of π:

$$\ln \frac{\pi_f - \pi_t}{\pi_f - \pi_0} = -k_i t \quad (71)$$

Where π_f, π_0 and π_t are the surface pressures at final adsorption time, at time t=0, and at any time t, respectively, and k_i is the first-order rate constant. In practice, a plot of equation 2 usually yields two or more linear regions. The initial slope is taken to correspond to a first-order constant of adsorption (k_{ads}), whereas the second slope is taken to correspond to a first-order constant of rearrangement (k_r), occurring among a more or less constant number of adsorbed molecules [Pèrez et al. (2009), Pèrez et al. (2010), Camino et al (2009)].

7. Interfacial behavior in dilation

7.1 Proteins

The interpretation of interfacial dilational data of proteins depends on the time-scale, i.e., on the strain rates or frequencies at which the measurements were performed [Bos and van Vliet (2001)]. Since protein molecules often appear to unfold slowly at the interface on adsorption, unfolding processes may contribute little to the dilatational moduli over short time-scales. In this respect the interfacial dilational moduli of proteins often appear to be relatively independent of film ageing [Benjamins and van Voorst Vader (1992)] compared to the corresponding shear measurements. Thus the dominant slow process in the dilatational rheology of adsorbed films may be the adsorption of new molecules to the interface rather than the rearrangements of existing molecules within the film.

For long time-scales/low frequency measurements these slow processes must be taken into account.

Micelle formation for proteins obviously need not be considered - though many proteins do aggregate to some extent depending on the conditions such as pH, ionic strength and metal ion concentration. Notable examples of aggregating proteins which are technologically important in foods include milk proteins (caseins and whey proteins) and many plant proteins [Murray (1998)]. In addition, for short time-scales and for small deformations, proteins are generally so strongly adsorbed that they may be considered as irreversibly adsorbed. It is seen that interpretation of the dilatational rheology of interfaces involving proteins cannot be dissociated from the issues surrounding the measurement and modelling of protein adsorption. Particularly important issues are the matter of energy barriers to adsorption [Miller et al. (1993)] and the effects of strong lateral interactions between adsorbed molecules at the interface. For an individual protein molecule to become adsorbed a certain, minimum length of the polypeptide chain must become associated with the surface. When the protein must change its conformation for this to occur, e.g., with a globular protein, then one might expect an energy barrier to adsorption. Alternatively this barrier may be of purely entropic (orientational) origin, where the protein molecule must rotate until the appropriate part of the molecule surface comes into contact with the interface. The more an interface is already covered with protein then the greater will be conformational change required and/or the greater will be the orientational restrictions imposed for further adsorption to occur [Murray (1998)].

Overall, compared to the wide-ranging interfacial shear rheology of proteins, the dilatational film properties of the food proteins examined so far are rather similar to each

other. [Murray (1998)] gave an explanation for this behavior some time ago in noting the similarity of many surface pressure - area isotherms for spread proteins at the A-W interface. Proteins at the surface can be considered to consist of strings of unit cells, each apparently equivalent to 6 – 8 amino acid residues. Once unfolded, all proteins simply behave as longer or shorter strings of these units. However, proteins which can adsorb quickly and/or rearrange quickly at interfaces, whether due to lower molecular weight (higher diffusion coefficient), or greater flexibility, are expected to give rise to lower dilatational moduli due to the more rapid recovery in γ possible at short times (high frequency). Thus the value of the dilational modulus for β-casein at the A-W interface is lower than that for BSA and β-lactoglobulin, following the general trend that the modulus increases with decreasing protein flexibility [Benjamins and van Voorst Vader (1992),Graham and Phillips, (1980)] . Numerous studies have found that globular proteins such as β-lactoglobulin, ovalbumin and lysozyme tend to form more viscoelastic films as compared to less ordered proteins such as β-casein [Bos and van Vliet (2001)]. This is explained by the flexible β-casein not transmitting force across the interface as efficiently as the more rigid, globular proteins.

Interfacial viscoelasticity of protein films depends on numerous factors including the type of protein, cosolutes present and thermal history of the solution. Due to the variety of amino acids contained in a typical protein, a range of intermolecular interactions are possible at the interface, including hydrogen bonding, hydrophobic contacts, electrostatics, disulfide bond formation and van der Waals interactions. Electrostatic interactions play a significant role in both protein adsorption and interfacial rheology [Bos and van Vliet (2001)].

Accordingly, protein adsorption to the interface is generally most rapid at this pH as electrostatic repulsion is minimized for the net neutrally charged proteins. Furthermore, viscoelasticity of interfacial films generally peaks for a range of proteins near their pI's [Bos and van Vliet (2001)].

Concerning the effect of the neighbouring phase, only very few data have been published on the dilational rheology of proteins at oil/water interfaces compared to those at air/water interface. This is mainly due to the greater experimental difficulties [Bos and van Vliet (2001)].

Oils used in the food industry (e.g. sunflower oil) however, probably influence the adsorbed layer in a different way. The polarity of the oil phase (hydrocarbon oil vs. triglyceride oil), the state of crystallisation and the presence of small impurities in the oil

will have an effect on the resulting interfacial rheological parameters [Dickinson and Tanai (1992), Stevenson et al. (1997)].

In figure 16 a comparison is given between the dilational moduli at an air/water and sunflower oil/water interface for various proteins.

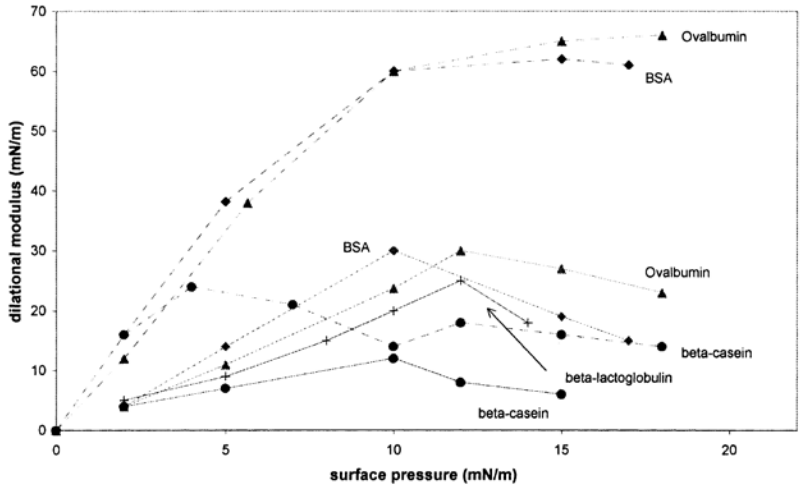


Figure 16. The interfacial dilational modulus of various proteins as a function of the interfacial pressure as determined with the dynamic drop tensiometer. The dashed lines are for air_water interfaces and the drawn lines are for sunflower oil_water interfaces. The frequency was 0.1 rad /s . [Bos and van Vliet (2001)]

For the air/water interfaces the moduli are higher than for the corresponding oil/water interfaces. All curves for the oil/water interfaces pass through a maximum, and at higher surface pressure decline more strongly than at the air/water interface, indicating that collapse-type phenomena, slow reconformations or exchange with multilayers probably play a role. Murray (1997) found a considerably more elastic behaviour for β-lactoglobulin films at the *n*-tetradecane/water interface than at the air/water interface by using a novel Langmuir trough-type apparatus. Williams and Prins (1996) found hardly any difference between the moduli at an oil/water (low viscosity paraffin oil) and an air/water interface for β-casein and β-lactoglobulin over a range of concentrations.

The differences observed in interfacial behavior at an oil/water interface between the various authors can be attributed to the different types of oil used in these studies. Another reason could be the very small linear region for adsorbed protein layers in dilation. It could well be that some reported measurements were not performed in the linear region [Bos and van Vliet (2001)].

7.2 Proteins +low molecular weight surfactant

Emulsifiers added to a protein solution can modify the adsorption layer properties at liquid/fluid interfaces significantly [Kotsmar et al. (2009)]. Proteins can interact with surfactant molecules in the bulk and at the interface in a different way, which results in complexes of different interfacial activity than the protein or emulsifier alone.

Then, protein-surfactant binding changes the adsorption energy of the protein for the interface by affecting the net charge or the overall hydrophobicity, and affects both the surface coverage and the conformation of adsorbing macromolecules. Since LMW surfactants can pack together more closely at interfaces than proteins, in general the former displace proteins when they are present at high enough bulk concentration with a different mechanism for ionic and nonionic surfactants respectively.

Nonionic surfactants in general exhibit a net repulsive interaction with adsorbed proteins, probably owing to steric repulsion, whereas ionic surfactants have a relatively more attractive interaction with adsorbed proteins [Hasenhuettl and Hartel (1998), Bos and van Vliet (2001), Dickinson (1998)].

This determines that ionic emulsifiers have a greater tendency to complex with the charged groups of proteins, thus, they bind to the protein forming a soluble protein-surfactant complex (solubilization mechanism), conversely nonionic emulsifiers tend to adsorb directly to the interface gradually replacing macromolecules (replacement mechanism) [Bos and van Vliet (2001), Dickinson (1998)].

In addition to the type of surfactant (water or oil soluble, ionic or nonionic) the simultaneous adsorption of proteins and surfactants at the interfaces depends on different factors, mainly including the native conformation of the biopolymer (random or globular), the nature of the interface (air/water, oil/water), the pH and the ionic strength of the solvent [Bos and van Vliet (2001), Maldonado-Valderrama and Rodrìguez Patino (2010)]. All these factors affect the rheological dilational properties of the adsorbed layers

The change observed in the dilational properties with increasing surfactant-protein ratio, are not as sharp as observed in interfacial shear rheology, owing to a smaller difference in the dilational elasticity and viscosity between proteins and LMW surfactants. However generally a decrease in the dilational elasticity and an increase in the dilational viscosity are found for various mixtures investigated by many authors [Krägel et al. (2003), Mackie and Wilde (2005), Rodrìguez Patino et al. (2003), Mackie at al. (1999), Petkov and Gurkov (1999), Maldonado-Valderrama and Rodrìguez Patino (2010), Bos and van Vliet (2001), Murray (1998)]

These decrease in the value of the interfacial rheology parameters depends on the surfactant-protein ratio and is different for every protein-surfactant combination [Bos and van Vliet (2001), Murray (1998)]. This decrease can be may result in a drastic loss of foams and emulsions stability which was attributed by many authors, to the different and incompatible stabilization mechanisms of the interface exhibited by proteins and LMW surfactants respectively. As a result surfactants weaken the visco-elasticity of the adsorbed protein layer, and the polymers retard the fluidity of the surfactants [Wilde (2000), Bos and van Vliet (2001), Dickinson (1998), Rouimi et al. (2005), Wilde et al. (2004), Mackie and Wilde (2005)].

8. Interfacial rheology and emulsion/foam stability

The term stability referred to emulsion and foam systems is broadly used to describe the their ability to resist changes in their properties with time. There are a variety of physicochemical mechanisms which may be responsible for alterations in the properties of an emulsion or foam.

A number of the most important physical mechanisms responsible for the instability of emulsions are shown schematically in figure (17). *Creaming* and *sedimentation* are both forms of *gravitational separation.* Creaming describes the upward movement of droplets due to the fact that they have a lower density than the surrounding liquid, whereas sedimentation describes the downward movement of droplets due to the fact that they have a higher density than the surrounding liquid. *Flocculation* and *coalescence* are both types of droplet aggregation. Flocculation occurs when two or more droplets come together to form an aggregate in which the droplets retain their individual integrity, whereas coalescence is the process where two or more droplets merge together to form a single larger droplet. Extensive droplet coalescence can eventually lead to the formation of a separate layer of oil on top of a sample, which is known as “oiling off.” *Phase inversion* is the process whereby an oil-in-water emulsion is converted into a water-in-oil emulsion or

vice versa. Similar mechanisms are responsible for the foam instability, and the differences existing between these two systems are linked to the fact that in the latter case the dispersed phase consists of bubbles.

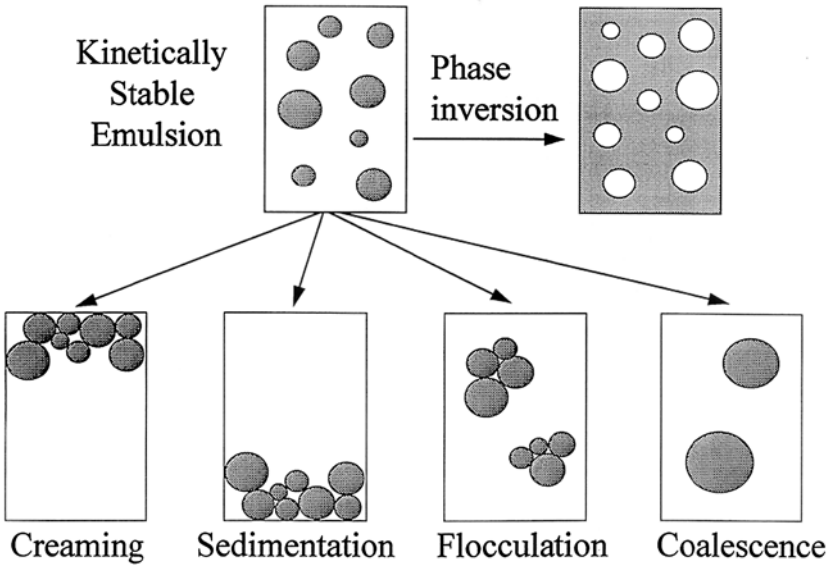


Figure 17. Physical mechanisms responsible for the instability of emulsions [McClements (1999)(a)]

In order to address the question as to whether a link exists between the interfacial rheological parameters and the actual stability of emulsions and foams one has to consider the interfacial experiment conditions. In fact, all measurements of interfacial rheology are made on macroscopic surfaces, and the range of stresses, strains and rates of strain applied certainly do not reflect the turbulent non-equilibrium conditions of practical foam formation or emulsification. This means that there may be no direct relationship between protein characteristics as determined by interfacial rheological measurements and emulsion and or foam formation capacity.

Regarding the stability of emulsions and foams during ‘quiescent’ conditions, such as on storage, one might expect that it is easier to establish a casual relation between the interfacial rheological parameters determined at macroscopic surfaces and certain physical stability or instability mechanisms.

In fact, there are many experimental evidences that interfacial rheological properties can be related to practical systems, especially to systems containing protein and LMW surfactants. Regarding these systems, it is often stated in the literature (and also in practice) that mixtures of emulsifiers and proteins can result in maximum or minimum stability characteristics for emulsions and foams which can be interpreted in terms of the mechanical characteristics of the interface.

An increase in interfacial properties is not always positively correlated with emulsion/foam stability [Bos and van Vliet (2001)]. The addition of the surfactant generally makes the rather rigid adsorbed protein layer more flexible and mobile. This could result in an adsorbed layer that is much better able to respond to deformations of varying size and rate, owing to a quicker recovery of the film which would prevent film rupture and thus have a positive effect on the stability of foams and emulsions [Bos and van Vliet (2001)]. Otherwise the surfactant addition to protein layer could cause a loss of the system stability owing to the process commonly known as competitive destabilization (figure 18) [Wilde et al. (2004), Mackie and Wilde (2005)].

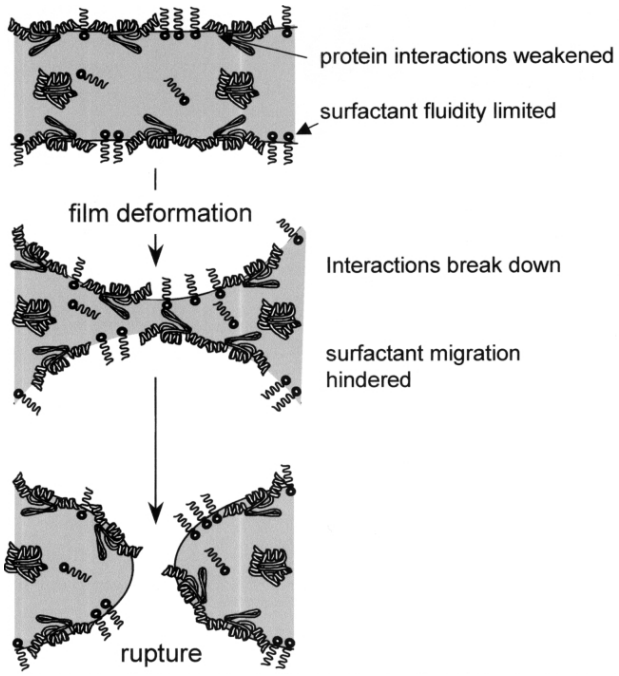


Figure 18. Competitive destabilization of protein-stabilized films by emulsifiers [Wilde et al. (2004)]

Specifically, during a competitive destabilization process the surfactants can disrupt the strong interactions developed between neighbouring protein molecules, effectively weakening the interface. The surfactants, which rely on rapid surface migration, are constrained by the presence of protein molecules still at the interface, and the protein component may still be in the form of a two dimensional network, effectively caging the surfactant molecules and seriously hampering their motion.

It is important to emphasize that in most surfactant-protein systems the relative stability to coalescence of a foam and emulsion decreases beyond a critical concentration of the surfactant, which can be determined by interfacial rheology experiments. The differences

in concentration required to destabilise these two systems are a function of the relative surface activities of the molecules at the two interfaces.

In this regard and as example, figure (19) shows the surface dilational modulus at an air/water interface, as a function of surfactant concentration [Wilde et al. (1998)]. The surface viscoelasticity decreases with surfactant concentration, thus correlating the interfacial rheology with the stability of foams created with the same solutions.

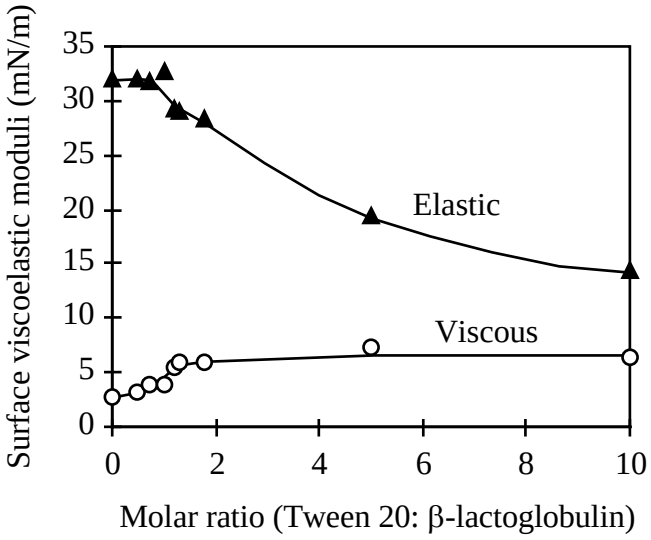


Figure 19. Elastic and viscous surface moduli of a protein stabilised air-water interface as a function of added surfactant (Tween 20) [Wilde (1998)]

Other properties of foams and emulsions may also be affected by the interfacial rheology of the system. These include foam drainage behavior, emulsion rheology and disproportionation.

Disproportionation is the mass transfer of gas from small bubbles to larger bubbles in a foam. The pressure inside smaller bubbles is greater due to the surface tension forces giving rise to a Laplace pressure which is proportional to $1/r$, where r is the bubble radius. This results in the time dependent coarsening of foam, where large bubbles become larger and small bubbles get smaller ad eventually disappear. If the bubble surface has a high dilational elasticity (|E|), it will resist shrinkage and expansion of the bubbles. It was suggested that if :

$$|E| > \frac{\gamma}{2} \tag{72}$$

where γ is the surface tension, then disproportionation could be significantly reduced.

The effect of interfacial rheology on foam drainage is a very interesting current area of research.

Moreover, there has been an interest in how interfacial rheology may affect the bulk viscosity of emulsions. The viscosity of emulsions is highly dependent on the interactions between emulsion droplets, and those interactions could well depend on the rheology of the interface. Figure 21[Wilde (1998)] shows the effect of a small amount of surfactant on the shear dependent viscosity of an emulsion stabilised by a protein.

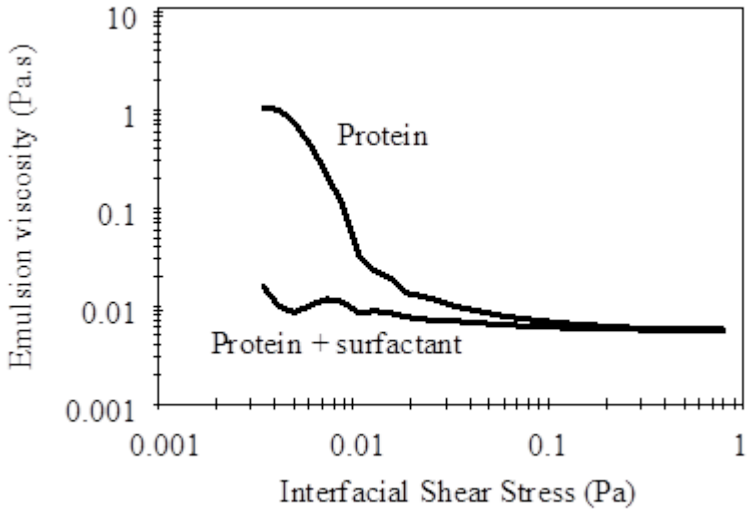


Figure 20. Stress dependent viscosity of emulsions stabilised by protein in the presence and absence of surfactant [Wilde (1998)]

Both emulsions, considered in figure (20) have the same particle size and phase volume, but the interfacial rheology of the two systems are quite different [Wilde (1998)]. It can be noticed that at high shear rates, both emulsions have the same viscosity, because the surface of the droplets are behaving in the same way, that is they proved have the same interfacial viscoelasticity at high stresses [Wilde (1998)]. At low shear rates however, the protein alone emulsion his a distinctly higher viscosity, there is no doubt that this is due to the droplets having a more rigid interface, but the precise mechanism is not known.

Despite all these considerations, it is important to emphasize that no reliable theory exists for the relationship between interfacial rheological properties and stability phenomena for protein stabilized emulsions and foams. Ideas and hypotheses are based on circumstantial evidence.

REFERENCES

1) McClements D.J. (a) (1999). Food emulsion: Principles, Practice and Techniques. CRC Press: Boca Raton, FL., chapter 5
2) Bos M.V. and van Vliet T. (2001), Interfacial rheological properties of adsorbed protein layers and surfactants: a review, *Advances in Colloid and Interface Science* (91), 437-471
3) Murray S.B. (1998), Interfacial rheology of mixed food protein and surfactant adsorption layers with respect to emulsion and foam stability in M*ö*bius D. and Miller R., Studies in interface science 7, Proteins at Liquid Interfaces, Elsevier editor, Amsterdam (The Netherlands), chapter 5
4) McClements D.J. (b) (1999), Food emulsion: Principles, Practice and Techniques. CRC Press: Boca Raton, FL., chapter 7
5) Wilde P.J. (2000), Interfaces: their role in foam and emulsion behavior, *Current Opinion in Colloid & Interface Science* (5), 176-181
6) Eastoe J. (2010), Surfactant Aggregation and Adsorption at Interfaces in T. Cosgrove Colloid Science Principle, Methods and Applications. Second Edition Wiley, U.K., chapter 4
7) Maldonado-Valderrama J. and Rodrìguez Patino J.M. (2010) Interfacial rheology of protein–surfactant mixtures, *Current Opinion in Colloid & Interface Science* (15), 271–282
8) Dickinson E. (1998), Proteins at interfaces and in emulsions. Stability, rheology and interactions. *Journal of the Chemical Society, Faraday Transactions* (94), 1657-1669
9) Benjamins J, Lucassen-Reynders EH. (1998), Surface dilational rheology of proteins adsorbed at air/water and oil/water interfaces. In: Mobius D, Miller R, editors. Proteins at liquid interfaces. Amsterdam: Elsevier;. p. 341–384
10) Rodríguez Patino J.M., Carrera C., Rodríguez Niño M.R. (2008), Implications of interfacial characteristics of food foaming agents in foam formulations. *Advances Colloid Interface Science* (140), 95–113
11) Rodríguez Patino J.M., Rodríguez Niño M.R., Carrera C. (2007), Physico-chemical properties of surfactant and protein films. *Current Opinion of Colloid Interface Science* (12), 187–95

12) Rodríguez Patino J.M., Rodríguez Niño M.R., Carrera C. (2003), Protein–emulsifier interactions at the air–water interface. *Current Opinion of Colloid Interface Science* (8), 387–95.

13) Wilde P., Mackie A., Husband F., Gunning P., Morris V.(2004), Proteins and emulsifiers at liquid interfaces, *Advances in Colloid and Interface Science* (108 – 109), 63-71

14) McClements D.J. (c) (1999), Food emulsion: Principles, Practice and Techniques. CRC Press: Boca Raton, FL., chapter 4

15) Murray BS. (1998), Interfacial Rheology of mixed food protein and surfactant adsorption layers with respect to emulsion and foam stability. In Mobius D, Miller R, eds. Proteins at Liquid Interfaces. Amsterdam: Elsevier Sciences, 179-220

16) W*ü*stneck R, Kragel J, Miller R, (1996) Dynamic surface tension and adsorption properties of beta- casein and beta-lactoglobulin. Food Hydrocolloids (4),395-405

17) Beverung, C. J., Randke, C. J., & Blanch, H. W. (1999) Protein adsorption at the oil water interface: characterization of adsorption kinetics by dynamic interfacial tension measurements. *Biophysical Chemistry* (81), 59–80

18) Miller R., Fainerman V.B., Makievski A.V., Kr*ä*gel J., Grigoriev D.O., Kazakov V.N., Sinyachenko O.V., (2000) Dynamics of protein and mixed protein/surfactant adsorption layers at the water fluid interface *Advances in Colloid and Interface Science* (86), 39-82

19) Davis P. (2005), Investigations into the mechanisms responsible for the yield stress of protein based foams, phD Thesis

20) Pèrez O.E., Carrera Sànchez C., Pilosof A.M.R., Rodrìguez Patino J.M. (2009) Kinetics of adsorption of whey proteins and hydroxypropyl-methyl-cellulose mixtures at the air-water. *Journal of Colloid and Interface Science* (336), 485-496

21) Pèrez A.A., Carrera Sànchez C., Rodrìguez Patino J.M., Rubiolo A.C., Santiago L.G. (2010).Milk whey proteins and xanthan gum interactions in solution and at the air-water interface: A rheokinetic study. *Colloids and Surface B*: Biointerfaces (81) 50-57

22) Van Hunsel, J. Bleys G. Joos P. (1986) Adsorption Kinetics at the Oil/Water Interface. *Journal of Colloid and Interface Science* (114), 424-430

23) Demeter-Vodnàr J., Salajan M., Lowy D.A. (1996) Kinetic Study of the diffusion and Adsorption of Fatty Acids at the Benzene-Water Interface. *Journal of Colloid and Interface Science* (183), 424-430

24) Rosen M.J., Surfactants and interfacial phenomena, Third Edition, John Wiley & Sons, New Jersey (U.S.A) 2004, 208-238

25) Tripp B.C., Magda J.J., Andrade J.D. (1995). Adsorption of globular proteins at the air / water interface as measured via dynamic surface tension. Concentration dependence, mass – transfer considerations, and adsorption kinetics. *Journal of Colloids Interface Science* (173), 16.

26) Ward A.F.H. and Tordai L. (1946) Time-dependence of Boundary Tensions of Solutions. *The Journal of chemical physics* (14) 453-461

27) Graham D.E. and Phillips M.C.(1979) Proteins at liquid interface III Molecular structures of adsorbed films. *Journal of Colloid and Interface Science* (70), 427-439

28) Camino N.A., Pèrez O.E., Carrera Sanchez C., Rodriguez Patino J.M., Pilosof A.M.R. (2009). Hydroxypropylmethylcellulose surface activity at equilibrium and adsorption dynamics at the air-water and oil-water interfaces. *Food Hydrocolloids* (23) 2359-2368

29) Lucassen-Reynders E.H. (1966), Surface equation of state for ionized surfactants. *Journal Physical Chemistry* (70), 1777

30) Joos P. (1999), Dynamic Surface Phenomena. VSP BV, Utrecht.

31) Führling C. (2004), Interactions between Proteins, Sugars and Surfactants – Dynamic Studies on Adsorption at Interfaces. phD Thesis

32) Mackie A, Wilde P., (2005) The role of interactions in defining the structure of mixed protein–surfactant interfaces, *Advances in Colloid and Interface Science* (117), 3-13

33) V.B. Galazka, E. Dickinson, D.A. Leward (1996), Effect of high pressure on the emulsifying behaviour of β-lactoglobulin, *Food Hydrocolloids* (10) 213-219

34) Arai T., Norde W. (1990) (a), The behavior of some model proteins at solid-liquid interfaces 2. Sequential and competitive adsorption. *Colloids and Surfaces* (51), 1-16

35) Arai T., Norde W. (1990) (b), The behavior of some model proteins at solid-liquid interfaces 2. Sequential and competitive adsorption. *Colloids and Surfaces* (51), 1-16

36) Gunning P.A., Mackie A.R., Gunning A.P., Wilde P.J., Woodward N.C., Morris V.J. (2004), The effect of surfactant type on protein displacement from the air-water interface. *Food Hydrocolloids* (18) 2359-2368

37) Dickinson, E., *Introduction to Food Colloids,* Oxford University Press, Oxford, 1992.

38) Murray B.S. (2002), Interfacial rheology of food emulsifiers and proteins. *Current Opinion in Colloid & Interface Science* (7), 426-431

39) Ravera F., Loglio G., Kovalchuck V. I.(2010), Interfacial dilational rheology by oscillating bubble/drop methods. *Current Opinion in Colloid & Interface Science,* article in press

40) Kokelaar J.J., Prins A.& De Gee M. (1991), A new method for measuring the surface dilational modulus of a liquid. *Journal of Colloid and Interface Science* (146), 507-511

41) Sherriff M. & Warburton B. (1974), Measurement of dynamic rheological properties using the principle of externally shifted and restored resonance. *Polymer* (15), 253-254

42) Benjamins, J. and van Voorst Vader, F. (1992) *Colloids Surf*, 65, 161

43) Miller R., Policova *Z.*, Sedev R. and Neumann A. W. (1993) *Colloids and Surface A: Physicochemical Engineering Aspects* (76), 179

44) Graham D. E. and Phillips, M. C. (1980), Journal of *Colloid Interface Science* (76), 227

45) Dickinson E. and Tanai S. (1992), *Journal of Agriculture Food Chemistry* (40), 179

46) Stevenson E.M., Horne D.S., Leaver J. (1997), Displacement of native and thiolated b-casein from oil-water interfaces-effect of heating, ageing and oil phase, *Food Hydrocolloids* (11), 3-6

47) Murray B.S. (1997), Equilibrium and dynamic surface pressure-area measurements on protein films at air-water and oil-water interfaces. *Colloids and Surface A: Physicochemical Engineering Aspects* (125) 73-83

48) Williams A. and Prins A. (1996), Comparison of the dilational behavior of adsorbed milk proteins at the air-water and oil-water interfaces. *Colloids and Surfaces A. Physicochemical and Engineering Aspects* (114), 267-275

49) Kotsmar C., Pradines V., Alahverdjieva V.S., Aksenenko E.V., Fainerman V.B. Kovalchuk V.I., Krägel J., Leser M.E., Noskov B.A., Miller R. (2009), Thermodynamics, adsorption kinetics and rheology of mixed protein–surfactant interfacial layers, *Advances in Colloid and Interface Science* (150), 41–54

50) Hasenhuettl G.and Hartel W.R., Food emulsifiers and their applications, Second Edition Springer editor, New York (USA) 1998, chapter 5

51) Dickinson E. (1998), Proteins at interfaces and in emulsions. Stability, rheology and interactions. *Journal of the Chemical Society, Faraday Transactions* (94), 1657-1669

52) Maldonado-Valderrama J. and Rodrìguez Patino J.M. (2010) Interfacial rheology of protein–surfactant mixtures, *Current Opinion in Colloid & Interface Science* (15), 271–282

53) Rouimi S., Schrorsch C., Valentini C., Vaslin S., (2005) Foam stability and interfacial properties of milk protein-surfactant systems. *Food Hydrocolloids* (19), 467-478

54) Mackie A, Wilde P., (2005) The role of interactions in defining the structure of mixed protein–surfactant interfaces, *Advances in Colloid and Interface Science* (117), 3-13

55) Krägel J., O'Neill M., Makievski A.V., Michel M., Leser M.E., Miller R., (2003) Dynamics of mixed protein-surfactant layers adsorbed at the water/air and water/oil interface, *Colloids and Surfaces B: Bionterfaces* (31), 107-114

56) Rodrìguez Patino J.M., Rodriguez Nino M.R., Carrera Sànchez C. (2003) Protein-emulsifiers at the air water interface. *Current Opinion in Colloid & Interface Science* (8), 387-395.

57) Mackie A.R., Gunning P., Wilde P.J., Morris V.J.(1999) Orogenic Displacement of Protein from the Air/Water Interface by Competitive Adsorption *Journal of Colloid and Interface Science* (210), 157-166

58) Petkov J.T., Gurkov T.D.(2000), Dilatational and Shear Elasticity of Gel-like Protein Layers on Air/Water Interface, *Langmuir* (16), 3703-3711

59) Maldonado-Valderrama J. and Rodrìguez Patino J.M. (2010) Interfacial rheology of protein–surfactant mixtures, *Current Opinion in Colloid & Interface Science* (15), 271–282

60) Wilde P. (1998), Interfacial Rheology in Industry, Camtel Ltd, Royston, UK

Chapter 2
Interfacial dilational rheology by Pendant Drop/Bubble Method

Abstract.

The dynamic properties of interfaces together with the equilibrium values of interfacial tension and adsorption are often used to describe the effect of interface-active molecules on the phenomena connected to colloidal stability, and then, to many practical applications of emulsification and foaming. In this area dilational rheology represents a powerful tool to investigate equilibrium and dynamic properties of simple and more complex interfacial layers containing interfacial active molecules such as emulsifiers, proteins and polymers and to obtain useful correlations of the macroscopic behavior to fundamental microscopic phenomena. Concerning the experimental techniques, which are the focus of this article for dilational rheology, drop/bubble tensiometers based on the acquisition of the drop/bubble profile and or of the capillary pressure are especially effective. Their fundamental physical and mathematical principles are here shortly summarized. More attention is devoted to the drop shape method for the determination of the dilational viscoelasticity, which this technique is today widely employed for dilational rheology investigations due to its simplicity, versatility and its always better applicability to both liquid/liquid and liquid/air interfaces.

The main topic of this article is to describe the oscillating drop/bubble methodologies where harmonic variations of the interfacial area are utilized for dilational rheology investigations in the frequency domain.

1. Introduction

The equilibrium value of surface tension and adsorption are often used to describe the effect of surface-active molecules on phenomena connected to colloidal stability. In many instances, when dealing with liquid-gas or liquid-liquid interfaces, these properties alone have been shown to be insufficient for correlation of the macroscopic behavior to microscopic phenomena [Myrvold and Hansen (1998), Bos and Van Vliet (1998)]. These observations are relevant to many practical applications of emulsification and foaming. In these systems it is often the dynamic properties of interfaces that are important as a result of surface tension gradients and surface mobility. Being these systems in most practical cases subjected to dynamic conditions, dynamic interfacial tension and interfacial rheology, are then important characteristics which may be the driving force for their evolution and the key-feature for stability [Murray (1998), Bos and Van Vliet (1998), Ravera et al. (2010)].

The surface shear viscosity and elasticity, the 2-dimensional equivalents to ordinary bulk rheology, have been shown to be closely correlated with the stability of emulsions and foams [Wilde (2000), Bos and Van Vliet (1998), Petkov and Gurkov (2000)]. However, probably more important for these processes are the surface dilatational viscosity and elasticity that are often several orders of magnitude higher that the shear parameters. In case of dilational, or compression/expansion, rheology, the stress is the variation of the interfacial tension while the corresponding surface modification is the area change. Dilational rheology is of special significance for systems containing surfactants or, more in general, for composite interfacial layers where the interfacial tension changes due to surface relaxation processes or diffusion. For these systems a viscoelastic modulus, or dilational viscoelasticity, can be attributed to the interface characterizing its dynamic response to expansions/compressions. Moreover, the close link existing between the dynamic properties of the interfacial layers and the adsorption mechanisms makes dilational rheology a unique tool to access the characteristics of the transport and the kinetic processes determinant for the adsorption re-equilibration and the physicochemical properties of the involved surfactant-interface system. However to obtain qualitative and quantitative information about such adsorption mechanisms, it is necessary to adopt accurate and effective experimental methods, to access broad scales of time, and to interpret the rheological data by means of appropriate theoretical models.

Concerning the experimental techniques for dilational rheology several methods have been developed for the measurement of the surface dilational properties such as methods utilizing the Langmuir surface balance, the elastic ring method, surface waves and the oscillating bubbles/drops [McClements (1999), Bos and Van Vliet (1998)]. Of these, the latter method, which is the main topic of this article, is especially effective and, thus, bubble/drop techniques are today widely employed for dilational rheology investigations due to their simplicity, versatility and their always better applicability to both liquid-liquid and liquid-air interfaces [Ravera et al. (2010), Lin et al. (1996), Myrvold and Hansen (1998), Arashiro and Demarquette (1998), Zeppieri et al. (2001), Chen et al. (1998), Ravera et al. (2009)].

Oscillating bubble/drop methods derive from classical methods, originally conceived for interfacial tension measurements. By oscillating the drop/bubble and simultaneously measuring the surface area and surface tension, the surface dilational moduli can be determined. Two main types of bubble/drop techniques characterized by two different ways of monitoring the interfacial tension and then, the interfacial tension response $\Delta\gamma$, are utilized to calculate the dilational viscoelasticity: one is based on the analysis of the drop profile and the other one on the capillary pressure measurement [Ravera et al. (2010), McClements (1999)]. Both techniques exploit the relationship existing for fluid interfaces between the interface curvature, the pressure difference across them and their interfacial tension, as stated by the Laplace equation, but are appropriate for different scale time. For this reason in many experimental works an enlargement of the frequency range which is useful to study different relaxation processes responsible for the dynamic behavior of fluid interfaces, is obtained by coupling the two techniques and by using different tensiometers [Ravera et al. (2010)].

This article focuses on oscillating drop/bubble methodologies where harmonic variations of the interfacial area are utilized for dilational rheology investigations in the frequency domain. [Ravera et al. (2010)].

2. Experimental techniques of Pendant Drop/Bubble

The fundamental physical and mathematical principles for drop shape and capillary pressure tensiometries are here shortly above summarized. The pendant drop/bubble method involves the determination of the profile of a drop/bubble suspended in another fluid at mechanical equilibrium or the measurement of the capillary pressure. Both techniques used to measure the interfacial tension exploit the relationship existing for fluid interfaces between the interface curvature, the pressure difference across them and their interfacial tension, as stated by the Laplace equation [Ravera et al. (2010), Ravera et al. (2009)].

However, the interfacial tension response $\Delta\gamma$, which is necessary to calculate the dilational viscoelasticity, is obtained in different ways. In case of drop/bubble profile tensiometry where sufficiently large drops and bubbles which are well deformed due to gravity are used, the local pressure difference across the interface is obtained from the drop/bubble shape because the main curvature appears not constant and the capillary pressure varies along the interface.

On the contrary, in the case of pressure tensiometry the drops and bubbles used are small and practically not deformed in the gravity field and, as a consequence, the shape of the liquid meniscus appears very close to a spherical shape and the capillary pressure in different points at the interface is almost constant. This allows one to measure this unique capillary pressure directly by a pressure sensor.

These techniques are effective for liquid/liquid and air/liquid interfaces and can be used according to different methodologies to investigate different dynamic aspects of the interfacial properties.

Concerning the dilational viscoelasticity measurements, as each tensiometer works properly in a given time scale, their coupling is very effective to cover a quite broad frequency range [Ravera et al. (2010)]. These methods are limited in frequency by the onset of non radial oscillations and also by other fluodynamic effects which, set a limit between 100 and 1000 Hz, depending on the experimental setup and conditions [Freer et al.(2005), Leser at al. (2005), Ravera et al. (2010), Miller et al. (2005), Ravera et al. (2009)]. Therefore, for higher frequencies, drop/bubble tensiometers are not longer appropriate and other methods such as those based on capillary waves damping are instead preferable [Ravera et al. (2010), Ravera et al. (2009)].

2.1 Theory of drop/bubble shape tensiometry

The drop/bubble shape tensiometry is a well-established technique for determining the mechanical properties of liquid/gas and liquid/liquid interfaces. Under gravity conditions, a bubble or a drop of one liquid inside another fluid assumes a shape which minimises the total energy of the system. Such shape is determined by a combination of surface tension and gravity effects: surface forces tend to make drops and bubbles spherical whereas gravity tends to vertically elongate or squeeze them. At each point of the surface of a drop/bubble, under mechanical equilibrium condition, the relation between the pressure difference across the interface, the surface tension and the surface curvature is provided by the Laplace equation (eq.1) [Ravera et al. (2009)].

$$\Delta P = \gamma\left(\frac{1}{R_1}+\frac{1}{R_2}\right) \qquad (1)$$

Where ΔP is the pressure difference across the interface, γ is the surface tension, R_1 and R_2 are the two principal radii of curvature of the interface defined in figure 1.

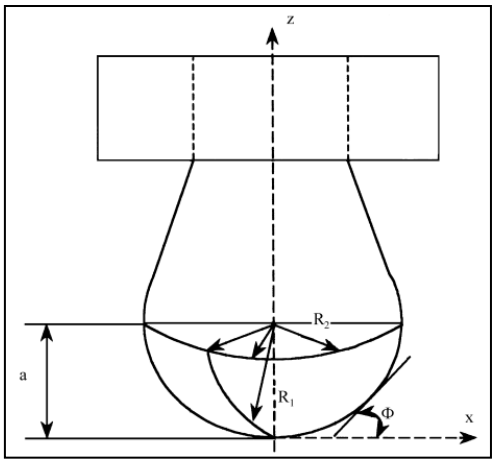


Figure 1 - The pedant drop geometry [Arashiro and Demarquette (1999)]

For axis-symmetric menisci, the Laplace equation together with the pressure dependence on the drop height (eq.2), due to the gravity field, lead to a differential equation (eq.3), in terms of geometrical parameters of the drop/bubble (fig.1) with no analytical solution. This is known as Laplace-Young equation.

$$\Delta P = \Delta\rho g h \qquad (2)$$

$$\Delta\rho g h = \gamma\left[\frac{d^2z/dx^2}{\left[1+(dz/dx)^2\right]^{3/2}} + \frac{dz/dx}{x\left[1+(dz/dx)^2\right]^{1/2}}\right] \tag{3}$$

Where Δρ is the difference in densities between the fluids in contact, g the acceleration of gravity, h the drop height, and x and z are the geometrical coordinates of the drop profile defined as in figure 1. The Bashforth and Adams equation (eq. 4) is given below, was the first equation to put forth a numerical solution to the Laplace-Young equation relating the drop profile to the interfacial tension and providing the theoretical profile of meridian section of the drop/bubble.

$$\frac{1}{\frac{R_1}{a}} + \frac{\sin\phi}{\frac{x}{a}} = -B\frac{z}{a} + 2 \tag{4}$$

Where B is a dimensionless shape factor relating the drop deformation due to gravity effect to the interfacial tension, and is given by

$$B = \frac{a^2 g \Delta\rho}{\gamma} \tag{5}$$

and a is the radius of curvature at the apex of the drop, and ϕ is the coordinate defined in figure 1. R_1 and ϕ can be defined geometrically by:

$$R_1 = \frac{\left[1+\left(\frac{dz}{dx}\right)^2\right]^{3/2}}{\frac{d^2z}{dx^2}} \tag{6}$$

$$\sin\phi = \frac{\frac{dz}{dx}}{\left[1+\left(\frac{dz}{dx}\right)^2\right]^{1/2}} \tag{7}$$

The theoretical profile of a pendant drop/bubble derived by Bashforth and Adams, results to be dependent on a dimensionless factor B, which is fundamental to calculate the contours of the drop and bubble, and then, to determine the interfacial tension. In fact, the working principle for the measurement is to determine this parameter by fitting the experimentally measured drop/bubble contour to the theoretical curve, and subsequently to find the interfacial tension [Ravera et al. (2010), Arashiro and Demarquette (1998), Chen et al. (1998)].

According to this principle, there are then two fundamental requirements for the application of this technique: the two involved fluids must have an appreciable density difference and the interface must be not far from the mechanical equilibrium. In fact if the liquids are isodense the drop is spherical whatever is the interfacial tension which then results undetermined. The dimensionless shape factor B gives an estimation of the level of drop deformation due to gravity effect and in practice determines the measurement accuracy. In many practical cases, with the most common acquisition systems, accurate data are obtained for $|B|>0.1$ [Ravera et al. (2010)].

The requirement of mechanical equilibrium of the interface implies that only slow variation of the surface area can be applied to obtain accurate measurements. The drop shape tensiometry is then commonly used for measuring interfacial tension at constant area to investigate for example adsorption kinetics or during slow variations of the surface area to investigate interfacial rheology at low frequency [Ravera et al. (2009), Ravera et al. (2010), Chen et al. (1998)].

On the other side the advantages of this tensiometric thecnique are numerous. Only very small amounts of the liquid are required, just enough to form one drop. It is suitable for both liquid/vapour and liquid/liquid interfaces, and applicable to materials ranging from organic liquids to molten polymers [Arashiro and Demarquette (1998)] and from pure solvents to concentrated solutions [Ravera et al. (2010), Lin et al. (1996), Myrvold and Knut Hansen (1998), Zeppieri et al. (2001), Chen et al. (1998)].

Operating at constant interfacial area, the time scale ranges from parts of a second up to hours and even days so that even extremely slow processes can be easily followed.

Under periodic perturbation of interfacial area, the technique allows the acquisition of the interfacial tension response for requencies spanning some decades in the low frequency range, i.e. 10^{-5}–10^{-1} Hz [Ravera et al. (2010)]. At higher oscillation frequencies hydrodynamic and viscous effects cause deviations of drop and bubble profiles from a Laplacian shape [Freer et al.(2005), Leser at al. (2005), Ravera et al. (2010), Miller et al. (2005), Ravera et al. (2009)]. Thus, high-frequency studies require other technique, such as capillary pressure method, which reaches frequencies up to 150 Hz, or the capillary wave damping techniques, which can work even at oscillation frequencies of up to 1000 Hz [Leser at al. (2005)].

A typical drop shape tensiometer apparatus which is shown in figure 2, consists of three parts: an experimental illuminating cell, a viewing system to visualize the drop and a data acquisition system to infer the interfacial tension from the pendant profile.

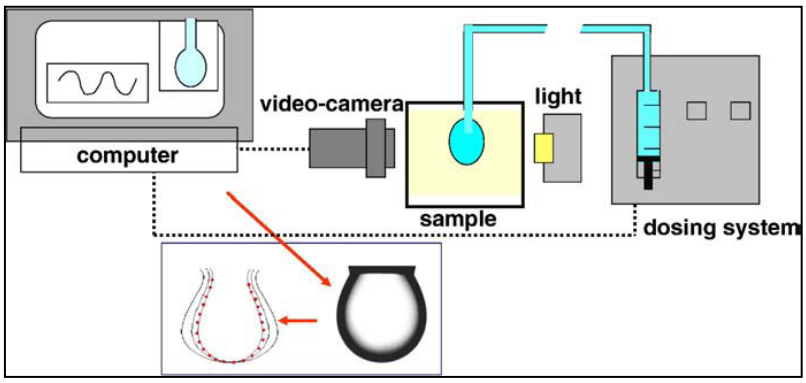


Figure 2 – Scheme of an experimental set-up of Drop Shape Analysis Pendant Drop/Bubble [Ravera at al. (2010)]

In the cell a drop or a bubble is formed inside the other fluid, at the tip of a vertical or of a U shaped capillary by using an automated pump that can be fitted with various sizes of syringes and needles to allow for control of pendant drop formation. According to the densities of the two adjoining phases, variant configurations are possible, namely pendant/emerging drops, sessile drops and captive/emerging bubbles.

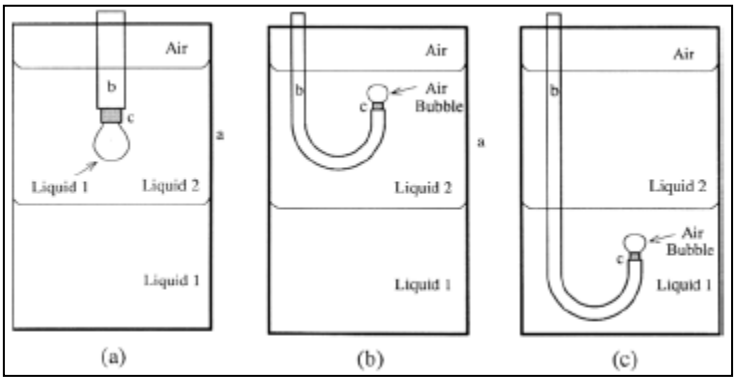


Figure 3 - Experimental set-up for (a) interfacial tension measurements of liquids 1 and 2, (b) surface tension measurements of liquid 2, and (c) surface tension measurements of liquid 1, where liquid 1 is heavier than liquid 2 [27]. a - quartz cuvette, b - metal tubing, and c - teflon capillary [Chen et al. (1998)]

The drop/bubble is continuously obtained by microscope and monitored by a video-camera coupled to a computer and its profile is acquired in an automatic way in order to

calculate the surface tension by means of a numerical fitting procedure. The video signal of the drop/bubble is transmitted to a digital video processor which performs the frame grabbing and digitization of the image to a prefixed number to pixels with 256 grey levels.

The automated image viewing and capturing system, with various image capture triggering options, can be used to capture drop image. The software allows for an automated drop shape analysis of the captured drop image, and for measuring the surface/interfacial tension. The whole process of digitalization and analysis of the drop/bubble consists often of four step: capture and digitalization of the image; extraction of the drop/bubble contour and determination of the radius of curvature at the apex; smoothing of the extracted contour of the drop/bubble using polynomial regression; shape comparison between the theoretical and experimental profile, inferring the interfacial tension value [Arashiro and Demarquette (1998)].

Modern versions of drop shape tensiometers and in particular some commercial tensiometers offer the option of controlling the interfacial area and all the geometrical characteristics of a drop/bubble as a function of time, by a feed-back loop comparing the observed drop area-value with the set-value in a pre-defined time-line. This feature is an essential instrumental tool for studying the dynamic interfacial tension and the interfacial responses to controlled area perturbations [Ravera et al. (2010), Chen et al. (1998)].

Different algorithms and procedures have been proposed and are available to obtain interfacial tension from drop/bubble profile measurements [Maze and Burnet (1969), Rotemberg et al. (1983), Pallas and Harrison (1990)]. In particular, in the axisymmetric drop shape analysis (ADSA) of Rotenberg et al. [Rotemberg et al. (1983)] a fitting procedure was developed which is based on minimization of the error function defined by the deviation of the theoretical curve from the experimental profile. In this procedure the parameter B, the coordinates of the drop apex, and the curvature at the drop apex (a) are used as adjustable parameters [Ravera et al. (2010)].

A critical aspect in drop/bubble shape analysis is an accurate edge detection procedure. The edge detection methods are continuously improved to provide better sensitivity, resistance against noise and to minimize possible errors. The derivative algorithms analysing the variation of light intensity near the edge are the most popular way to

develop of an edge detector. In particular, in gradient edge detections the position of the local maximum of the gradient across an edge is assumed to be the drop edge coordinate. The edge detection can be improved by using special correction and smoothening procedures [Hoorfar and Neumann (2006)].

2.2 Theory of capillary pressure tensiometry

The capillary pressure tensiometry exploits in a direct way the Laplace equation being based on the direct measurement of the pressure difference across the interface of a spherical, or nearlyspherical, drop/bubble. Indeed, according to the Laplace equation, such pressure, P, is directly linked to the interfacial tension

$$P = \frac{2\gamma}{R} + P_0 \tag{8}$$

where R is the drop radius (or the curvature radius at the apex of the drop) and P_0 can be either an hydrostatic constant or a fluid-dynamic term, depending on the adopted experimental methodology. The interfacial tension can be then inferred from the above relationship by knowing P, R and P_0 versus the time [Ravera et al. (2010)].

A typical capillary pressure tensiometer is composed by two chambers connected by a capillary tube, as schematically sketched in figure 4. One of the chambers is closed and contains both the pressure sensor and a piezoelectric rod. The latter is utilised to control the volume of the drop/ bubble formed at a tip of a glass capillary. A video camera allows for a continuous monitoring of the drop. Sub-millimetric droplets are typically utilised to obtain measurable capillary pressure values, which are typically of the order of a hundred Pascal. The drop radius is either measured by direct imaging or calculated from the injected liquid volume, if the compressibility of the closed phase is known. The other cell either is open to atmospheric pressure or, if closed, contains another pressure sensor. Practically, two configurations are usually utilized as sketched in figure 4, in which the closed cell may contain either the liquid phase forming the drop or the liquid surrounding the drop [Liggieri and Ravera (1998), Liggieri et al. (2002), Ferrari et al. (1998)].

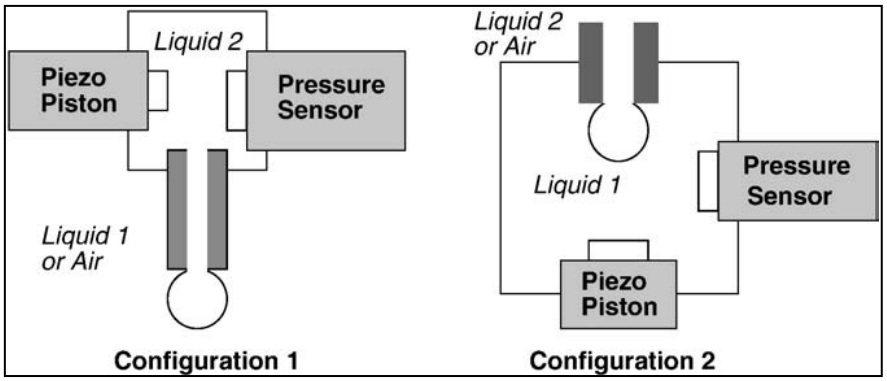


Figure 4 - Sketch of the measurement cell of a capillary pressure tensiometer according to two possible configurations [Ravera at al. (2010)]

An important characteristics of this technique is that it does not require gravity deformed droplet but it works preferably with spherical interfaces, which makes it suitable both for liquid/liquid and liquid/air systems, using either small drops or small density difference.

The capillary pressure tensiometry has been widely utilised in many experimental investigations of adsorbed layers based on the measurement of equilibrium and dynamic interfacial tension and of the dilational visco-elasticity [Liggieri et al. (2002)], both at water/air and water/oil interfaces.

The capillary pressure tensiometry is a versatile technique which can be used according to different experimental methodologies, to investigate dynamic and equilibrium aspects of pure and composite surfactant systems.

The method has also been proposed to investigate adsorption kinetics. This method does not require the direct drop imaging, being possible to calculate the drop curvature from the volume rate. The growing drop experiments are also used for side measurements in dilational studies to evaluate the compressibility of the system [Liggieri et al. (2002)].

Rheological studies can be performed in the capillary pressure tensiometry where the response of the interfacial tension to small amplitude perturbation of the surface area are investigated. These perturbations can be aperiodic functions, such as in the stress-relaxation experiment, or periodic with different shapes of the forced signal, such as pulsed, trapezoidal or sinusoidal [Ravera et al. (2010)]. In this method harmonic perturbations are applied to the drop/ bubble interfacial area by means of controlled displacement of liquid volume while the oscillating pressure response, related to the response of the interfacial tension, is acquired.

3. The oscillating drop/bubble method

3.1 Generalities on the dilational visco-elasticity

The rheological properties of adsorption layers are expressed by the relationship between the variation of the interfacial tension γ, from its initial value γ_0, and the expansion or contraction of the surface area A.

The dilational stress of a system subjected to expansion or contraction of the surface area A can be written as the sum of two terms [Ravera et al. (2009)]:

$$\Delta\gamma = E_0 a + \eta \dot{a} \tag{9}$$

The first one, purely elastic, is proportional to the relative variation of the area *a*

$$a = \frac{\Delta A}{A_0} = \frac{\left(A(t) - A_0\right)}{A_0} \tag{10}$$

The second one is instead a viscous term proportional to the rate of the area variation $\dot{a}$

$$\dot{a} = da/dt \tag{11}$$

This viscous character arises from the relaxation processes in the adsorbed layer or from the adsorption re-equilibration.

The coefficient E_0 and η, are then termed as the dilational surface elasticity and viscosity respectively. Equation 9 leads to a definition of the complex dilational visco-elastic modulus or the dilational viscoelasticty, E. In fact for a low amplitude harmonic perturbation of angular frequency ω, the area perturbation can be expressed in terms of the amplitude of area oscillation $\tilde{A}$, ω and the time *t* as

$$\Delta A = \tilde{A} t^{i\omega t} \tag{12}$$

From equation 9 one obtains

$$E = \frac{\Delta\gamma}{\Delta A / A_0} = E_0 + i\omega\eta \tag{13}$$

The dilational viscoelasticity E is then a frequency dependent complex quantity, where the real part $E' = E_0$ is the dilational elasticity and the imaginary part $E'' = 2\pi\nu\eta$ is directly related to the dilational viscosity. Considering only small amplitude perturbation, the systems behaves linear, and it can be expressed, according to the Fourier formalism for linear systems, as asuperposition of harmonic components in the domain of frequency.

When a purely harmonic perturbation is applied to the surface at a given frequency, the interfacial tension and the other quantities influenced by this perturbation vary according also a harmonic variation at the same frequency. Thus, the variation of a time dependent quantity can be written as a superposition of harmonic functions. Using the Fourier formalism, from equation 9, the response of the interfacial tension to an arbitrary area variation of the adsorbed layer is given by [Ravera et al. (2009), Ravera et al. (2010)]

$$\Delta\gamma(t) = \int_0^t \hat{\varepsilon}(\tau)a(t-\tau)d\tau \qquad (14)$$

where $\hat{\varepsilon}$ e is the inverse Fourier transform of E. Thus the complex modulus E(ω) can be considered as the transfer function of the interfacial layer which is assumed to be a linear system. Equation 14 evidences that the interfacial tension response of an interfacial layer can be assessed in a way as accurate as large is the frequency range where E(ω) is determined.

3.2 Measurement method

Drop/bubble tensiometers are particularly suitable to investigate the dynamic behaviour of the adsorbed layers when they are used according to the oscillating drop/bubble methodology to measure the visco-elasticity versus frequency.

These tensiometers allow precise measurements of this complex quantity for various types of fluid interfaces. Any deviation from equilibrium causes various relaxation processes at the interface and in the adjacent bulk solution. As a result the interfacial tension varies periodically with the same frequency and with a certain delay with respect to the external disturbance.

For small-amplitude harmonic perturbations of the surface area the magnitude and phase of the interfacial tension response is directly related to the dilational viscoelasticity through equations (13) or (14).

Whatever is the tensiometer employed, in order to get the frequency trend of the dilational viscoelasticity, the oscillating drop experiments are usually performed by applying a frequency sweep to the surface area A. Thus for each frequency ω it holds:

$$A = A_0 + \tilde{A}\sin(\omega t) \tag{15}$$

Where A_0 is the reference surface area and $\tilde{A}$ the amplitude of the area oscillations. The harmonic response of the surface tension γ can be described by the function:

$$\gamma = \gamma_0 + \tilde{\gamma}\sin(\omega t + \delta) \tag{16}$$

Where γ_0 is the equilibrium reference surface tension and $\tilde{\gamma}$ the measured amplitude of the surface tension oscillations. The phase shift δ between the area perturbation and the response of the interfacial tension, is the phase of the complex dilational modulus. In the usual manner, equation 16 may be written as

$$\Delta\gamma = \tilde{\gamma}\sin(\omega t)\cos\delta + \tilde{\gamma}\cos(\omega t)\sin\delta \tag{17}$$

And by using the Gibbs definition of the surface elasticity (eq.18) [Myrvold and Hansen (1998), Chen et al. (1998), Ravera et al. (2009)]

$$E = \frac{d\gamma}{d\ln A} \tag{18}$$

we see that the complex surface dilational modulus is the expressed by [Ravera et al. (2009), Ravera at al. (2010), Freer et al. (2004)]

$$E^*(\omega) = \frac{\tilde{\gamma}}{\tilde{A}/A_0}e^{i\delta(\omega)} \tag{19}$$

Equation 19 provides an expression for E in terms of quantities that can be determined experimentally as frequency functions, either in a direct way or by an appropriate calculation procedure, depending on the utilized tensiometer.

Because the drop area oscillates periodically, the dilatational modulus exhibits two elements: an elastic part accounting for the recoverable energy stored in the interface (storage modulus, *E'*) and a viscous part accounting for energy lost through relaxation processes (loss modulus, *E''*). The interfacial storage and loss moduli correspond to the real and imaginary components of the complex dilatational elasticity [Freer at al. (2004), Dicharry et al. (2009)]:

$$E^* = E' + iE'' = |E|\cos\delta + i|E|\sin\delta \tag{20}$$

where

$$|E| = \frac{\tilde{\gamma}}{\tilde{A}/A_0} \tag{21}$$

Then the absolute modulus $|E|$ is a measure of the total material dilational resitance to deformation (elastic and viscous). For a perfectly elastic material stress and strain are in phase $\delta = 0$ and the imaginary term is zero. In the case of perfectly viscous material $\delta = 90°$ and the real part is zero. The loss angle tangent can be defined by equation (22); thus, if the film is purely elastic, the loss angle tangent is zero:

$$\tan\delta = E''/E' \qquad (22)$$

When the oscillating drop/bubble method is applied in a drop shape tensiometer, γ against time is directly acquired while a controlled harmonic perturbation is applied to the surface area. The amplitudes $\tilde{A}$ and $\tilde{\gamma}$ and the phase shift δ are obtained as amplitudes and phase of the components of frequency ω, extracted by the experimental signals via DFT (Discrete Fourier Transform) algorithms [Loglio et al. (2005), Loglio et al. (2004)].

At each frequency the complex dilational viscoelasticity is then calculated according to equation 19. In figure 5 data acquired during the surface oscillation are reported as an example with the extracted theoretical harmonics utilised for the calculation of E*.

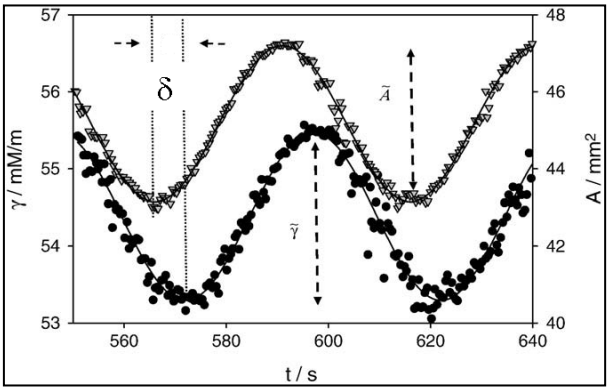


Figure 5 - Example of acquired surface tension (○) and surface area (▽) by the oscillating drop method in the drop shape tensiometry [Ravera at al. (2010)]

When oscillating drop/bubble techniques are applied in a capillary pressure tensiometry the measurable quantities are the pressure inside the closed cell and the elongation of the piezoelectric rod which, due to the compressibility of the system does not necessarily correspond to the variation of the drop volume. In fact, while at low frequency the geometrical characteristics of the drop (radius and surface area) can be directly measured by imaging techniques, such direct acquisition of the drop profile

variation becomes more and more unreliable at increasing frequencies. To get dilational viscoelasticity for frequency of the order of 1 Hz and higher, a specific experiment theory is necessary allowing for the calculation of E(ω) from the acquired signals [Ravera et al. (2010)].

3.3 Oscillatory experimental signals

The real oscillating signals are the surface tension and surface area, in the case of drop shape tensiometer, and the pressure and piezo volume for capillary pressure tensiometer. As a matter of fact, even under experimental conditions ensuring the linearity of the system, a real output signal may contain harmonics with frequencies different from that imposed due, for example, to some external disturbances (drift or low and high frequency noise) or to a weak non linearity of the system [Loglio et al. (2004)]. For this reason it is preferable to adopt a procedure of harmonics extraction with respect to a fitting procedure with a sinusoidal function which, when the experimental signal contains other frequency components, may definitely ensue in meaningless amplitude and phase values. The mathematical procedure for the harmonics extraction is based on the Discrete Fourier analysis. The component at frequency ω of a generic experimental signal *g(t)* presenting a phase δ is

$$g = g^0 + \tilde{g}\sin(\omega t + \delta) \tag{23}$$

Considering the discretisation of this experimental signal, at the generic time t_j, using the Fourier series expansion

$$\Delta g_j = A\cos(\omega t_j) + B\sin(\omega t_j) \tag{24}$$

Where $A = 1/N\sum_{K=1}^{N} g_k 2\cos(\omega t_k)$, $B = 1/N\sum_{K=1}^{N} g_k 2\sin(\omega t_k)$, N is the number of experimental points and g_k is the measured value at the time t_k. Thus one obtains

$$\tilde{g} = \sqrt{A^2 + B^2} \tag{25}$$

$$\delta = \arctan\left(\frac{B}{A}\right) \tag{26}$$

In a typical oscillating drop experiment the oscillating signal is acquired for a number of cycles sufficient to warrant the achievement of stationary oscillations and a significant statistics.

The validity of the linearity hypothesis is of crucial importance for such analysis because the magnitude of the dilational modulus, which is a transfer function, should be independent of the oscillation amplitude. In [Ravera et al. (2010)] the total harmonic distortion (THD) parameter was proposed for a quantitative estimation of the goodness of the linearity hypothesis in oscillation experiments with drops and bubbles. It can be defined as

$$THD = \frac{\left(a_2^2 + a_3^2 + ... + a_n^2\right)^{1/2}}{a_1} \qquad (27)$$

where a_1 is the amplitude value at the fundamental frequency and a_2, a_3, …, an amplitude values of the higher harmonics. Under condition of periodic oscillations with different amplitudes at the same frequency, the THD parameter shows the existence of a linearity range in the relationship between an imposed interfacial area variation and the resulting interfacial tension response.

The condition of the mechanical equilibrium of the drop/bubble to have Laplacian profile is very important especially when a drop shape tensiometer is used for dilational rheology measurements, i.e. when periodic oscillation are applied to the surface area.

Increasing the frequency of the area perturbation in fact a threshold may be overcome where the interface is no more at mechanical equilibrium as shape distortion occurs due to viscous forces and to triggering of drop/bubble normal oscillation modes [Ravera et al. (2010), Freer et al. (2005)]. About this upper limit in the frequency range, specific investigations have shown [Ravera et al. (2010)] that, for amplitude of the area oscillations below 10%, the drop can be considered at mechanical equilibrium for frequencies below 1 Hz. This condition holds for water–air systems while for more viscous liquids or liquid–liquid interfaces, the limit frequency reduces to about 0.1 Hz.

Similar results on this frequency limit are found in Ref. [Freer et al. (2005)]where the effects of oscillating a viscous oil drop in water, on the drop profile based measurements, is experimentally explored.

4. Conclusions

Dilational rheology represents a powerful tool to investigate equilibrium and dynamic properties of simple and more complex interfacial layers containing surfactants, proteins, polymers or micronano sized particles.

The development of oscillating bubble and drop techniques during the last years responded to the need of obtaining increasingly accurate measurements of the parameters charactering dilational rheology. The oscillating drop/bubble methods provide today the possibility to obtain accurate data of dilational viscoelasticity as a function of the frequency in a quite broad frequency range. This is especially true when the two kinds of tensiometers available, drop shape tensiometry and capillary pressure tensiometry, are coupled to investigate the same system.

Many improvements of the efficiency of these techniques have been obtained during the last ten years due, from one side, to the implementation of advanced instrumentations which make faster the drop/bubble control and the data acquisition and, on the other side, to the application of new theoretical approaches for data acquisition and interpretation. Further improvements of these techniques are however expected especially concerning the enlargement of the frequency range and the field of applicability. In this way systematic and accurate rheological investigations should be undertaken aimed at deepening the understanding of the relationship expected between the interfacial rheology and the stability conditions and the behaviour of liquid films, emulsions and foams.

REFERENCES

1) Myrvold R. and Knut Hansen F. (1998), Surface Elasticity and Viscosity from Oscillating Bubbles Measured by Automatic Axisymmetric Drop Shape Analysis, *Journal of Colloid and Interface Science* (207) 97–105
2) Bos M.V. and van Vliet T. (2001), Interfacial rheological properties of adsorbed protein layers and surfactants: a review, *Advances in Colloid and Interface Science* (91), 437-471
3) Murray S.B., (1998) Interfacial rheology of mixed food protein and surfactant adsorption layers with respect to emulsion and foam stability in M*ö*bius D. and Miller R., Studies in interface science 7, Proteins at Liquid Interfaces, Elsevier editor, Amsterdam (The Netherlands), chapter 5
4) Ravera F., Loglio G., Kovalchuck V. I.(2010), Interfacial dilational rheology by oscillating bubble/drop methods, *Current Opinion in Colloid & Interface Science*, doi:10.1016/j.cocis.2010.04.001
5) Wilde P.J. (2000), Interfaces: their role in foam and emulsion behavior, *Current Opinion in Colloid & Interface Science* (5), 176-181
6) Petkov J.T., Gurkov T.D.(2000). Dilatational and Shear Elasticity of Gel-like Protein Layers on Air/Water Interface, *Langmuir* (16), 3703-3711
7) McClements D. J.,Food Emulsions Principles, Practice and Techniques, CRC Press LLC (1999), chapter 5;
8) Lin S.Y., Wang W.J., Lin L.W., Chen L.J. (1996) Systematic effects of bubble volume on the surface tension measured by pendat bubble profiles *Colloids and Surfaces A: Physicochemical and Engineering Aspects* (114), 31-39
9) Arashiro E. Y. and Demarquette N.R. (1999) Use of the Pendant Drop Method to Measure Interfacial Tension between Molten Polymers, *Materials Research*, (2), 23-32;
10) Zeppieri S., Rodrìguez J., Lòpez de Ramos A.L. (2001), Interfacial Tension of Alkane+ Water Systems, *Journal of Chemical Engineering Data* (46), 1086-1088

11) Chen P., Kwok D.Y., Prokop R.M., del Rio O.I., Susnar S.S., Neumann A.W., Axisymmetric Drop Shape Analysis (ADSA) and its applications. In: M*ö*bius D. and Miller R. Editors, Drops and Bubbles in Interfacial Research, Elsevier Science (1998), 61-136.

12) Ravera F., Liggieri L., Loglio G. Dilational rheology of adsorbed layers by oscillating drops and bubbles. In: R. Miller, L. Liggieri Interfacial Rheology Volume 1 , Brill Academic Pub, Leiden Boston 2009, 138-177

13) Freer E.M., Wong H., Radke C.J. (2005), Oscillating drop/bubble tensiometry: effect of viscous forces on the measurement of interfacial tension, *Journal of Colloid and Interface Science* (282) 128–132

14) Leser M.E., Acquisrapace S., Cagna A., Makievski A.V., Miller R. (2005), Limits of oscillations frequencies in drop and bubble shape tensiometry, *Colloids and Surfaces A: Physicochemical and Engineering Aspects* (261), 25-28

15) Maze C. and Burnet G. (1969), A non-linear regression method for calculating surface tension and contact angle from the shape of a sessile drop. *Surface Science* (13),451–70.

16) Rotenberg Y., Boruvka L., Neumann A.W. (1983), Determination of surface tension and
contact angle from the shapes of axisymmetric fluid interfaces, *Journal of Colloid Interface*
Science (93),169–73.

17) Pallas N.R., Harrison Y. (1990)An automated drop shape apparatus and the surface tension of pure water, *Colloids Surface* (43),169–94.

18) Hoorfar M. and Neumann A.W. (2006), Recent progress in Axisymmetric Drop Shape Analysis (ADSA). *Advances in Colloid Interface Science* (121), 25–49.

19) Liggieri L., Ravera F., Capillary pressure tensiometry and applications in microgravity. In: M*ö*bius D. and Miller R. Editors, Drops and Bubbles in Interfacial Research, Elsevier Science (1998), 239-278.

20) Liggieri L., Attolini V., Ferrari M., Ravera F. (2002), Measurement of the surface dilational viscoelasticity of adsorbed layers with a capillary pressure tensiometer. *Journal of Colloid Interface Science* (252), 225–35.

21) Ferrari M., Liggieri L., Ravera F. (1998), Adsorption properties of C10E8 at the water–hexane interface. *Journal of Physical Chemical B* (102), 10521-10527.

22) Freer E. M., Sub Yim K., Fuller G. G., Radke C. J. (2004), Shear and Dilatational Relaxation Mechanisms of Globular and Flexible Proteins at the Hexadecane/Water Interface. *Langmuir* ***(****20),*10159-10167

23) Dicharry C., Arla D., Sinquin A., Graciaa A., Bouriat P. (2006) Stability of water/crude oil emulsions based on interfacial dilatational rheology. *Journal of Colloid and Interface Science* (297) 785–791

24) Loglio G., Pandolfini P., Miller R., Makievski A.V., Krägel J., Ravera F.(2005), Perturbation–response relationship in liquid interfacial systems: non-linearity assessment by frequency-domain analysis. *Colloids Surface A* (261), 57–63.

25) Loglio G., Pandolfini P., Miller R., Makievski A.V., Kr¨agel J., Ravera F. (2004), Oscillation of interfacial properties in liquid systems: assessment of harmonic distortion. Physical Chemistry (6), 1375–1379.

Chapter 3
The effect of surfactant type on rheology of Ovalbumin layers adsorbed at the air/water and oil/water interfaces

Abstract

The aim of this research is to quantify the competitive adsorption of Ovalbumin protein and two different types of food emulsifiers, nonionic Tween60 and anionic Admul Datem, respectively, at the air-water and sunflower oil-water interfaces by means of dynamic interfacial tension measurements and harmonic drop oscillation experiments on a time scale of some seconds. Axialsymmetric drop shape analysis (ADSA) was used to calculate drop volume, area and interfacial tension. One value of protein concentration was studied (10^{-2}% wt), corresponding to the typical one used in many literature studies, whereas the emulsifier/protein weight ratio was changed in the range between 0 and 0.6. Temperature and pH of aqueous systems were kept constant at 22°C and 6.5 respectively.

Adsorption behavior at both interfaces was discussed from a kinetic point of view in terms of molecular diffusion and penetration of adsorbed protein molecules in the presence of low molecular weight surfactants.

The influence of the neighbouring phase was recorded and differences between air-water and oil-water interfaces were individuated for each studied system. A dependence of the dilational parameters on the frequency of drop volume oscillation was determined and air-water and oil-water rheological properties were studied by using a gel critical approach, assuming that the interfaces were a critical gel composed of molecules which prevent film rupture.

Different effects both on adsorption behavior and the rheological properties of the Ovalbumin layers were found in the presence of Tween60 and Admul Datem emulsifiers respectively, owing to dissimilar competitive phenomena.

1. Introduction

Protein-stabilized food dispersions often contain a range of surface active species, many of them are of low molecular weight and include lipids and/or food emulsifiers.

Owing to the widespread use of proteins and low molecular weight surfactants, and their different colloid-stabilizing properties, much attention has been paid to their interfacial behavior over the last half century.

Proteins are co-polymers of amino acids with hydrophobic and hydrophilic residues, which give them a strong tendency to adsorb at both types of interface [Bos and van Vliet (2001)].

Conversely, low-molecular-weight (LMW) surfactants, which may be natural components in foods, such as phospholipids, glycerides, fatty acids, etc., or synthetic molecules such as the Spans and Tweens, are amphipilic molecules, which may adsorb strongly or weakly at the interface depending on the chemical structure, i.e., the relative size of the hydrophilic and hydrophobic portions of the molecule (or HLB) [Murray (1998), Bos and van Vliet (2001)]. Owing to the higher molecular weight of proteins and the co-operative nature of their adsorption, protein adsorption and desorption tend to be considerably slower than those of LMW surfactants and may involve considerable changes in the three-dimensional structure of the molecule, unlike with LMW surfactants, where such structural changes are minimal.

These differences can lead to very dissimilar interfacial rheological properties for proteins and LMW surfactants and, consequently, there are large variations in behavior possible when the two are present in admixture, since both will compete for adsorption at the interface [Murray (1998)].

Proteins can interact with LMW surfactant molecules in the bulk and at the surface/interface in a different way. This interaction can be of hydrophobic and/or electrostatic nature and can change the conformation of the protein molecules in the bulk and at the surface/interface, respectively [Kotsmar et al. (2009), Krägel et al. (2003), Maldonado-Valderrama and Rodrìguez Patino (2010)].

Then protein-surfactant binding changes the adsorption energy of the protein for the interface by affecting the net charge or the overall hydrophobicity, and affects both the surface coverage and the conformation of adsorbing macromolecules. Since LMW surfactants can pack together more closely at interfaces than proteins, in general the former displace proteins when they are present at high enough bulk concentration with a different mechanism for ionic and nonionic surfactants respectively. Nonionic surfactants in general

exhibit a net repulsive interaction with adsorbed proteins, probably owing to steric repulsion, whereas ionic surfactants have a relatively more attractive interaction with adsorbed proteins [Hasenhuettl and Hartel (1998), Bos and van Vliet (2001), Dickinson (1998)].

This determines that ionic emulsifiers have a greater tendency to complex with the charged groups of proteins, thus, they bind to the protein forming a soluble protein-surfactant complex (solubilization mechanism), conversely nonionic emulsifiers tend to adsorb directly to the interface gradually replacing macromolecules (replacement mechanism) [Bos and van Vliet (2001), Dickinson (1998)].

In addition to the type of surfactant (water or oil soluble, ionic or nonionic) the simultaneous adsorption of proteins and surfactants at the interfaces depends on different factors, mainly including the native conformation of the biopolymer (random or globular), the nature of the interface (air/water, oil/water), the pH and the ionic strength of the solvent [Bos and van Vliet (2001), Maldonado-Valderrama and Rodrìguez Patino (2010)]. These factors affect the rheological properties of such complex at different interfaces.

Starting from this evidence, it clearly appears that the interfacial properties of such mixed adsorption layers, given by the components used and by their interactions, can strongly influence the formation and stabilization of respective foams and emulsions [Kotsmar et al. (2009), Bos and van Vliet (2001), Dickinson (1998)].

Particularly, it was found that competition processes between the protein and LMW surfactants can cause complex behavior with contrasting implications for foam and emulsion stability.

Mixtures of LMW surfactants and proteins can result often in maximum stability characteristics for dispersions, owing to a greater capacity of these systems than single components, to respond more readily to a wide range of rates and extents of deformations, and to recover a surface film capable of preventing film rupture more quickly [Murray (1998)]. At the same time, protein and surfactant combinations may result in a drastic loss of foams and emulsions stability [Wilde (2000), Bos and van Vliet (2001), Dickinson (1998), Rouimi et al. (2005), Wilde et al. (2004), Mackie and Wilde (2005)], which was attributed by many authors, to the different and incompatible stabilization mechanisms of the interface exhibited by proteins and LMW surfactants respectively. As a result surfactants weaken the visco-elasticity of the adsorbed protein layer, and the polymers retard the fluidity of the surfactants.

Rheological interfacial properties of fluid interfaces in the presence of macromolecular and small-area surfactants have been extensively investigated, in order to obtain any quantitative link of the shear or dilational moduli and the measured emulsion or foam stability.

Particularly, dilational rheology, which implies a deformation in the interfacial area is a very sensitive technique to monitor the interfacial structure, the concentration of single emulsifiers at the interface or the relative concentration, the competitive adsorption, and the magnitude of interactions between different surface active molecules at the interface [Maldonado-Valderrama and Rodrìguez Patino (2010), Ravera et al. (2010)].

Moreover, since the optimum use of proteins and emulsifiers in emulsion and foam design may depend on the knowledge of their interfacial rheological characteristics and the kinetics of film formation at the fluid interface, dilational rheology and dynamic tensiometry analysis proves to be a useful tool to determine indirectly the existence of protein-surfactant interactions, the mechanical behavior and the composition of mixed films at the interfaces.

This work compares the adsorption behavior of mixed protein-surfactant systems at the air-water (A/W) and sunflower oil-water (O/W) interfaces, focusing on Ovalbumin protein in the presence of two food and water soluble emulsifiers, Tween60 (*Polyoxyethylene Sorbitan Esters of Monoglycerides*) and Admul Datem (*Diacetyl Tartaric Acid Ester of Mono-Diglycerides*), which are very different for their headgroup nature and ability to dissolve in the water phase (HLB number 14.9 and 8 respectively) [Bos and van Vliet (2001)].

Ovalbumin, the major protein in egg white, is commonly used in the food industry because of its foaming ability and gelation on heating [Galazka et al. (2000), Croguennec et al.(2007)]. This globular protein has been well characterized structurally and, thus, is an excellent candidate for testing the effects of a number of different chemical variables on its adsorption dynamics. It has a molecular weight of 42 KDa, one disulfide bon, and four free sulphydryl groups. The isoelectric point is 4.6 and, thus, the protein is negatively charged at neutral pH [Beverung et al. (1999)]. Ovalbumin is almost completely involved in a secondary structure, with nine α-helices and three β-sheets [Beverung et al. (1999)].

Their capacity to form an entangled gel-like structure at the A/W and O/W interfaces has been extensively studied by determining time-dependent interfacial dilational moduli [Benjamins et al. (1996), Lucassen-Reynders et al. (2010)], but there are no literature

works, which have studied Ovalbumin behavior in the presence of LMW emulsifiers at different interfaces.

Rheological parameters and kinetics of film formation of Ovalbumin alone and in the presence of Tween60 and Admul Datem emulsifiers, were studied at the A/W and O/W interfaces by means of dynamic interfacial tension measurements and harmonic drop oscillation experiments in a time scale of some seconds.

The objective of this research was to investigate the effect of Ovalbumin-emulsifier interactions on the interfacial behavior of protein, as a function of the surfactant type competing with protein at the interface and of the type of interface where the molecules adsorption occurs.

It is important to note, moreover, that for the sake of simplicity, in this work the word “interface” will be used to denote both A/W and O/W interfaces.

2. Materials and methods

2.1 Samples preparation

Sample solutions were prepared by dissolving the protein Ovalbumin (from hen egg white, grade II, A5253, Sigma Aldrich) and the emulsifiers, Tween 60 (*polyoxyethylene sorbitan esters of monoglycerides*) (P1629-1Ga, Sigma Aldrich) and Admul Datem (*diacetyl tartaric acid ester of mono- and di-glycerides*) (1915, 5Z10712, Kerry Company) in twice-distilled water, in imidazole buffer 20 mM at pH=6.5 [Galazka et al. (2000)]. The buffer pH was chosen to correspond with the intermediate value of pH range characteristic of many commercial egg albumin food products [Mleko et al. (2007)].

The twice distilled water used throughout all experiments was obtained from a Milli-Q purification system (Millipore, USA), and it was checked for contaminants before each experiment, measuring the surface tension of the buffer solution at the air/water boundary at ambient temperature. No aqueous solutions with a surface tension other than accepted in the literature (72-73 mN/m at 20°C) were used.

The samples containing a mixture of Ovalbumin and emulsifiers, with different weight ratio emulsifier/protein, were indicated according to Tables (1) and (2) .

SAMPLE ID	**Emulsifier bulk concentration [%wt]**	**Weight ratio Emulsifier/Protein [/]**
TW/OV (0.01)	10^{-4}	*0.01*
TW/OV (0.3)	$3 \cdot 10^{-3}$	*0.3*
TW/OV (0.6)	$6 \cdot 10^{-3}$	*0.6*

Table 1. Samples containing Ovalbumin (0.1 g/l) and Tween60 molecules with different emulsifier- protein weight ratio

SAMPLE ID	**Emulsifier bulk concentration [%wt]**	**Weight ratio Emulsifier/Protein [/]**
DA/OV (0.01)	10^{-4}	*0.01*
DA/OV (0.3)	$3 \cdot 10^{-3}$	*0.3*
DA/OV (0.6)	$6 \cdot 10^{-3}$	*0.6*

Table 2. Samples containing Ovalbumin (0.1 g/l) and Admul Datem molecules with different emulsifier-protein weight ratio

In the samples the total amount of protein was kept constant and corresponding to the final concentration of 0.1 g/l, typical value of Ovalbumin concentration used in many literature studies [Benjamins et al. (1996), Bos and van Vliet (2001)], whereas the weight ratio emulsifier/protein was changed in the same way for both emulsifier types, to investigate the effect of this ratio and of the surfactant type, on the rheological properties of Ovalbumin layers.

Ovalbumin and emulsifiers were dispersed separately in the buffer solutions at ambient temperature (20-23°C), and at 70°C respectively, stirring for 1 hour by using heated magnetic device (ARE, Velp scientific, Italy). Afterwards the protein and emulsifier aqueous solutions were mixed at room temperature and were stirred for a further 30 minutes before the interfacial measurements were performed.

In order to make comparisons between the protein-emulsifier mixtures and the individual molecules, samples containing single surfactants, Ovalbumin (indicated with OV), and Tween60 and Admul Datem (indicated with TW and DA, respectively) were also prepared, as described above. Their bulk concentrations were chosen to correspond, respectively, to the same protein concentration (0.1 g/l) and to the emulsifier maximum one (0.06 g/l) used in the mixture samples (Tables 1-2).

All solutions were freshly prepared (within no more than 24 h) for the characterization and two replicates were prepared for each sample.

Sunflower oil (Carlo Erba Reagents-356241) without further purification was used as the oil phase in this research. It contains triglycerides and free saturated and unsaturated fatty acid (0.4%) which are responsible for the interfacial tension reduction measured between the pure oil and water phases and also measured with no added surfactants.

2.2 Dynamic interfacial properties measurements

Individual molecules films and Ovalbumin-emulsifiers mixed layers adsorbed at the A/W and O/W interfaces were characterized by dynamic interfacial tension measurements and harmonic drop oscillation experiments in a time scale of some seconds.

Axialsymmetric drop shape analysis (ADSA) was used to calculate drop volume, area and interfacial tension by using an automated pendant drop tensiometer (FTA200 First Ten Angstroms, USA) equipped with the *fta32 v2.0* software. Details of this apparatus are given by Biresaw et al. (2008).

The instrument comprises an automated pump that can be fitted with various sizes of syringes and needles to allow for control of pendant drop formation and of sinusoidal variations in the drop volume or surface area by software.

An automated image viewing and capturing system, with various image capture triggering options, was used to capture drop image.

The computer hardware and software also provide the capability for data capture, storage, analysis and transfer. The software allows for an automated drop shape analysis of the captured drop image, and for measuring the surface-interfacial tension of the drop formed in air or in a second fluid at rest and in periodic motion conditions of the interface respectively.

Image acquisition and regression of the interfacial tension were performed directly with commercially available drop-image software by fitting the Bashforth-Adams equation to the drop shape [Biresaw et al. (2008)]. Drop-image software also controlled an automatic pipetting system that maintained constant drop volume with time period over which dynamic tensions were measured.

2.2.1 Dynamic interfacial tension

The method adopted to measure the interfacial tension of the investigated solutions with time (γ *vs t*), by using an automatic drop tensiometer, involved the analysis of the profile of the drops created in the air/oil phases respectively, and kept at rest (constant volume).

The experiments were carried out at room temperature (22°C within ± 1 °C), placing sample aqueous solutions in a 100 µl glass Hamilton syringe equipped with a 20 Gauge stainless steel needle, and delivering drops in a rectangular quartz cuvette (5ml) containing the desired phase. In the measurements with air as external phase, the cuvette environment was also closed with parafilm and vapor saturated to reduce water evaporation, and then, to

avoid an excessive drop volume reduction, which can compromise the validity of the measure.

Drop volumes of 9-12 μl were used in every test in order to measure interfacial tension values, independent of the drop size [Lin et all, 1996]. High drop volumes of the kind chosen, proved to be generally more suitable for these tests, because they increased the number of profile points, which can be used for drop shape analysis [Lin et all, 1996].

The experiments started with the creation of the drop; then, drop images were continuously taken from a CCD camera and digitalized, registering the surface tension values over the test time.

Drop profile was monitored up to a maximum time of 180 min, which assures the molecules adsorption at the interface and the reaching of a quasi-equilibrium interfacial tension value.

Equilibrium of γ was assumed when the interfacial tension did not change by more than 0.5 mN/m in 30 min [Camino et al. (2009)].

The interfacial pressure values π with time were also calculated as the difference between the pure water interfacial tension in the absence of macromolecules γ_0 (equal to 72.8 ± 0.3 mN/m and 24 ± 0.35 mN/m for A/W and O/W interfaces respectively), and that registered for the analyzed solutions during the test time γ.

2.2.2 Dilational rheological properties

The method adopted to determine the dilational rheological properties of the interface, by using an automatic drop tensiometer, involved the profile analysis of the drop formed in the air/oil phases and subjected to periodic motion conditions, according to the oscillating drop methodology, able to measure the interfacial visco-elasticity versus frequency.

Oscillating drop experiments are usually performed subjecting the interface to an infinitesimal sinusoidal compression and expansion.

The surface dilational modulus derived from the change in surface tension γ (dilational stress) (Eq. 1), resulting from a small change in surface area A (dilational strain) (Eq.2), may be described by equation 3 [Lucassen and van den Tempel (1972)].

$$\gamma = \gamma_0 + \Delta\gamma \sin(\omega t + \delta) \qquad (1)$$

$$A = A_0 + \Delta A \sin(\omega t) \tag{2}$$

$$E^* = \frac{d\gamma}{d \ln A} \tag{3}$$

Where γ_0 and A_0 are the equilibrium reference surface tension and the unperturbed interfacial area of the drop respectively, Δγ and ΔA are the stress and strain amplitude respectively, and δ is the phase angle between stress and strain, measure of the relative film viscoelasticity.

Since the drop area periodically oscillates, the dilational modulus exhibits two contributions: an elastic part accounting for the recoverable energy stored in the interface (storage modulus, *E'*) and the dissipative part accounting for energy lost through relaxation processes (loss modulus, *E''*).

$$E^* = E' + iE'' = \frac{\Delta\gamma}{\Delta A / A_0}\cos(\delta) + i\frac{\Delta\gamma}{\Delta A / A_0}\sin(\delta) \tag{4}$$

Then the surface dilatational modulus, E*, as a measure of the total material resistance to dilatational deformation (elastic and viscous), is a complex quantity composed of real and imaginary part [Freer et al. (2003), Ravera et al. (2009), Myrvold and Hansen (1998)].

For a perfectly elastic material stress and strain are in phase $\delta = 0$ and the imaginary term is zero. In the case of perfectly viscous material $\delta = 90°$ and the real part is zero. The loss angle tangent can be defined by equation (5):

$$\tan\delta = E'' / E' \tag{5}$$

In this work, we applied a periodic strain by differentially oscillating the drop area at a prefixed frequency value, and we measured the periodic stress response with time.

Then the dilational viscoelastic parameters of interface, the dilational complex modulus (E*), its elastic (E') and viscous (E'') components and the loss angle tangent were measured as a function of the adsorption time *t*.

The *Time Sweep Tests* were carried out by using deformation amplitude ($\Delta A/A_0$) values of 6-15% and angular frequency ones varying in the 0.005 Hz-0.1 Hz range.

The percentage area change was determined before each time sweep test by performing *Amplitude Sweep Experiments* (data not shown) realized at the extreme frequency values of

the investigated range. The latter experiments were useful to assure that system response was not influenced by perturbation amplitude (linear viscoelastic behavior) and to be in the linear region, so as to avoid oscillation amplitude that causes disruption of the supramolecular organization or provides adequate measurement sensitivity.

The duration of each test was established so as to register equilibrium values of the dilational moduli with time (maximum variation of 3% was accepted).

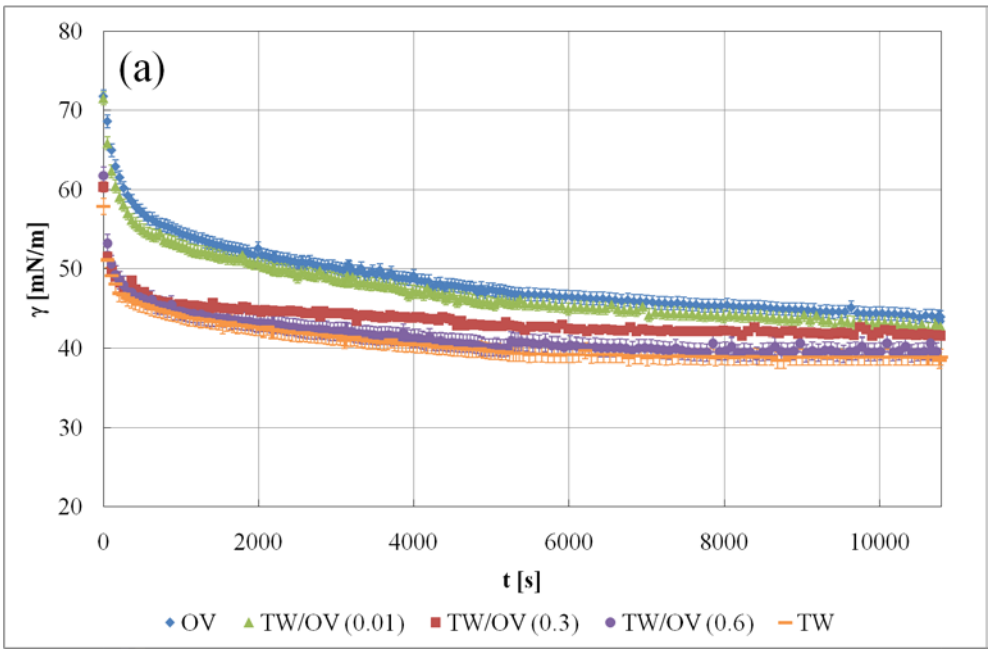
The E* *vs* ω curves were then obtained by using the latter values in the frequency range investigated.

3. Results and discussion

3.1 Dynamic interfacial tension trends

In order to understand the dynamic behavior of the systems studied and to study the effects of Ovalbumin-emulsifier interactions on the protein adsorption, time dependence interfacial tensions were shown and discussed below.

Dynamic interfacial tension for TW/OV and DA/OV adsorbed films as a function of Tween 60 and Admul Datem bulk concentration were shown comparatively in figures (1) and (2) respectively, at the A/W (a) and O/W (b) interfaces. For comparison, the values obtained for the OV, TW and DA solutions were also shown.



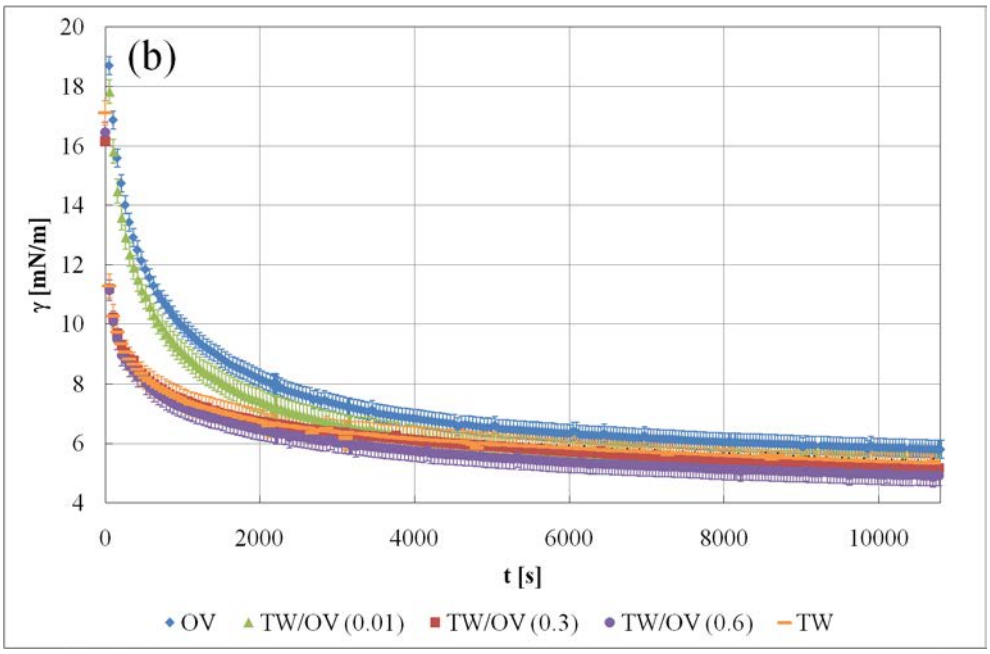
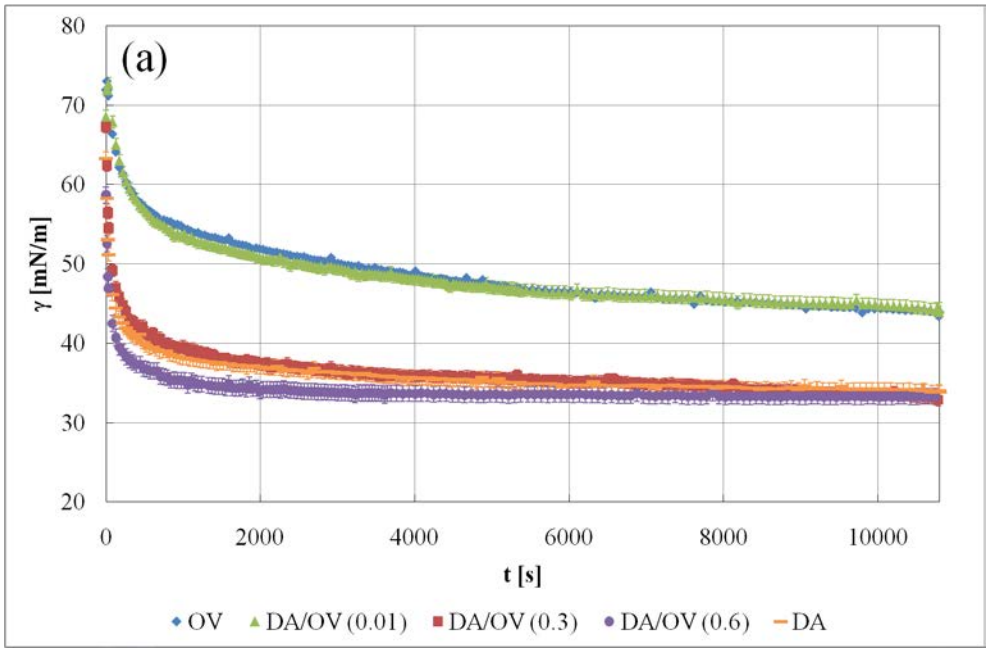


Figure 1. Time dependent surface tension of OV, TW, and TW/OV films adsorbed at the A/W (a) and O/W (b) interfaces as a function of Tween60 bulk concentration



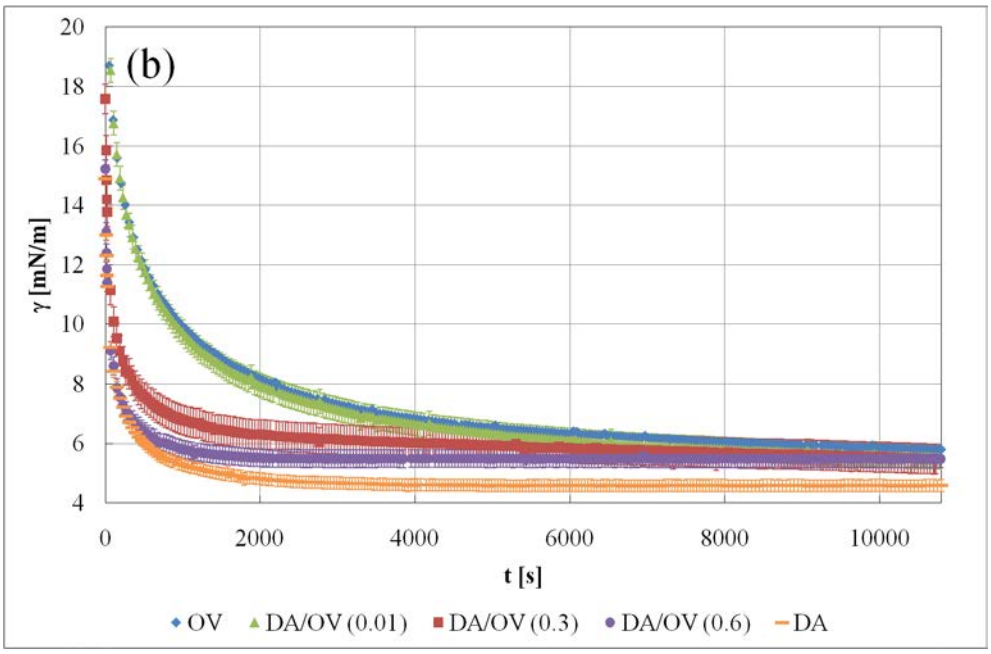


Figure 2. Time dependent surface tension of OV, DA, and DA/OV films adsorbed at the A/W (a) and O/W (b) interfaces as a function of Admul Datem bulk concentration

Typical behaviour of surface active biopolymers and low molecular weight surfactants, respectively, was observed at both interfaces [Miller et al. (2000)].

It can be noticed that the emulsifiers Tween 60 and Admul Datem, at the investigated concentration values, were more superficial active and more rapid to adsorb at interface than Ovalbumin.

In fact, differently from protein, whose surface tension value decreased with time, tending to pseudo-equilibrium, true equilibrium adsorption of emulsifiers proved to be possible (no changes in γ values upon time) in the time scale investigated.

The adsorption kinetic of TW was finished after about 5000 s at both interfaces studied, whereas that of DA was finished in times lower than TW ones, and different at the A/W and O/W interfaces (4000s and 2000s respectively).

It is possible to notice that both mixed systems exhibited both more surfactant-like adsorption kinetics, and a bigger absolute change in surface tension, with increasing surfactant concentration. Actually, the surface/interfacial tensions for the mixed systems TW/OV and DA/OV tended to the value characteristic of OV at sufficiently low emulsifier concentrations (10^{-4}% wt), while an approach to the Tween60 and Admul Datem ones, respectively, was observed when increasing these concentrations.

Then, the Ovalbumin appeared to play little part in the surface properties when increasing the emulsifier concentration or emulsifier/protein weight ratio, and this could be

interpreted as a result of the considerable extent of protein replacement from the interface by emulsifiers or by surfactant/protein complexes, owing to their higher surface activity and affinity for the interface [Miller et al. (2000), Bos and van Vliet (2001), Wilde (2000), Mackie and Wilde (2005), Rodrìguez Patino et al. (2003), Wilde et al. (2004), Mackie et al. (1999)].

This is in agreement with the present technical literature and in particular with the study of Petkov et al.(2000) and Krägel et al. (2003) on the β–lactoglobulin protein adsorption in presence of nonionic (Tween20) and anionic (SDS) emulsifiers respectively .

Ionic and nonionic surfactants, usually exhibit quite different surface activity and, thus, a reasonable comparison of their effect on protein/surfactant adsorption layers is very difficult [Wilde (2004)].

It is well known that ionic surfactants interact with protein molecules via electrostatic interaction, whereas nonionic surfactants via hydrophobic ones, and this difference affects the dynamic of adsorption.

For mixed protein/nonionic emulsifier systems competitive adsorption phenomena are often evident only at sufficiently high surfactant concentration, and in this case, the dynamic curve (γ(t)) is completely controlled by small molecule surfactants; on the contrary, for mixed protein/ionic emulsifier, generally, both protein-surfactant complexes and surfactants dominate the interface in dependence on the relative surface activity [Wilde (2004), Mackie and Wilde (2005), Krägel et al. (2003)].

From figures (1) and (2) it is possible to observe differences in the adsorption behaviour of each studied system at the A/W and O/W interfaces, and then, some influence of the neighbouring phase on the interfacial properties. These differences were essentially relative to the final absolute reduction of interfacial tension registered in the observation time used, the shape of the γ-t curve in the first few seconds of the test.

Specifically, the absolute change of interfacial tension or interfacial pressure π resulted significantly larger at the A/W interface (figures a) than O/W one (figures b), whereas the initial slope of the interfacial tension versus the time was steeper at the latter.

It could obey the different nature of the hydrophobic phase, which in the case of sunflower oil is formed by different interfacial active components, whose content is considered responsible for its charge and interfacial behaviour [Wüsteneck at al. (1999)].

It is well known actually, that the nature of the interface significantly affects the dynamics of the adsorption process and the equilibrium adsorption characteristics of surface active molecules [Beverung et al. (1999), Miller et al. (2000), Krägel et al. 2003), Benjamins and

Lucassen-Reynders (1998)]. Moreover, triacylglycerol phases, which are more polar than the hydrocarbon one, may result therefore in an interfacial behaviour that is much more distinct than in the hydrocarbon-water interface and the air-water one [Benjamins and Lucassen-Reynders (1998)].

Lower π values at the sunflower O/W interface than at the A/W one, were also reported by Wüsteneck at al. (1999) studying β-lactoglobulin adsorption, by Rotureau et al. (2004) working with amphiphilic derivates of dextran, by Ganzevles et al. (2007) with mixtures of β-lactoglobulin and pectins and by Camino et al. (2009) with the polysaccharide hydroxypropylmethylcelluloses.

The better solvency offered by oil, for hydrophobic groups, compared with air, can explain the lower surface activity and, then, the lower efficiency of OV, TW, DA and of their mixed systems to reduce interfacial tension at the O/W interface [Camino et al. (2009)]. Regarding this, Medrzycka and Zwierzykowski (2000) proposed the existence of more cohesion between carbon chains of surfactants molecules at the A/W interface. Such cohesions, known as trains and loops, provoke surface tension decrease. These cohesions would be absent, or present in a lower number, at the O/W interface.

The initial slope of the interfacial tension versus time observed higher at the O/W interface than the A/W one is in line with similar trends obtained by many authors who studied protein layers adsorption [Beverung et al. (1999), Miller et al. (2000), Graham and Phillips (1979), Krägel et al. 2003)]. These differences were ascribed to the different structure of the adsorption layers in the air and oil phase, which are thicker in the latter phase, owing to the possibility of adsorbing molecules to protrude into this phase, leading to an earlier onset of interfacial tension decrease than in air [Beverung et al. (1999), Miller et al. (2000)].

Moreover, it is interesting to note that DA and DA/OV proved to be more efficient to reduce interfacial tension than TW and TW/OV respectively, at both interfaces.

The best performance of the first emulsifier, in particular at the O/W interface, may be explained, probably, by its higher hydrophobicity than the second one, which allowed it to anchor strongly between the glycerides of the oil phase (Camino et al. (2009)).

3.2 Adsorption kinetics

3.2.1 Data analysis

It is well known in the open literature that the surface active molecules adsorption at the gas/fluid and fluid/fluid interfaces can be monitored by measuring changes in interfacial tension, from which adsorption kinetics parameters can be obtained directly.

The main features of the adsorption kinetics of proteins and LMW surfactants can include the diffusion of the molecules from the bulk onto the interface, the adsorption and penetration of molecules at the interface, and the interfacial aggregation and rearrangement of molecules adsorbed within the interfacial layer [Pérez et al. (2009), Pérez et al. (2010), Camino et al. (2009), Van Hunsel et al. (1986), Demeter-Vodnàr et al. (1996), Rosen (2004), Davis (2005)].

The adsorption of LMW surfactants is different from that of proteins in many respects: i) proteins unfold at the interfaces due to interfacial denaturation, in particular, at low surface pressures or surface coverage, ii) the interfacial area occupied by the adsorbed protein molecule is large compared to that of a small surfactant and it cannot be assumed constant, iii) the number of configurations of adsorbed protein molecules exceeds that of proteins in the bulk solution, iv) protein adsorption and subsequent changes within the adsorbed layer take place on a time scale several orders of magnitude higher than for small molecular weight surfactants [Wilde (2000)], v) protein adsorption can be considered irreversible, differently from low molecular weight surfactants, which can leave the interface and penetrate into the bulk phase [Van Hunsel et al. (1986), Demeter-Vodnàr et al. (1996), Rosen (2004)].

Nevertheless, the same kinetic approach was generally used in the technical literature to study the adsorption behavior of these different surface active agents [Rosen (2004), Pérez et al. (2009), Demeter-Vodnár et al. (1996)].

This approach was used in this work to study Ovalbumin, Tween60 and Admul Datem adsorption at A/W and O/W interfaces. It includes the analysis of at least two adsorption steps characterized by low and high values of interfacial pressure respectively, which can be studied with specific models.

During the first adsorption step, at relatively low surface pressures, when diffusion is rate-determining step, a modified form of the Ward-Torday [Ward and Torday (1946)] equation can be used to correlate the change in the interfacial pressure π with time (eq.6):

$$\pi = \gamma - \gamma_0 = C_0 KT\left(D_{dif}\, t / 3.14\right)^{\frac{1}{2}} \qquad (6)$$

Where γ and γ_0 are the interfacial tension at the same time t and t=0, C_0 is the concentration in the aqueous phase, K is the Botzmann constant, T the absolute temperature, D_{dif} is the diffusion coefficient and t the adsorption time. If the diffusion at the interface controls the adsorption process, a plot of π against $t^{1/2}$ will then be linear [Pèrez et al. (2009), Pèrez et al. (2010), Camino et al. (2009)] and the slope of this plot will be the diffusion rate (K_{dif}).

At higher adsorption time, in the period after that affected by the diffusion, there is an energy barrier for protein-emulsifiers adsorption, which can be attributed to adsorption, penetration, unfolding and rearrangements of the molecules at the interface.

Since the interfacial concentration of adsorbed molecules is several times higher than that in the bulk phase, the molecular unfolding and rearrangements steps are magnified processes happening at the interface, especially for high molecular weight macromolecules [Pèrez et al. (2009)].

To analyze adsorption and probable unfolding/rearrangement of adsorbed molecules, the approach proposed by Graham and Philips [Graham. and Phillips (1978)] was used. Thus, the rate of these processes can be analyzed by a first-order equation:

$$\ln \frac{\pi_{180} - \pi_t}{\pi_{180} - \pi_0} = -k_i t \qquad (7)$$

Where π_{180}, π_0 and π_t are the surface pressures at 180 min of adsorption time, at time t=0, and at any time t, respectively, and k_i is the first-order rate constant. In practice, a plot of equation 2 usually yields two or more linear regions. The initial slope is taken to correspond to a first-order constant of adsorption (k_{ads}), whereas the second slope is taken to correspond to a first-order constant of rearrangement (k_r), occurring among a more or less constant number of adsorbed molecules [Pèrez et al. (2009), Pèrez et al. (2010), Camino et al (2009)]. As an example, the application of equation 7 to Ovalbumin adsorption at the A/W and O/W interfaces is given in figure 3. Dynamic interfacial tension data for all samples at the both interfaces were fitted by equations 6 and 7 by using Table Curve 2D v4 Systat Software.

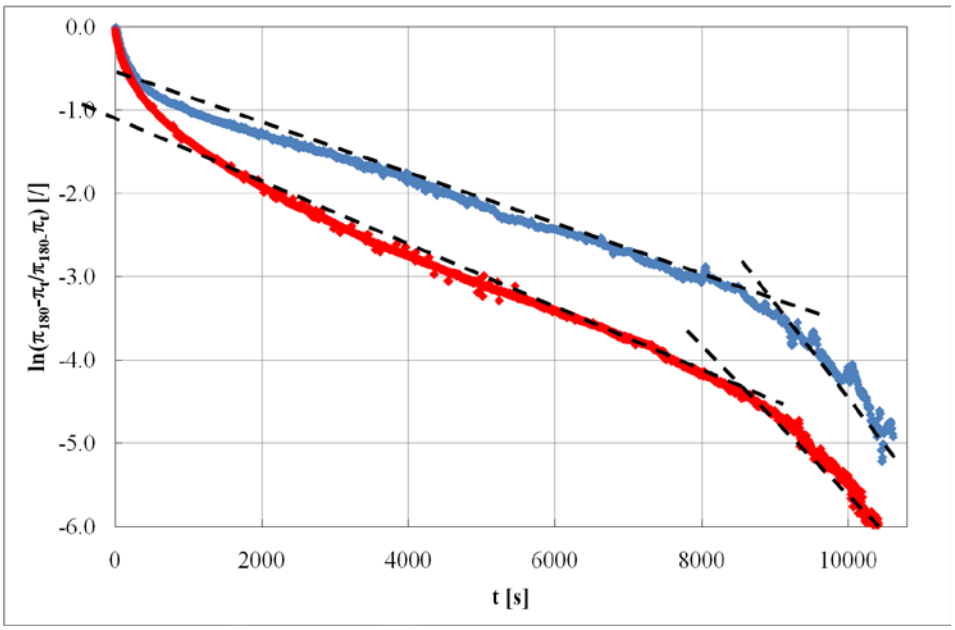


Figure 3. Kinetic model applied to the adsorption mechanism for Ovalbumin layers adsorbed at the A/W (blue data) and O/W (red data). Ovalbumin concentration 10^{-2} wt%

It is important to emphasize that molecular penetration and rearrangement steps are magnified processes just for macromolecules such as proteins and biopolymers, which generally exhibit considerable changes in the three-dimensional structure of the molecule, owing to alteration or modification of intermolecular and intramolecular bonds distribution at interface. On the contrary, the rearrangement processes for the small surface active molecules such us emulsifiers can be negligible and little evident and their structural changes are generally minimal at the interface [Murray (1998)].

For this reason and in order to compare consistently the effect of the Ovalbumin-emulsifiers interaction on the protein adsorption kinetics, only one first-order constant (k_{ads}) was obtained from the experimental data, which is, then, a measure of the only penetration process of the molecules at the interface.

3.2.2 Diffusion

According to the short-time approximation of the Ward–Tordai equation (eq. 6) for diffusion-controlled adsorption, the kinetics of adsorption were deduced from $\pi - t^{1/2}$ curves for the individual surface active molecules studied and for their mixtures, the slope of these plots being constant (K_{dif}).

It was found that the diffusion step for these systems, except for the OV and TW-DA/OV(0.01) ones, was too fast ($\pi > 10mN/m$) at both interfaces to be detected by the experimental technique used in this work, as deduced from $\pi - t^{1/2}$ plots (calculated from data reported in figures (1) and (2)) [Camino et al.(2009), Pèrez et al. (2009), Pèrez et al. (2010)].

Anyway, it is possible to obtain from them an estimation of the diffusion rate constant (K_{dif}) from the slope at the beginning of the adsorption (at 15 s).

K_{dif} values for individual and mixed layers adsorbed at the A/W and O/W interfaces were compared in figures (4) and (5) respectively.

The diffusion step obtained for the individual surface active components, permitted apparent diffusion coefficients D_{dif} values of the order of 10^{-10} m^2/s to be calculated, which proved to be close to the physically reasonable ones for simple conventional surfactants [Rosen (2004), Wierenga (2005)]. Furthermore, the apparent diffusion coefficient of Ovalbumin, calculated as the average value from the slope of $\pi - t^{1/2}$ plots, obtained at the A/W and O/W interfaces, was calculated equal to $9.42 \cdot 10^{-10} \pm 6.5 \cdot 10^{-11}$ m^2/s, which is not far from the actual native protein value ($7 \cdot 10^{-10}$ m^2/s) [Wierenga (2005)].

It is necessary to point out that differences in the apparent diffusion coefficient values of proteins were obtained by different authors, and were ascribed to changes of protein diffusion steps strongly dependant on solution conditions, molecular conformation, aggregate formation and measuring method. [Dickinson (1999), Miller et al. (2000)]. Specifically speaking, with decreasing protein concentration, values of D_{dif} were found, in other works, several orders of magnitude higher than expected from the size and shape of the molecules, and these discrepancies were attributed to the models used, not containing assumption of any changes in the conformation of adsorbed molecules [Miller et al. (2000)].

Independently of the type of surface active molecule, it can be seen in figures (4) and (5) that $k_{dif}^{W/A} \approx k_{dif}^{W/O}$, indicating clearly that diffusion kinetics in the time scale considered

were practically equivalent at the A/W and O/W interfaces. Then, the nature of the neighbouring phase (air or oil) did not influence this step [Van Hunsel et al. (1986)].

Van Hunsel et al. (1986) obtained the same result for LMW surfactants insoluble in the oil phase, and thus, similar considerations can be extended to Ovalbumin adsorption for its strong hydrophilic nature.

The results also indicated the existence of competitive phenomena between the emulsifiers and Ovalbumin, which proved to play little part in the diffusion rate of mixed systems when increasing the emulsifier/protein weight ratio.

In fact, diffusion-controlled adsorption was strongly affected by this ratio at both the interfaces. This can be deduced from k_{dif} dependence exhibited by TW/OV and DA/OV mixed systems, on the relative concentration of Tween 60 and Admul Datem present in the mixtures.

It can be seen, from figures (4) and (5), that the increment of emulsifier/protein weight ratio in the mixed systems clearly caused an increasing of k_{dif} for all these systems, in agreement with the higher values of the apparent diffusion rates exhibited by both emulsifiers than protein ones.

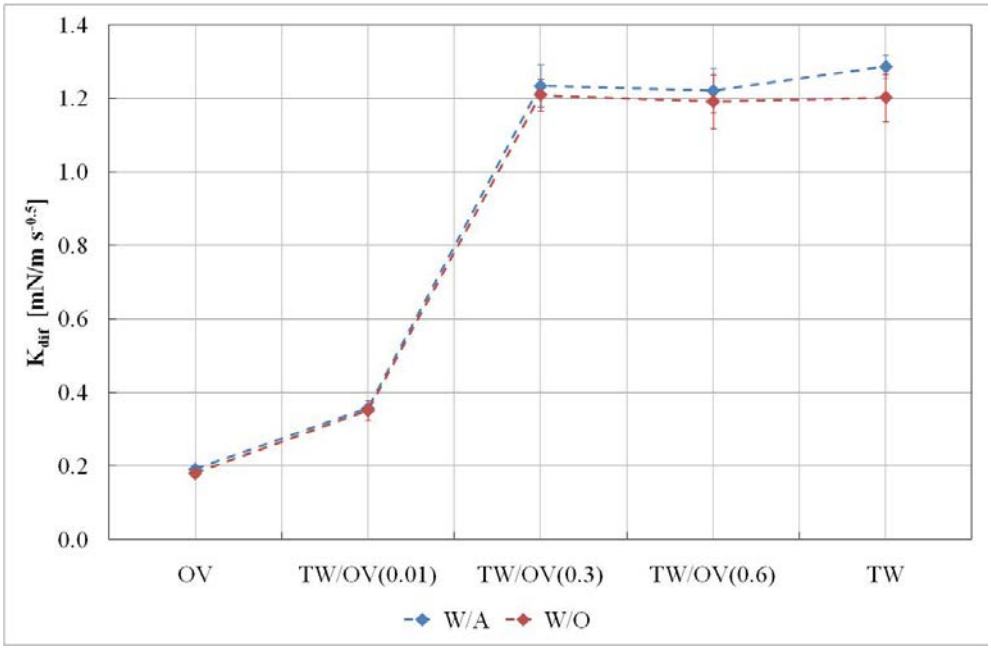


Figure 4. The evolution of the apparent diffusion rate constant k_{dif} for TW/OV mixed systems as a function of the Tween60/Ovalbumin weight ratio in the mixture

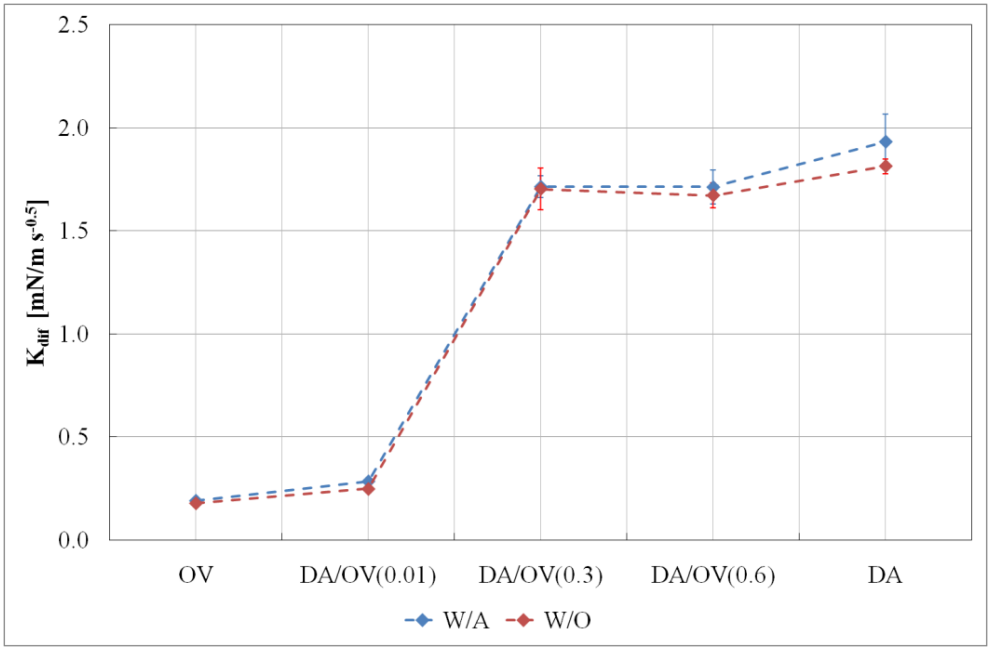


Figure 5. The evolution of the apparent diffusion rate constant k_{dif} for DA/OV mixed systems as a function of the Admul Datem/Ovalbumin weight ratio in the mixture

Then the results obtained might suggest that molecular dynamics occurring in the solution of mixed systems due to protein-emulsifier interaction could play a decisive role in the Ovalbumin diffusion step toward A/W and O/W interfaces.

Differences in the behaviour exhibited by the two emulsifiers studied during diffusion-controlled adsorption can be identified in the absolute value of k_{dif}, which resulted higher for DA than TW. This behavior can be justified by the differences in the molecular dimensions and hydrophobicity, between the Admul Datem and Tween60 emulsifiers. Actually the former has both HLB and molecular weight lower than Tween 60, resulting more hydrophobic and quicker to reach the interface.

The same differences were found for the mixed protein-emulsifier systems, which showed higher values of k_{dif} in presence of Admul Datem at each emulsifier/protein weight ratio used in the mixtures.

On the contrary of the above, the type of small surfactant present in the mixtures with protein, did not affect the k_{dif} dependence on the emulsifier concentration. Actually, k_{dif} values of mixed systems for the lowest Tween 60 and Admul Datem concentrations studied, proved to be more or less close to the value exhibited by pure protein. Conversely, mixed systems with the other emulsifier /protein weight ratios (*0.3 and 0.6*) showed more emulsifier-like diffusion kinetics with k_{dif} values being the same as single emulsifiers.

3.2.3 Adsorption or penetration

The adsorption rate constants calculated by fitting the only first slope of the equation 7 plots, were shown in the figures (6) and (7) for TW/OV and DA/OV systems respectively.

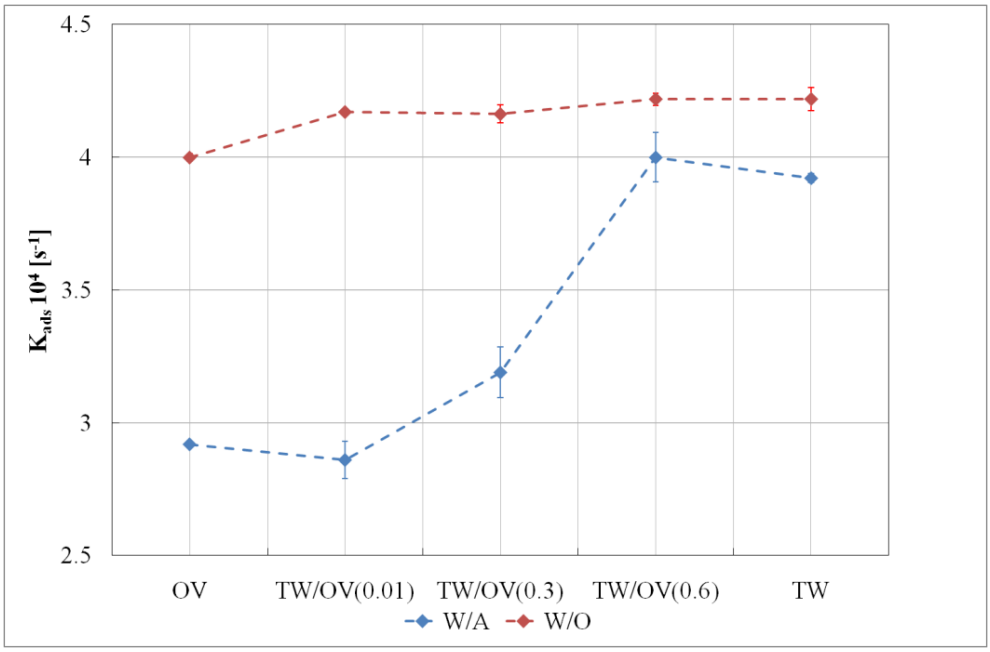


Figure 6. The evolution of the adsorption rate constant k_{ads} for TW/OV mixed systems as a function of the Tween60/Ovalbumin weight ratio in the mixture

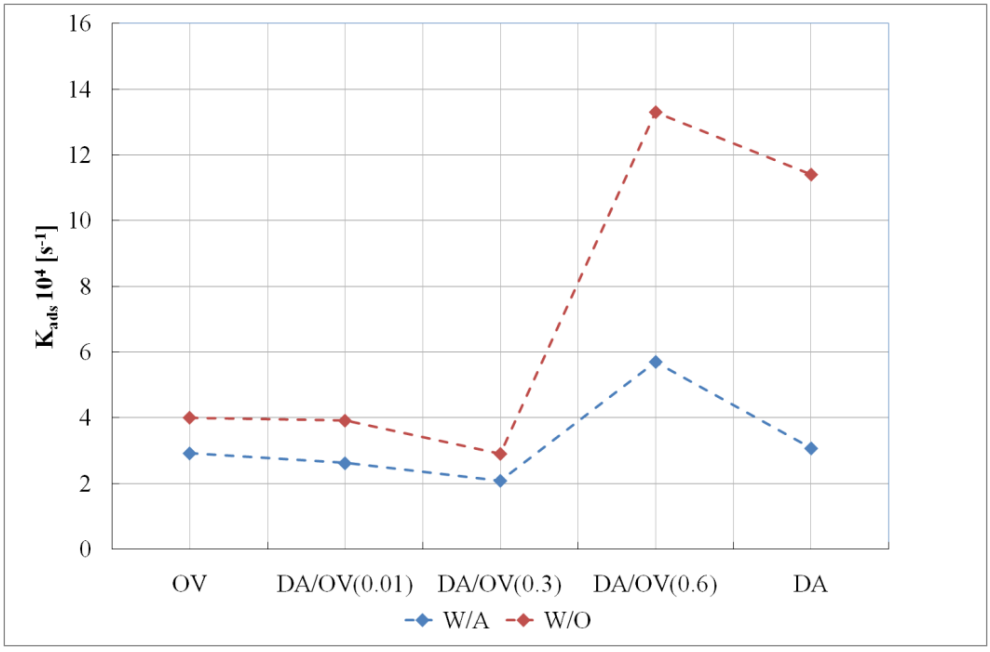


Figure 7. The evolution of the adsorption rate constant k_{ads} for DA/OV mixed systems as a function of the Admul Datem/Ovalbumin weight ratio in the mixture

Differently from the trend discussed above, regarding the equivalent diffusion velocities of the investigated systems at both interfaces, a strong influence of the neighbouring phase,

air or sunflower oil, was found on the interfacial adsorption kinetics when molecular penetration was the step control.

Actually, it can be seen from figures (6) and (7) that the adsorption rate constants k_{ads} were greater in the presence of the oil than the air phase, for each studied system.

More rapid adsorption kinetics at the O/W than A/W interface were obtained by many authors in their studies of protein adsorption at both interfaces [Beverung et al. (1999), Miller et al. (2000), Krägel et al. 2003), Benjamins and Lucassen-Reynders (1998)]. This behavior was generally ascribed to a greater affinity of the hydrophobic residues on the proteins for the oil and to the different structures of the adsorption layers forming as a result of the neighbouring phase polarity.

Polarity and electrostatic interaction effects can probably also explain the greater differences of k_{ads} exhibited by Admul Datem and their mixtures, between the O/W and A/W interfaces than the Tween60 ones.

Despite these differences, the dependence of k_{ads} values of mixed systems on the emulsifier concentration present in the mixture led to the identification of competitive adsorption phenomena between Ovalbumin and both the emulsifiers at the O/W and A/W interfaces.

In general, it can be observed that the presence of the Tween 60 and Admul Datem surfactants in the subphase caused, in most cases, an increment of the adsorption rate of the components at both interfaces with increasing emulsifier/protein weight ratio used in the mixture. On the contrary, with decreasing this ratio k_{ads} values of mixed systems proved to be more or less identical to pure protein ones.

These results are consistent with the hypothesis that low molecular weight surfactants can dominate the interface only when present with high concentrations in the mixed protein-emulsifier systems, and in a way that is different according to the type of emulsifier competing with the protein for the interface [Miller et al. (2000)].

TW/OV systems exhibited intermediate k_{ads} values between the pure protein and emulsifier ones, whereas the DA/OV systems showed values also different from these. This result could be attributed to a different displacement mechanism of the protein by low molecular weight emulsifier at the interface and, then, to different interactions between them either in solution or at the interface.

Then, the competitive adsorption proceeding via the formation of protein-surfactant complexes having surface activity different from the pure components ones could be recognised for the systems constituted of Ovalbumin and ionic surfactant Admul Datem, while the competitive adsorption involving mainly via the replacement of protein by the

surfactant molecules could explain the Ovalbumin adsorption behavior in the presence of the nonionic emulsifier Tween60 [Miller et al. (2000), Bos and van Vliet (2001), Krägel et al. (2003), Mackie and Wilde (2005), Mackie et al. (1999)].

3.3 Dilational rheological properties

The impact of Tween60 and Admul Datem surfactants on the dilational rheological properties of Ovalbumin layers adsorbed at the A/W and O/W interfaces was explored in order to obtain additional information on the protein-emulsifier interaction.

The dependence of the rheological parameters, and in particular of the complex interfacial dilational modulus (E^*) and the loss angle tangent ($tan\delta$), on the adsorption time was monitored, and then, from this, their frequency dependence was obtained as described above (*2.2.2*). In this regard a typical trend registered in a time sweep test was reported in figure (8).

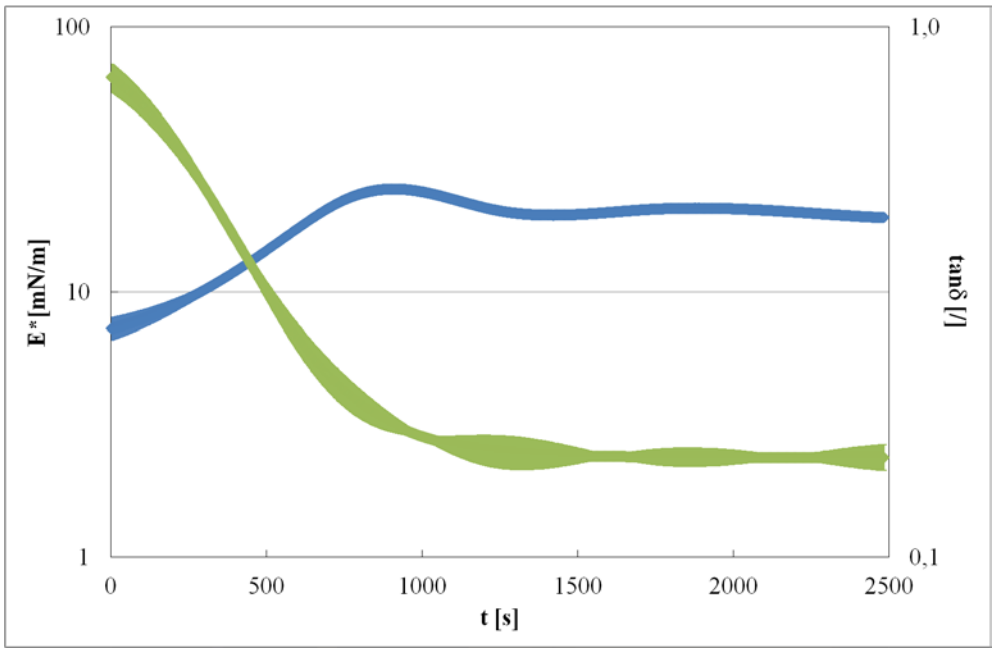


Figure 8. Trend of E* and tanδ with time registered for OV film adsorbed at the O/W interface (ω=0.005Hz, $\Delta A/A_0$=10%)

The frequency behavior being the main focus of this work, the rheological parameters were reported as a function of drop oscillation frequency in the following figures. Specifically, the E^* *vs* ω (figures **a**) and $tan\delta$ *vs* ω(figures **b**) curves of the TW/OV films adsorbed at the

A/W and O/W interfaces were shown in figures (9) and (10) respectively, whereas figures (11) and (12) showed the same data for the DA/OV systems at both the interfaces.

Moreover, for a comparison, rheological parameters obtained for the individual components (OV, TW and DA samples) were also shown in figures (9)-(12).

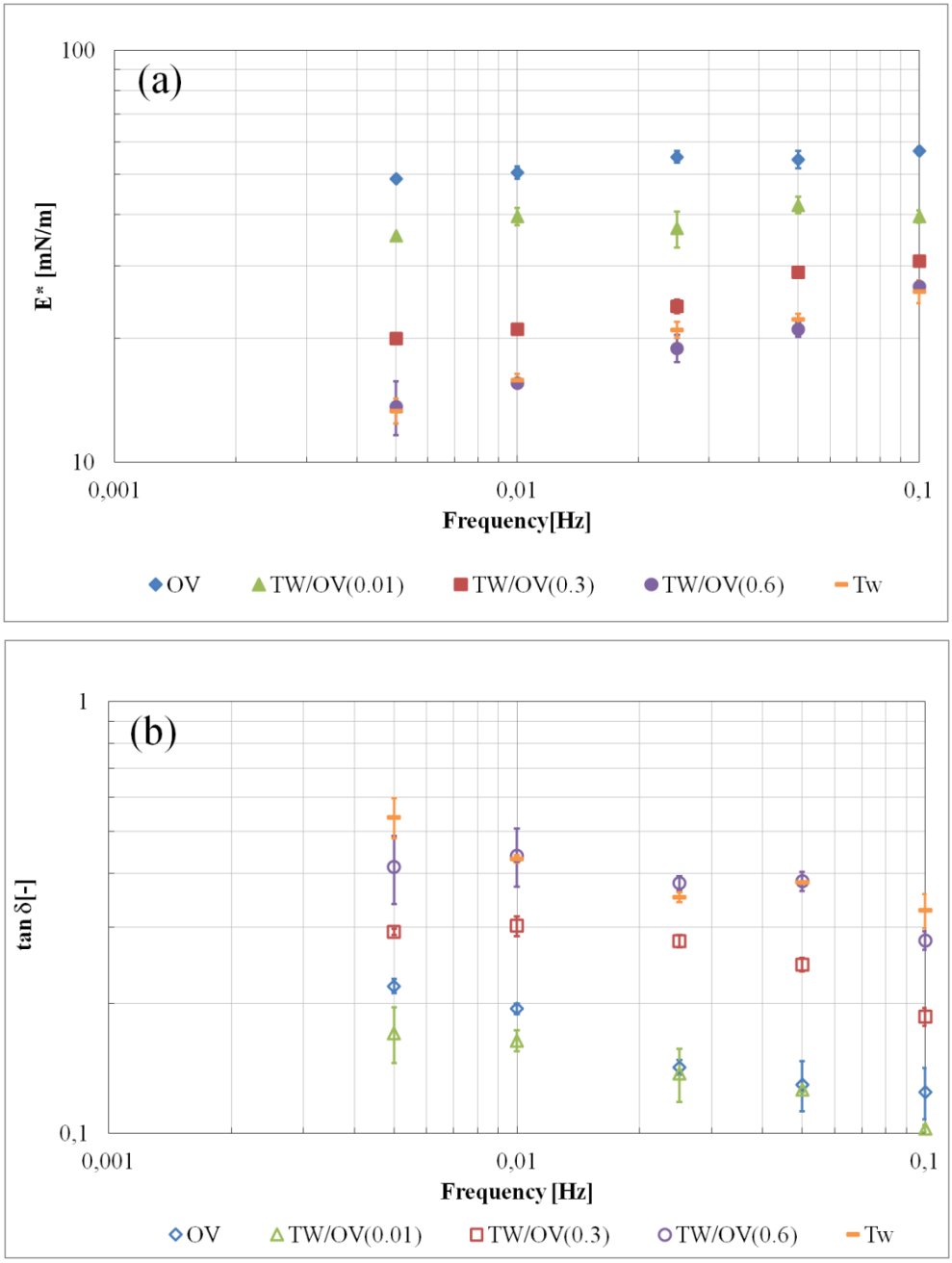


Figure 9. (a) Surface dilational modulus, E* , and **(b)** phase angle tangent, tan δ , as a function of frequency for OV, TW, and TW/OV films adsorbed at the A/W interface

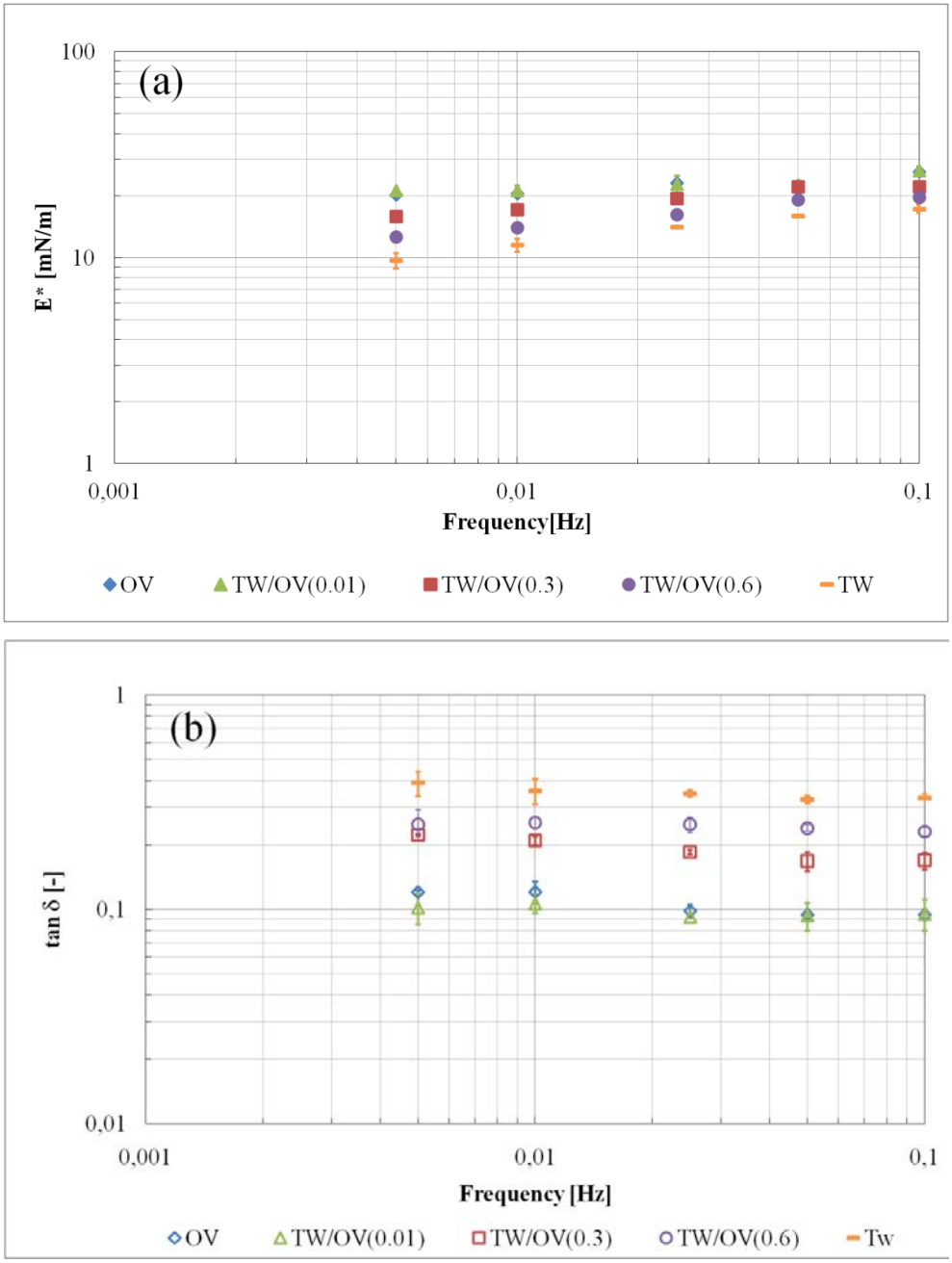


Figure 10. (a) Surface dilational modulus, E* , and **(b)** phase angle tangent, tan δ , as a function of frequency for OV, TW, and TW/OV films adsorbed at the O/W interface

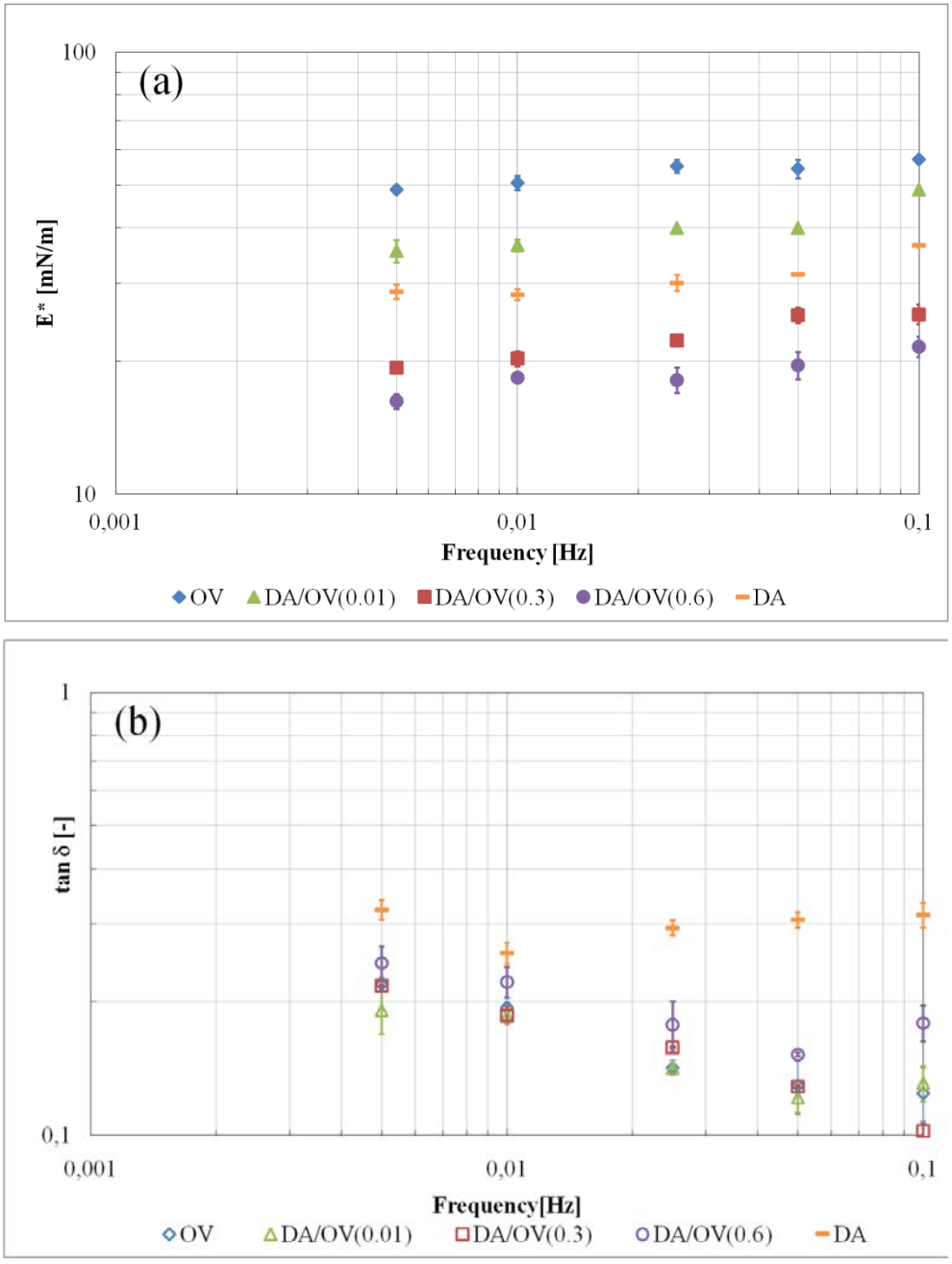


Figure 11. (a) Surface dilational modulus, E* , and **(b)** phase angle tangent, tan δ , as a function of frequency for OV, DA, and DA/OV films adsorbed at the A/W interface

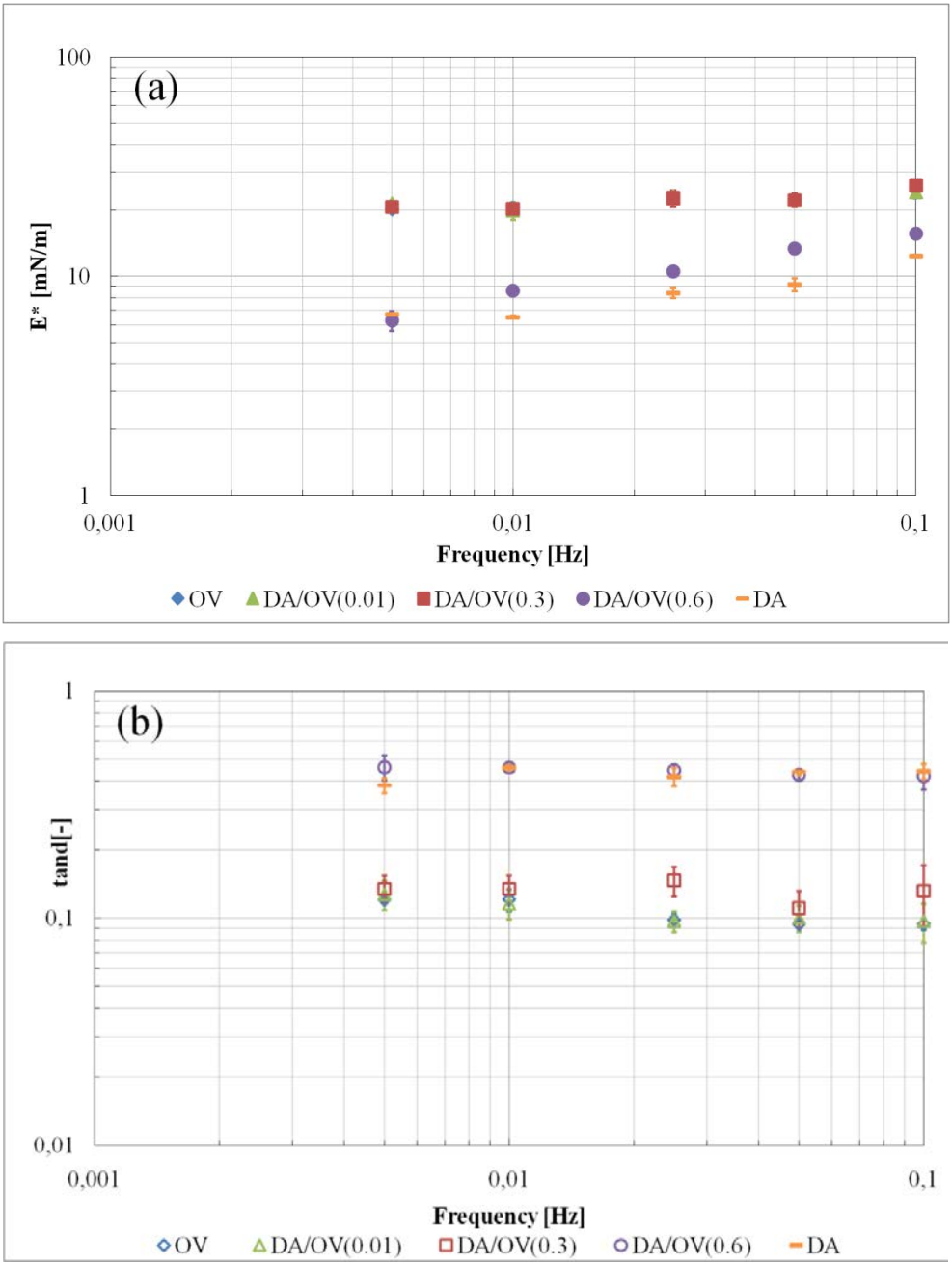


Figure 12. (a) Surface dilational modulus, E* , and **(b)** phase angle tangent, tan δ , as a function of frequency for OV, DA, and DA/OV films adsorbed at the O/W interface

It is possible to see in figures (9)-(12) that for each investigated system the E* modulus proved to be higher at the interface with air than with sunflower oil. This result agrees with the findings by many authors [Bos and Van Vliet (2001), Wüstneck et al. (1999), Krägel et al.(2003), Williams and Prins (1996), Camino et al.(2009), Murray (1998), Dickinson (1998), Valderrama and Rodrìguez Patino (2010)].

The differences of the dilational rheological parameters between the A/W and O/W interfaces, were typically ascribed to the restriction of the hydrophobic interamolecular interaction of the adsorbed molecules by their salvation in the oil phase.

Then, in accordance with the technical literature the conditions to form an interfacial structure of high mechanical stability are best when conformation changes are not restricted, i.e. at the interface with air, where the adsorbed molecules exhibit higher resistance against unfolding [Bos and Van Vliet (2001), W*ü*stneck et al. (1999), Benjamins et al. (1996)].

However, at both the A/W and O/W interfaces, the E* and tanδ of each sample system showed small variations in the investigated frequency range, which became more important with rising emulsifier/protein weight ratio of the mixtures analyzed .

The almost independence of E* on the frequency combined with the low values of $E^{"}$, as indicated by the very small value of the loss tangent (tanδ <1), therefore means that interfacial relaxation processes, attributed to the exchange of matter between the bulk solution and the interface, and to conformational changes in the interface, were negligible for the studied systems, and that the investigated adsorbed layers could present the typical rheological characteristics of a 2D critical gel [Bouriat et al. (2004), Dicharry et al. (2006), Kopperud and Hansen (2001)].

In fact, it is possible to see in figures (9)-(12) that the log−log plot of the complex interfacial dilational modulus *E* vs* ω was a straight line and followed a scaling law of form:

$$E^* \approx \omega^n \tag{8}$$

The loss tangent tanδ remained practically unvaried with pulsation for both the investigated interfaces. Moreover, in some cases (O/W interface), the loss angle δ was also related to the slope *n* of the power law curve by the equation (9):

$$\delta = n\frac{\pi}{2} \tag{9}$$

Equations (8) and (9) are relevant to the rheology of 3D-gel near its gelation point (critical gel), as demonstrated by Winter and Chambon for polymers [Winter and Chambon, 1986], and were considered still valid by Bouriat et al. (2004) and Dicharry et al. (2006) to

interpret 2D-rheology measurements assuming that the interface can be modelled as a parallel coupling of continuous Maxwellian blocks with relaxation times, τ, and elasticity:

$$k(\tau) = \alpha\tau^{-(n+1)} \qquad (10)$$

where α, which can identified as the strength of the gel, is proportional to the number of aggregates at interface relaxing with the characteristic time τ.

According the equation (10) the complex elasticity modulus E* can then be calculated by equation (11), from which (8) and (9) can be deduced.

$$E^*(\omega) == \int_0^\infty k(\tau)\frac{i\omega\tau}{1+i\omega\tau}d\tau = \frac{\alpha\pi}{\sin(n\pi)}\omega^n e^{in\pi/2} \qquad (11)$$

In agreement with Bouriat et al. (2004) and Dicharry et al. (2006), in the present work the gel critical approach was used, and the *E*vs*ω data were fitted and interpreted with a power law equation (8). According this equation the parameter *n* can be considered an indirect measure of structuring degree of interface, which proves to be as high as the *n* value is small, while the E* value extrapolated at the frequency of 1 Hz (*k* parameter), is a measure of the strength of the interfacial gel.

It is important to point out that for the investigated systems equation (8) proved to be satisfied at both the investigated interfaces, whereas equation (9) only at the O/W interface. It can be deduced from the figure (13), where the differences between measured and calculated δ values relative to the A/W **(a)** and O/W **(b)** interfaces were shown for only the OV, TW and DA systems. The same discrepancies were obtained for the other investigated systems but were not shown.

Nevertheless, although the rheological approach described can be considered valid only for the molecules adsorbed at the O/W interface, it was used also to study the dilational behavior registered at the A/W interface where, anyway, the loss tangent tanδ remained practically little varied with pulsation, indicating a behavior not too far from that of a critical gel.

This rheological approach may be useful to evaluate and to differentiate the interfacial network behavior of Ovalbumin in the presence of LMW emulsifiers, on the basis of rheological parameters, which are a measure of its structuring. The rheological *n* and *k* ,

calculated according to equation (8) were reported in tables (3) and (4) respectively, and discussed below.

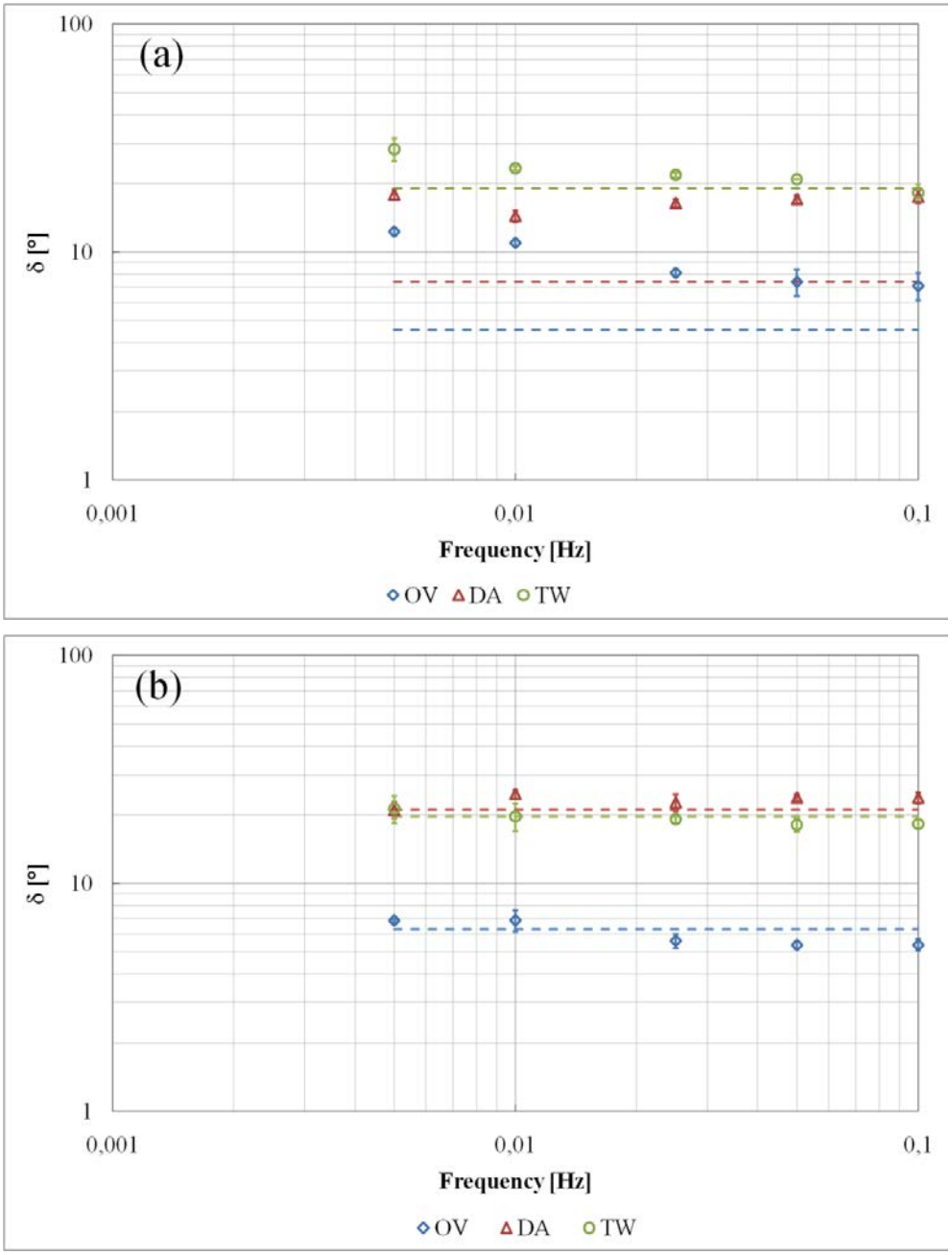


Figure 13. Measured (points) and calculated (dashed line) values of phase angle δ(°), for the OV, TW and DA systems adsorbed at the A/W **(a)** and O/W **(b)** interfaces

Actually since it well known that the steric effect due to a presence of large amphiphilic materials at interface can prevent droplets from coalescing, one may expect that there is a correlation between the presence of a critical gel at interface and emulsion or foam stability [Dicharry et al.(2006)].

The rheological *n* and *k* , calculated according to equation (8) were reported in the tables (3) and (4) respectively, and discussed below.

3.3.1 Tween60-Ovalbumin interaction

To analyze the effect of Twee60-Ovalbumin interactions on the dilational rheological behavior of protein layers adsorbed at the A/W and O/W interfaces, figures (9) and (10) can be examined.

From these figures it can be seen that the addition of Tween60 to Ovalbumin solutions, determined a substantial reduction of the E* modulus and an increasing of tanδ at both the interfaces investigated in the frequency range investigated. This behavior, representative of a considerable fluidization of the adsorbed protein layer became more evident and important when emulsifier/protein weight ratio increased in the mixture.

It could be interpreted as a result of a competitive adsorption phenomenon, combined with weak hydrophobic interaction between the protein and emulsifier and with the prevalent presence of Tween60 molecules at the interfaces [Bos and Van Vliet (2001), Maldonado-Valderrama and Rodrìguez Patino (2010)].

This is in agreement with Mackie et al. (1999) who demonstrated that Tween 20 replaced the proteins by the so-called Orogenic mechanism. It provides that LMW surfactants are first adsorbed at defects in the interfacial protein network, and that the nucleated patches grow, compressing the gel-like structure until the latter is destroyed and finally protein desorption occurs.

Manifestation of the dominance of Tween60 at the interface can be seen from the comparison of E* values exhibited by TW/OV mixtures and those of the pure emulsifier and protein respectively. In fact, a monotonous decrease of E* was registered for TW/OV systems with increasing the emulsifier concentration in the mixture, at both the interfaces, in agreement with Maldonado-Valderrama and Rodrìguez Patino (2010) and Petkov et al (2000).

However, the type of neighboring phase air/oil strongly affected the interfacial rheological behavior of the investigated molecules [Valderrama and Rodrìguez Patino (2010), Kragel et al.(2003)]. In fact, the E* decrease was less important at the O/W than A/W interface, where already the pure components exhibited very different E* values, probably owing to different molecular interactions and to a different conformation of the adsorbed molecules.

Then at the O/W interface, as opposed to the W/A interface at which the replacement of protein was clearly observed, this substitution could be more difficult probably due to a different protein conformation and to a salvation of the hydrophobic protein groups in the oil phase [Valderrama and Rodrìguez Patino (2010), Kragel et al.(2003)].

The rheological behavior of mixed Twee60-Ovalbumin layers adsorbed at the interfaces was investigated assuming that it was a 2D critical gel *(3.3),* and then, calculating and analyzing the gel critical model parameters *n* and *k* of these systems. They were reported in table (3) together with the values obtained for OV and TW systems for comparison.

	A/W		O/W	
	k **[mN/m ·s^n]**	***n*** **[/]**	***k*** **[mN/m ·s^n]**	***n*** **[/]**
OV	64.3± 2.2	0.05 ± 0.01	28.9 ± 1.5	0.07 ± 0.01
TW/OV(0.01)	42.6 ± 1.0	0.05 ± 0.01	29.8 ± 2.1	0.07 ± 0.02
TW/OV(0.3)	42.6 ± 2.0	0.15 ± 0.01	30.5 ± 1.4	0.12 ± 0.01
TW/OV(0.6)	43.5 ± 2.5	0.21 ± 0.01	29.4 ± 1.2	0.16 ± 0.01
TW	42.7 ± 2.5	0.21 ± 0.02	27.2 ± 1.4	0.20 ± 0.02

Table 3. Rheological parameters characteristic of interfacial gel model n and k , obtained by equation (8) for OV, TW/OV and TW layers adsorbed at the A/W and O/W interfaces

Data in table (3) relative to *n* parameter partially confirmed the existence of competitive adsorption phenomenon between Ovalbumin and Tween60 resulting in a probable replacement of protein by emulsifier.

Actually, an increasing of the *n* parameter value, which corresponds to a reduction of structuring degree of the interfacial film, was registered for almost all TW/OV samples, with the exception of TW/OV(0.01), which showed *n* values more or less identical to those of pure Ovalbumin at both the interfaces. On the contrary the other sample systems had *n* values very similar to those of pure emulsifier, which revealed a predominant influence on the structure of mixture layers adsorbed.

Then with increasing the emulsifier concentration in the mixture, the TW/OV adsorbed layers presented a substantial reduction of structuring degree of the interfacial film, which underwent a substantial fluidization.

Concerning the parameter *k*, which is a measure of the strength of the interfacial gel, a different trend between the A/W interface and the O/W one can be seen in table (3), which

is in agreement with what discussed above about to different molecular interactions and to a different conformation of the adsorbed molecules at the two interfaces.

Actually, at the A/W interface the *k* values of the TW/OV layers proved to decrease with increasing the emulsifier concentration in the mixture, confirming the weakening of the interfacial film due to the dominance of Tween 60 molecules and then, the replacement of Ovalbumin at the interface. On the contrary, at the O/W interface no trend was registered for the parameter *k* , whose values for the mixed and pure systems were in deviation.

3.3.2 Admul Datem/Ovalbumin interaction

To analyze the effect of Admul Datem-Ovalbumin interactions on the dilational rheological behavior of protein layers adsorbed at the A/W and O/W interfaces, figures (11) and (12) can be examined.

From these figures different trends of complex dilational modulus E* can be seen at the A/W and O/W interfaces in the investigated frequency range.

Actually, at the former E* of mixed systems DA/OV exhibited values lower than that of pure protein film OV at every concentration of emulsifier used in the mixture, and also lower than that of the DA pure emulsifier system at the highest ones (DA/OV(0.3) and DA/OV(0.6)). On the contrary, at the O/W interface a reduction of surface dilational modulus for mixed systems was registered only for the DA/OV(0.6) system, which anyway assumed elasticity higher than the pure emulsifier.

The differences registered between the investigated interfaces could be ascribed to a dissimilar competitive phenomena occurring between Ovalbumin and Admul Datem molecules, characterized by the formation of emulsifier-protein complexes with surface activity different from that of pure protein and pure emulsifier [K*r*ägel et al. (2003)], or ascribed to different conformations taken by protein adsorbed at the interfaces in presence of an ionic emulsifier [Maldonado-Valderrama and Rodrìguez Patino (2010)].

Similar variations were found also by K*r*ägel et al. (2003) studying the adsorption layer structure of mixed βlactoglobulin and anionic emulsifier sodium dodecyl sulphate at A/W and O/W(hexane) interfaces. For these mixtures a competitive process between protein/surfactant complexes and free surfactant molecules was found at the former interface, while the O/W interface was recognized essentially to be covered by the complexes.

Different competitive phenomena of βlactoglobulin protein in the presence of ionic surfactants occurring at the A/W and O/W interfaces were also described by Maldonado-Valderrama and Rodrìguez Patino (2010) and ascribed to different conformations of protein adsorbed which are responsible for the differences encountered in the dilational response of mixtures.

To complete the analysis of the rheological behavior of mixed Admul Datem-Ovalbumin layers adsorbed at the interfaces, the gel critical model parameters *n* and *k* of these systems were calculated and analyzed as for the TW/OV ones.

They were reported in table (4) together with the values obtained for OV and TW systems for comparison.

	A/W		O/W	
	***k* [mN/m ·s^n]**	***n* [/]**	***k* [mN/m ·s^n]**	***n* [/]**
OV	64.3 ± 2.3	0.05 ± 0.01	28.9 ±1.4	0.07 ± 0.01
DA/OV(0.01)	57.5 ± 3.8	0.09 ± 0.01	29.8 ± 2.3	0.08 ± 0.02
DA/OV(0.3)	29.8 ± 2.0	0.08 ± 0.01	30.5 ± 2.0	0.08 ± 0.02
DA/OV(0.6)	25.4 ± 2.4	0.08 ± 0.02	30.7 ± 1.4	0.26 ± 0.01
DA	42.0 ± 2.4	0.08 ± 0.01	19.8 ± 1.9	0.24 ± 0.03

Table 4. Rheological parameters characteristic of interfacial gel *n* and *k* , obtained by equation (8), for OV, DA/OV and DA layers adsorbed at the A/W and O/W interfaces

The *n* parameter, in particular, proved to be useful to confirm the effect of the addition of Admul Datem on the rheological characteristics of the Ovalbumin layers adsorbed at the O/W interface.

From table (4), considering the trend of *n* values, a substantial reduction of structuring degree of interface of mixed systems can be seen at both the interfaces when increasing the emulsifier concentration in the mixture.

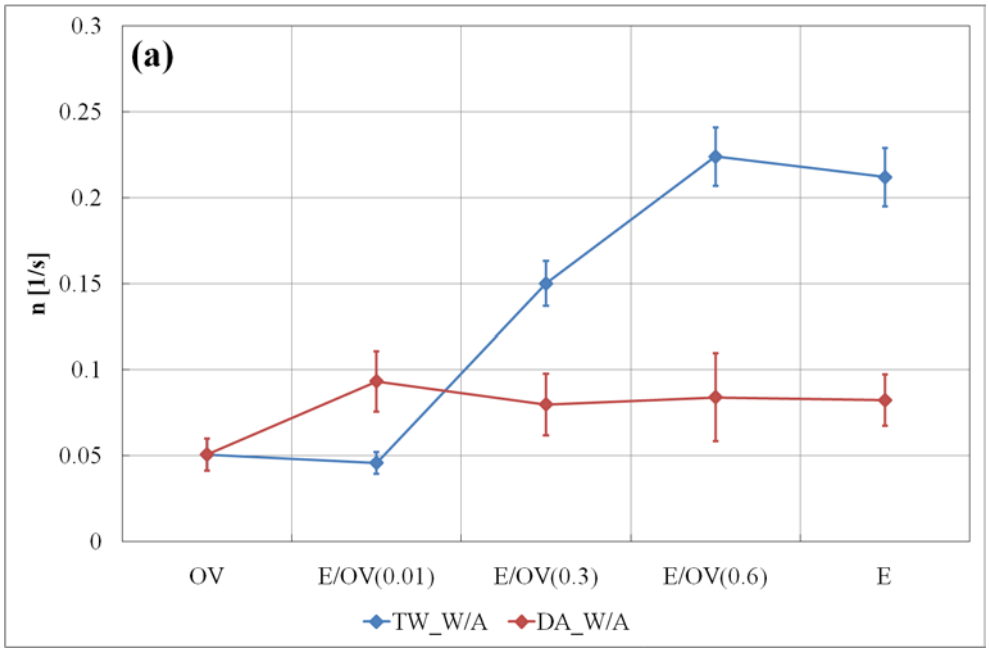
Regarding the *k* values no variations with respect to pure protein systems were found at the O/W interface, indicating that the addition of emulsifier to the protein system did not affect the strength of the interfacial gel.

On the contrary, at the A/W interface, for each mixed systems *k* values much lower than the pure protein one can be observed in table (4). These values proved to be also lower than pure emulsifier in the case of DA/OV(0.3) and DA/OV(0.6) system, confirming the

probable presence of emulsifier-protein complexes at the interface with characteristics different from pure components due to electrostatic interactions [Krägel et al. (2003)].

3.3.3 Effect of the emulsifier type on the structure of the Ovalbumin layer

The effect of the addition of Tween60 and Admul Datem emulsifiers on Ovalbumin layers adsorbed at the A/W and O/W interfaces was characterized by a substantial weakening of the protein network, which in both the cases could result in a stability reduction of dispersion containing these substances [Dicharry et al.(2006), Wilde et al.(2004)]. The weakening of the protein network assumed different characteristics according to the type of emulsifier added and to the type of interface, which caused a different trend of the *n* parameter, as can be seen in figure (14).



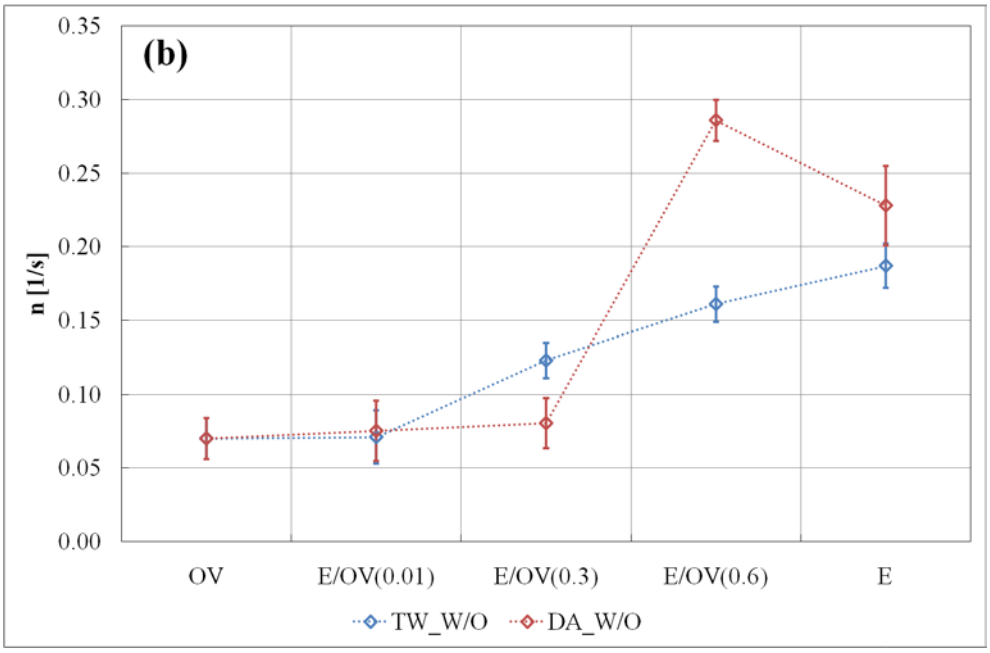


Figure 14. Rheological parameter characteristic of interfacial gel model *n*, obtained by equation (8), for OV, TW/OV, DA/OV, TW and DA layers adsorbed at A/W **(a)** and O/W **(b)** interfaces as a function of emulsifier/ovalbumin ratio (E/OV) used in the mixture

It showed that the nonionic emulsifier Tween60 affected the protein interfacial structure layer more than the ionic emulsifier Admul Datem at the A/W interface, whereas the opposite behavior was registered at the O/W interface. This effect could be probably be ascribed to a different influence of the oil-air phase on the adsorption and to a different hydrophobicity among the surfactant molecules studied, which allowed them to have a different capacity to anchor to interface.

While the competitive phenomena between Tween60 and Ovalbumin were probably of the same type at both interfaces, as indicated by the same trend of n parameter at the interfaces as a function of emulsifier/Ovalbumin ratio in the mixture, in the presence of Admul Datem differences of the absolute variation of structuring degree of interfacial film were found. This result indicated that hydrophobic interactions occurring between nonionic Tween60 and the Ovalbumin protein did not vary in the presence of the air or oil phase, whereas electrostatic ones characteristic of ionic Admul Datem and protein were strongly influenced.

4. Conclusions

Ovalbumin adsorption layers in the presence of nonionic Tween60 and ionic Admul Datem emulsifiers respectively, at the air/water and sunflower oil/water interfaces, were characterized by dynamic interfacial tension measurements and harmonic drop oscillation experiments on a time scale of some seconds. Axialsymmetric drop shape analysis (ADSA) was used to calculate drop volume, area and interfacial tension.

The adsorption behavior was discussed from a kinetic point of view in terms of molecular diffusion and penetration of adsorbed protein molecules in the presence of emulsifier ones. Surface tension vs time trend led to identify competitive adsorption phenomena between Ovalbumin and both the emulsifiers. Adsorption kinetics at long time, more rapid at the O/W than A/W interface, was found for each investigated system, differently from the apparent diffusion rates, which were equivalent.

Dependence of the dilational parameters on frequency of drop oscillation was determined at the A/W and O/W interfaces. Purely elastic behavior, having an almost frequency-independent storage module and a low loss module, was found at both the interfaces..

The effect of the addition of Tween60 and Admul Datem emulsifiers on Ovalbumin layers adsorbed at the A/W and O/W interfaces was characterised by a substantial weakening of the protein network, which was analyzed assuming that the film adsorbed behavior was a 2D ge*l* and considering the rheological model used for the 3D critical gel valid at the interface. This rheological approach proved to be useful to evaluate and to differentiate the interfacial network behavior of Ovalbumin in the presence of LMW emulsifiers, on the basis of rheological parameters, which are a measure of its structuring properties.

REFERENCES

1) Bos M.V. and van Vliet T. (2001), Interfacial rheological properties of adsorbed protein layers and surfactants: a review, *Advances in Colloid and Interface Science* (91), 437-471
2) Murray S.B., (1998) Interfacial rheology of mixed food protein and surfactant adsorption layers with respect to emulsion and foam stability in M*ö*bius D. and Miller R., Studies in interface science 7, Proteins at Liquid Interfaces, Elsevier editor, Amsterdam (The Netherlands), chapter 5
3) Kotsmar C., Pradines V., Alahverdjieva V.S., Aksenenko E.V., Fainerman V.B. Kovalchuk V.I., Krägel J., Leser M.E., Noskov B.A., Miller R. (2009), Thermodynamics, adsorption kinetics and rheology of mixed protein–surfactant interfacial layers, *Advances in Colloid and Interface Science* (150), 41–54
4) Maldonado-Valderrama J. and Rodrìguez Patino J.M. (2010) Interfacial rheology of protein–surfactant mixtures, *Current Opinion in Colloid & Interface Science* (15), 271–282
5) Krägel J., O'Neill M., Makievski A.V., Michel M., Leser M.E., Miller R., (2003) Dynamics of mixed protein-surfactant layers adsorbed at the water/air and water/oil interface, *Colloids and Surfaces B: Bionterfaces* (31), 107-114
6) Hasenhuettl G.and Hartel W.R., Food emulsifiers and their applications, Second Edition Springer editor, New York (USA) 1998, chapter 5
7) Dickinson E. (1998), Proteins at interfaces and in emulsions. Stability, rheology and interactions. *Journal of the Chemical Society, Faraday Transactions* (94), 1657-1669
8) Wilde P.J. (2000), Interfaces: their role in foam and emulsion behavior, *Current Opinion in Colloid & Interface Science* (5), 176-181
9) Rouimi S., Schrorsch C., Valentini C., Vaslin S., (2005) Foam stability and interfacial properties of milk protein-surfactant systems. *Food Hydrocolloids* (19), 467-478
10) Wilde P., Mackie A., Husband F., Gunning P., Morris V.(2004), Proteins and emulsifiers at liquid interfaces, *Advances in Colloid and Interface Science* (108 – 109), 63-71

11) Mackie A, Wilde P., (2005) The role of interactions in defining the structure of mixed protein–surfactant interfaces, *Advances in Colloid and Interface Science* (117), 3-13

12) Ravera F., Loglio G., Kovalchuck V. I.(2010), Interfacial dilational rheology by oscillating bubble/drop methods, *Current Opinion in Colloid & Interface Science,* article in press

13) Galazka V.B., Dickinson E., Ledward D.A. (2000) Emulsifying properties of ovalbumin in mixtures with sulphated polysaccharides: effects of pH, ionic strength, heat and high pressure treatment, *Journal of the Science and Agriculture* (80), 1219-1229

14) Croguennec T. , Renault A. , Beaufils S., Dubois J.J., Pezennec S. (2007), Interfacial properties of heat-treated ovalbumin, *Journal of Colloid and Interface Science* (315) 627–636

15) Benjamins J., Cagna A. Lucassen-Reynders E.H.(1996), Viscoelastic properties of triacyglycerol/water interfaces covered by protein, *Colloids and Surfaces A: Physicochemical and Engineering Aspects* (114), 245-254

16) Lucassen-Reynders E.H., Benjamins J., Fainerman V.B. (2010), Dilational rheology of protein films adsorbed at fluid interfaces, *Current Opinion in Colloid & Interface Science* (15), 264-270

17) Mleko S., Kristinsson H.G., Liang Y., Gustaw W. (2007), Rheological properties of foams generated from egg albumin after pH treatment, *LWT* (40) 908–914

18) W*ü*stneck R., Kr*ä*gel J., Miller R., Wilde P., Clark D.C. (1996), The adsorption of surface-active molecules between β-casein, β-lactoglobulin and ionic surfactants and their rheological shear behavior. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* (114), 255-265

19) Camino N.A., Pèrez O.E., Carrera Sanchez C., Rodriguez Patino J.M., Pilosof A.M.R. (2009). Hydroxypropylmethylcellulose surface activity at equilibrium and adsorption dynamics at the air-water and oil-water interfaces. *Food Hydrocolloids* (23) 2359-2368

20) Biresaw G., Liu Z. S., Erhan S.Z.(2008), Investigation of the Surface Properties of Polymeric Soaps Obtained by Ring-Opening Polymerization of Epoxidized Soybean Oil, Wiley InterScience (www.interscience.wiley.com)

21) W*ü*stneck, R., Moser, B., Muschiolik, G. (1999). Interfacial dilational behaviour of adsorbed b-lactoglobulin layers at the different fluid interfaces. *Colloids and Surface B: Biointerfaces,* (15) 263–273.

22) Lin S.Y., Wang W.J., Lin L.W., Chen L.J. (1996) Systematic effects of bubble volume on the surface tension measured by pendat bubble profiles *Colloids and Surfaces A: Physicochemical and Engineering Aspects* (114), 31-39

23) Pèrez O.E., Carrera Sànchez C., Pilosof A.M.R., Rodrìguez Patino J.M. (2009) Kinetics of adsorption of whey proteins and hydroxypropyl-methyl-cellulose mixtures at the air-water. *Journal of Colloid and Interface Science* (336), 485-496

24) Pèrez A.A., Carrera Sànchez C., Rodrìguez Patino J.M., Rubiolo A.C., Santiago L.G. (2010).Milk whey proteins and xanthan gum interactions in solution and at the air-water interface: A rheokinetic study. *Colloids and Surface B*: Biointerfaces (81) 50-57

25) Van Hunsel, J. Bleys G. Joos P. (1986) Adsorption Kinetics at the Oil/Water Interface. *Journal of Colloid and Interface Science* (114), 424-430

26) Demeter-Vodnàr J., Salajan M., Lowy D.A. (1996) Kinetic Study of the diffusion and Adsorption of Fatty Acids at the Benzene-Water Interface. *Journal of Colloid and Interface Science* (183), 424-430

27) Rosen M.J., Surfactants and interfacial phenomena, Third Edition, John Wiley & Sons, New Jersey (U.S.A) 2004, 208-238

28) Davis J. P., (2005), Investigations into the mechanisms responsible for the yield stress of protein based foams, PhD Thesis

29) Ward A.F.H. and Tordai L. (1946) Time-dependence of Boundary Tensions of Solutions. *The Journal of chemical physics* (14) 453-461

30) Graham D.E. and Phillips M.C.(1979) Proteins at liquid interface III Molecular structures of adsorbed films. *Journal of Colloid and Interface Science* (70), 427-439

31) Lucassen J. and van den Tempel M. (1972), Dynamic measurements of dilational properties of a liquid interface, *Chemical Engineering Science* (27) 1283-1291

32) Freer E. M., Sub Yim K., Fuller G. G., Radke C. J. (2004), Shear and Dilatational Relaxation Mechanisms of Globular and Flexible Proteins at the Hexadecane/Water Interface. *Langmuir (20),*10159-10167

33) Ravera F., Liggieri L., Loglio G. Dilational rheology of adsorbed layers by oscillating drops and bubbles. In: R. Miller, L. Liggieri Interfacial Rheology Volume 1 , Brill Academic Pub, Leiden Boston 2009, 138-177

34) Myrvold R. and Knut Hansen F. (1998), Surface Elasticity and Viscosity from Oscillating Bubbles Measured by Automatic Axisymmetric Drop Shape Analysis,

35) Dicharry C., Arla D., Sinquin A., Graciaa A., Bouriat P. (2006) Stability of water/crude oil emulsions based on interfacial dilatational rheology *Journal of Colloid and Interface Science* (297) 785–791

36) Bouriat,P., El Kerri N., Graciaa A., Lachaise J. (2004) Properties of a Two-Dimensional Asphaltene Network at the Water-Cyclohexane Interface Deduced from Dynamic Tensiometry *Langmuir* (20) 7459-7464

37) Winter H.H. and Chambon F.(1986) Analysis of linear viscoelasticity of a cross-linking polymer at the gel point *Journal of Rheology* (30) 367-382

38) Miller R., Fainerman V.B., Makievski A.V., Krägel J., Grigoriev D.O., Kazakov V.N., Sinyachenko O.V., (2000) Dynamics of protein and mixed protein/surfactant adsorption layers at the water fluid interface *Advances in Colloid and Interface Science* (86), 39-82

39) Rodrìguez Patino J.M., Rodriguez Nino M.R., Carrera Sànchez C. (2003) Protein-emulsifiers at the air water interface. *Current Opinion in Colloid & Interface Science* (8), 387-395.

40) Mackie A.R., Gunning P., Wilde P.J., Morris V.J.(1999) Orogenic Displacement of Protein from the Air/Water Interface by Competitive Adsorption *Journal of Colloid and Interface Science* (210), 157-166

41) Petkov J.T., Gurkov T.D.(2000). Dilatational and Shear Elasticity of Gel-like Protein Layers on Air/Water Interface, *Langmuir* (16), 3703-3711

42) Rotureau, E., Leonard, M., Dellacherie, E., Durand, A. (2004). Amphiphilic derivates of dextran: adsorption at air/water and oil/water interfaces. *Journal of Colloid and Interface Science* (279) 68–77.

43) Ganzevles, R. A., van Vliet, T., Cohen Stuart, M. A., de Jongh, H. H. J. (2007). Manipulation of adsorption behaviour at liquid interfaces by changing protein–polysaccharide electrostatic interactions. *Food Hydrocolloids (*21) 195–208.

44) Medrzycka K.,and Zwierzykowski W. (2000). Adsorption of alkyltrimethylammonium bromides at the various interfaces. *Journal of Colloid andInterface Science* (230) 67–72.

45) Wierenga P.A. (2005), Basics of macroscopic properties of adsorbed protein layers formed at air-water interfaces, based on molecular parameters, PhD thesis, Wageningen University, The Netherlands, chapter 2

46) Beverung, C. J., Randke, C. J., & Blanch, H. W. (1999) Protein adsorption at the oil water interface: characterization of adsorption kinetics by dynamic interfacial tension measurements. *Biophysical Chemistry* (81), 59–80

47) Kopperud H. B. M. and Hansen F.K. (2001), Surface Tension and Surface Dilational Elasticity of Associating Hydrophobically Modified Polyacrylamides in Aqueous Solutions. *Macromolecules* (34), 5635-5643

Chapter 4
Comparison of the dilational behavior and adsorption dynamics of milk protein mixtures at the oil-water interface

Abstract

The adsorption of milk proteins β-Casein and β-Lactoglobulin, both alone and in binary mixtures, at the sunflower oil-water interface was investigated by dynamic interfacial tension measurements and harmonic drop oscillation. Axialsymmetric drop shape analysis (ADSA) was used to calculate drop volume, area and interfacial tension.

The main goal of this research is to investigate the interfacial mechanisms and properties underlying the emulsion functionality of milk protein mixtures, which are classically based ingredients of dairy systems.

The β-Casein and β-Lactoglobulin adsorbed layers composed of their individual molecules and binary mixtures of them were characterized by varying the bulk protein concentration and the protein weight ratio used in the mixture, respectively.

The adsorption of milk proteins at the O/W interface was discussed from a kinetic point of view considering the rate of the molecular penetration and rearrangement phenomena of the adsorbed surfactants at the interface.

The dependence of the dilational parameters on the drop oscillation frequency was determined and interfacial rheological properties similar to those of three-dimensional protein gels were obtained.

Then, the rheological models used classically for the 3D critical gel and, moreover, applied to the bulk behavior of composite systems were considered valid at the O/W interface. They were used to derive some structural interfacial information and to interpret data of protein mixtures in terms of the interfacial properties of the individual components

The results suggested that the adsorption and the interfacial mechanical properties of the β-Casein/β-Lactoglobulin adsorbed layers were dominated by the β-casein molecules at each protein weight ratio used in the mixture.

1. Introduction

The stability and the rheological properties of emulsion systems are largely determined by the interactions between the droplets. The nature and the strength of these interactions are dependent, in turn, on the structure and composition of adsorbed layer at the oil/water interface, which tend to be very complex in food colloids [Bos and Van Vliet (2001), Murray (1998), Dickinson (1998)].

The majority of food emulsions are created and stabilized by proteins representing a class of surface active components very important from the interfacial science point of view. Particularly, the proteins derived from milk are widely used for these purposes being valued as food ingredients with excellent surface-active, emulsifying and colloid-stabilizing characteristics [Dickinson (1998), Rouimi et al. (2005), Dickinson (1999)(a), Dickinson (2001), Raikos (2010), Williams and Prins (1996), Cornec et al. (1996)].

Milk proteins are, traditionally, classified in two major categories, caseins and whey proteins [Raikos (2010)]; their specific concentrations in bovine milk were reported in Table (1) [Jensen (1995)].

Major milk proteins	Grams/liter
Total proteins	*36*
Total caseins	*29.5*
αs1	*11.9*
αs2	*3.1*
β	***9.8***
κ	*3.5*
y	*1.2*
Total whey proteins	*6.5*
α-Lactalbumin	*1.2*
β-Lactoglobulin	***3.1***
Serum Albumin	*0.4*
Immunoglobulins	*0.8*
Proteose peptone	*1*

Table 1. Protein composition of bovine milk from Jensen (1995)

Milk protein based ingredients tend to be complex mixtures of proteins having compositions and functionalities largely dependent on the extraction, purification and subsequent processing procedures [Ridout et al. (2004)]. The functional behavior of these mixtures can, therefore, be complex and often unpredictable.

Progress in understanding their properties have been made by studying the interfacial properties of simpler systems or "model systems", which typically contain only one protein type. These studies proved to be necessary in many cases to control and predict the mechanisms underlying the functionality of protein mixtures. Although the surface activity is certainly an important attribute, the lowering of interfacial tension does not by itself explain the stability of protein-based emulsions. The essential stabilizing function of proteins is that they enable the fluid interface to resist tangential stresses from the adjoin flowing liquids [Dickinson (1999)]. For this reason, surface rheological techniques have been used extensively in the area of food colloids, to study protein capacity to form viscoelastic interfaces, with rheological properties similar to those of three-dimensional protein gels [Dickinson (1998), Bos and van Vliet (2001), Dickinson (2001), W*ü*stneck et al. (1996), Dickinson and Matsumara (1994), Freer et al. (2004)].

The technique is very useful for studying interactions at interfaces between proteins and other surface active ingredients such as low molecular weight emulsifiers and lipids, which have very little surface viscolelasticity [Dickinson (1999), Murray (1998), Bos and van Vliet (2001)].

Although many proteins exhibit similar surface tension behavior, their surface rheological characteristics can be very different [Bos and van Vliet (2001), W*ü*stneck et al. (1996), Ridout et al. (2004)]. Gross structural differences such as in the case of globular proteins versus disordered proteins [Bos and van Vliet (2001), Mackie and Wilde (2005), Dickinson and Matsumara (1994), Freer et al. (2004), W*ü*stneck et al. (1996)], but also very small structural changes, such as differences between genetic variants of the same protein, can cause measurable differences in the surface rheological behavior, and then in the resulting functionality [Ridout et al.(2004)].

The protein molecular structure strongly affects the protein affinity toward the interface, the ability to adsorb at the interface and the tendency to be partially or wholly displaced from the latter by other more surface active protein species or by small-molecule surfactants [Cornec et al. (1996), Maldonado-Valderrama and Rodrìguez Patino (2010), Kr*ä*gel et al.(2003), Murray (1998), Rouimi et al. (2005), Wilde at al. (2004), Mackie and Wilde (2005)]. Specifically, the mutual displacement between proteins has been ascribed to the difference in the proteins ability to change their conformation during the adsorption [Bos and van Vliet (2001)]. Flexible proteins change their conformation more easily than globular proteins and, for this reason, they could be more able to dominate the interface

[Arai and Norde (1990) (a), Arai and Norde (1990) (b), Dickinson (1999) (b), Bos and van Vliet (2001)].

Competitive adsorption phenomena between different protein species adsorbed at both air/water and oil/water interfaces proved to be very complex being affected by many factors other than just specific affinity for the interface, such as hydrophobicity, electric charge, the molecular mass, and the structure stability [Ridout et al. (2004), Arai and Norde (1990) (a), Arai and Norde (1990) (b), Dickinson (1999) (b), Bos and van Vliet (2001)].

In this work mixtures of the flexible β-Casein and the globular β-Lactoglobulin proteins were characterized by interfacial properties analysis in order to investigate the interfacial mechanisms and the properties underlying the emulsion functionality of protein mixtures, which are classically based ingredients of dairy systems. Individual protein systems were also characterized in order to obtain a link between the interfacial properties of protein mixtures and those of the pure components.

It is important to emphasize that despite the great complexity of milk its surface was dominated by free β-Casein and β-Lactoglobulin [Dickinson (1999) (b), W*ü*stneck et al. (1996)], and as a consequence, a quantitative investigation of the interfacial rheology of systems composed of these proteins could be a very useful tool to control and predict the functionality of milk product.

Then, milk and sunflower oil being two ingredients often used for the preparation of food dairy emulsions, the sunflower oil/water interface with adsorbed β-Casein and β-Lactoglobulin proteins, which are one of the major caseins and the most abundant whey protein present in the milk respectively, was selected as “model protein interface” for dairy emulsions [Raikos et al.(2010), Gabriele et al. (2009)].

The adsorption behavior of pure β-Casein and β-Lactoglobulin proteins, has been well characterized at different interfaces specially at the air/water and paraffin oil/water interfaces [Williams and Prins (1996), Ridout et al. (2004), Dickinson (1999) (b), W*ü*stneck et al. (1996)]. However, little attention has been paid to the interfacial behavior of their mixtures, in particular at the interface with tracylglycerol oil phases, such as sunflower oil.

2. Materials and methods

2.1 Samples preparation

Sample solutions were prepared by dissolving the proteins derived from bovine milk β-Casein (C6905, Sigma Aldrich, Lot 029K7430) and β-Lactoglobulin (L3908, Sigma Aldrich, Lot 097K7012) in twice-distilled water in imidazole buffer 75 mM at pH=6.8 ± 0.2 [Williams and Prins (1996)]. The pH was chosen to correspond with the conditions in milk [Jensen (1995)].

The twice distilled water used throughout all the experiments was obtained from a Milli-Q purification system (Millipore, USA), and it was checked for contaminants before each experiment, measuring the surface tension of the buffer solution at the air/water boundary at ambient temperature. No aqueous solutions with a surface tension other than accepted in the literature (72-73 mN/m at 20°C) were used.

Solutions with single proteins and with a protein binary mixtures were prepared at ambient temperature (20-23°C) stirring for one hour by using a heating magnetic device (ARE, Velp Scientific, Italy). In the former solutions protein concentration was changed in the range 10^{-4}- 1 g/l, in the latter the total amount of proteins was kept constant equal to the maximum concentration investigated (1 g/l) and the weight ratio between the two analyzed proteins was varied producing three samples: L+C (1:1), L+C (1:3) and L+C (3:1). The sample ID identifies the weight ratio between β–Lactoglobulin:β–Casein (L:C w/w); for instance L+C (1:3) refers to the weight ratio 1:3 w/w, which is that characteristic of the bovine milk.

All solutions were freshly prepared (within no more than 24 h) for the characterization and two replicates were prepared for each sample.

Sunflower oil (Carlo Erba Reagents-356241) without further purification was used as the oil phase in this research. It contains triglycerides and free saturated and unsaturated fatty acid (0.4%) which are responsible of the interfacial tension reduction of 10 mN/m measured between the pure phases oil and buffer solution without the addition of surfactants in accordance with Wüstneck et al.(1996) and Camino et al. (2009)].

2.2 Dynamic interfacial properties measurements

Pure and mixed β-Casein and β-Lactoglobulin layers adsorbed at the O/W interface were characterized by dynamic interfacial tension measurements and harmonic drop oscillation experiments on a time scale of some seconds.

Axialsymmetric drop shape analysis (ADSA) was used to calculate drop volume, area and interfacial tension by using an automated pendant drop tensiometer (FTA200 First Ten Angstroms, USA) equipped with the *fta32 v2.0* software. Details of this apparatus are given by Biresaw et al. (2008).

The instrument comprises an automated pump that can be fitted with various sizes of syringes and needles to allow for control of pendant drop formation and of sinusoidal variations in the drop volume or surface area by software.

An automated image viewing and capturing system, with various image capture triggering options, was used to capture the drop image.

The computer hardware and software also provide the capability of data capture, storage, analysis and transfer. The software allows for an automated drop shape analysis of the captured drop image, and for measuring the surface-interfacial tension of the drop formed in air or in a second fluid at rest and in periodic motion conditions of the interface respectively.

Image acquisition and regression of the interfacial tension were performed directly with commercially available drop-image software by fitting the Bashforth-Adams equation to the drop shape [Biresaw et al. (2008)]. Drop-image software also controlled an automatic pipetting system that maintained constant drop volume with time period over which dynamic tensions were measured.

2.2.1 Dynamic interfacial tension

The method adopted to measure the interfacial tension of the investigated solutions with time (γ *vs* t), by using an automatic drop tensiometer, involved the analysis of the profile of the drops created in the air/oil phases respectively, and kept at rest (constant volume).

The experiments were carried out at room temperature (22°C within ± 1 °C), placing sample aqueous solutions in a 100 µl glass Hamilton syringe equipped with a 20 Gauge stainless steel needle, and delivering drops in a rectangular quartz cuvette (5ml) containing the oil phase.

Drop volumes of 9-12 μl were used in each experiment, in order to measure interfacial tension values independent of the drop size [Lin et all, 1996]. High drop volumes of the kind chosen, proved to be generally more suitable for these tests, because they increased the number of profile points, which can be used for drop shape analysis [Lin et all, 1996].

The experiments started with the creation of the drop; then, drop images were continuously taken from a CCD camera and digitalized, registering the surface tension values over the test time.

Drop profile was monitored up to maximum time of 180 minutes, which assures the molecules adsorption at interface and the reaching of a quasi-equilibrium interfacial tension value.

Equilibrium of γ was assumed when the interfacial tension did not change by more than 0.4 mN/m in 30 minutes [Camino et al. (2009)].

From γ values measured with time the corresponding interfacial pressure was also calculated as difference between the pure water interfacial tension in the absence of macromolecules γ_0 (equal to 24 ± 0.35 mN/m), and that registered for the analyzed solutions during the test time γ.

Interfacial pressure isotherms were then obtained for the pure β-casein and β-lactoglobulin layers in the bulk protein concentration range of 10^{-4} - 1 g/l.

All experiments were repeated at least twice and the experimental data are reported as mean value ± standard deviation.

2.2.2 Interfacial dilational properties and data analysis

The method adopted to determine the dilational rheological properties of the interface involved the profile analysis of the drop formed in the oil phase subjected to periodic motion conditions, according to the oscillating drop methodology, able to measure the interfacial visco-elasticity versus frequency.

Oscillating drop experiments are usually performed subjecting the interface to an infinitesimal sinusoidal compression and expansion, then by applying a frequency sweep to the surface area A, in order to measure the interfacial dilational modulus.

The surface dilational modulus derived from the change in surface tension γ (dilational stress) (eq. 1), resulting from a small change in surface area A (dilational strain) (eq.2), may be described by equations [Lucassen and van den Tempel, 1972]:

$$\gamma = \gamma_0 + \Delta\gamma \sin(\omega t + \delta) \quad (1)$$

$$A = A_0 + \Delta A \sin(\omega t) \quad (2)$$

$$E^* = \frac{d\gamma}{d \ln A} \quad (3)$$

Where γ_0 and A_0 are the equilibrium reference surface tension and the unperturbed interfacial area of the drop respectively, $\Delta\gamma$ and ΔA are the stress and strain amplitude respectively, and δ is the phase angle between stress and strain, measure of the relative film viscoelasticity.

Since the drop area periodically oscillates, the dilational modulus exhibits two contributions: an elastic part accounting for the recoverable energy stored in the interface (storage modulus, *E'*) and the dissipative part accounting for energy lost through relaxation processes (loss modulus, *E''*).

$$E^* = E' + iE'' = \frac{\Delta\gamma}{\Delta A / A_0}\cos(\delta) + i\frac{\Delta\gamma}{\Delta A / A_0}\sin(\delta) \quad (4)$$

Then the surface dilatational modulus, E*, as a measure of the total material resistance to dilatational deformation (elastic and viscous), is a complex quantity composed of real and imaginary part [Freer et al. (2003), Ravera et al. (2009), Myrvold and Hansen (1998)].

For a perfectly elastic material stress and strain are in phase $\delta = 0$ and the imaginary term is zero. In the case of perfectly viscous material $\delta = 90°$ and the real part is zero. The loss angle tangent can be defined by equation (5):

$$\tan\delta = E'' / E' \quad (5)$$

In this work, we applied a periodic strain by differentially oscillating the drop area at a prefixed frequency value, and we measured the periodic stress response with time.

Then, the dilational viscoelastic parameters of interface, the dilational complex modulus (E*), its elastic (E') and viscous (E'') components and the loss angle tangent were measured as a function of the adsorption time *t*.

The *Time Sweep Tests* were carried out by using deformation amplitude ($\Delta A/A_0$) values of 5-10% and angular frequency ones varying in the 0.005 Hz-0.1 Hz range.

The percentage area change was determined before each time sweep test by performing *Amplitude Sweep Experiments* (data not shown) realized at the extreme frequency values of the investigated range. The latter experiments were useful to assure that system response was not influenced by perturbation amplitude (linear viscoelastic behavior) and to be in the linear region, so as to avoid oscillation amplitude that causes disruption of the supramolecular organization or provides adequate measurement sensitivity.

The duration of each test was established so as to register equilibrium values of the dilational moduli with time (maximum variation of 3% was accepted).

The E* *vs* ω curves were then obtained by using the latter values in the frequency range investigated.

3. Results and discussion

3.1. Interfacial tension of individual proteins

Time dependence interfacial tensions of adsorbed β-Casein and β-Lactoglobulin films at the O/W interface with different protein bulk concentrations (10^{-4} –1 g/l) were shown in figures (1) and (2) respectively.

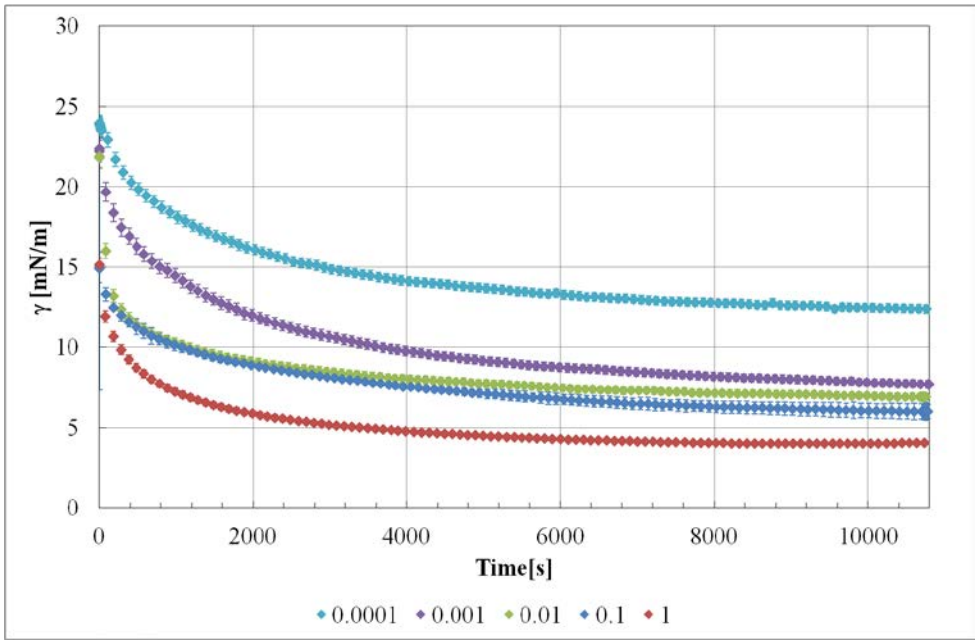


Figure 1. Time dependent surface tension, γ(t), of β-Casein film layer (imidazole buffer, pH 6.8) at the O/W interface as a function of bulk protein concentration (g/l)

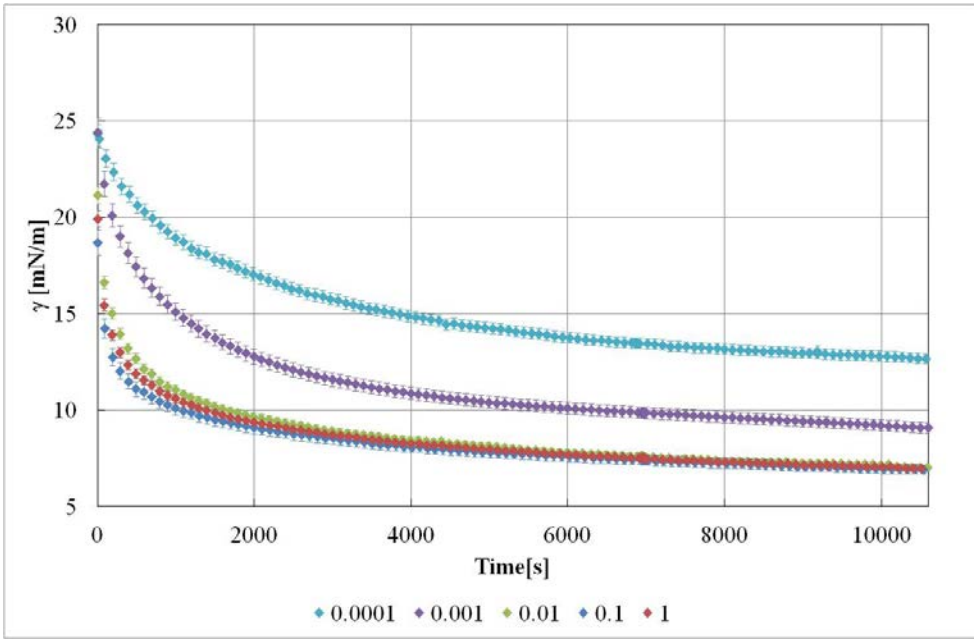


Figure 2. Time dependent interfacial tension, γ(t), of β-Lactoglobulin film layer (imidazole buffer, pH 6.8) at the O/W interface as function of bulk protein concentration (g/l)

The decrease of the interfacial tension was weak at low concentrations and became, however, remarkable starting from a definite protein concentration up to the reaching the interfacial saturation value.

At low protein concentrations, the interfacial tension measured in the first few seconds, was nearly the same as that found for the buffer solution alone (24 ± 0.35 mN/m), whereas at high ones this value is lower, indicating faster adsorption phenomena.

It can be easily seen that after a certain time, depending on the protein concentration value, the interfacial tension became constant - indicating the steady state.

From the figures (1) and (2) it can be noticed that the bulk protein concentration affected the shape of the γ *vs* t, indicating its obvious influence on the diffusion process of surfactant molecules toward the interface [W*ü*steneck et al. (1996)]

Specifically, the protein concentration affected the slope of the curves before the reaching the interfacial tension value of the steady state and, at the same time, the position of the steep decrease of the interfacial tension. In fact, with increasing protein concentration the former became greater, and the latter was shifted to shorter time.

It is important to point out that no induction period, characterized by the constant interfacial tension values prior to the onset of the decrease, was observed in the adsorption process at each given protein concentration, in agreement with Benjamins et al. (1996).

On the contrary, induction periods for β-casein and β-lactoglobulin film layers adsorbed at the air/water interface were reported by many authors [W*ü*steneck et al. (1996), Beverung et al. (1999), Miller et al. (2000), Graham and Phillips (1979), Kr*ä*gel et al. 2003)].

The absence of lag time and the fast adsorption kinetics, indicated by the high slope of the γ *vs* t curve at the initial time, can be attributed certainly to the oil type used in the study [Bos and van Vliet (2001)] containing triglycerides and free saturated-unsaturated fatty acids (0.4%), which could be responsible for the initial reduction of interfacial tension recorded.

Different charges of sunflower oil, indicating a dissimilar content of impurities and interfacial active components, are known to differ in time establishing constant interfacial tension [W*ü*stneck et al.(1999)] and in the absolute interfacial tension values [Camino et al. 2009].

Differences in the adsorption behavior between the two milk proteins investigated were also found in accordance with the findings of many authors [W*ü*stneck et al.(1996), Bos and van Vliet (2001), Graham and Phillips (1979), Dickinson (1999) (b), Ridout et al. (2004)].

In fact, β-Casein proved to adsorb more quickly at the interface than β-Lactoglobulin, exhibiting interfacial tension values dropping more rapidly over the first hour and showing interfacial tension values more or less smaller as shown in the figures (1) and (2).

This behavior can be ascribed to its random, flexible structure in solution [Wüstneck et al.(1996), Bos and van Vliet (2001) Dickinson (1999) (b), Ridout et al. (2004)], which as opposed to that globular of the β-Lactoglobulin, tends to accelerate the adsorption at the interface [Dickinson (1999) (b), Ridout et al. (2004)].

In this regard it is interesting to compare the adsorption isotherms of these proteins, π-log C (figure (3)), constructed for an adsorption time of 3 hours, by using data shown in figures (1) and (2).

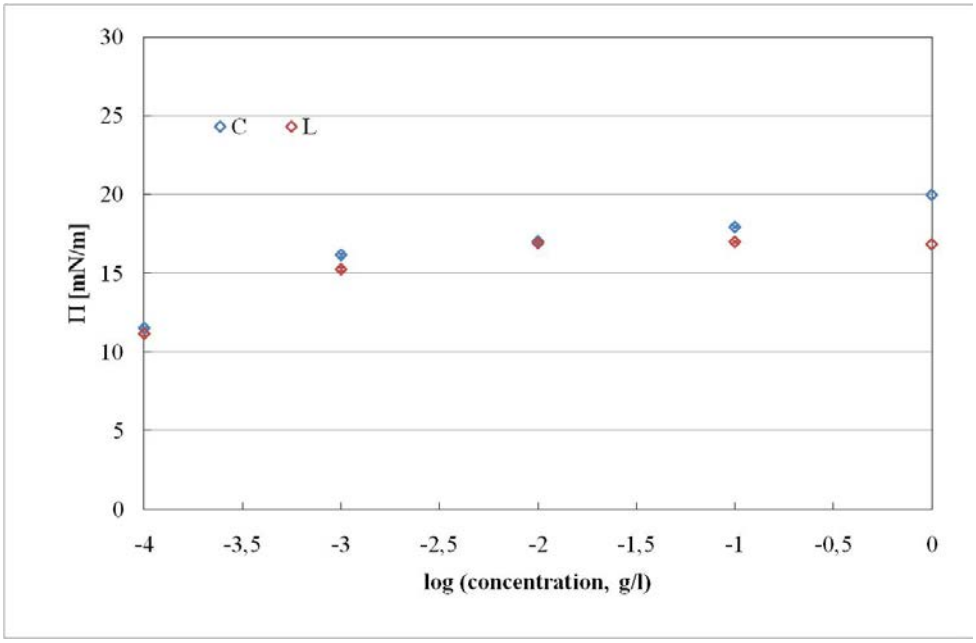


Figure 3. Interfacial pressure isotherms of β-Lactoglobulin and β-Casein at the sunflower oil-water interface for adsorption time of 3 hours. Imidazole buffer pH 6.8 and temperature 22 °C

The adsorption isotherms showed a saturation concentration of 10^{-3} g/l for both the proteins and small differences in the absolute values of interfacial pressure with varying protein concentration. Thus, π-logC isotherms were not sigmoidal and no strong inflections in the adsorption isotherm were observed for both the proteins studied, suggesting that structural changes do not take place in the concentration range studied [Wüstneck et al.(1999), Camino et al. 2009].

This behavior characterized by low concentration dependant change of the interfacial pressure and by a substantial shift of the saturation concentration to low protein

concentrations can be ascribed to the polarity of the neighboring phase, which is well known to affect strongly the saturation concentration of the surface active molecules at the interfaces, in accordance with the findings of Wüstneck et al.(1999) and Camino et al. 2009.

Finally, it is interesting to note also that in the case of the β-Casein protein adsorption, the saturation of the O/W interface was accompanied by a further interfacial pressure increase after saturation (10% difference). This can be interpreted by changes of the molecular aggregation in the bulk phase or by the presence of impurities [Wüstneck et al. (1996)].

3.2 Interfacial tension of β-Lactoglobulin and β-Casein mixtures

In order to understand the dynamic behavior of the mixed β-Lactoglobulin and β-Casein systems and to study the effect of their interactions on the adsorption phenomena, time dependence interfacial tensions were shown and discussed below.

Specifically, in figure (4) dynamic interfacial tensions of β-Lactoglobulin and β-Casein systems, (L+C), were reported as a function of protein weight ratio characteristic of the mixture. For comparison the results obtained for pure β-Lactoglobulin and β-Casein adsorption were also reported.

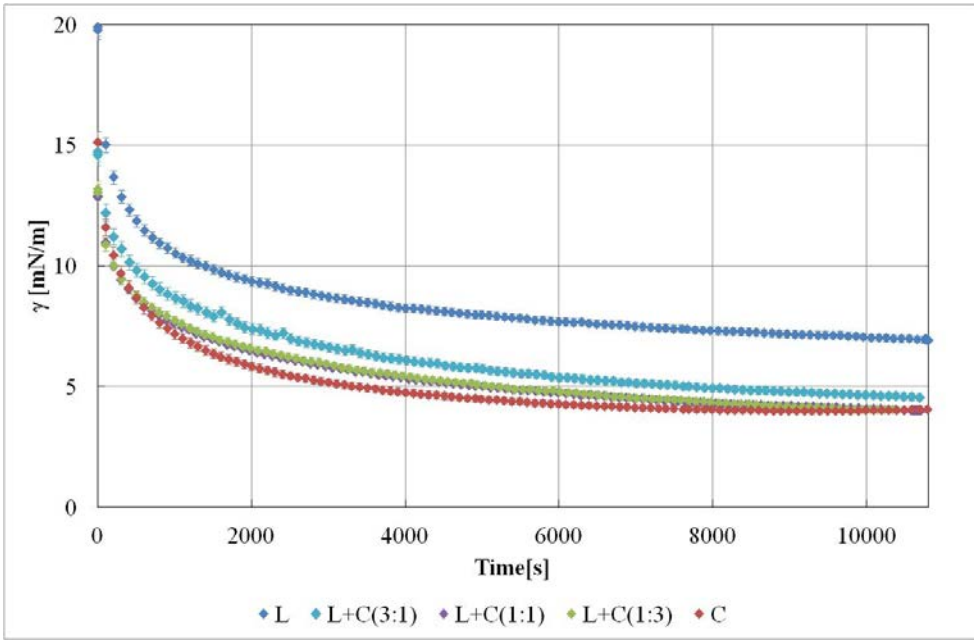


Figure 4. Time dependent interfacial tension, γ(t), of β-Lactoglobulin/ β-Casein film layer (imidazole buffer, pH 6.8) at the O/W interface as function of protein mixing ratio used in the solution (total protein concentration of 1 g/l)

From the figure (4) it can be noticed that although the interfacial tensions of the mixed protein samples fell between the values of the individual components in all adsorption time considered, they were very similar to value exhibited by the β-Casein protein at each mixing ratio investigated. This could indicate that in the system containing a mixture of these proteins the composition of the interfacial layer was strongly influenced by the adsorption of the β-Casein, which proved to dominate the O/W interface of the mixed systems also when its bulk concentration was smaller than that of β-Lactoglobulin, owing its capacity to adsorb more rapidly. In fact, it is well known that the adsorption dynamics of each protein from the mixture are usually determined by the order and rate of arrival at the interface [Dickinson (2010)] and by the specific composition of the mixture.

In order to investigate this result and to understand better the effect of protein mixing ratio on the interfacial activity of the mixtures investigated, the adsorption processes of pure and mixed β-casein/β-lactoglobulin systems were analyzed also from a kinetic point of view, by using the approach proposed by Graham and Philips [Graham. and Phillips (1979)]. This is useful to analyze the rate of adsorption of surface active molecules at the interface and moreover the velocity of molecular unfolding/rearrangement processes (conformational reorganization) happening at the interface especially for high molecular weight macromolecules [Pèrez et al. (2009)].

The rate of these processes can be analyzed by a first-order equation reported below:

$$\ln \frac{\pi_{180} - \pi_t}{\pi_{180} - \pi_0} = -k_i t \qquad (6)$$

Where π_{180}, π_0 and π_t are the surface pressures at 180 min of adsorption time, at time t=0, and at any time t, respectively, and k_i is the first-order rate constant.

In practice, a plot of equation (6) usually yields two or more linear regions. The initial slope is taken to correspond to a first-order constant of adsorption (k_{ads}), while the second slope is taken to correspond to a first-order constant of rearrangement (k_r), occurring among a more or less constant number of adsorbed molecules [Pèrez et al. (2009), Pèrez et al. (2010), Camino et al (2009)].

Specifically, a plot of equation (6) calculated from the γ vs t data of the investigated systems, yielded only two linear regions (data not shown) and then, two first-order rate constants were obtained. They were calculated for all protein mixtures considered, and for the pure systems only with the same total protein concentration used in the mixtures (1 g/l),

in order to interpret consistently the kinetic behavior of mixed systems as a function of that of the individual proteins.

Table (2) summarized the results of the kinetic data analysis, thus, the adsorption kinetic constants k_{ads} and k_r as well as the time at which the rearrangement started (or time at which the adsorption/penetration ended).

Samples	$k_{ads} \cdot 10^4$ [s^{-1}]	R^2	$t_{end\ ads.}$	$k_r \cdot 10^4$ [s^{-1}]	R^2
L	3.38 ± 0.01	0.98	9900	20.25 ± 0.45	0.91
L+C (3:1)	3.57± 0.01	0.99	9900	24.82 ± 0.82	0.88
L+C (1:1)	3.63 ± 0.01	0.99	9500	28.62 ± 1.08	0.88
L+C (1:3)	3.79 ± 0.01	0.98	9400	31.58 ± 0.45	0.97
C	5.42 ± 0.02	0.98	8100	37.80 ± 0.72	0.91

Table 2. Kinetic parameters k_{ads} (rate constant of adsorption/penetration) and k_r (rate constant of rearrangement) for β-Casein (C) and β-Lactoglobulin (L) and their mixture with different protein weight mixing ratio (total protein concentration of 1 g/l)

Kinetic data obtained for the pure β-Casein and β-Lactoglobulin systems confirmed the above considerations about the different adsorption velocities exhibited by these proteins at the O/W interface owing to their structures and consequent surface hydrophobicity [Bos and van Vliet (2001) Dickinson (1999) (b),W*ü*stneck et al.(1999), Ridout et al. (2004), Brun and Dalgleish (1999)]. In fact, the data relative to globular β-Lactoglobulin protein, indicated dynamics of adsorption and conformation rearrangement extremely slow [Freer et al. (2004), Bos and van Vliet (2001) Dickinson (1999) (b)], whereas β-Casein (C) showed kinetic constant values higher than β-Lactoglobulin (L) at both short and long adsorption times and, then, adsorption times smaller, and as a consequence of a more rapid overall adsorption rate.

According the behavior of mixed β-Casein and β-Lactoglobulin systems, from table (2) it can be seen that both k_{ads} and k_r values of these systems were intermediate to those of pure proteins and, at the same time, they increased with increased bulk concentration of the β-Casein in the mixture.

The influence of the mixing ratio on the adsorption kinetics led to observe that the co-adsorption process from the investigated mixtures followed a kinetically controlled non-competitive mechanism [Dickinson (2010)], where the protein which was present with a higher level in the mixture was able to influence the interfacial composition and the

interfacial activity more in terms of velocity in reducing interfacial tension. In fact, from the data obtained it can be deduced that β-Lactoglobulin in the presence of β-casein began to dominate the O/W interface when present with sufficiently high level in the mixture, and moreover, that the flexible β-Casein, once adsorbed was not able to displace the rigid globular protein β-Lactoglobulin [Ridout et al.(2004)].

In fact, when the amount of these proteins in the mixture is the same (sample L+C(1:1)) the kinetic constants at both short and long adsorption times were intermediate to those of the both proteins. Then, this did not confirm the dominance of β-Casein at the O/W interface hypothesized by only observing the time dependent interfacial tension, γ(t), of β-Lactoglobulin/ β-Casein film layer in figure (4).

3.3. Dilational rheological properties of individual proteins

The dependence of the dilational rheological properties on the angular frequency of pure β-Casein and β-Lactoglobulin layers adsorbed at the interface with sunflower oil was determined as a function of protein bulk concentration above the saturation one (figure. (3)), as described in *2.2.2*, and the results were reported in figures (5) and (6) respectively.

From these figures it can be noticed that both proteins showed very low frequency dependence at each bulk concentration studied, as indicated by the invariance of the dilational complex modulus, E*, and of the loss tangent, tanδ, in the frequency range investigated.

The very low values of tanδ allow us to detect an almost purely elastic behavior of the protein interfaces where relaxation processes, often connected with molecular conformational changes, were rather limited in the time scale investigated [Williams and Prins (1996), Benjamins and Lucassen-Reynders (1998), Kopperud and Hansen (2001)].

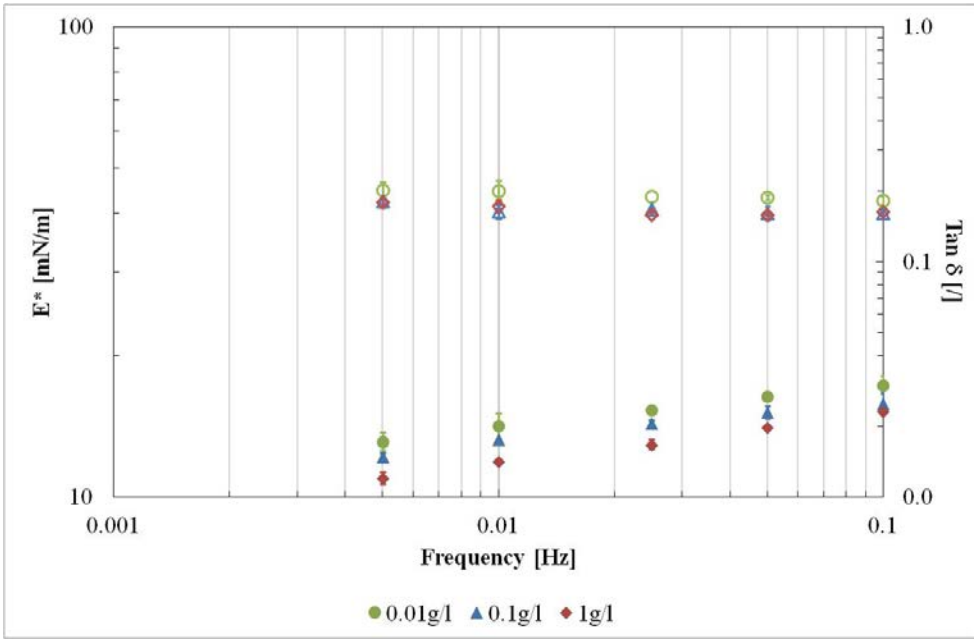


Figure 5. Interfacial dilational modulus, E* (full), and loss tangent, tanδ (empty s) as a function of drop oscillation frequency at different β-Casein concentration (g/l), 0.01 (red), 0,1 (blue), and 1 (red)

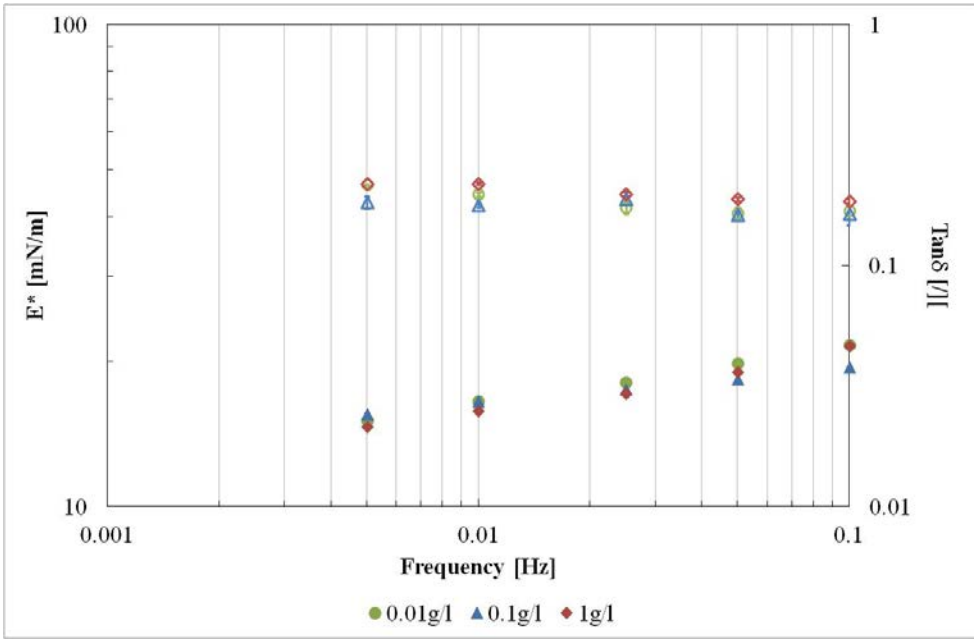


Figure 6. Interfacial dilational modulus, E* (full), and loss tangent, tanδ (empty) as a function of drop oscillation frequency at different β-Lactoglobulin concentration (g/l), 0.01 (red), 0,1 (blue), and 1 (red)

Values of E* for β-Casein slightly smaller than for β-lactoglobulin were registered at each concentration, according with the trend reported by many authors. [Murray (1998), Williams and Prins (1996), Benjamins and Lucassen-Reynders (1998)]. In fact, the complex interfacial dilational modulus of disordered and globular proteins, was known to

increase with decreasing protein flexibility due to the different intramolecular and intermolecular interactions.

Proteins which can adsorb quickly and/or rearrange quickly at the interfaces, whether owing to lower molecular weight (higher diffusion coefficient), or greater flexibility, are expected to give rise to lower dilational moduli due to the more rapid recovery in γ possible at short and long times [Murray (1998), Williams and Prins (1996), Benjamins and Lucassen-Reynders (1998)].

Concerning the dependence of the dilational rheological properties on the protein bulk concentration values, from the above figures it can be noticed that the behavior of both the proteins did not depend very much on the concentration in the range considered.

In particular, a slightly decrement of E* with increasing protein concentration was observed for β-Casein, whereas a substantial invariance of this parameter was obtained for β-Lactoglobulin. Similar values of E* and some concentration independence of dilational parameters were also reported by Benjamins and Lucassen-Reynders (1998) for the same proteins at the interface with sunflower oil.

At the same time, very different results were obtained by Williams and Prins (1996) studying the dilational behavior of β-Casein and β-Lactoglobulin at the paraffin oil/water interface with concentrations ranging from 10^{-4} to 1 g/l at only one frequency (1Hz). They indicated similar values of both the dilational modulus (around 25 mN/m) and the loss tangent for the two proteins at low bulk concentrations, and very different ones with increasing protein concentration. These differences can be attributed to the oil type (tryacylglicerols phase) used in the study, which is more polar than the hydrocarbons phase [Bos and van Vliet (2001)], and, as a consequence led the formation of a interfacial network with a smaller resistance to a change in its area than that obtained with paraffin oil.

3.4 Dilational properties of β-lactoglobulin and β-Casein mixtures

The dependence of the dilational rheological properties on the angular frequency of β-Casein and β-Lactoglobulin mixed layers adsorbed at the interface with sunflower oil was determined on a time scale of some seconds (0.005-0.1 Hz) as a function of the protein bulk mixing ratio, as described in *2.2.2*,, and the results were reported in figure (7).

The values obtained for the individual systems at the same total protein concentration of mixtures were also reported for comparison, in order to interpret data in terms of the rheological interfacial properties of the pure components and to evaluate their influence on the mixture behavior.

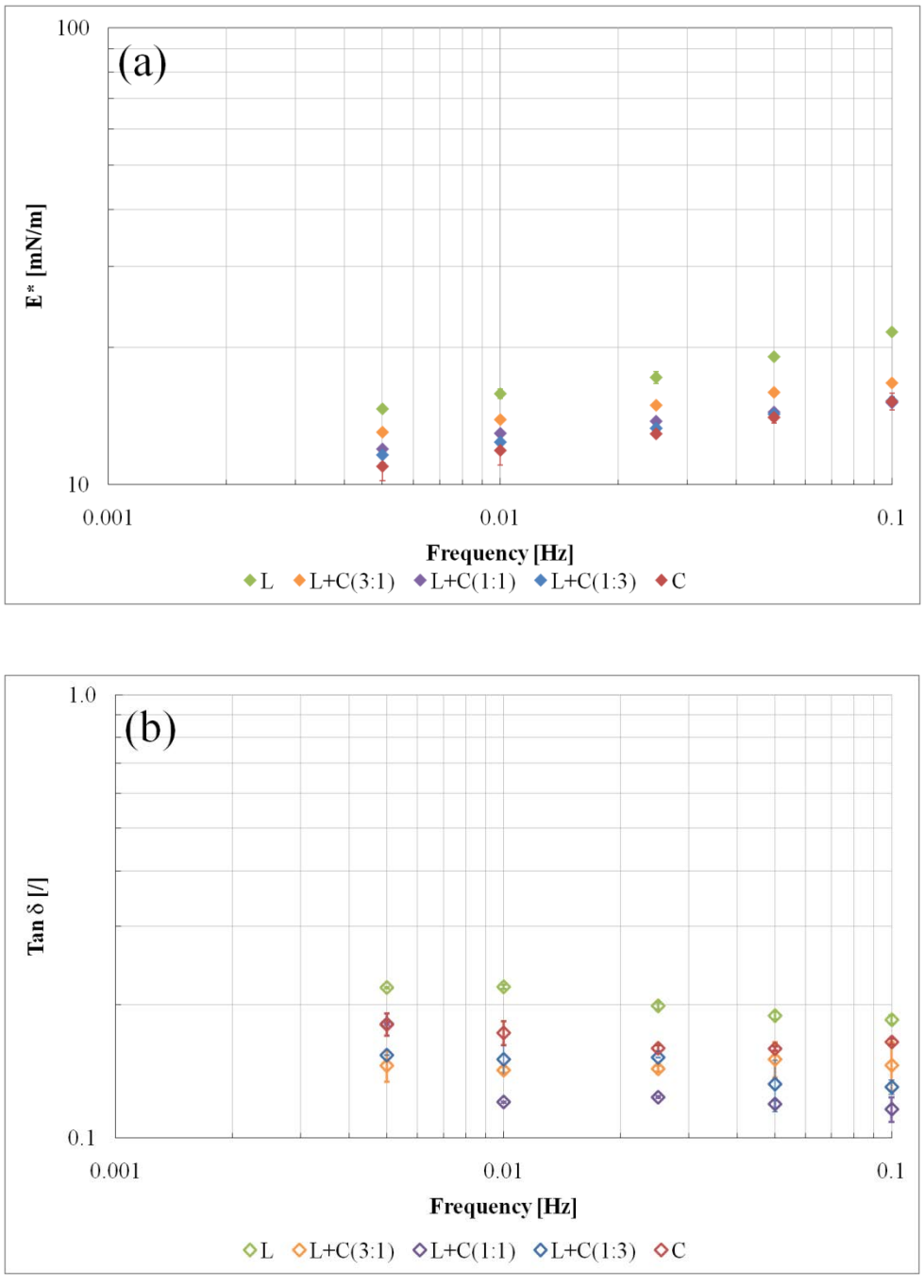


Figure 7. (a) Interfacial dilational modulus, E*, and (b) loss tangent , tanδ, measured values , as a function of drop oscillation frequency for β-Casein (C) and β-Lactoglobulin (L) and their mixture with different protein weight mixing ratio (total protein concentration of 1 g/l)

From the plots of the complex interfacial dilational modulus E* *vs* drop oscillation frequency ω, it can be seen that all data of the mixed protein systems fell between those of the individual components. Particularly L+C(1:3) and L+C(3:1) systems exhibited E* values more similar to the that of β-Casein (C) and β-Lactoglobulin (L) respectively, indicating the predominance at interface of the protein specie more present in the bulk solution, and the absence of competitive replacement phenomena at the O/W interface between the investigated proteins. Then the flexible casein molecules proved not to be able to displace the rigid globular protein molecules from the fluid interfaces [Dickinson (2010), Ridout et al. (2004), Brun and Dalgleish (1999].

When the bulk concentration is the same in the mixture between the proteins (L+C(1:1)) the system exhibited, in turn, intermediate values to those of the other mixed systems at the low frequencies considered, whereas values more or less identical to those of β-Casein were recorded at the higher frequencies. This indicates that when the time scale of compression-dilation cycle decreases, β-Casein more greatly influenced the interfacial behavior of the mixed layer adsorbed. This influence was much greater than expected from the bulk solution composition, especially at high drop oscillation frequency and it can be explained considering that the protein adsorbing more quickly will usually have a greater chance of forming a continuous network at the interface and dominating the surface rheological properties. On the contrary, the protein that adsorbs more slowly either fills in the available space or, if the bulk concentration is great enough, will continue to adsorb, and perhaps even disrupt or displace the first protein [Ridout et al. (2004)]. As a consequence β-Casein was found to dominate the rheological dilational and shear parameters, also at a relative low concentrations in the presence of other globular proteins [Ridout et al. (2004)].

Moreover, observing the figure (7b) it can be noticed that for the loss tangent values of the protein mixed systems proved to be lower than those of the pure protein systems at each investigated mixing ratio and in all the frequency range considered, indicating a substantially increase of the elasticity of the interface. This could be ascribed to a formation of a new interfacial network structure owing to the presence of both the proteins, with a higher resistance to compression and expansion of the drop area.

Anyway, it is important to point out that the pure and mixed protein layers adsorbed at the sunflower oil/water interface exhibited very small variations of the complex modulus E*, and the loss tangent in the frequency range investigated, showing a rheological behavior in a dimension 2D which typically characterize a strong 3D gel. In fact, the almost

independence of E* on the frequency combined with the low values of E", as indicated by the very small value of the loss tangent (tanδ <1), therefore means that the investigated adsorbed layers could present the rheological properties of a 2D critical gel [Bouriat et al. (2004), Dicharry et al. (2006), Kopperud and Hansen (2001)].

As it can be noticed in figure (7) the log−log plot of the complex interfacial dilational modulus *E* vs* the frequency ω, was a straight line and followed a scaling law of form:

$$E^* \approx \omega^n \tag{7}$$

The loss tangent tanδ remained practically little variant with pulsation, and it was also related to the slope *n* of the power law curve by the equation (8), as it can be seen in figure (8):

$$\tan(\delta) = \tan(n\frac{\pi}{2}) \tag{8}$$

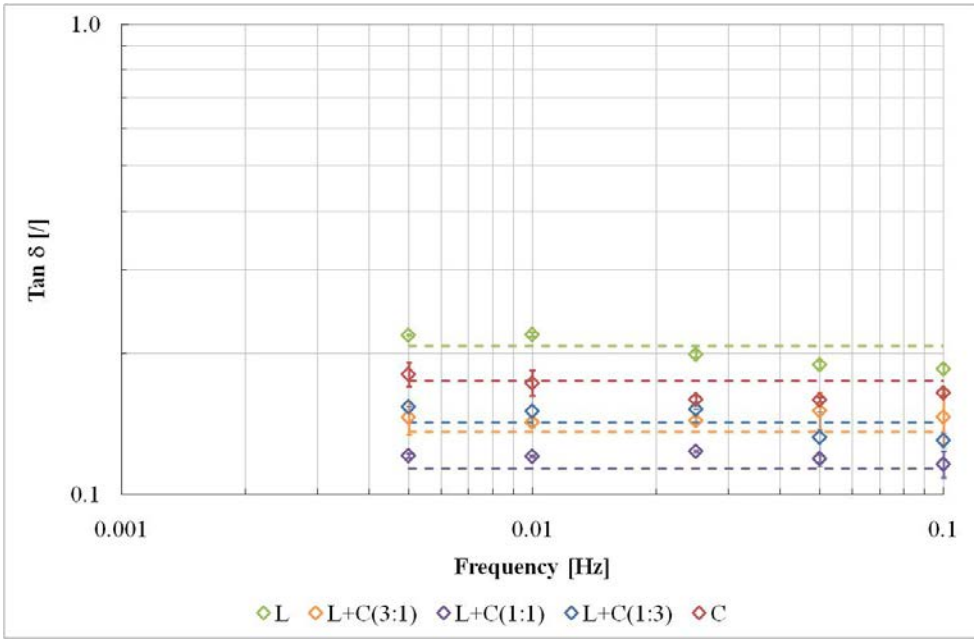


Figure 8. Loss tangent, tanδ, values calculated by equation (8) (dashed line) and measured (point) , as a function of drop oscillation frequency for β-Casein (C) and β-Lactoglobulin (L) and their mixture with different protein weight mixing ratio (total protein concentration of 1 g/l)

Equations (7) and (8) are relevant to the rheology of 3D-gel near its gelation point (critical gel), as demonstrated by Winter and Chambon for polymers [Winter and Chambon, 1986], and were considered still valid by Bouriat et al. (2004) and Dicharry et al. (2006) to

interpret 2D-rheology measurements assuming that the interface can be modeled as a parallel coupling of continuous Maxwellian blocks with relaxation times, τ, and elasticity:

$$k(\tau) = \alpha\tau^{-(n+1)} \tag{9}$$

where α, which can identified as the strength of the gel, is proportional to the number of aggregates at interface relaxing with the characteristic time τ.

According the equation (9) the complex elasticity modulus E* can then be calculated by equation (10), from which (7) and (8) can be deduced.

$$E^*(\omega) == \int_0^\infty k(\tau)\frac{i\omega\tau}{1+i\omega\tau}d\tau = \frac{\alpha\pi}{\sin(n\pi)}\omega^n e^{in\pi/2} \tag{10}$$

In agreement with Bouriat et al. (2004) and Dicharry et al. (2006), which considered interfacial protein films in a similar way as bulk systems, but restricted to two dimensions, in the present work gel critical model was used to investigate the nature of the interface and the protein interaction effects on the interfacial mechanical properties.

The *E* vs* ω curves of pure and mixed protein sample systems were fitted and interpreted with a power law equation (7). According to this equation the parameter *n* can be considered an indirect measure of structuring degree of interface, which proves to be as high as the *n* value is small, whereas the E* value extrapolated at the frequency of 1 Hz (*k* parameter), is a measure of the strength of the interfacial gel.

These model parameters estimated by using the equation (7) were reported in table (3) for the systems analyzed.

Sample ID	*k* [mN/m ·sn]	*n* [/]	R^2
C	*19.5 ± 0.3*	*0.11 ± 0.01*	*0.99*
L+C(1:3)	*18.7± 0.1*	*0.09 ± 0.01*	*0.99*
L+C(1:1)	*18.1 ± 0.5*	*0.07 ± 0.01*	*0.93*
L+C(3:1)	*20.3 ± 0.2*	*0.08 ± 0.01*	*0.99*
L	*28.6 ± 0.7*	*0.12 ± 0.01*	*0.98*

Table 3. Gel 2D rheological model parameters k and n obtained according to equation (7) for β-Casein (C) and β-Lactoglobulin (L) and their mixture with different protein weight mixing ratio (total protein concentration of 1 g/l)

From the data in table (3) it can be noticed that the pure and mixed protein layers adsorbed at the O/W interface exhibited different values of both the interfacial film strength and the structuring degree, indicating that the protein composition and the protein molecular structure strongly affected the mechanical properties of the interface. [Cornec et al. (1996), Maldonado-Valderrama and Rodrìguez Patino (2010), Krägel et al.(2003), Murray (1998), Rouimi et al. (2005), Wilde at al. (2004), Mackie and Wilde (2005)].

The protein molecular structure influenced much more the resistance of interface to area expansion and compression than to interfacial network structure as indicated by the bigger change of the *k* parameter than the *n* one with varying the protein type and the mixing ratio used in the solutions.

It can be seen that the interfacial strength was higher for the pure β-Lactoglobulin than the β-Casein, owing its characteristic rigid molecular structure [Ridout et al.(2004), Williams and Prins (1996)].

In fact, the globular protein is thought to form a strong cohesive network within the interface, which limits the amount of both diffusional and conformational relaxation, whereas β-Casein forms a weak network at the interface compared with that formed by the globular protein.

In the case of the mixed systems, a substantial increase of the structuring degree of interface than those of pure protein films was registered, as can be seen by comparing the *n* model parameter. In fact, these values proved to be smaller than those of β-Lactoglobulin and β-Casein at each protein mixture ratio investigated, indicating the formation of a new interfacial network which can be ascribed to the presence at the interface of both proteins in a way independent of the bulk protein amount in the mixture.

Considering the *k* parameter, it can be noticed that the mixed systems exhibited values of interfacial strength which were more similar to those of β-Casein than β-Lactoglobulin ones indicating a probable much greater influence of β-Casein protein on the dilational properties of β-Casein/β-Lactoglobulin adsorbed layers.

In order to investigate this result and derive some structural information of mixtures from those relative to pure components two models applied classically to the bulk rheology of non-interacting composites and known as the series and parallel models were applied [Ridout et al. (2004)]. A similar approach was used by Ridout et al. (2004) studying β-Casein and β-Lactoglobulin films adsorbed at the air/water interface in order to verify the possibility to predict their relative surface concentrations.

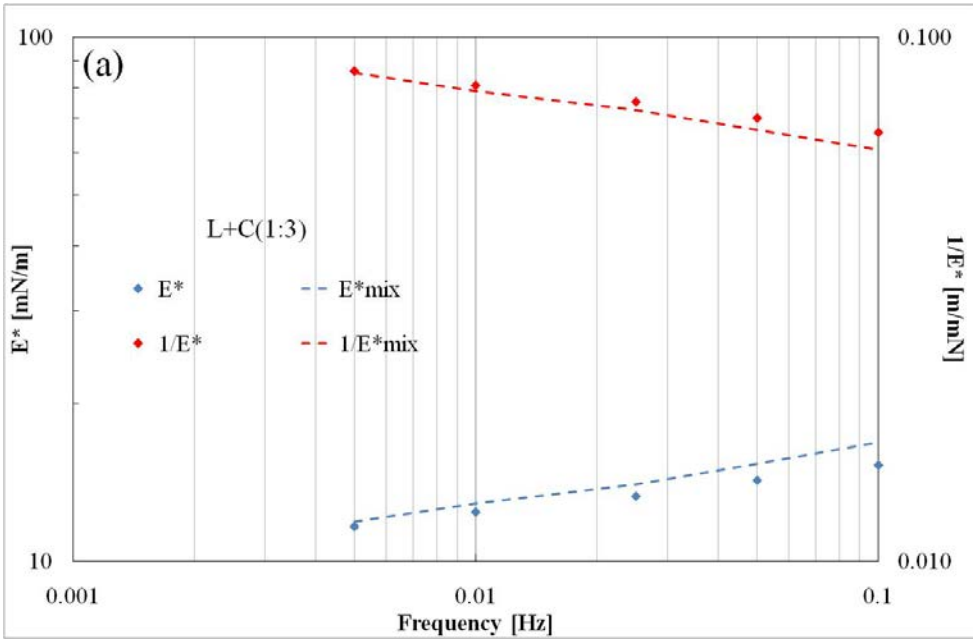
Considering the interfacial protein films in a similar way as bulk system, but restricted to two dimensions, these model were rewritten in term of dilational parameters representing two extremes of composite modulus, E^*_{mix} , for (11) equal stress distribution or (12) equal strain distribution by the composite, respectively:

$$E^*_{mix} = \varphi_1 E^*_1 + \varphi_2 E^*_2 \tag{11}$$

$$\frac{1}{E^*_{mix}} = \left(\frac{\varphi_1}{E^*_1}\right) + \left(\frac{\varphi_2}{E^*_2}\right) \tag{12}$$

Where E^*_{mix}, E^*_1 and E^*_2 are the moduli of the mixture and individual components, and φ_1 and φ_2 are the bulk volume fractions of the individual components in the mixture, respectively. Then, in the equation (11) the stronger components is assumed to make up the interfacial network and, therefore, dominates the mechanical behavior, whereas in the equation (12) is the weaker component. φ_1 and φ_2 were taken as the protein mixing weight fractions of β-Casein and β-Lactoglobulin respectively.

Predicted complex dilational moduli (dashed line) calculated by using the equations (11) and (12) were reported in figure (9) and compared with the measured values (point), in order to verify the validity of the fitting models.



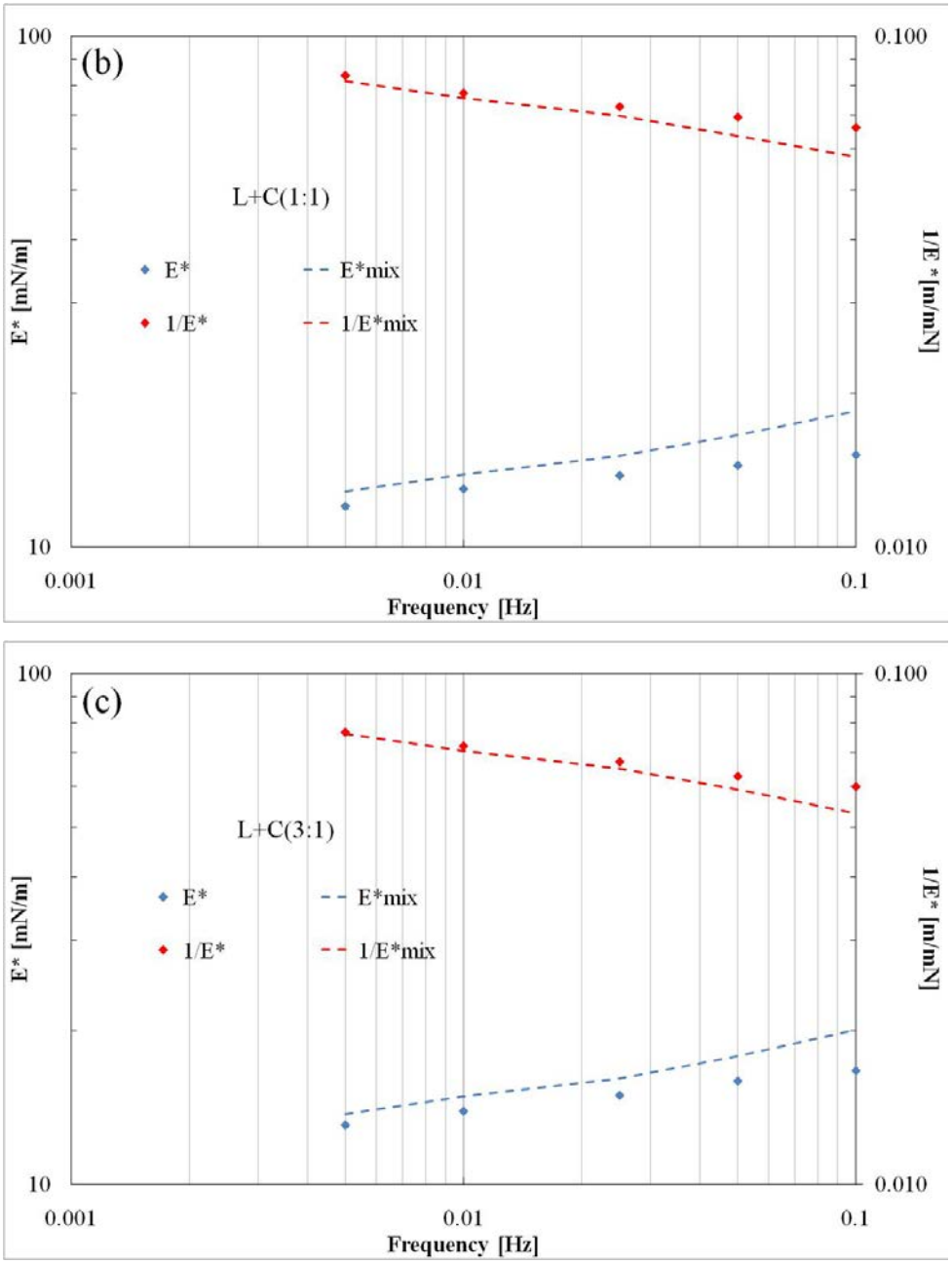


Figure 9. Comparison between the complex dilational modulus measured point) E*, and predicted E^*_{mix} (dashed line) by using the equations 11 (blue) and 12 (red) for the mixture systems analysed L+C(1:3) (a), L+C(1:1) (b), L+C(3:1) (c),

From the figure (9) it can be seen the equation (11) always predicted higher E* values than measured ones in the frequency range investigated, indicating the inability of this model to predict interfacial rheological behavior. On the contrary, the other model (eq.12) proved to fit the majority of the data more accurately for all mixture systems. A slight error between numerical and experimental results when increasing drop oscillation frequency were registered owing to probably too low time scale of compression-dilation cycle to have a response very sensitive to the molecular structure of the individual components.

In any case, the model represented by the equation (12) was in good agreement with experimental data in the frequency range of 0.005-0.025 Hz, and proved to be better than the other to predict rheological behavior of mixed protein adsorption at the oil/water interface.

It is important to underline that in the equation (12) the weaker component (β-Casein) was assumed to make up the interfacial network and to dominate the interfacial behavior, differently from equation (11) where the component dominant was the stronger β-Lactoglobulin. Then the greater validity of the former model than the latter, confirmed that the interfacial dilational moduli of mixed protein solutions appeared to be more dominated by β-Casein.

4.Conclusions

The adsorption of milk proteins β-Casein and β-Lactoglobulin, both alone and in binary mixtures to the sunflower oil/water interface was studied by dynamic interfacial tension measurements and harmonic drop oscillation experiments, to investigate the interfacial mechanisms and properties underlying the emulsion functionality of milk protein mixtures.

The interfacial tensions of the mixed protein systems were found intermediate between the values of the individual components in all adsorption time considered. The influence of the mixing ratio, studied from a kinetic point of view, led to observe that the co-adsorption process from the investigated mixtures followed a kinetically controlled non-competitive mechanism, where the protein which was present with higher level in the bulk solution was able to more influence the interfacial activity and the velocity in reducing interfacial tension.

Pure and mixed protein layers adsorbed at the sunflower oil/water interface exhibited very small variations of the complex modulus E* and the loss tangent in the frequency range investigated, showing a rheological behavior in a dimension 2D which typically characterize a strong 3D gel. The influence of the β-Casein protein on the interfacial rheological properties of the protein mixtures was discovered much greater than those of that of β-Lactoglobulin.

Finally, two models usually applied to the bulk rheology of non-interacting composites, were used to predict the interfacial rheological behavior from those relative to pure components. Only one of them was found useful, allowing us to recognize the β-casein protein able to dominate the interfacial network and to strongly influence the mechanical response of the mixed adsorbed layers at the O/W interface .

References

1) Bos M.V. and van Vliet T. (2001), Interfacial rheological properties of adsorbed protein layers and surfactants: a review, *Advances in Colloid and Interface Science* (91), 437-471
2) Murray S.B., (1998) Interfacial rheology of mixed food protein and surfactant adsorption layers with respect to emulsion and foam stability, in M*ö*bius D. and Miller R., Studies in interface science 7, Proteins at Liquid Interfaces, Elsevier editor, Amsterdam (The Netherlands), chapter 5
3) Dickinson E. (1998), Proteins at interfaces and in emulsions. Stability, rheology and interactions. *Journal of the Chemical Society, Faraday Transactions* (94), 1657-1669
4) Rouimi S., Schrorsch C., Valentini C., Vaslin S., (2005) Foam stability and interfacial properties of milk protein-surfactant systems. *Food Hydrocolloids* (19), 467-478
5) Dickinson E. (1999) (a), Caseins in emulsions: interfacial properties and interactions. *International Dairy Journal* (9), 305-312
6) Dickinson E. (2001), Milk protein interfacial layers and the relationship to emulsion stability and rheology Review. *Colloids and Surfaces B: Biointerfaces* (20), 197-210
7) Raikos V. (2010), Effect of heat treatment on milk protein functionality at emulsion interfaces. A review. *Food Hydrocolloids* (24), 259-265
8) Williams A. and Prins A. (1996), Comparison of the dilational behavior of adsorbed milk proteins at the air-water and oil-water interfaces, *Colloids and Surfaces A: Physicochemical and Engineering Aspects* (114), 267-275
9) Cornec M., Mackie A.R., Wilde P.J., Clark D.C. (1996), Competitive adsorption of β-lactoglobulin and β-casein with Span80 at the oil-water interface and the effect on emulsion behavior. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* (114), 237-244
10) Jensen R.G., Handbook of Milk Composition, Academic Press, New York (U.S.A) 1995, chapter 5
11) Ridout M.J., Mackie A.R., Wilde P.J. (2004) Rheology of Mixed *β-casein/β-lactoglobulin* Films at the Air-Water Interface, Journal of Agricultural and food chemistry (52), 3930-3937

12) W*ü*stneck R., Kragel J., Miller R., Wilde P., Clark D.C. (1996), The adsorption of surface-active molecules between β-casein, β-lactoglobulin and ionic surfactants and their rheological shear behavior. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* (114), 255-265

13) Dickinson E., Matsumara Y. (1994), Review Proteins at liquid interfaces: role of the molten globule state. *Colloids and Surfaces B: Biointerfaces* (3), 1-17

14) Freer E. M., Yim K. S., Fuller G.,. Radke C. J. (2004), Interfacial Rheology of Globular and Flexible Proteins at the Hexadecane/Water Interface: Comparison of Shear and Dilatation Deformation. *Journal of Physical Chemistry B* (108), 3835-3844

15) Mackie A, Wilde P. (2005), The role of interactions in defining the structure of mixed protein–surfactant interfaces, *Advances in Colloid and Interface Science* (117), 3-13

16) Maldonado-Valderrama J. and Rodrìguez Patino J.M. (2010) Interfacial rheology of protein–surfactant mixtures, *Current Opinion in Colloid & Interface Science* (15), 271–282

17) Krägel J., O'Neill M., Makievski A.V., Michel M., Leser M.E., Miller R., (2003) Dynamics of mixed protein-surfactant layers adsorbed at the water/air and water/oil interface, *Colloids and Surfaces B: Biointerfaces* (31), 107-114

18) Wilde P., Mackie A., Husband F., Gunning P., Morris V.(2004), Proteins and emulsifiers at liquid interfaces, *Advances in Colloid and Interface Science* (108 – 109), 63-71

19) Arai T., Norde W. (1990) (a), The behavior of some model proteins at solid-liquid interfaces 2. Sequential and competitive adsorption. *Colloids and Surfaces* (51), 1-16

20) Arai T., Norde W. (1990) (b), The behavior of some model proteins at solid-liquid interfaces 2. Sequential and competitive adsorption. *Colloids and Surfaces* (51), 1-16

21) Dickinson E. (1999) (b), Review Adsorbed protein layers at fluid interfaces: interactions, structure and surface rheology. *Colloids and Surfaces B: Biointerfaces* (15), 161-176

22) Gabriele D., Migliori M., Di Sanzo R., Oliviero Rossi C., Ruffolo S. A., de Cindio B. (2009), Characterisation of dairy emulsions by NMR and rheological techniques, Food *Hydrocolloids* (23), 619-628

23) Camino N.A., Pèrez O.E., Carrera Sanchez C., Rodriguez Patino J.M., Pilosof A.M.R. (2009). Hydroxypropylmethylcellulose surface activity at equilibrium and adsorption dynamics at the air-water and oil-water interfaces. *Food Hydrocolloids* (23) 2359-2368

24) Biresaw G., Liu Z. S., Erhan S.Z. (2008), Investigation of the Surface Properties of Polymeric Soaps Obtained by Ring-Opening Polymerization of Epoxidized Soybean Oil, Wiley InterScience (www.interscience.wiley.com)

25) W*ü*stneck R., Moser B., Muschiolik G. (1999), Interfacial dilational behavior of adsorbed b-lactoglobulin layers at the different fluid interfaces. *Colloids and Surfaces B: Biointerfaces* (15), 263-273

26) Lin S.Y., Wang W.J., Lin L.W., Chen L.J. (1996) Systematic effects of bubble volume on the surface tension measured by pendant bubble profiles *Colloids and Surfaces A: Physicochemical and Engineering Aspects* (114), 31-39

27) Pèrez O.E., Carrera Sànchez C., Pilosof A.M.R., Rodrìguez Patino J.M. (2009) Kinetics of adsorption of whey proteins and hydroxypropyl-methyl-cellulose mixtures at the air-water. *Journal of Colloid and Interface Science* (336), 485-496

28) Pèrez A.A., Carrera Sànchez C., Rodrìguez Patino J.M., Rubiolo A.C., Santiago L.G. (2010).Milk whey proteins and xanthan gum interactions in solution and at the air-water interface: A rheokinetic study. *Colloids and Surface B*: Biointerfaces (81) 50-57

29) Graham D.E. and Phillips M.C.(1979) Proteins at liquid interface III Molecular structures of adsorbed films. *Journal of Colloid and Interface Science* (70), 427-439

30) Lucassen J. and van den Tempel M. (1972), Dynamic measurements of dilational properties of a liquid interface, *Chemical Engineering Science* (27) 1283-1291

31) Dicharry C., Arla D., Sinquin A., Graciaa A., Bouriat P. (2006) Stability of water/crude oil emulsions based on interfacial dilatational rheology *Journal of Colloid and Interface Science* (297) 785–791

32) Bouriat, P., El Kerri N., Gracia A., Lachaise J. (2004) Properties of a Two-Dimensional Asphaltene Network at the Water-Cyclohexane Interface Deduced from Dynamic Tensiometry *Langmuir* (20) 7459-7464

33) Winter H.H. and Chambon F.(1986) Analysis of linear viscoelasticity of a cross-linking polymer at the gel point *Journal of Rheology* (30) 367-382

34) Benjamins J., Cagna A. Lucassen-Reynders E.H.(1996), Viscoelastic properties of triacyglycerol/water interfaces covered by protein, *Colloids and Surfaces A: Physicochemical and Engineering Aspects* (114), 245-254

35) Miller R., Fainerman V.B., Makievski A.V., Kr*ä*gel J., Grigoriev D.O., Kazakov V.N., Sinyachenko O.V., (2000) Dynamics of protein and mixed protein/surfactant adsorption layers at the water fluid interface *Advances in Colloid and Interface Science* (86), 39-82

36) Beverung, C. J., Randke, C. J., & Blanch, H. W. (1999) Protein adsorption at the oil water interface: characterization of adsorption kinetics by dynamic interfacial tension measurements. *Biophysical Chemistry* (81), 59–80

37) Graham D.E. and Phillips M.C.(1979) Proteins at liquid interface III Molecular structures of adsorbed films. *Journal of Colloid and Interface Science* (70), 427-439

38) Benjamins J., Lucassen-Reynders E.H., (1998) Surface dilational rheology of proteins adsorbed at air/water and oil/water interfaces in M*ö*bius D. and Miller R, Studies in interface science 7, Proteins at Liquid Interfaces, Elsevier editor, Amsterdam (The Netherlands) 1998, chapter 5

39) Dickinson E., (2010) Mixed biopolymers at interfaces: Competitive adsorption and multilayer structures *Food Hydrocolloids* (xxx), 1-18 article in press

40) Brun J.M., Dalgleish D.G. (1999). Some effects of heat on the competitive adsorption of caseins and whey proteins in oil-in-water emulsions, *International Dairy Journal* (9). 323-327

41) Kopperud H. B. M. and Hansen F.K. (2001), Surface Tension and Surface Dilational Elasticity of Associating Hydrophobically Modified Polyacrylamides in Aqueous Solutions. *Macromolecules* (34), 5635-5643.

Chapter 5
Interfacial behavior of milk protein mixtures in the presence of Tween 60 and Admul Datem at the oil-water interface

Abstract

The effect of the nonionic Tween 60 and anionic Admul Datem water-soluble emulsifiers on the interfacial properties of the mixture β-Casein-β-Lactoglobulin (1:1 w/w) at the sunflower oil-water was investigated by varying the emulsifier/protein weight ratio used in the mixture.

Here we aim to obtain systematic information on the interfacial mechanisms and properties underlying the emulsion functionality of milk protein mixtures, which are typically used with small-molecule surfactants in the food dairy systems.

Transient interfacial tension measurements and dilational dynamic tests were performed out by using a "pendant drop" tensiometer. Axisymmetric drop shape analysis (ADSA) was used to calculate drop volume, area and interfacial tension. Differences between milk proteins-Tween 60/Admul Datem were individuated probably because of dissimilar competitive phenomena among protein and tested emulsifiers, which result in a different extent of protein replacement from the interface by emulsifiers or by surfactant/protein complexes.

The ionic emulsifier Admul Datem proved to be affect the protein adsorption and interfacial structure layer more than the nonionic emulsifier Tween 60.

The dependence of the dilational parameters on the drop oscillation frequency was determined and interfacial dilatational moduli evidenced the potential formation of a 2D critical gel. They were analyzed assuming the validity at the interface of rheological model used for the 3D critical gel.

1. Introduction

Proteins in general, and milk proteins in particular, are widely valued as the main functional ingredients for the formation and stabilization of food emulsions, but they are in many applications used with low molecular weight surfactants to this purpose [Bos and van Vliet (2001)]. Both of them are characterized by surface activity and, therefore, potential competitive adsorption phenomena at interface can establish. As a consequence, the macroscopic properties of the final emulsion are strongly affected by the characteristics of the obtained interfacial layers.

In this regard, fundamental knowledge of the adsorbed layer behavior has been derived from experiments involving one or more proteins and one or more surfactants at the oil/water interface [Cornec at al. (1996), Dickinson (1999), Bos and van Vliet (2001), Dickinson (1998), Murray (1998), Rouimi et al. (2005)]. When small-molecule surfactant are present in protein-containing systems, the adsorption of the protein is affected by the binding of surfactant to both the protein and the fluid interface [Dickinson (1999)]. Milk proteins saturate fluid interfaces at much lower bulk concentrations than do small-molecule emulsifiers. But a converse situation occurs at high bulk concentrations, where the small-molecule surfactant gives a lower tension and a more densely packed layer. In addition to the preferential binding of surfactant to the interface at high bulk concentrations, surfactant binding to hydrophobic sites on the protein may also reduce its surface affinity. Therefore the protein can be removed from the interface as a consequence of two distinct mechanisms [Bos and van Vliet (2001), Dickinson (1999)]: (i) the solubilization mechanism (desorption of protein arises as a result of solubilization into the aqueous phase in the form of a protein–surfactant complex); (ii) the replacement mechanism (displacement of protein arises because surfactant lowers the interfacial free energy more effectively than does protein or protein–surfactant complex). Ionic surfactants bind strongly to proteins, and so competitive adsorption involving charged amphiphiles can be regarded as proceeding mainly by the solubilization mechanism. With more weakly interacting non-ionic surfactants, however, the replacement mechanism can be regarded as predominant.

This work focuses on the interfacial properties of mixed milk proteins (β-Lactoglobulin and β-Casein) at the sunflower oil-water (O/W) interface in the presence of two food and water soluble emulsifiers, Tween60 (*Polyoxyethylene Sorbitan Esters of Monoglycerides*) and Admul Datem (*Diacetyl Tartaric Acid Ester of Mono-Diglycerides*), which are very

different for their headgroup nature and ability to dissolve in the water phase (HLB number 14.9 and 8 respectively) [Bos and van Vliet (2001)].

The sunflower oil/water interface with adsorbed β-Casein and β-Lactoglobulin proteins, was selected as "model protein interface" for dairy emulsions because they are one of the major caseins and the most abundant whey protein present in the milk respectively, and milk and sunflower oil in turn are two ingredients often used for the preparation of food dairy emulsions [Raikos et al.(2010), Gabriele et al. (2009)]. The emulsifiers Tween 60 and Admul Datem were chosen owing to wide use in food dairy emulsions and owing to their different nature.

Despite the great complexity of milk, its surface proved to be dominated by free β-Casein and β-Lactoglobulin [Dickinson (1999) (b), W*ü*stneck et al. (1996)], and as a consequence, a quantitative investigation of the interfacial rheology of systems composed of these proteins in the presence of some emulsifiers could be a very useful tool to control and predict the functionality of milk product.

Then, the main goal of this research is to investigate the effect of protein-emulsifier interactions on the rheological properties of interface by varying the surfactant type competing with protein and the emulsifier/protein ratio in the mixture as to define the surfactant pairs which guarantees the best interfacial properties.

2. Materials and methods

2.1 Sample preparation

Sample solutions were prepared by dissolving the proteins derived from bovine milk β-Casein (C6905, Sigma Aldrich, Lot 029K7430) and β-Lactoglobulin (L3908, Sigma Aldrich, Lot 097K7012) and the emulsifiers, Tween 60 (*polyoxyethylene sorbitan esters of monoglycerides*) (P1629-1Ga, Sigma Aldrich) and Admul Datem (*diacetyl tartaric acid ester of mono- and di-glycerides*) (1915, 5Z10712, Kerry Company) in twice-distilled water in imidazole buffer 75 mM at pH=6.8 ± 0.2 [Williams and Prins (1996)]. The pH was chosen to correspond with the conditions in milk [Jensen (1995)].

The twice distilled water used throughout all the experiments was obtained from a Milli-Q purification system (Millipore, USA), and it was checked for contaminants before each experiment, measuring the surface tension of the buffer solution at the air/water boundary at ambient temperature. No aqueous solutions with a surface tension other than accepted in the literature (72-73 mN/m at 20°C) were used.

In the samples the total amount of proteins and the weight ratio between β–Lactoglobulin (L):β–Casein (C) proteins were kept constant and corresponding to the final concentration of 1 g/l and to the 1:1 respectively, whereas the weight ratio emulsifier/protein was changed in the same way for both emulsifier types, to investigate the effect of this ratio and of the surfactant type, on the rheological properties of protein layers.

The bulk emulsifier concentrations used in the samples were chosen to correspond with higher values than that of CMC for both Tween 60 (TW) and Admul Datem (DA) and, then, two emulsifier/protein weight ratios were analyzed, 0.3 and 0.6, producing 4 samples indicated by according the table (1).

In order to make comparisons between the protein-emulsifier mixtures and the individual molecules, samples containing single type of surfactants, β–Lactoglobulin and β–Casein proteins (indicated with L+C(1:1)), and Tween60 and Admul Datem (indicated with TW and DA, respectively) were also prepared (table (1)).

Sample ID	*(%wt) protein*	*(% wt) emulsifier*	
	β-Lactoglobin+ β-Casein	*Tween 60*	*Admul Datem*
L+C(1:1)	0.1	0	0
L+C(1:1)+TW (0.3)	0.1	0.03	0
L+C(1:1)+TW (0.6)	0.1	0.06	0
L+C(1:1)+DA (0.3)	0.1	0	0.03
L+C(1:1)+DA (0.6)	0.1	0	0.06
TW	0	0.06	0
DA	0	0	0.06

Table 1. Pure and mixed samples containing milk proteins (1 g/l) and Tween60 /Admul Datem emulsifiers

Milk proteins and emulsifiers were dispersed separately in the buffer solutions at ambient temperature (20-23°C), and at 70°C respectively, stirring for 1 hour by using heated magnetic device (ARE, Velp scientific, Italy). Afterwards the protein and emulsifier aqueous solutions were mixed at room temperature and were stirred for a further 30 minutes before the interfacial measurements were performed.

All solutions were freshly prepared (within no more than 24 h) for the characterization and two replicates were prepared for each sample.

Sunflower oil (Carlo Erba Reagents-356241) without further purification was used as the oil phase in this research. It contains triglycerides and free saturated and unsaturated fatty acid (0.4%) which are responsible of the interfacial tension reduction of 10 mN/m measured between the pure phases oil and buffer solution without the addition of surfactants in accordance with Wüstneck et al.(1996) and Camino et al. (2009)].

2.2 Dynamic interfacial properties measurements

Pure and mixed β-Casein and β-Lactoglobulin layers adsorbed at the O/W interface were characterized by dynamic interfacial tension measurements and harmonic drop oscillation experiments on a time scale of some seconds.

Axialsymmetric drop shape analysis (ADSA) was used to calculate drop volume, area and interfacial tension by using an automated pendant drop tensiometer (FTA200 First Ten Angstroms, USA) equipped with the *fta32 v2.0* software. Details of this apparatus are given by Biresaw et al. (2008).

The instrument comprises an automated pump that can be fitted with various sizes of syringes and needles to allow for control of pendant drop formation and of sinusoidal variations in the drop volume or surface area by software.

An automated image viewing and capturing system, with various image capture triggering options, was used to capture the drop image.

The computer hardware and software also provide the capability of data capture, storage, analysis and transfer. The software allows for an automated drop shape analysis of the captured drop image, and for measuring the surface-interfacial tension of the drop formed in air or in a second fluid at rest and in periodic motion conditions of the interface respectively.

Image acquisition and regression of the interfacial tension were performed directly with commercially available drop-image software by fitting the Bashforth-Adams equation to the drop shape [Biresaw et al. (2008)]. Drop-image software also controlled an automatic pipetting system that maintained constant drop volume with time period over which dynamic tensions were measured.

2.2.1 Dynamic interfacial tension

The method adopted to measure the interfacial tension of the investigated solutions with time (γ *vs* t), by using an automatic drop tensiometer, involved the analysis of the profile of the drops created in the air/oil phases respectively, and kept at rest (constant volume).

The experiments were carried out at room temperature (22°C within ± 1 °C), placing sample aqueous solutions in a 100 µl glass Hamilton syringe equipped with a 20 Gauge stainless steel needle, and delivering drops in a rectangular quartz cuvette (5ml) containing the oil phase.

Drop volumes of 9-12 µl were used in each experiment, in order to measure interfacial tension values independent of the drop size [Lin et all, 1996]. High drop volumes of the kind chosen, proved to be generally more suitable for these tests, because they increased the number of profile points, which can be used for drop shape analysis [Lin et all, 1996].

The experiments started with the creation of the drop; then, drop images were continuously taken from a CCD camera and digitalized, registering the surface tension values over the test time.

Drop profile was monitored up to maximum time of 180 minutes, which assures the molecules adsorption at interface and the reaching of a quasi-equilibrium interfacial tension value.

Equilibrium of γ was assumed when the interfacial tension did not change by more than 0.4 mN/m in 30 minutes [Camino et al. (2009)].

All experiments were repeated at least twice and the experimental data are reported as mean value ± standard deviation.

2.2.2 Interfacial dilational properties and data analysis

The method adopted to determine the dilational rheological properties of the interface involved the profile analysis of the drop formed in the oil phase subjected to periodic motion conditions, according to the oscillating drop methodology, able to measure the interfacial visco-elasticity versus frequency.

Oscillating drop experiments are usually performed subjecting the interface to an infinitesimal sinusoidal compression and expansion, then by applying a frequency sweep to the surface area A, in order to measure the interfacial dilational modulus.

The surface dilational modulus derived from the change in surface tension γ (dilational stress) (eq. 1), resulting from a small change in surface area A (dilational strain) (eq.2), may be described by equations [Lucassen and van den Tempel, 1972]:

$$\gamma = \gamma_0 + \Delta\gamma \sin(\omega t + \delta) \quad (1)$$

$$A = A_0 + \Delta A \sin(\omega t) \quad (2)$$

$$E^* = \frac{d\gamma}{d \ln A} \quad (3)$$

Where γ_0 and A_0 are the equilibrium reference surface tension and the unperturbed interfacial area of the drop respectively, Δγ and ΔA are the stress and strain amplitude respectively, and δ is the phase angle between stress and strain, measure of the relative film viscoelasticity.

Since the drop area periodically oscillates, the dilational modulus exhibits two contributions: an elastic part accounting for the recoverable energy stored in the interface

(storage modulus, *E'*) and the dissipative part accounting for energy lost through relaxation processes (loss modulus, *E''*).

$$E^* = E' + iE'' = \frac{\Delta\gamma}{\Delta A/A_0}\cos(\delta) + i\frac{\Delta\gamma}{\Delta A/A_0}\sin(\delta) \qquad (4)$$

Then the surface dilatational modulus, E*, as a measure of the total material resistance to dilatational deformation (elastic and viscous), is a complex quantity composed of real and imaginary part [Freer et al. (2003), Ravera et al. (2009), Myrvold and Hansen (1998)].
For a perfectly elastic material stress and strain are in phase $\delta = 0$ and the imaginary term is zero. In the case of perfectly viscous material $\delta = 90°$ and the real part is zero. The loss angle tangent can be defined by equation (5):

$$\tan\delta = E''/E' \qquad (5)$$

In this work, we applied a periodic strain by differentially oscillating the drop area at a prefixed frequency value, and we measured the periodic stress response with time.
Then, the dilational viscoelastic parameters of interface, the dilational complex modulus (E*), its elastic (E') and viscous (E'') components and the loss angle tangent were measured as a function of the adsorption time *t*.
The *Time Sweep Tests* were carried out by using deformation amplitude ($\Delta A/A_0$) values of 4-5% and angular frequency ones varying in the 0.005 Hz-0.1 Hz range.
The percentage area change was determined before each time sweep test by performing *Amplitude Sweep Experiments* (data not shown) realized at the extreme frequency values of the investigated range. The latter experiments were useful to assure that system response was not influenced by perturbation amplitude (linear viscoelastic behavior) and to be in the linear region, so as to avoid oscillation amplitude that causes disruption of the supramolecular organization or provides adequate measurement sensitivity.
The duration of each test was established so as to register equilibrium values of the dilational moduli with time (maximum variation of 3% was accepted).
The E* *vs* ω curves were then obtained by using the latter values in the frequency range investigated.

3. Results and discussion

3.1. Interfacial tension

In order to understand the transient behavior of the mixed β-Lactoglobulin and β-Casein layers in the presence of the Tween 60 and Admul Datem emulsifiers and to study the effect of their interactions on the adsorption phenomena at the O/W interface, time dependence interfacial tensions were shown (figures (1) and (2)) and discussed below.

For comparison the results obtained for pure emulsifier (TW, DA) and protein (L+C(1:1)) systems were also reported.

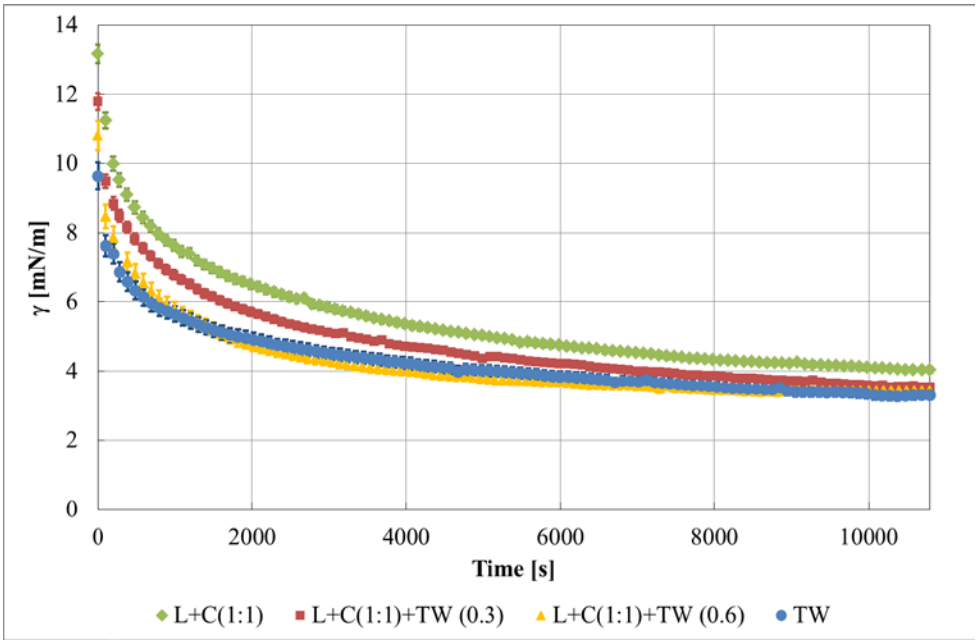


Figure 1. Time dependent interfacial tension, γ(t), of the pure and mixed systems containing milk proteins, β-Lactoglobulin and β-Casein (1 g/l), and Tween 60 emulsifier (imidazole buffer, pH 6.8) at the O/W interface as a function of emulsifier/protein weight ratio

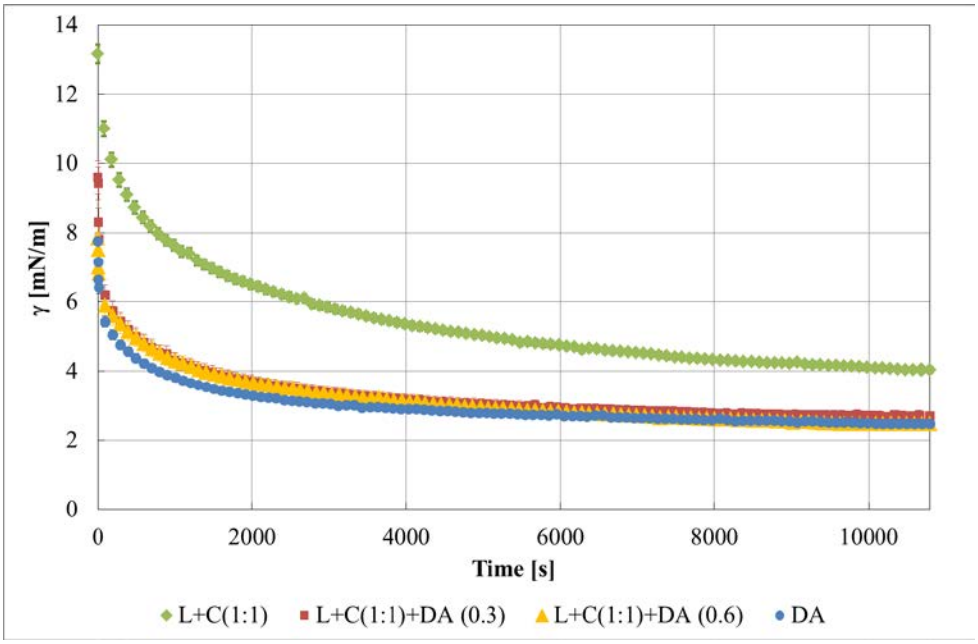


Figure 2. Time dependent interfacial tension, γ(t), of the pure and mixed systems containing milk proteins, β-Lactoglobulin and β-Casein (1 g/l), and Admul Datem emulsifier (imidazole buffer, pH 6.8) at the O/W interface as a function of emulsifier/protein weight ratio

It is possible to notice that both mixed systems investigated exhibited both more surfactant-like adsorption kinetics, and a bigger equilibrium interfacial tension values, with increasing the emulsifier amount in the mixture.

Actually, the interfacial tensions for the L+C(1:1)+TW and L+C(1:1)+DA systems especially at long adsorption times tended more to the values characteristic of TW and DA samples, respectively, rather thse of L+C(1:1) at each emulsifier/protein ratio studied.

Then, the milk proteins appeared to play little part in the interfacial properties when small-molecule surfactant are present in protein-containing systems indicating that the adsorption of the proteins is affected by the binding of surfactant to both the protein and the fluid interface [Dickinson (1999)]

This behavior could be interpreted as a result of the considerable extent of protein replacement from the interface by emulsifiers or by surfactant/protein complexes, owing to their higher surface activity and affinity for the interface, in agreement with the findings of many authors [Miller et al. (2000), Bos and van Vliet (2001), Wilde (2000), Mackie and Wilde (2005), Rodrìguez Patino et al. (2003), Wilde et al. (2004), Mackie et al. (1999), Petkov et al.(2000), Krägel et al. (2003)].

It is interesting to note that DA and L+C(1:1)+DA systems proved to be more efficient to reduce the interfacial tension with time than TW and L+C(1:1)+TW ones respectively.

The best performance of the Admul Datem emulsifier at the O/W interface may be explained, probably, by its higher hydrophobicity from that of Tween 60, which allowed it to anchor strongly between the glycerides of the oil phase (Camino et al. (2009)).

Also dissimilar protein-emulsifier interactions can be noticed at the O/W interface between nonionic Tween 60 and ionic Admul Datem surfactants with β-Lactoglobulin and β-Casein proteins, from a comparison of the adsorption behavior of the mixed systems. In fact, whereas the γ values of L+C(1:1)+DA systems proved to be similar to those of DA with time at each emulsifier/protein ratio investigated, those of L+C(1:1)+TW systems became more similar to those of TW only with increasing the emulsifier concentration in the mixture and, especially, at long adsorption times. These differences in the dynamic of adsorption could be representative of dissimilar protein removal mechanism from the interface realized by the emulsifiers, and precisely of replacement and solubilisation mechanisms for nonionic Tween 60 and ionic Admul Datem surfactants, respectively.

For mixed protein/nonionic emulsifier systems competitive replacement adsorption phenomena are often evident only at sufficiently high surfactant concentration, and in this case, the dynamic curve (γ(t)) is completely controlled by small molecule surfactants; on the contrary, for mixed protein/ionic emulsifier, generally, both protein-surfactant complexes and surfactants dominate the interface in dependence on the relative surface activity [Wilde (2004), Mackie and Wilde (2005), Krägel et al. (2003)].

3.2 Dilational rheology

The dependence of the dilational rheological properties on the angular frequency for the systems investigated was determined in the 0.005-0.1 Hz frequency range (as described in *2.2.2*), and the results were reported in figure (3) and (4) in terms of complex dilational modulus, E*(**a**), and loss tangent, tanδ, (**b**) as a function of drop oscillation frequency .

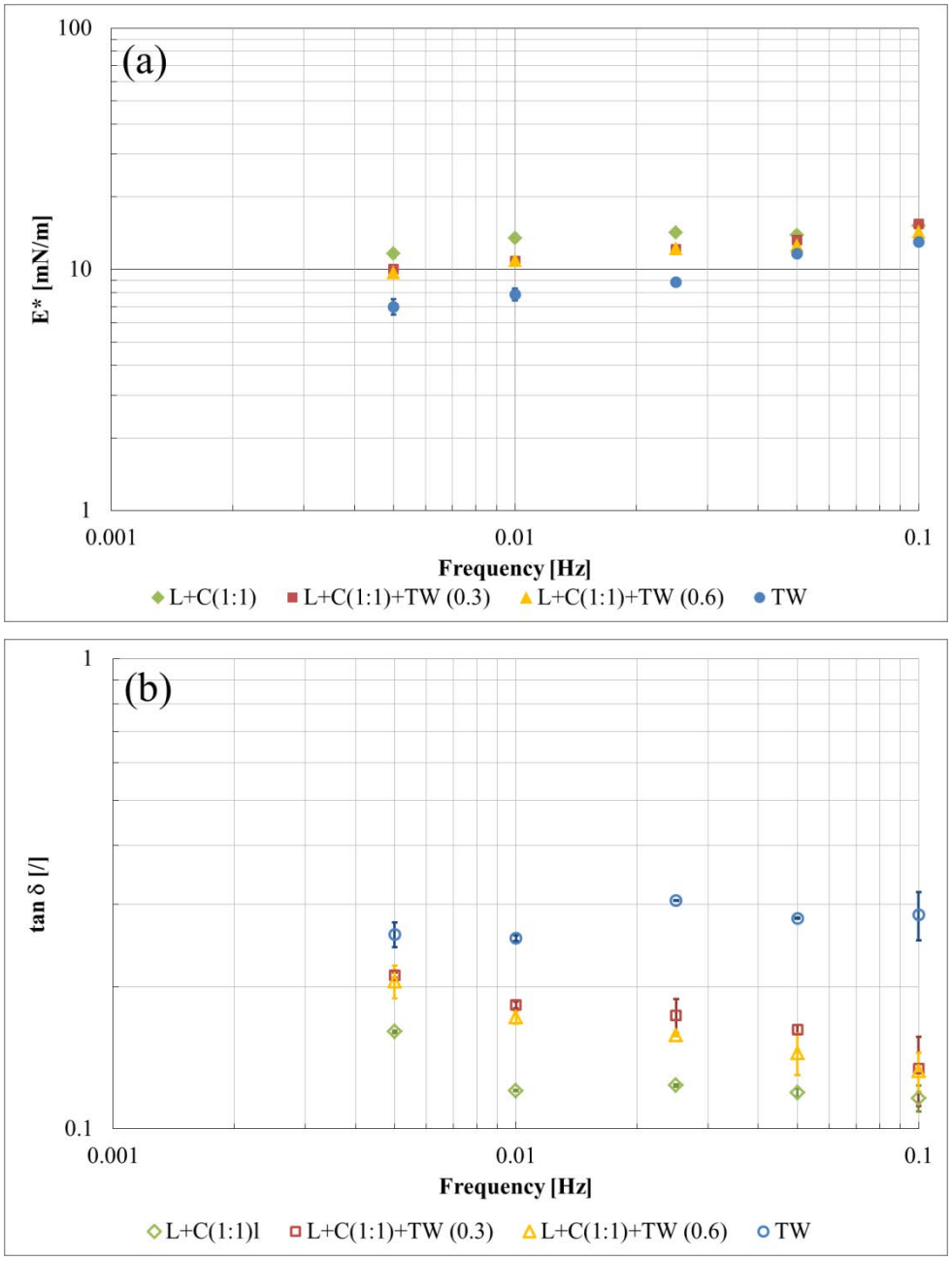


Figure 3. (a) Interfacial dilational modulus, E*, and (b) loss tangent , tanδ, values , as a function of drop oscillation frequency for the pure and mixed systems containing milk proteins, β-Lactoglobulin and β-Casein (1 g/l), and Tween 60 emulsifier at the O/W interface (imidazole buffer, pH 6.8)

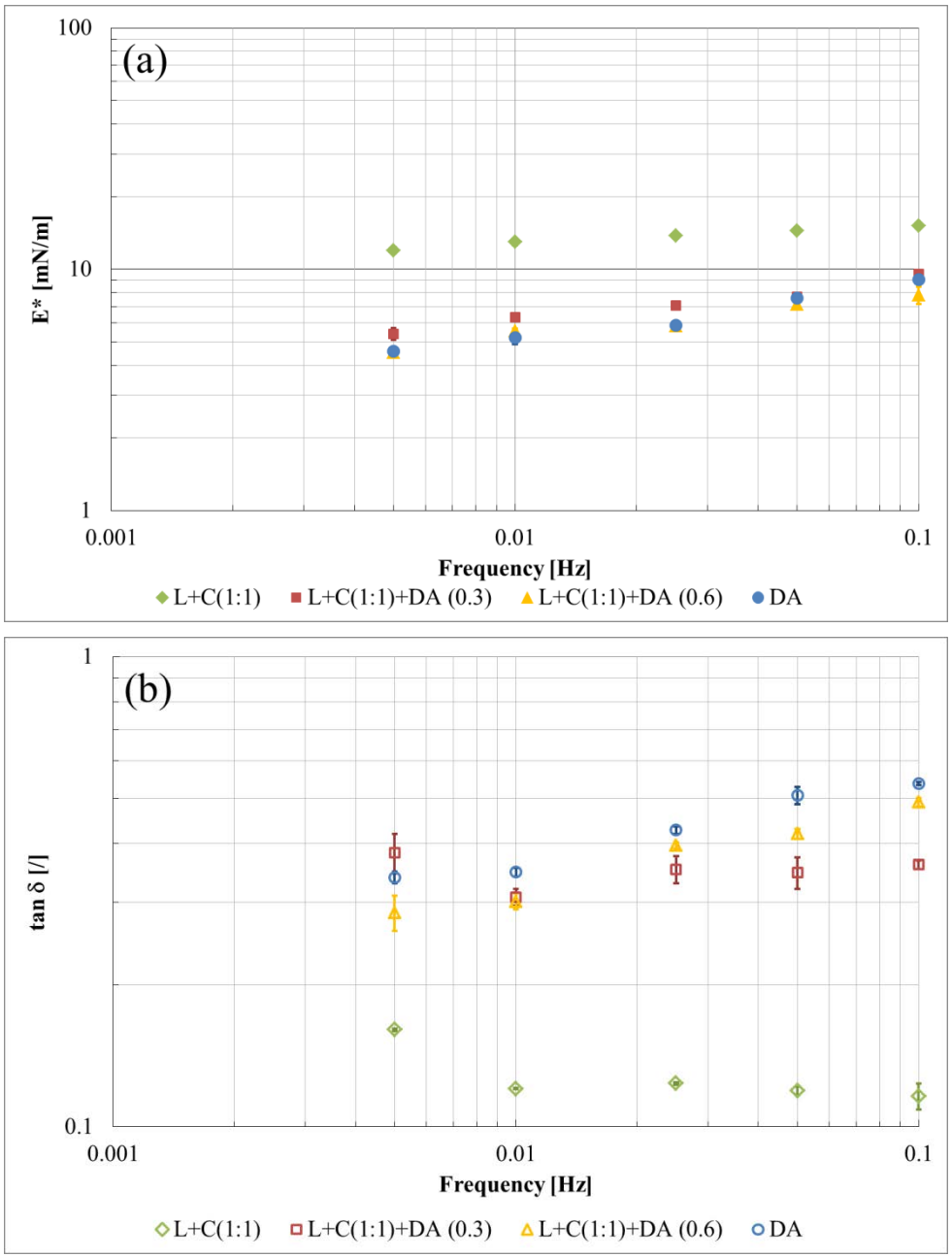


Figure 4. (a) Interfacial dilational modulus, E*, and (b) loss tangent , tanδ, values , as a function of drop oscillation frequency for the pure and mixed systems containing milk proteins, β-Lactoglobulin and β-Casein (1 g/l), and Admul Datem emulsifier at the O/W interface (imidazole buffer, pH 6.8)

From the figures (3) and (4) it can be noticed that for each system considered the interface showed a viscoelastic behavior with marked interfacial solid-like properties as shown by the low values of tanδ (tanδ <1) in all the frequency range studied indicating E' larger than E'' by about more or less one order of magnitude.

Moreover, it can be seen that the addition of both Tween 60 and Admul Datem emulsifiers to the milk protein system caused a relevant weakening of the interfacial protein network. This is indicated by the reduction of the E*modulus and the simultaneous increase of the

loss tangent tanδ values from those of β-Lactoglobulin and β-Casein system, which was recorded for the L+C(1:1)+TW and L+C(1:1)+DA samples.

Then, observing also the frequency behavior obtained for the emulsifier alone adsorbed at the interface, it can be said that the rheological properties of mixed emulsifier-protein layer proved to be more surfactant-like

This behavior, representative of a considerable fluidization of the adsorbed protein layer became more evident and important when emulsifier/protein weight ratio increased in the mixture, although all rheological data of the mixed protein-emulsifier systems fell between those of the individual components.

It could be interpreted as a consequence of a competitive adsorption phenomena, ascribed to hydrophobic and or electrostatic interactions between the proteins and emulsifiers, which result in the replacement of protein from the interface and the prevalent presence of the emulsifier or emulsifier-protein complexes at the O/W interface [Bos and Van Vliet (2001), Maldonado-Valderrama and Rodrìguez Patino (2010)].

Furthermore, from a comparison among the above figures it appears clearly that the weakening of the milk protein network assumed different characteristics according to the type of emulsifier added. In fact, the ionic emulsifier Admul Datem affected the protein interfacial structure layer more than the nonionic emulsifier Tween 60, causing a greater increasing of tanδ (and also reduction of E*) at each emulsifier/protein considered.

Anyway, it is important to point out that the pure and mixed protein layers adsorbed at the sunflower oil/water interface exhibited very small variations of the complex modulus E*, and the loss tangent in the frequency range investigated, showing a rheological behavior in a dimension 2D which typically characterize a strong 3D gel.

The almost independence of E* on the frequency combined with the low values of $E^{"}$, as indicated by the very small value of the loss tangent (tanδ <1), therefore means that the investigated adsorbed layers could present the rheological properties of a 2D critical gel [Bouriat et al. (2004), Dicharry et al. (2006), Kopperud and Hansen (2001)].

As it can be noticed in figure (3a) and (4a) the log−log plot of the complex interfacial dilational modulus *E* vs* the frequency ω, was a straight line and followed a scaling law of form:

$$E^* \approx \omega^n \qquad (6)$$

The loss tangent tanδ remained practically little variant with pulsation, and it was also related to the slope *n* of the power law curve by the equation (7), as it can be seen in figure (8):

$$\tan(\delta) = \tan(n\frac{\pi}{2}) \qquad (7)$$

Equations (7) and (8) are relevant to the rheology of 3D-gel near its gelation point (critical gel), as demonstrated by Winter and Chambon for polymers [Winter and Chambon, 1986], and were considered still valid by Bouriat et al. (2004) and Dicharry et al. (2006) to interpret 2D-rheology measurements assuming that the interface can be modeled as a parallel coupling of continuous Maxwellian blocks with relaxation times, τ, and elasticity:

$$k(\tau) = \alpha\tau^{-(n+1)} \qquad (8)$$

where α, which can identified as the strength of the gel, is proportional to the number of aggregates at interface relaxing with the characteristic time τ.

According the equation (8) the complex elasticity modulus E* can then be calculated by equation (9), from which (6) and (7) can be deduced.

$$E^*(\omega) == \int_0^\infty k(\tau)\frac{i\omega\tau}{1+i\omega\tau}d\tau = \frac{\alpha\pi}{\sin(n\pi)}\omega^n e^{in\pi/2} \qquad 9)$$

In agreement with Bouriat et al. (2004) and Dicharry et al. (2006), which considered interfacial protein films in a similar way as bulk systems, but restricted to two dimensions, in the present work gel critical model was used to investigate the nature of the interface and the protein-emulsifier interaction effects on the interfacial mechanical properties.

Then the *E* vs* frequency curves of pure and mixed protein sample systems were interpreted with a power law equation (6), and according to this equation the parameters *n* and *k* were evaluated. *n* can be considered an indirect measure of structuring degree of interface, which proves to be as high as the *n* value is small, whereas *k* parameter is the E* value extrapolated at the frequency of 1 Hz and, then, a measure of the strength of the interfacial gel.

The model parameters estimated for the mixed milk proteins-Tween 60/Admul Datem systems were reported in figures (5) and (6), respectively, together with those obtained for the pure surfactant layer (L+C(1:1), TW, DA).

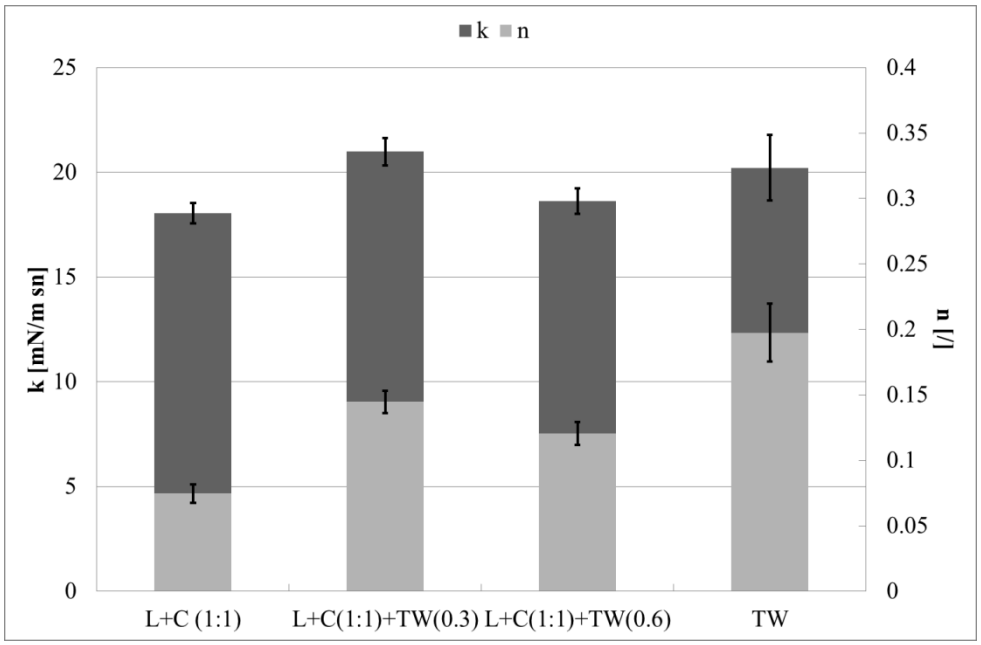


Figure 5. Gel 2D rheological model parameters k and n obtained according to equation (7) for the milk proteins-Tween 60 systems

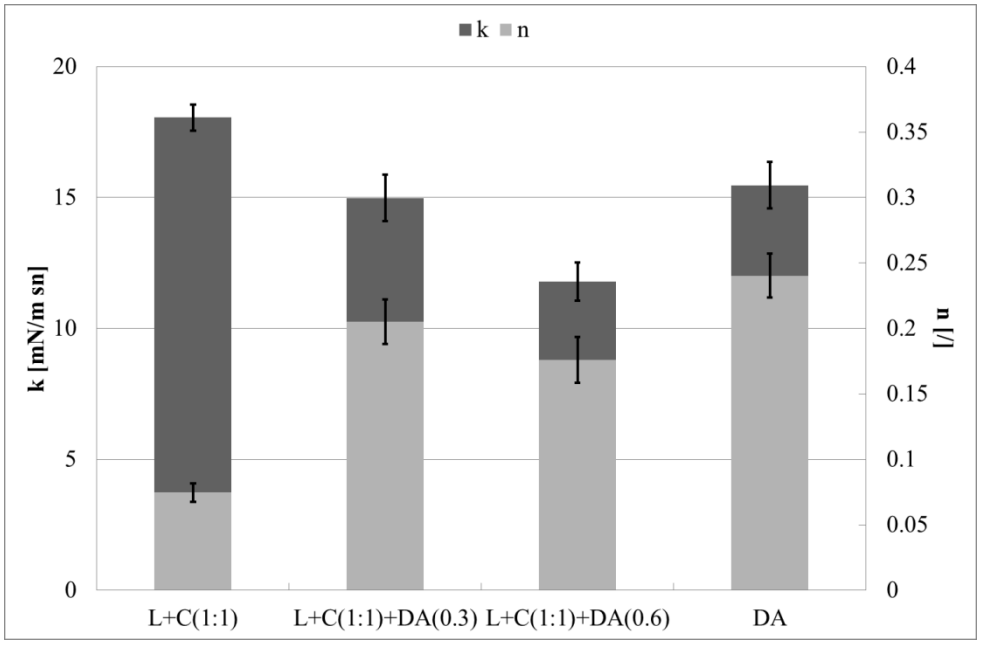


Figure 6. Gel 2D rheological model parameters k and n obtained according to equation (7) for the milk proteins-Admul Datem systems

From the figure it appears clearly that the rheological approach used, which allow us to evaluate rheological parameters being a measure of the interfacial layer structure , proved

to be very useful to confirm the existence of competitive adsorption phenomena between milk proteins and Tween60/Admul Datem emulsifiers resulting in a probable replacement of protein by emulsifier, and, thereby to differentiate the protein network behavior by varying the emulsifier/protein weight ratio in the solution.

In fact, an increasing of the *n* parameter value than that of pure protein films, which corresponds to a reduction of structuring degree of the interfacial film, was registered for all L+C(1:1)+TW and L+C(1:1)+DA systems. They, anyway, both in the Tween 60 case and in the Admul Datem one, showed *n* values smaller than the TW and DA samples respectively, at each emulsifier/protein ratio investigated, indicating the synergic influence of β-Lactoglobulin and β-Casein and emulsifiers on the mechanical properties of the interfacial network.

Considering the *k* parameter, it can be noticed that the protein-Tween 60 mixed systems exhibited little differences in the values of interfacial network strength at each emulsifier/protein ratio used, compared to that of the proteins alone. On the contrary the addition of Admul Datem on the protein layer caused a relevant reduction of k parameter, which became more important with increasing the emulsifier amount in the mixture.

Then, the higher influence of the ionic emulsifier Admul Datem on the interfacial protein structure layer than that of nonionic emulsifier Tween 60, discussed above, was also confirmed by the rheological model results, which allow us to find a larger increasing of *n* value and reduction of *k* parameter for the L+C(1:1)+DA sample than L+C(1:1)+TW one.

This result indicates that probably different competitive phenomena occurred between the two emulsifiers investigated and the milk proteins at the O/W interface, which result in a different extent of protein replacement from the interface by emulsifiers or by surfactant/protein complexes. This proved to be more relevant in the presence of Admul Datem emulsifier owing to probably its higher hydrophobicity which allowed it to oil-water interface [Camino et al. (2009)].

4.Conclusions

The adsorption of milk proteins β-Casein and β-Lactoglobulin at the sunflower oil/water interface in the presence of food emulsifiers Tween 60 and Admul Date was successfully investigated by dynamic interfacial tension measurements and harmonic drop oscillation experiments, in order to study the interfacial mechanisms and properties underlying the emulsion functionality of milk protein-emulsifier mixtures.

Differences between milk proteins-Tween 60/Admul Datem were individuated probably because of dissimilar competitive phenomena among protein and tested emulsifiers, which result in a different extent of protein replacement from the interface by emulsifiers or by surfactant/protein complexes.

The ionic emulsifier Admul Datem proved to be affect the protein adsorption and interfacial structure layer more than the nonionic emulsifier Tween 60.

Interfacial dilatational moduli evidenced the potential formation of a 2D critical gel at the oil/water interface and they were analysed assuming the validity at the interface of rheological model used for the 3D critical gel.

This rheological approach proved to be very useful to evaluate and to differentiate the protein network behaviour by varying the emulsifier/protein weight ratio in the solution, on the basis of rheological parameters which are a measure of the interfacial layer structure.

References

1) Cornec M., Mackie A.R., Wilde P.J., Clark D.C. (1996), Competitive adsorption of β-lactoglobulin and β-casein with Span80 at the oil-water interface and the effect on emulsion behavior. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* (114), 237-244
2) Dickinson E. (1999), Review Adsorbed protein layers at fluid interfaces: interactions, structure and surface rheology. *Colloids and Surfaces B: Biointerfaces* (15), 161-176
3) Bos M.V. and van Vliet T. (2001), Interfacial rheological properties of adsorbed protein layers and surfactants: a review, *Advances in Colloid and Interface Science* (91), 437-471
4) Murray S.B., (1998) Interfacial rheology of mixed food protein and surfactant adsorption layers with respect to emulsion and foam stability, in M*ö*bius D. and Miller R., Studies in interface science 7, Proteins at Liquid Interfaces, Elsevier editor, Amsterdam (The Netherlands), chapter 5
5) Dickinson E. (1998), Proteins at interfaces and in emulsions. Stability, rheology and interactions. *Journal of the Chemical Society, Faraday Transactions* (94), 1657-1669
6) Rouimi S., Schrorsch C., Valentini C., Vaslin S., (2005) Foam stability and interfacial properties of milk protein-surfactant systems. *Food Hydrocolloids* (19), 467-478
7) Williams A. and Prins A. (1996), Comparison of the dilational behavior of adsorbed milk proteins at the air-water and oil-water interfaces, *Colloids and Surfaces A: Physicochemical and Engineering Aspects* (114), 267-275
8) Jensen R.G., Handbook of Milk Composition, Academic Press, New York (U.S.A) 1995, chapter 5
9) W*ü*stneck R., Kragel J., Miller R., Wilde P., Clark D.C. (1996), The adsorption of surface-active molecules between β-casein, β-lactoglobulin and ionic surfactants and their rheological shear behavior. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* (114), 255-265
10) Camino N.A., Pèrez O.E., Carrera Sanchez C., Rodriguez Patino J.M., Pilosof A.M.R. (2009). Hydroxypropylmethylcellulose surface activity at equilibrium and

adsorption dynamics at the air-water and oil-water interfaces. *Food Hydrocolloids* (23) 2359-2368

11) Biresaw G., Liu Z. S., Erhan S.Z. (2008), Investigation of the Surface Properties of Polymeric Soaps Obtained by Ring-Opening Polymerization of Epoxidized Soybean Oil, Wiley InterScience (www.interscience.wiley.com)
12) Lin S.Y., Wang W.J., Lin L.W., Chen L.J. (1996) Systematic effects of bubble volume on the surface tension measured by pendant bubble profiles *Colloids and Surfaces A: Physicochemical and Engineering Aspects* (114), 31-39
13) Lucassen J. and van den Tempel M. (1972), Dynamic measurements of dilational properties of a liquid interface, *Chemical Engineering Science* (27) 1283-129
14) Freer E. M., Yim K. S., Fuller G.,. Radke C. J. (2004), Interfacial Rheology of Globular and Flexible Proteins at the Hexadecane/Water Interface: Comparison of Shear and Dilatation Deformation. *Journal of Physical Chemistry B* (108), 3835-3844
15) Ravera F., Liggieri L., Loglio G. Dilational rheology of adsorbed layers by oscillating drops and bubbles. In: R. Miller, L. Liggieri Interfacial Rheology Volume 1 , Brill Academic Pub, Leiden Boston 2009, 138-177
16) Myrvold R. and Knut Hansen F. (1998), Surface Elasticity and Viscosity from Oscillating Bubbles Measured by Automatic Axisymmetric Drop Shape Analysis,
17) Miller R., Fainerman V.B., Makievski A.V., Krägel J., Grigoriev D.O., Kazakov V.N., Sinyachenko O.V., (2000) Dynamics of protein and mixed protein/surfactant adsorption layers at the water fluid interface *Advances in Colloid and Interface Science* (86), 39-82
18) Wilde P.J. (2000), Interfaces: their role in foam and emulsion behavior, *Current Opinion in Colloid & Interface Science* (5), 176-181
19) Mackie A, Wilde P. (2005), The role of interactions in defining the structure of mixed protein–surfactant interfaces, *Advances in Colloid and Interface Science* (117), 3-13
20) Rodrìguez Patino J.M., Rodriguez Nino M.R., Carrera Sànchez C. (2003) Protein-emulsifiers at the air water interface. *Current Opinion in Colloid & Interface Science* (8), 387-395.
21) Mackie A.R., Gunning P., Wilde P.J., Morris V.J.(1999) Orogenic Displacement of Protein from the Air/Water Interface by Competitive Adsorption *Journal of Colloid and Interface Science* (210), 157-166

22) Petkov J.T., Gurkov T.D.(2000). Dilatational and Shear Elasticity of Gel-like Protein Layers on Air/Water Interface, *Langmuir* (16), 3703-3711

23) Krägel J., O'Neill M., Makievski A.V., Michel M., Leser M.E., Miller R., (2003) Dynamics of mixed protein-surfactant layers adsorbed at the water/air and water/oil interface, *Colloids and Surfaces B: Biointerfaces* (31), 107-114

24) Maldonado-Valderrama J. and Rodrìguez Patino J.M. (2010) Interfacial rheology of protein–surfactant mixtures, *Current Opinion in Colloid & Interface Science* (15), 271–282

25) Dicharry C., Arla D., Sinquin A., Graciaa A., Bouriat P. (2006) Stability of water/crude oil emulsions based on interfacial dilatational rheology *Journal of Colloid and Interface Science* (297) 785–791

26) Bouriat, P., El Kerri N., Gracia A., Lachaise J. (2004) Properties of a Two-Dimensional Asphaltene Network at the Water-Cyclohexane Interface Deduced from Dynamic Tensiometry *Langmuir* (20) 7459-7464

27) Winter H.H. and Chambon F.(1986) Analysis of linear viscoelasticity of a cross-linking polymer at the gel point *Journal of Rheology* (30) 367-382

28) Kopperud H. B. M. and Hansen F.K. (2001), Surface Tension and Surface Dilational Elasticity of Associating Hydrophobically Modified Polyacrylamides in Aqueous Solutions. *Macromolecules* (34), 5635-5643.

Chapter 6
Milk proteins-polysaccharide interactions at the oil-water interface

Abstract

This work studies the interfacial behavior of mixed milk protein β-casein/β-lactoglobulin system in the presence of various polysaccharides to gain knowledge on the interactions between these biopolymers at the sunflower oil/water interface under dynamic conditions at pH=6.8 , corresponding with the conditions in milk, where a limited incompatibility between macromolecules can occur. The polysaccharides used were: k-carrageenan (kcar), ι-carrageenan (ιcar) and guar gum (guar). Bulk protein concentration in the systems was 0.1% w/w with a weight ratio between β-casein and β-lactoglobulin of 1:1, whereas polysaccharide concentration was varied in the 0.01-0.1% w/w range. The dynamic interfacial tension and rheological properties of films were evaluated with a drop tensiometer on a time scale of some seconds. Axialsymmetric drop shape analysis (ADSA) was used to calculate drop volume, area and interfacial tension.

Differences in the interaction of carrageenans and guar with the protein were obtained and should be mainly ascribed to different degrees of incompatibility and to the fact that guar is not charged.

1. Introduction

Proteins and polysaccharides are frequently present together in food dispersed systems, with both kinds of biopolymers contributing to stability, texture and shelf life of food emulsions or foams.

Proteins are best known for their emulsifying and foaming properties, playing a major role in the formation and stabilization of emulsions by a combination of electrostatic and steric mechanisms [Bos and Van Vliet (2001), McClements (2005)], whereas polysaccharides are best known for water-holding and thickening properties, which allow them to control the

rheology and network structure of the continuous phase, hence retarding phase separation and gravity-induced creaming [Dickinson (2003), Rodrìguez Patino and Pilosof (2011)].

Although traditionally associated with thickening and gelation behavior, polysaccharides can also influence the interfacial properties of dispersed systems, affecting the protein adsorption at the interface, exhibiting its own interfacial activity generally ascribed to the presence of protein impurities, or through different interactions with protein molecules resulting from blending these biopolymers [Dickinson (2003), Rodrìguez Patino and Pilosof (2011), Perez et al. (2009)]. In the first case described, the polysaccharides can be considered surface active molecules, SA-PS, (pectin, cellulose derivates), and in the second one, which is the most diffuse case, they are non surface active molecules, Non SA-PS, (carrageenan, guar gum, xanthan, etc.)

The different mechanisms involved in the adsorption and/or interactions between proteins and the polysaccharide were schematized in figure (1).

When a protein adsorbs at a fluid interface in the presence of polysaccharides under conditions of thermodynamic compatibility in the bulk, three phenomena can occur :

(i) the polysaccharide (SA-PS) adsorbs at the interface on its own in competition with the protein for the interface (competitive adsorption) (ii) the polysaccharide (SA-PS or Non-SA-PS) complexes with the adsorbed protein mainly by electrostatic interactions or hydrogen bonding and (iii) because of the existence of a limited thermodynamic compatibility between the protein and polysaccharide, the polysaccharide concentrates the adsorbed protein [Rodrìguez Patino and Pilosof (2011)].

Anchorage of the polysaccharide at the interfacial film may occur by mechanism (i) or (ii), depending on the chemical structure of the polysaccharide and on the pH. Once the polysaccharide is into the interface or attached by complexation, exclusion volume effects between both biopolymers at neutral pH could lead to a rise of chemical potential or, in other words, to a modification of the thermodynamic activity of the protein at the interface. Therefore, the protein at the interface would perform as a more concentrate film, leading to an increase in the surface pressure.

Even if the polysaccharide does not participate in the interface (i.e., it does not adsorb by its own or does not complex with adsorbed protein) the existence of a limited thermodynamic compatibility between the protein and polysaccharide in the vicinity of the interface could lead to concentration of adsorbed protein by a depletion mechanism ((iii) in figure(1)). There is an osmotic driving force that favors protein aggregation that could result in a variation of surface pressure [Rodrìguez Patino and Pilosof (2011)].

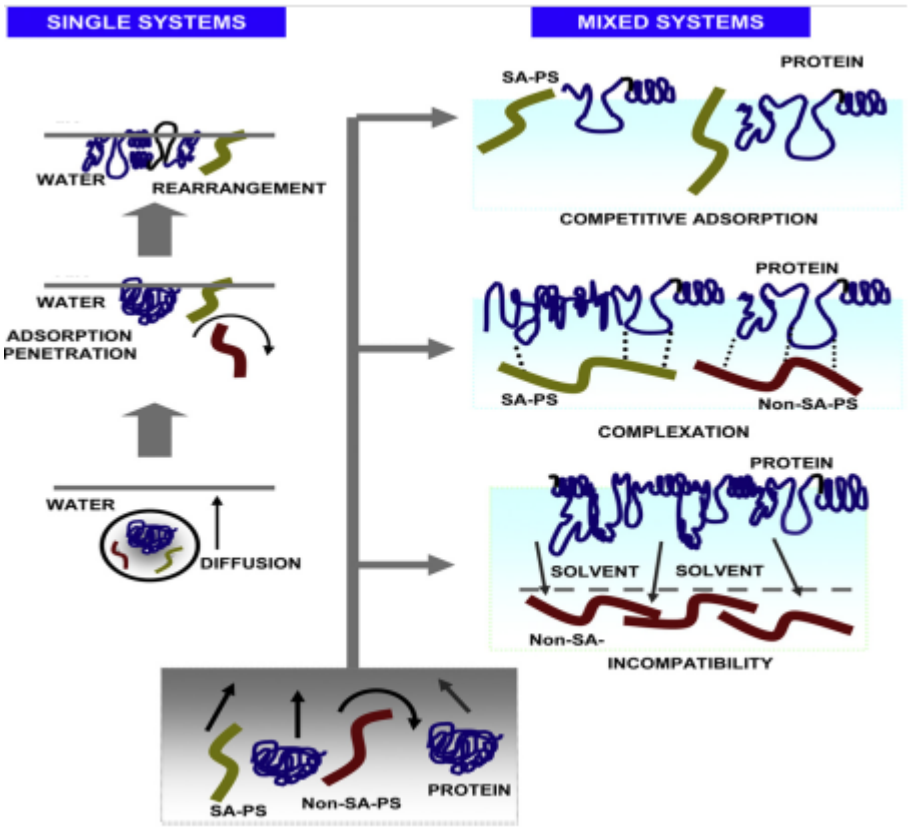


Figure 1. Scheme of different mechanisms involved in the adsorption and or interactions between biopolymers (proteins, surface active polysaccharide SA-PS, non surface active polysaccharide NSA-PS) [Rodrìguez Patino and Pilosof (2011)]

Then, protein-polysaccharide interactions play a significant role in the structure and stability of many emulsions, influencing the composition and the structure of the adsorbed layers at the interface.

Protein and polysaccharide molecules can link together by a covalent bond giving a specific, strong and essentially permanent “conjugate”, and on the other hand, protein and polysaccharide molecules can also associate via physical interactions, or non-covalent interactions (electrostatic and hydrophobic interactions, steric exclusion, hydrogen bonding, etc.).

With charged polysaccharides, the contribution of electrostatic interactions is predominant [Dickinson (2003), Rodrìguez Patino and Pilosof (2011)].

Strong attractive electrostatic complexes are typically formed with mixtures of positively charged proteins (pH<pI) and negatively charged polysaccharide.

Weaker reversible complexes tend to be formed between anionic polysaccharides and

proteins carrying nearly zero overall charge (pH=pI) or a net negative charge (pH> pI). Thus, on adjusting the pH and/or ionic strength of the aqueous phase, the strength of the protein polysaccharide interactions may vary substantially [Dickinson (2003), Rodrìguez Patino and Pilosof (2011)].

In order to understand the functionality of milk protein mixture of β-casein and β-lactoglobulin adsorbed at the sunflower-oil/water interface in the presence of three different polysaccharides k-carrageenan, *k-car*, ι-carrageenan, *ι-car*, and guar gum, *guar*, the interactions between these biopolymers at the O/W interface were studied under dynamic conditions at pH=6.8, a value close to neutral pH, where a limited incompatibility can occur [Martinez et al. (2007), Rodrìgeuz and Patino (2011), Benichou et al. (2002), Dickinson (2003)].

This contribution complements previous studies on intermolecular interactions between milk proteins and food hydrocolloid interactions [Syrbe et al. (1998), Singh et al. (2003), Suk Gu et al. (2004)], focusing on the interfacial properties of non-commercial milk protein, by using dynamic interfacial tension measurements and harmonic drop oscillation experiments in a time scale of some seconds.

The investigated polysaccharides are used in the dairy emulsion industry as thickening and stabilizing agents, being commonly considered as non-surface-active polysaccharides [Piculell, (1995)].

Specifically, carrageenans are anionic polysaccharides extracted from red seaweed (Rhodophyta), consisting of linear polymers of alternating β-1,3 and α-1,4 linked galactose residues. There are three generic carrageenan families (*k*, *ι* and λ) with varying number and position of sulphate groups on the galactose dimer.

Schematic representation of the different idealized repeating units of *k*- and, *ι* -carrageenans were showed in figure (2).

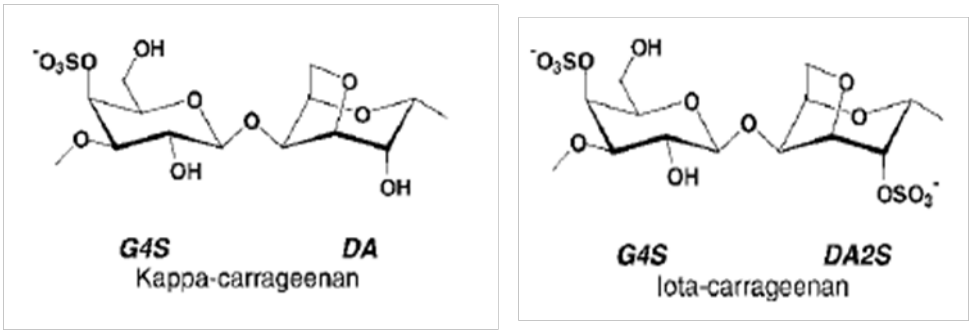


Figure 2. Alternating 3-linked β-D-galactopyranose (G-units) and 4-linked β-D -galactopyranose (D-units) or 4- linked 3,6-anhydrogalactose (DA-units),forming the "ideal" disaccharide-repeating unit of k- and ι-carrageenans, taken from van de Velde and de Ruiter (2005)

When dispersed in aqueous solution, *k*- and, *ι* -carrageenans undergo a coil (disordered state) to helix (ordered) transition, depending on the temperature and ionic strength [Piculell (1995), van de Velde and de Ruiter (2005)].

Guar Gum is made up of neutral polysaccharides with high molecular weights (10^3 kDa), and can be used as a bulk agent to increase viscosity, but not generally lead to gel formation [Miquelim et al. (2010), Narchi et al. (2009)]. Guar gum is a hydrocolloid obtained from the endosperm of the *Cyanmopsisvtetragonolobus* belonging to the family Leguminosae. The structure of guar gum consists of a linear backbone of β(1,4)-linked D-mannose units with various amounts of α(1,6)-linked D-galactose side chains, and the ratio of mannose to galactose is 2:1 [Srichamroen (2007)].

Finally, β-casein and β-lactoglobulin adsorbed layers at the sunflower oil/water interface were selected as a "model protein interface" representative of dairy emulsion, because these proteins are one of the major caseins and the most abundant whey protein present in milk, respectively. In fact, despite the great complexity of milk its surface proved to be dominated by free β-casein and β-lactoglobulin molecules owing to the stability of casein micelles [Dickinson (1999), W*ü*stneck et al. (1996)], and as a consequence, a quantitative investigation of the interfacial rheology of systems composed of these proteins could be a very useful tool to control and predict the functionality of milk products.

The main goal of this research was to study the interfacial properties of a β-casein / β-lactoglobulin protein mixture upon addition of high-molecular weight polysaccharides at constant pH (above the isoelectric point of both proteins). The results could be used to interpret the real behaviour of dairy emulsions stabilized by corresponding systems.

2. Materials and methods

2.1 Materials

All ingredients used were purchased from Sigma Aldrich, Inc (Germany): β-casein (Ref: C6905), β-lactoglobulin (Ref: L3908), ι-carrageenan (Ref:C1138), κ-carrageenan (Ref: C1013) and guar (Ref: G4129). Imidazole (Ref. A1073,05) used as buffer for adjusting the pH of the water-protein-polysaccharide solutions was obtained by AppliChem (Germany). Sunflower oil used as oil phase in the pendant drop experiments (Rif:356241) without further purification was purchased from Carlo Erba Reagents (Italy) . It contains triglycerides and free saturated and unsaturated fatty acid (0.4%) .

2.2 Sample preparation

Protein solutions were prepared by adding the protein powder to twice-distilled water in imidazole buffer 75 mM at pH=6.8 $\pm$ 0.2 [Williams and Prins (1996)], corresponding to the conditions in milk [Jensen (1995)], and then stirring for 30 min at room temperature to ensure complete dispersion.

Carrageenan and guar solutions were prepared by dissolving the powder in the same buffer at room temperature then heating in a water bath at 75 °C and 95° respectively for 30 min [Singh et al. (2003), Srichamroen (2007)]. Appropriate quantities of protein and polysaccharide solutions were then mixed at room temperature to give the total protein concentration of 0.1% w/w with a β-casein and β-lactoglobulin weight ratio of 1:1, and to give polysaccharide concentrations varying between 0.01-0.1% w/w. The twice distilled water used throughout all the experiments was obtained from a Milli-Q purification system (Millipore, USA), and it was checked for contaminants before each experiment, measuring the surface tension of the buffer solution at the air/water boundary at ambient temperature. No aqueous solutions with a surface tension other than accepted in the literature (72-73 mN/m at 20°C) were used.

All sample solutions were freshly prepared (within no more than 24 h) for the characterization and two replicates were prepared for each sample.

2.3 Dynamic interfacial properties measurements

Mixed protein-polysaccharides systems adsorbed at the O/W interface were characterized by dynamic interfacial tension measurements and harmonic drop oscillation experiments on a time scale of some seconds.

Axialsymmetric drop shape analysis (ADSA) was used to calculate drop volume, area and interfacial tension by using an automated pendant drop tensiometer (FTA200 First Ten Angstroms, USA) equipped with the *fta32 v2.0* software. Details of this apparatus are given by Biresaw at al. (2008).

The instrument comprises an automated pump that can be fitted with various sizes of syringes and needles to allow for control of pendant drop formation and of sinusoidal variations in the drop volume or surface area by software.

An automated image viewing and capturing system, with various image capture triggering options, was used to capture drop image.

Computer hardware and software also provide the capability for data capture, storage, analysis, and transfer. The software allows for an automated drop shape analysis of the captured drop image, and for measuring the surface-interfacial tension of the drop formed in air or in a second fluid at rest and in periodic motion conditions of the interface respectively.

Image acquisition and regression of the interfacial tension were performed directly with commercially available drop-image software by fitting the Bashforth-Adams equation to the drop shape [Biresaw at al. (2008)].

Drop-image software also controlled an automatic pipetting system that maintained constant drop volume with time period over which dynamic tensions were measured.

2.3.1 Dynamic interfacial tension

The method adopted to measure the interfacial tension of the investigated solutions with time (γ *vs* t), by using an automatic drop tensiometer, involved the analysis of the profile of the drops created oil phase, and kept at rest (constant volume).

The experiments were carried out at room temperature (22°C within ± 1 °C), placing sample aqueous solutions in a 100 µl glass Hamilton syringe equipped with a 20 Gauge stainless steel needle, and delivering drops in a rectangular quartz cuvette (5ml) containing the desired phase.

Drop volumes of 9-12 µl were used in every test in order to measure interfacial tension values, independent of the drop size [Lin et all, 1996]. High drop volumes of the kind

chosen, proved to be generally more suitable for these tests, because they increased the number of profile points, which can be used for drop shape analysis [Lin et all, 1996].

The experiments started with the creation of the drop; then drop images were continuously taken from a CCD camera and digitalized, registering the interfacial tension values over the test time.

Drop profile was monitored up to a maximum time of 180 min, which assures the molecules adsorption at the interface and the reaching of a quasi-equilibrium interfacial tension value.

Equilibrium of γ was assumed when the interfacial tension did not change by more than 0.5 mN/m in 30 min [Camino et al. (2009)].

2.3.2 Dilational rheological properties

The method adopted to determine the dilational rheological properties of the interface, by using an automatic drop tensiometer, involved the profile analysis of the drop formed in the air/oil phases and subjected to periodic motion conditions, according to the oscillating drop methodology, able to measure the interfacial visco-elasticity versus frequency.

Oscillating drop experiments are usually performed subjecting the interface to an infinitesimal sinusoidal compression and expansion, then by applying a frequency sweep to the surface area A, in order to measure the interfacial dilational modulus.

The surface dilational modulus derived from the change in surface tension γ (dilational stress) (Eq. 1), resulting from a small change in surface area A (dilational strain) (Eq.2), may be described by equation 3 [Lucassen and van den Tempel, 1972].

$$\gamma = \gamma_0 + \Delta\gamma \sin(\omega t + \delta) \quad (1)$$

$$A = A_0 + \Delta A \sin(\omega t) \quad (2)$$

$$E^* = \frac{d\gamma}{d \ln A} \quad (3)$$

Where γ_0 and A_0 are the equilibrium reference surface tension and the unperturbed interfacial area of the drop respectively, Δγ and ΔA are the measured stress and strain amplitude respectively, and δ is the phase angle between stress and strain: themeasure of the relative film viscoelasticity.

Since the drop area periodically oscillates, the dilational modulus exhibits two contributions: an elastic part accounting for the recoverable energy stored in the interface (storage modulus, *E'*) and the dissipative part accounting for energy lost through relaxation processes (loss modulus, *E''*).

$$E^* = E' + iE'' = \frac{\Delta\gamma}{\Delta A/A_0}\cos(\delta) + i\frac{\Delta\gamma}{\Delta A/A_0}\sin(\delta) \qquad (4)$$

Then the surface dilatational modulus, E*, as a measure of the total material resistance to dilatational deformation (elastic and viscous), is a complex quantity composed of a real and an imaginary part [Freer et al. (2004), Ravera et al. (2009), Myrvold and Hansen (1998)].

To obtain a perfectly elastic material, stress and strain are in phase $\delta = 0$ and the imaginary term is zero. In the case of perfectly viscous material $\delta = 90°$ the real part is zero. The loss angle tangent can be defined by equation (5):

$$\tan\delta = E''/E' \qquad (5)$$

In this work, we applied a periodic strain by differentially oscillating the drop area at a prefixed frequency value, and we measured the periodic stress response with time.

Then the dilational viscoelastic parameters of interface, the dilational complex modulus (E*), its elastic (E') and viscous (E'') components and the loss angle tangent were measured as a function of the adsorption time *t*.

The *Time Sweep Tests* were carried out by using deformation amplitude ($\Delta A/A_0$) values of 3-5% and angular frequency ones varying in the 0.005 Hz-0.1 Hz range.

The percentage area change was determined before each time sweep test by performing *Amplitude Sweep Experiments* (data not shown) realized at the extreme frequency values of the investigated range. The latter experiments were useful to assure that system response was not influenced by perturbation amplitude (linear viscoelastic behavior) and to be in the linear region, so as to avoid oscillation amplitude that causes disruption of the supramolecular organization or provides adequate measurement sensitivity.

The duration of each test was established so as to register equilibrium values of the dilational moduli with time (maximum variation of 3% was accepted).

The E* *vs* ω curves were then obtained by using the latter values for the frequency range investigated.

3. Results and discussion

3.1 Effect of polysaccharides on protein film interfacial tension

The interfacial tension evolution with time for milk proteins and protein-polysaccharide mixed films adsorbed at the sunflower oil/water interface plotted in figure (3), shows that except for guar, the presence of polysaccharide in the bulk phase led to an increase of interfacial tension γ when compared to the proteins alone.

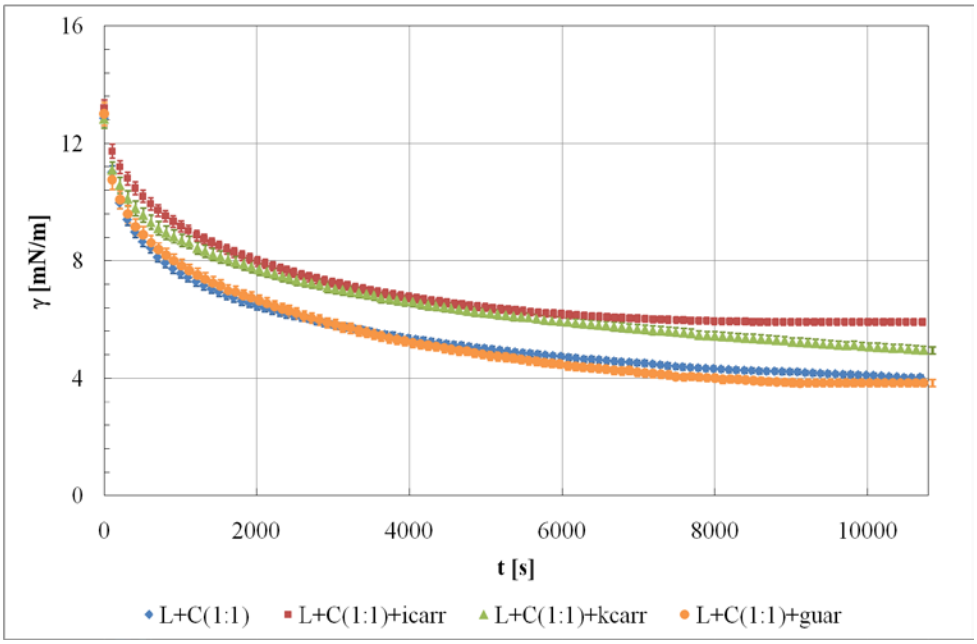


Figure 3. Interfacial tension versus time measured by pendant drop experiments for β-lactoglobulin/β-Casein mixed proteins, L+C(1:1) (0.1% w/w), and milk proteins +polysaccharides (0.01%w/w), L+C(1:1)+ιcar, L+C(1:1)+kcar, L+C(1:1)+guar, adsorbed at the O/W interface

Plateau values of interfacial tension were also reported in table (1) for all systems considered in order to compare the effect of polysaccharide addition on the mixed proteins adsorption.

	γ_{eq} [mN/m]
L+C(1:1)	*4.1 ± 0.1*
L+C(1:1)+icar	*5.9 ± 0.1*
L+C(1:1)+kcar	*5.1 ± 0.2*
L+C(1:1)+guar	*3.9 ± 0.1*

Table 1. Plateau values of interfacial tension of β-lactoglobulin/β-Casein mixed proteins, L+C(1:1) (0.1% w/w), and milk proteins +polysaccharides (0.01%w/w), L+C(1:1)+ιcar, L+C(1:1)+kcar, L+C(1:1)+guar, adsorbed at the O/W interface

Guar little influenced the interfacial tension of L+C(1:1), only producing a slight decrease of the interfacial tension plateau value or equilibrium value, which anyway remained in deviation with that exhibited by the protein film.

On the contrary very different values of the equilibrium interfacial tension (and also of the rate of the interfacial tension decrease) from those of proteins alone, were obtained for the protein+carrageenans systems.

Specifically, kcar and icar decreased the surface activity of mixed β-lactoglobulin and β-casein film adsorbed at interface, probably owing to changes of the conformation of the adsorbed protein molecules at interface.

This behavior allows us to identify some differences in the interactions of guar and carrageenans with milk proteins adsorbed at O/W interface, probably linked to dissimilar charges of polysaccharide molecules studied, and to different degree of incompatibility resulting between these biopolymers.

In fact, at pH of 6.8 the milk proteins are negatively charged and the i-carrageenan, k-carrageenan and guar are respectively negatively, negatively and neutrally charged. Thus at these conditions, repulsive electrostatic interactions can occur between the protein and carrageenan molecules alone, whereas hydrophobic interactions and hydrogen bonding are the possible types of interaction for the protein and guar molecules [Miquelim et al. (2010)]. Attractive electrostatic interactions are inhibited in every case.

Analyzing these conditions, it clearly appears that the electrostatic interactions had a greater effect on the interfacial tension of the protein film than those characterizing protein

and neutral polysaccharide, such as guar. This is in accordance with many authors [Martinez et al. (2007), Miquelim et al. (2010), Rodrìguez Patino and Pilosof (2011)].

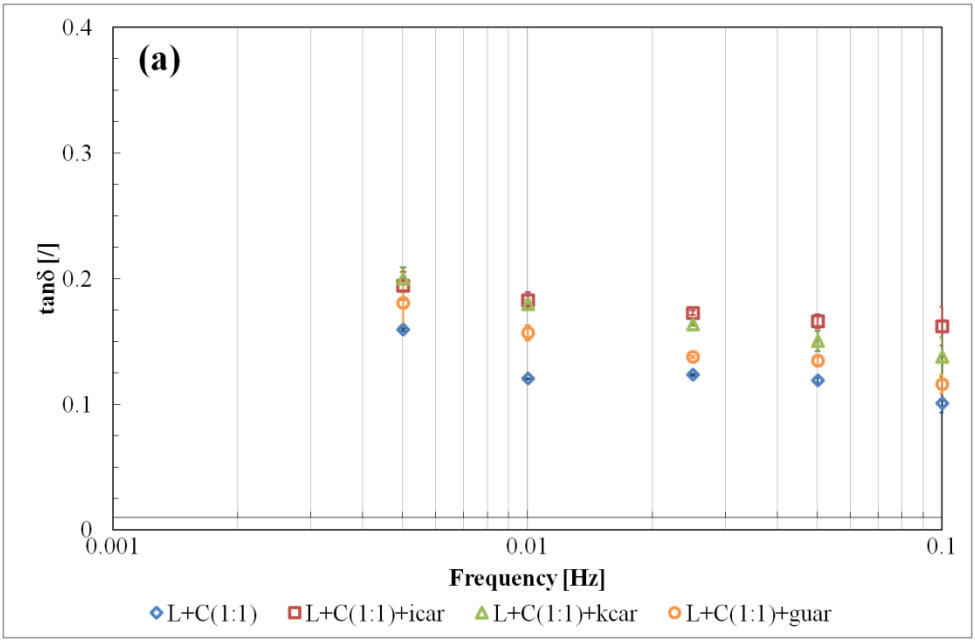
In fact, even if the carrageenans and guar gum did not directly adsorb the interface, the presence of latter biopolymers in the vicinity of the interface proved to affect the protein adsorption more strongly, and probably affects the structure of adsorbed molecules by a depletion mechanism [Rodrìguez Patino and Pilosof (2011)].

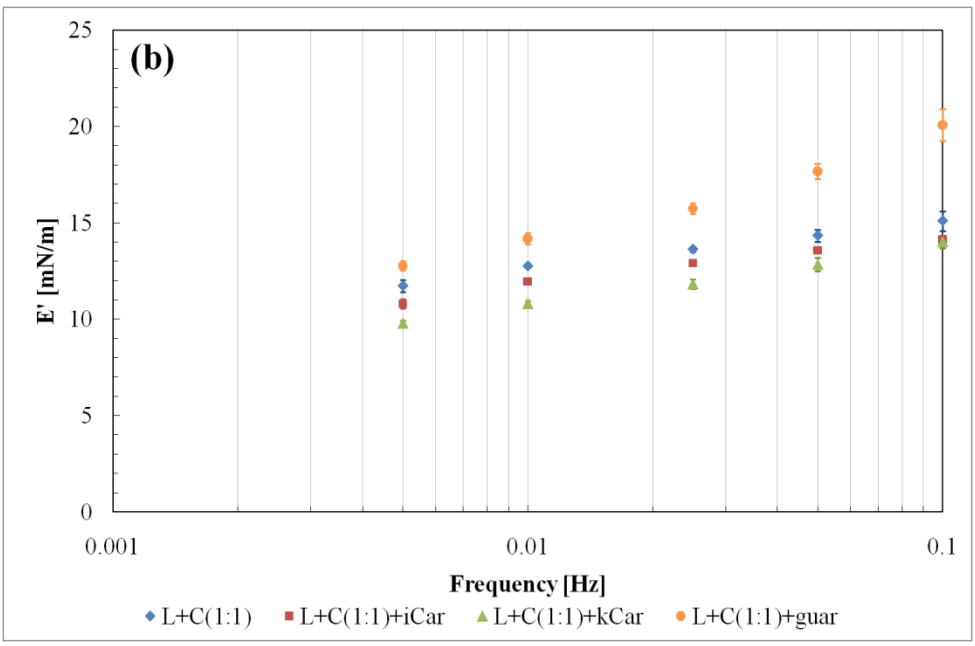
When milk proteins alone adsorb from bulk, and not in the presence of high-molecular weight molecules, they probably have the time to adsorb assuming a more favorable conformation at the interface, whereas on adsorption in the presence of carrageenan molecules in the bulk, the system is jammed at interface before these conformational changes can occur.

As a consequence, the latter structure proved to be probably less ordered and less compact at high adsorption time, allowing for less interfacial tension decrease [Ganzevles et al. (2006)].

3.2 Effect of polysaccharides on the rheological properties of protein film adsorbed at the interface

The frequency behavior being the main focus of this work , oscillatory interfacial dilational rheometry results in terms of loss tangent tanδ, elastic E' and viscous E'' components of complex interfacial dilational modulus were plotted in figure (4) for mixed films adsorbed at the sunflower oil/water interface as a function of drop oscillation frequency ω.They provided additional information on the strength of interfaces in the systems considered.





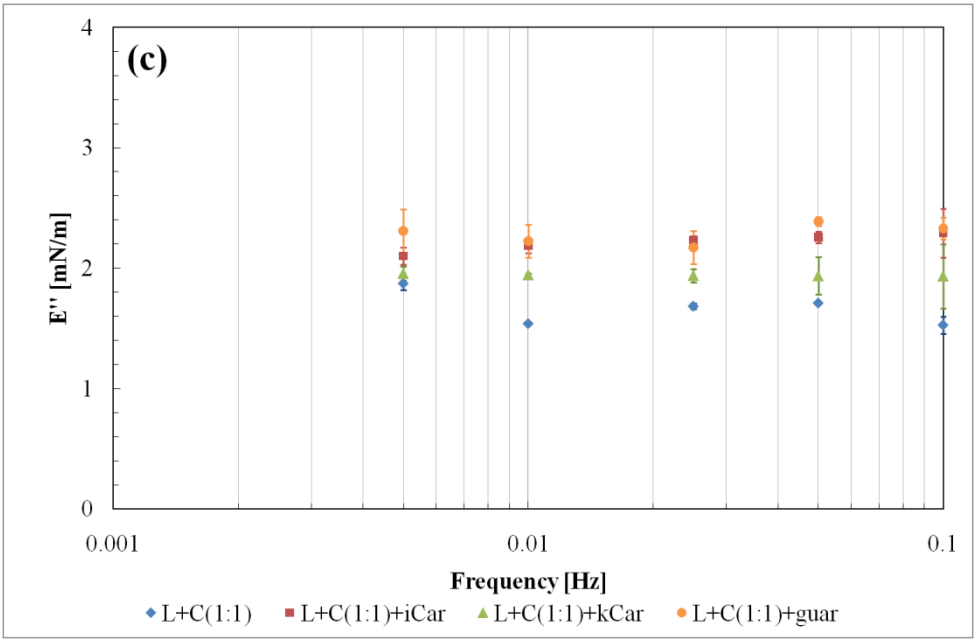


Figure 4. Drop oscillation frequency-dependent interfacial dilational properties for β-lactoglobulin/β-Casein mixed proteins, L+C(1:1) (0.1% w/w), and milk proteins +polysaccharides (0.01%w/w), L+C(1:1)+ıcar, L+C(1:1)+kcar, L+C(1:1)+guar adsorbed at the O/W interface. **(a)** Loss tangent; **(b)** Interfacial dilational elastic component; **(c)** Interfacial dilational viscous component.

From the figure (4), it can be noticed that for each system considered, the interface showed a viscoelastic behavior, with E' larger than E'' by about more or less one order of magnitude in the frequency range studied. This is consistent with an interfacial solid-like behavior [Miquelim et al. (2010)].

Furthermore, the presence of the polysaccharide molecule in the bulk solution proved to affect the rheological behaviour of L+C(1:1) milk proteins adsorbed, in a way depending on the type and charge of polysaccharide used.

In fact, guar exhibited higher values of both E' and E'' moduli than those of the protein layer, whereas for kcar and icar greater values of E'' and smaller ones of E'' than those of L+C(1:1) were obtained [Martinez et al. (2007), Ganzevles et al (2006), Rodrìguez Patino and Pilosof (2011)].

Anyway, for each protein-polysaccharide system a decreasing of interfacial rigidity and then, a substantial weakening of the protein network were measured as indicated by the substantial increase of loss tangent values of mixed protein–polysaccharide system than those of proteins alone. This could be attributed to conformational changes of the protein molecules at the interface, or to rearrangements of the protein–polysaccharide complexes or a combination of both [Ganzevles et al (2006), Rodrìguez Patino and Pilosof (2011)].

Protein weakening proved to be more important in the presence of anionic carragenan molecules than that recorded in the presence of guar gum, neutral biopolymer, indicating differences in the interactions between these biopolymers and milk proteins adsorbed at the O/W interface, probably owing to dissimilar charges of the polysaccharide molecules considered, and to a different degree of incompatibility resulting. [Martinez et al. (2007), Miquelim et al. (2010), Rodrìguez Patino and Pilosof (2011)].

These differences can be interpreted in term of layer structure [Ganzevles et al (2006)] considering that the higher the polysaccharide charge density, the more the protein molecules are hindered to form a adsorbed layer at the O/W interface, having little time to adopt a more favorable conformation at the interface, allowing for less increase of elasticity.

At this point it very interesting to consider the complex interfacial dilational moduli, E*, of each system considered, which were compared in figure (5) in a log−log plot of E^* *vs* ω.

It can be noticed that carrageenans decreased the dilational modulus of mixed β-lactoglobulin and β-casein film adsorbed at the interface, whereas guar gum molecules increased it in the frequency range considered.

However, it important to emphasize that the E*, as with tanδ of each sample system, exhibited small variations in the investigated frequency range.

The almost independence of E* on the oscillation frequency combined with the low values of $E^{''}$, as indicated by the very small value of the loss tangent (tanδ <1), therefore means that interfacial relaxation processes, attributed to the exchange of matter between the bulk solution and the interface, and to conformational changes in the interface, were negligible for the systems studied, and that the investigated adsorbed layers could present the typical rheological characteristics of a 2D critical gel [Bouriat et al. (2004), Dicharry et al. (2006), Kopperud and Hansen (2001)].

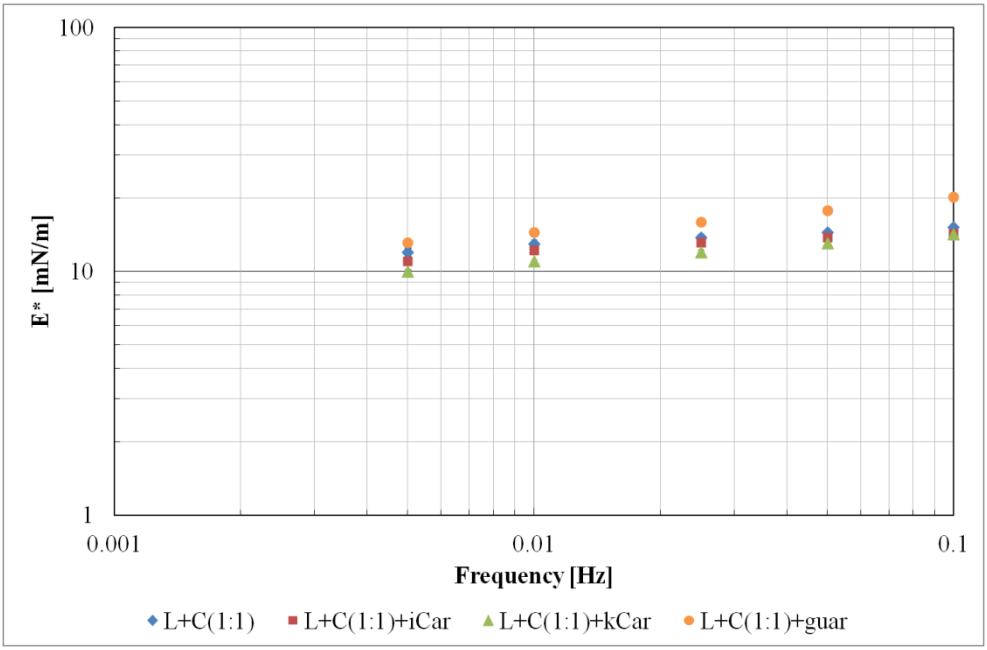


Figure 5. Drop oscillation frequency-dependent interfacial complex dilational modulus E* for β-lactoglobulin/β-Casein mixed proteins, L+C(1:1) (0.1% w/w), and milk proteins+polysaccharides (0.01%w/w), L+C(1:1)+ιcar, L+C(1:1)+kcar, L+C(1:1)+guar adsorbed at the O/W interface

In fact, for the investigated interfaces the loss tangent tanδ remained practically unvaried with pulsation and the log−log plot of the complex interfacial dilational modulus *E* vs* ω was a straight line and followed a scaling law of form:

$$E^* \approx \omega^n \qquad (1)$$

These conditions are relevant to the rheology of 3D-gel near its gelation point (critical gel), as demonstrated by Winter and Chambon for polymers [Winter and Chambon, 1986], and were considered still valid by Bouriat et al. (2004) and Dicharry et al. (2006) to interpret 2D-rheology measurements assuming that the interface can be modelled as a parallel coupling of continuous Maxwellian blocks with relaxation times, τ, and elasticity:

$$k(\tau) = \alpha\tau^{-(n+1)} \qquad (10)$$

where α, which can be identified as the strength of the gel, is proportional to the number of aggregates at interface relaxing with the characteristic time τ.

In agreement with Bouriat et al. (2004) and Dicharry et al. (2006), in the present work the gel critical approach was used, and the *E*vs*ω data were fitted and interpreted with a power law equation (1). According this equation the parameter *n* can be considered an indirect measure of structuring degree of interface, which proves to be as high as the *n* value is

small, while the E* value extrapolated at the frequency of 1 Hz (*k* parameter), is a measure of the strength of the interfacial gel.

This rheological approach may be useful to evaluate and to differentiate the interfacial network behavior of milk proteins in the presence of polysaccharide polymers, on the basis of rheological parameters, which are a measure of structuring properties of the interfacial network. The rheological *n* and *k*, calculated according to equation (1) were reported in table (2) and discussed below.

Sample ID	***k*** **[mN/m·sn]**	***n*** **[/]**	$\mathbf{R^2}$
L+C(1:1)	*18.1 ± 0.5*	*0.07± 0.007*	*0.93*
L+C(1:1)+ιcar	*17.5 ± 0.8*	*0.08 ± 0.005*	*0.96*
L+C(1:1)+kcar	*18.3 ± 0.1*	*0.1± 0.006*	*0.98*
L+C(1:1)+guar	*27.7 ± 0.9*	*0.1± 0.008*	*0.97*

Table 2. Rheological parameters characteristic of interfacial gel model n and k, obtained by equation (1) for β-lactoglobulin/β-Casein mixed proteins, L+C(1:1) (0.1% w/w), and milk proteins +polysaccharides (0.01%w/w), L+C(1:1)+ιcar, L+C(1:1)+kcar, L+C(1:1)+guar adsorbed at the O/W interface.

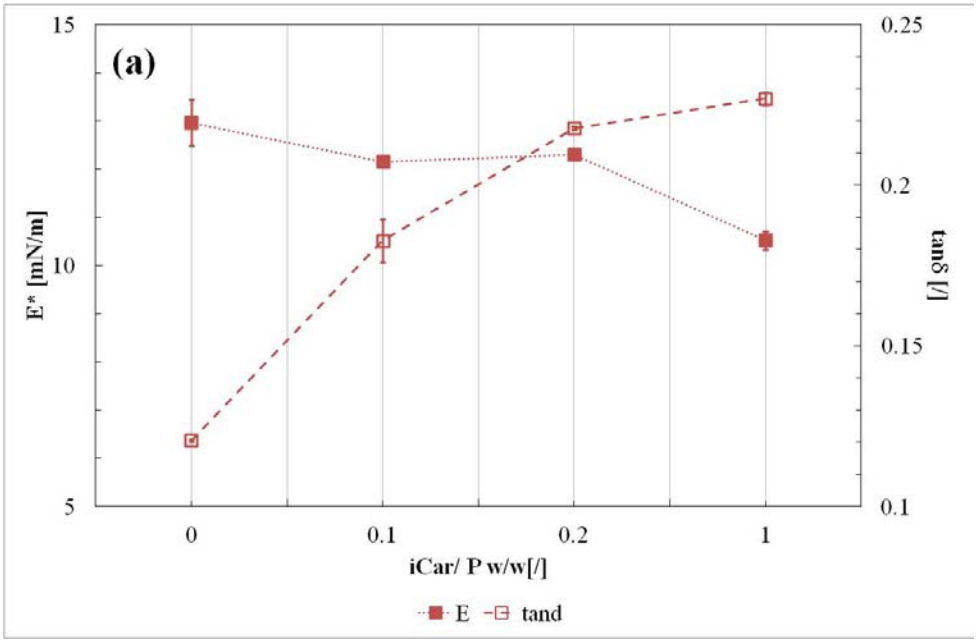
Data in table (2) relative to *n* parameter allowed us to identify a conformation change in the protein film adsorbed at the O/W interface owing to polysaccharide addition in solution, which can be deduced from the increase of *n* parameter of each mixed protein+polysaccharide system when compared to that of proteins alone.

An increasing of *n* parameter indicates clearly a significant reduction of structuring degree of interfacial gel structure, which was, then, affected by complexation of proteins with polysaccharide in the bulk solution and by protein-polysaccharide interactions. The latter probably lead to a formation of a less ordered layer structure at the interface than as with that of milk proteins alone.

A different trend from that of the *n* parameter, was obtained for the k one, which allowed us to estimate the strength of interfacial network. In fact, *k* was invariant in the presence of kcarrageenan and icarrageenan, whereas it varied in the presence of guar gum polysaccharide, exhibiting some increase there. Then the repulsive electrostatic interactions which occur between protein and carrageenan molecules did not influence the degree of hardness of the protein film at the interface, differently from the protein-guar interactions that improved this latter, allowing for more interfacial dilational moduli increase, than found with milk proteins alone.

Then, the higher strength of interfacial protein network in the presence of guar gum polysaccharide with the protein-polysaccharide bulk mixing ratio considered, can be explained by the formation of more compact layer structure, which anyway proved to be less ordered [Ganzevles et al. (2006)].

Since the protein-polysaccharide interactions and their effects on the rheological behavior of the adsorbed layer depend on the system mixing ratio, oscillatory experiments at 0.01 Hz were carried out on mixed layers also for different bulk mixing ratios with the same protein amount used in the solution analyzed above (0.1% w/w and a constant weight ratio between β-casein and β-lactoglobulin of 1:1) (figure (6))



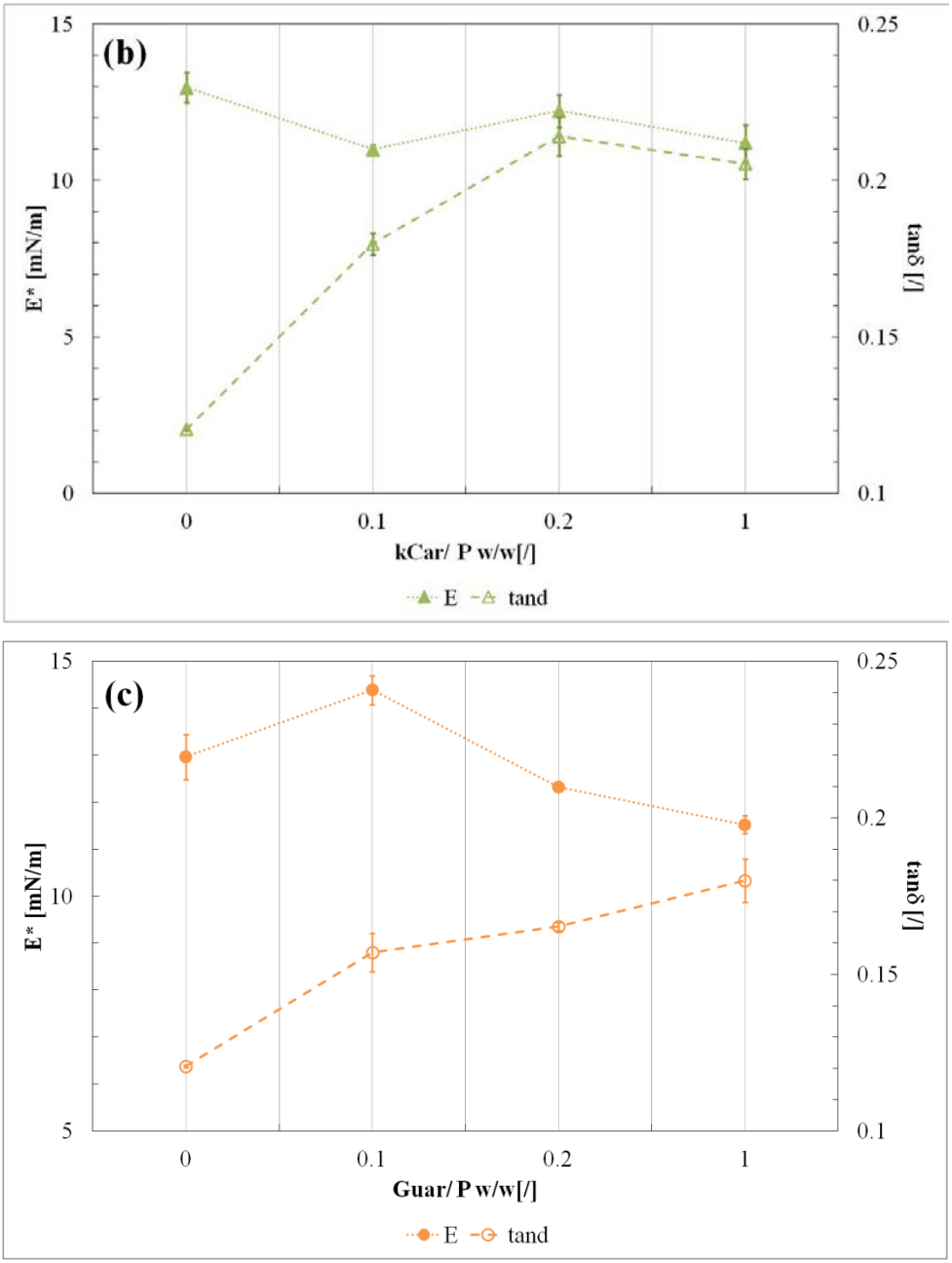


Figure 6. Complex interfacial dilational modulus, E* , and phase angle tangent, tan δ , as a function of polysaccharide/protein ratio used in the mixture: **(a)** icar/p; **(b)** kcar/p; **(c)** guar/p; at a fixed protein amount in the solution of 0.1% w/w , p, and constant weight ratio between β-casein and β-lactoglobulin of 1:1

Figure (6) shows that the higher the bulk polysaccharide/protein mixing ratio, the greater the weakening of protein film adsorbed at the O/W interface, exhibiting a more liquid/like behavior than that of mixed milk proteins alone. In fact, a monotonous decrease of E* and increase of tanδ was registered for all mixed biopolymer systems with increasing the bulk polysaccharide/protein mixing ratio above the 0.1 value, in agreement with Ganzevles et al. (2006).

This behavior, representative of a considerable fluidization of the adsorbed protein layer became more evident and important when the emulsifier/protein weight ratio increased in the mixture. This effect might suggest that the presence of high molecular weight molecules in the bulk which develop more viscous solutions can hinder the protein adsorption at the interface and the formation of a more elastic interfacial network [Leroux et al (2003)].

4. Conclusion

Mixed milk protein β-casein and β-lactoglobulin system adsorption in the presence of various polysaccharides at the sunflower oil/water interface was characterised by dynamic interfacial tension measurements and harmonic drop oscillation experiments to gain knowledge on the interactions between these biopolymers.

Differences in the interaction of carrageenans and guar with the protein were obtained in terms of interfacial tension values and rheological dilational properties. They should be mainly ascribed to different degrees of incompatibility between these biopolymers and to the fact that guar is not charged. For each protein-polysaccharide system a decreasing of interfacial rigidity and then, a substantial weakening of the protein network were measured as indicated by the substantial increase of loss tangent values of mixed protein–polysaccharide system than those of proteins alone. This could be attributed to conformational changes of the protein molecules at the interface, or to rearrangements of the protein–polysaccharide complexes or a combination of both. The protein weakening proved to be more important in the presence of anionic carrageenan molecules than that recorded in the presence of guar gum, neutral biopolymer, indicating differences in the interactions between these biopolymers and milk proteins adsorbed at the O/W interface.

The rheological behavior of each mixed system investigated was affected by the protein/polysaccharide bulk mixing ratio. The higher the bulk polysaccharide/protein mixing ratio, the greater the weakening of the protein film adsorbed at the O/W interface, which exhibited a more liquid/like behavior than that of mixed milk proteins alone.

REFERENCES

1) Bos M.V. and van Vliet T. (2001), Interfacial rheological properties of adsorbed protein layers and surfactants: a review, *Advances in Colloid and Interface Science* (91), 437-471
2) McClements, D. J. (2005). Food emulsions (2nd ed.). Boca Raton, FL: CRC Press.
3) Dickinson E. (2003). Hydrocolloids at interfaces and the influence on the properties of dispersed systems Review. *Food Hydrocolloids* (17), 25-39
4) Rodríguez Patino J. M. and Pilosof A.M.R. (2011). Protein-polysaccharide interactions at fluid interfaces. *Food Hydrocolloids* (25), 1925-1937
5) Perez A.A., Carrara C.R., Carrera Sánchez C., Rodríguez Patino J. M., Santiago L. G. (2009). Interactions between milk whey protein and polysaccharide in solution. *Food Chemistry* (116) 104–113
6) Martinez K.D., Carrera Sanchez C., Pizones Ruiz-Henestrosa, Rodríguez Patino J. M., Pilosof A.M.R. (2007). Soy protein-polysaccharides interactions at the air-water interface. *Food Hydrocolloids* (21), 804-812
7) Benichou A., Aserin A., Garti N. (2002). Protein-Polysaccharide Interactions for Stabilization of Food Emulsions. *Journal of Dispersion Science and Technology* (23), 1-3, 93-123
8) Syrbe A., Bauer W. J. and Klostermeyer H. (1998). Polymer Science Concepts in Dairy Systems-An Overwiew of Milk protein and food hydrocolloid interaction. *Dairy Journal* (8) 179-193
9) Singh H., Tamehana M., Hemar Y., Munro P. A.(2003). Interfacial compositions, microstuctures and properties of oil-in-water emulsions formed with mixtures of milkproteins and k-carrageenan: 1. Sodium caseinate. *Food Hydrocolloids* (17), 539-548
10) Suk Gu Y., Decker E.A., McClements D.J. (2004). Influence of pH and ι-Carrageenan Concentration on Phisicochemical Properties and Stability of β-Lactoglobulin-Stabilized Oil-in-Water Emulsions. *Journal of Agricultural Food Chemistry* (52),3626-3621
11) Piculell, L. (1995). Gelling carrageenans. In A. M. Stephen (Ed.), Food polysaccharides and their applications (pp. 205–243). New York: Dekker (Marcel).

12) van de Velde1 F. and, De Ruiter G. A. (2005). In A. Steinbuchel and S. Ki Rhee (Ed.), Polysaccarides and Polyamides in food industry, volume 1, (pp.245-270). Germany

13) Miquelim J.N., Lannes S.C.S, Mezzenga R. (2010). pH Influence on the stability of foams with protein-polysaccharide complexes at their interfaces. *Food Hydrocolloids* (24), 398-405

14) Narchi I., Vial C.D. (2009). Effect of protein-polysaccharide mixtures on the continuous manufacturing of foamed food products. *Food Hydrocolloids* (23), 188-201

15) Srichamroen A. (2007). Influence of Temperature and Salt on Viscosity Property of Guar Gum. *Naresuan University Journal* (15), 55-62

16) Dickinson E. (1999), Review Adsorbed protein layers at fluid interfaces: interactions, structure and surface rheology. *Colloids and Surfaces B: Biointerfaces* (15), 161-176

17) W*ü*stneck R., Kragel J., Miller R., Wilde P., Clark D.C. (1996), The adsorption of surface-active molecules between β-casein, β-lactoglobulin and ionic surfactants and their rheological shear behavior. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* (114), 255-265

18) Williams A. and Prins A. (1996), Comparison of the dilational behavior of adsorbed milk proteins at the air-water and oil-water interfaces, *Colloids and Surfaces A: Physicochemical and Engineering Aspects* (114), 267-275

19) Jensen R.G., Handbook of Milk Composition, Academic Press, New York (U.S.A) 1995, chapter 5

20) Biresaw G., Liu Z. S., Erhan S.Z.(2008), Investigation of the Surface Properties of Polymeric Soaps Obtained by Ring-Opening Polymerization of Epoxidized Soybean Oil, Wiley InterScience (www.interscience.wiley.com)

21) Lin S.Y., Wang W.J., Lin L.W., Chen L.J. (1996) Systematic effects of bubble volume on the surface tension measured by pendat bubble profiles *Colloids and Surfaces A: Physicochemical and Engineering Aspects* (114), 31-39

22) Camino N.A., Pèrez O.E., Carrera Sanchez C., Rodriguez Patino J.M., Pilosof A.M.R. (2009). Hydroxypropylmethylcellulose surface activity at equilibrium and adsorption dynamics at the air-water and oil-water interfaces. *Food Hydrocolloids* (23) 2359-2368

23) Lucassen J. and van den Tempel M. (1972), Dynamic measurements of dilational properties of a liquid interface, *Chemical Engineering Science* (27) 1283-1291

24) Freer E. M., Sub Yim K., Fuller G. G., Radke C. J. (2004), Shear and Dilatational Relaxation Mechanisms of Globular and Flexible Proteins at the Hexadecane/Water Interface. *Langmuir (20),*10159-10167

25) Ravera F., Liggieri L., Loglio G. Dilational rheology of adsorbed layers by oscillating drops and bubbles. In: R. Miller, L. Liggieri Interfacial Rheology Volume 1 , Brill Academic Pub, Leiden Boston 2009, 138-177

26) Myrvold R. and Knut Hansen F. (1998), Surface Elasticity and Viscosity from Oscillating Bubbles Measured by Automatic Axisymmetric Drop Shape Analysis, *Journal of Colloid and Interface Science* (207) 97–105

27) Ganzevles R.A., Zinoviadou K., van Vliet T., Cohen Stuart M.A., de Jongh H.H.J. (2006). Modulating Surface Rheology by Electrostatic Protein/Polysaccharide Interactions. *Langmuir* (22), 10089-10096

28) Dicharry C., Arla D., Sinquin A., Graciaa A., Bouriat P. (2006) Stability of water/crude oil emulsions based on interfacial dilatational rheology *Journal of Colloid and Interface Science* (297) 785–791

29) Bouriat,P., El Kerri N., Graciaa A., Lachaise J. (2004) Properties of a Two-Dimensional Asphaltene Network at the Water-Cyclohexane Interface Deduced from Dynamic Tensiometry. *Langmuir* (20) 7459-7464

30) Winter H.H. and Chambon F.(1986) Analysis of linear viscoelasticity of a cross-linking polymer at the gel point *Journal of Rheology* (30) 367-382

31) Leroux J., Langendorff V., Schick G., Vaishnav V., Mazoyer J. (2003). Emulsion stabilizing properties of pectin. *Food Hydrocolloids* (17) 455-462

Chapter 7

Short-term stability of oil-in-water emulsions formed with milk whey proteins: influence of i- carrageenan and *k*-carrageenan

Abstract

The influence of the i-carrageenan and the k-carrageenan concentration on the properties of oil-in-water (O/W) emulsions stabilized by milk whey protein was investigated by measuring the particle size distribution, the creaming stability and the bulk and interfacial rheological properties. The main goal is to determine the experimental conditions where these polysaccharides can be used to improve emulsion stability.

Emulsions were formed using 3 wt % of commercial milk whey proteins (P) in the aqueous phase, to have a considerable amount of protein remaining in the aqueous phase after emulsion formation, whereas bulk carrageenan concentration was varied from 0 up to 0.2 wt%. The emulsions formed with carrageenan proved to be more stable compared with those stabilized with milk whey proteins alone, but the stability improved considerably by adding i-carrageenan (icar) rather than k-carrageenan (kcar). The excellent stability towards phase separation found for the stored i-carrageenan emulsion, and its bulk and interfacial properties, all together suggested the ability of the i-carrageenan and the inability of the k-carrageenan to reduce partial coalescence either by providing a sufficiently thick continuous phase or by acting as a protective coating for oil droplets. The behavior against the creaming phenomena of these systems proved to be independent of their droplet size distribution for all emulsions studied, but strongly affected by their bulk and interfacial properties. These were, in turn, highly influenced by the type and concentration of the carrageenan present in the continuous phase .

1.Introduction

Many food products can be categorized as oil-in-water (O/W) emulsions, which consist of small lipid droplets dispersed in an aqueous medium, e.g., milk, cream, beverages, sauces, dressings, dips, and desserts [McClements (1999)]. Emulsion-based products are easily destabilized during processing and storage because they are thermodynamically unstable systems. Emulsion destabilization may occur through a variety of physicochemical processes, including gravitational separation, flocculation, coalescence, and Ostwald ripening [McClements (1999)]. Many factors contribute to the destabilization of O/W emulsions, including the specific type of ingredients present, the way that the emulsion was produced, and the environmental conditions that they experience.

Emulsifiers are commonly used in food emulsion systems to increase their short and long term stability [McClements (1999), Bos and Van Vliet (1998), McClements (2005), Dickinson (2003)]. They are surface active ingredients that facilitate the production of small droplets during homogenization by lowering the interfacial tension and improving the emulsion stability by forming protective membranes around the droplets and/or by generating repulsive forces between droplets [McClements (1999), Bos and Van Vliet (1998), Murray (1998)].

The majority of food emulsions are created and stabilized by proteins representing a class of surface active components that are very important from the interfacial science point of view. Particularly, the proteins derived from milk are widely used for these purposes since they are valued as food ingredients with excellent surface-active, emulsifying and colloid-stabilising characteristics [Dickinson (1998), Rouimi et al. (2005), Dickinson (1999), Dickinson (2001), Raikos (2010), Williams and Prins (1996), Cornec et al. (1996)]. These proteins can be used in small quantities to prepare stable emulsions, but the long-term stability of protein-stabilized emulsions is highly sensitive to different factors such as pH, ionic strength and temperature [Gu et al. (2004)].

To improve the emulsion stability polysaccharides species are frequently used together with proteins in food systems, owing to their known thickening properties which allow them to control the rheology and network structure of the continuous phase via viscosity modification or gelation in the aqueous continuous phase, hence retarding phase separation and gravity-induced creaming [McClements (1999), Dickinson (2003), Rodrìguez Patino and Pilosof (2011)]. Emulsion stability induced by polysaccharides could generally be obtained at a sufficiently high polysaccharide concentration at which they interact strongly with the adsorbed protein layer, thus producing the repulsive forces [McClements (1999),

Dickinson (2003), Nor Hayati et al. (2009)] In this way, the emulsion becomes sterically stabilized by the secondary polysaccharide layer.

On the contrary, at certain low concentrations, polysaccharides may destabilize the emulsion by means of depletion and bridging flocculation. The depletion flocculation causes a phase separation between the adsorbed protein and the dispersed polysaccharide component.

Although traditionally associated with thickening and gelation behavior, polysaccharides can also influence the interfacial properties of dispersed systems, affecting the protein adsorption at the interface, exhibiting their own interfacial activity generally ascribed to the presence of protein impurities, or through different interactions with protein molecules resulting from blending these biopolymers [Dickinson (2003), Rodrìguez Patino and Pilosof (2011), Perez et al. (2009)]. Then, some polysaccharides have been shown to improve emulsion properties by forming an interfacial complex with adsorbed protein layer after homogenization [Rodrìguez Patino and Pilosof (2011), Suk Gu et al. (2004)].

Although much is known about functional properties of individual food proteins and polysaccharides in many model systems, our knowledge of the protein-polysaccharide interactions in food systems is still limited [Dickinson (2003), Rodrìguez Patino and Pilosof (2011), Syrbe et al. (1998), Singh et al. (2003), Suk Gu et al. (2004)].

It is well known that aqueous solutions of some mixtures of specific proteins and polysaccharides undergo phase separation. Two kinds of behavior have been recognized; coacervation and incompatibility. Complex coacervation involves coprecipitation of protein and polysaccharide under the influence of net attractive interactions between proteins and polysaccharides. Thermodynamic incompatibility, on the other hand, involves separation of protein and polysaccharide-rich phases under the influence of repulsive interactions [Dickinson (2003), Rodrìguez Patino and Pilosof (2011), Benichou et al. (2002)].

In food emulsions containing protein and polysaccharide, any of these interactions may take place in the aqueous phase of the system, with consequences for the structure, rheology and stability of the emulsion [Dickinson (2003), Rodrìguez Patino and Pilosof (2011), Syrbe et al. (1998), Singh et al. (2003), Gu et al. (2004)].

In addition, it is important to consider the interfacial behavior of these biopolymers, the interactions between protein and polysaccharides at the droplet surface and the consequences of these two factors for emulsion stability.

Carrageenans are anionic polysaccharides extracted from red seaweed (Rhodophyta), consisting of linear polymers of alternating β-1,3 and α-1,4 linked galactose residues. There are three generic carrageenan families (k-, i- and l-) with varying number and position of sulphate groups on the galactose dimer. When dispersed in aqueous solution, k-carrageenan and i-carrageenan undergo a coil (disordered state) to helix (ordered) transition, depending on the temperature and ionic strength [Piculell (1995)]. They are commonly used as stabilizers, thickeners and gelling agents in milk-based products [Gu et al. (2004)].

Whey proteins are very important nutritional ingredients, as well as being excellent emulsifiers, and are widely used emulsifiers in many food emulsions. They are particularly useful in this dual role when they are used to stabilize oil droplets in food emulsions. Whey proteins are globular proteins present in bovine milk with concentration of about 6.5 g/l (of which 3.1, 1.2, 0.4, and 0.8 g/l are made up of β-lactoglobulin, α-lactalbumin, serum albumin, and immunoglobulins, respectively)[Jansen (1995)].

There have been many studies of interactions of carrageenan polysaccharides with milk proteins, but little information is available on the details of carrageenans interactions in milk protein stabilized emulsions [Gu et al. (2004), Gu et al. (2005), Singh et al. (2003)].

The aim of this work is to understand the behavior of emulsions formed with commercial milk whey protein products, and i/k-carrageenans. Emulsions were formed using 3 wt% protein in the aqueous phase, which corresponds to about the total protein amount in the milk, in order to have a considerable amount of protein remaining in the aqueous phase after emulsion formation, whereas bulk carrageenan concentration was varied from 0 up to 0.2 wt %. Then, the influence of the latter concentration on the stability of milk whey stabilized emulsions was investigated by measuring particle size distribution, creaming, interfacial tension, bulk and interfacial rheological properties, so as to determine the range of experimental conditions where both polysaccharides can be used to improve emulsion stability.

2. Material and methods

2.1 Materials

Commercial milk whey proteins (90% purity) obtained by cross flow microfiltration process were purchased from Enervit S.p.A. (Italy) and was used without further purification. The protein product contains ≈ 92% dry matter of which about 98% was protein, 1% carbohydrates and 1% fat.

Sunflower oil (Rif:356241) was purchased from Carlo Erba Reagents (Italy). It contains triglycerides and free saturated and unsaturated fatty acid (0.4%) .

Both i-carrageenan (Ref:C1138), k-carrageenan (Ref: C1013) were obtained from Sigma Aldrich, Inc (Germany).

2.2 Solution preparation

Protein solutions were prepared by adding the protein powder to twice-distilled water and then stirring for 60 minutes at room temperature to ensure complete dispersion.

Carrageenan solutions were prepared by dissolving the powder in to twice-distilled water at room temperature and, then, heating up to 75 °C for 30 minutes [Singh et al. (2003)]. Appropriate quantities of protein and polysaccharide solutions were then mixed at room temperature and stirred for further 30 minutes, to give the total protein concentration of 3 wt% and the polysaccharide concentrations varying between 0.1-0.2 wt %.

The samples prepared and analyzed were indicated by according the table (1).

Sample ID	*wt % carrageenan*	
	i-carrageenan	*k-carrageenan*
P	0	0
P+icar(0.1)	0.1	0
P+icar(0.2)	0.2	0
P+kcar(0.1)	0	0.1
P+kcar(0.2)	0	0.2

Table 1. Carrageenan amount used in the aqueous solution containing milk whey protein (3 wt%)

The twice distilled water used throughout all experiments was obtained from a Milli-Q purification system (Millipore, USA), and it was checked for contaminants before each experiment, measuring the surface tension of the buffer solution at the air/water boundary at ambient temperature. No aqueous solutions with a surface tension other than accepted in the literature (72-73 mN/m at 20°C) were used.

All sample solutions were freshly prepared (within no more than 24 h) for the characterization and two replicates were prepared for each sample.

2.3 Emulsion preparation

O/W emulsions, identified with the same ID of the aqueous solutions used for the their preparation (*paragraph 2.2*), were prepared by homogenizing 5 wt% sunflower oil and 95 wt% surfactant aqueous solution at room temperature.

The oil and emulsifier solution were blended with a rotor stator system (Ultra-Turrax T 25, IKA, Germany) by using the same emulsification step for all samples. Emulsions were prepared by adding the oil phase to the aqueous one at 1500 rpm at room temperature for a mixing time of 260 seconds. Homogenization conditions were established experimentally in order to produce a stable protein emulsions for a period of at least 20 minutes. The pH was measured (micropH 2002, Alessandrini , Italy) and it was equal to 6.4 ± 0.1 for all samples. The emulsions were stored for 24 hours at room temperature and were analyzed in this time period.

2.4 Emulsion droplet size measurements and microscopic characterization (DSD)

The morphology of the emulsion droplets was observed with a contrast phase microscopy (MX5000, Meiji, Japan), equipped with phase contrast objective 40X. In order to produce photomicrographs without droplets aggregates, difficult to be analyzed, samples were diluted in bi-distilled water with a ratio 1:10, verifying no effects of dilution on DSD characterization.

A drop of diluted emulsion was then placed on a microscope slide, covered by a cover slip, and observed. An image of the emulsion was acquired using an image database software (*dhs* image database, Germany), which by greyscale detection gives as a result the number-based surface equivalent diameters.

The DSD was interpreted by the lognormal model [Hollingsworth and Johns (2003), Melle et al. (2005), Egger and McGrath (2006), Lupi (2009)] reported in the equation (1).

$$f(d) = \frac{1}{d \cdot \sigma_{\ln} \cdot \sqrt{2\pi}} \exp\left[\frac{-\left(\ln d - \overline{d_g}\right)^2}{2\sigma_{\ln}^{\ 2}} \right] \qquad (1)$$

Where $\overline{d_g}$ and $\sigma_{\ln}$ (the geometrical mean droplet diameter and the standard deviation respectively) are the model parameters. A mean diameter $\overline{d_s}$ and variance σ_s^2 can be

derived from the model parameters by the equation (2) and (3) respectively [Walpole at al. (2007)].

$$\overline{d_s} = e^{\overline{d_g} + \sigma_{\ln}^2/2} \quad (2)$$

$$\sigma_s^2 = e^{2\overline{d_g} + \sigma_{\ln}^2\left(e^{\sigma_{\ln}^2} - 1\right)} \quad (3)$$

The square root of the variance is the standard deviation σ_s, and it is commonly considered as an index of polydispersity.

2.5 Creaming stability measurements

Creaming stability was determined as described by Ye and Singh (2006). 10 ml of freshly prepared emulsions was poured in triplicate into graduated tubes (1 cm diameter and 13 cm high) and the samples were kept at room temperature (22°C within ± 1 °C) for 1 day. The separated cream layer was read after storage for different times (1, 4, 10 and 24 hours after the filling of the tubes). Creaming stability was represented by a creaming index defined as:

$$Cream.\% = \frac{H_c}{H_e} * 100 \quad (4)$$

Where H_c is the height of cream layer and H_t is the height of total emulsion.

The creaming index provided indirect information about the extent of droplet aggregation in an emulsion.

2.6 Viscosity measurements

Rheological measurements were performed by using a controlled strain rheometer ARES-RFS (TA Instruments, U.S.A.) The rheometer was equipped with parallel plates geometry (parallel plates ϕ=50mm, gap=1.5mm) to characterize *P+icar(0.2)* emulsion sample whereas for all other samples it was equipped with a coaxial cylinders geometry (cup diameter=34mm, bob diameter=32mm, bob length=33mm).

All the measurements were performed at 20°C. They were carried out at 1 hour and at 1 day after the homogenization for all samples and for the more stable emulsions respectively.

2.7 Interfacial properties measurements

Mixed protein-polysaccharides systems adsorbed at the O/W interface were characterized by transient interfacial tension measurements and dilational dynamic tests.

Axialsymmetric drop shape analysis (ADSA) was used to calculate drop volume, area and interfacial tension by using an automated pendant drop tensiometer (FTA200 First Ten Angstroms, USA) equipped with the *fta32 v2.0* software. Details of this apparatus are given by Biresaw at al. (2008).

The instrument comprises an automated pump that can be fitted with various sizes of syringes and needles to allow for control of pendant drop formation and of sinusoidal variations in the drop volume or surface area by software.

An automated image viewing and capturing system, with various image capture triggering options, was used to capture drop image.

The computer hardware and software also provide the capability for data capture, storage, analysis, and transfer. The software allows for an automated drop shape analysis of the captured drop image, and for measuring the surface-interfacial tension of the drop formed in air or in a second fluid at rest and in periodic motion conditions of the interface respectively.

Image acquisition and regression of the interfacial tension were performed directly with commercially available drop-image software by fitting the Bashforth-Adams equation to the drop shape [Biresaw at al. (2008)].

Drop-image software also controlled an automatic pipetting system that maintained constant drop volume with time period over which dynamic tensions were measured.

2.7.1 Dynamic interfacial tension

The method adopted to measure the interfacial tension of the investigated solutions with time (γ *vs* t), by using an automatic drop tensiometer, involved the analysis of the profile of the drops created in the oil phase, and kept at rest (constant volume).

The experiments were carried out at room temperature (22°C within ± 1 °C), placing sample aqueous solutions in a 100 µl glass Hamilton syringe equipped with a 20 Gauge stainless steel needle, and delivering drops in a rectangular quartz cuvette (5ml) containing the desired phase.

Drop volumes of 7-8 μl were used in every test in order to measure interfacial tension values, independent of the drop size [Lin et all, 1996]. High drop volumes of the kind chosen, proved to be generally more suitable for these tests, because they increased the number of profile points, which can be used for drop shape analysis [Lin et all, 1996].

The experiments started with the creation of the drop; then drop images were continuously taken from a CCD camera and digitalized, registering the interfacial tension values over the test time.

Drop profile was monitored up to a maximum time of 180 min, which assures the molecules adsorption at the interface and the reaching of a quasi-equilibrium interfacial tension value.

Equilibrium of γ was assumed when the interfacial tension did not change by more than 0.5 mN/m in 30 min [Camino et al. (2009)].

2.7.2 Dilational rheological properties

The method adopted to determine the dilational rheological properties of the interface, by using an automatic drop tensiometer, involved the profile analysis of the drop formed in the air/oil phases and subjected to periodic motion conditions, according to the oscillating drop methodology, able to measure the interfacial visco-elasticity vs frequency.

Oscillating drop experiments are usually performed subjecting the interface to an infinitesimal sinusoidal compression and expansion, then by applying a frequency sweep to the surface area A, in order to measure the interfacial dilational modulus.

The surface dilational modulus derived from the change in surface tension γ (measured dilational stress) (equation 5), resulting from a small change in surface area A (dilational strain) (equation 6), may be described by equation (7) [Lucassen and van den Tempel, (1972)]:

$$\gamma = \gamma_0 + \Delta\gamma \sin(\omega t + \delta) \tag{5}$$

$$A = A_0 + \Delta A \sin(\omega t) \tag{6}$$

$$E^* = \frac{d\gamma}{d \ln A} \tag{7}$$

Where γ_0 and A_0 are the equilibrium reference surface tension and the unperturbed interfacial area of the drop respectively, Δγ and ΔA are the stress and strain amplitude

respectively, and δ is the phase angle between stress and strain, measure of the relative film viscoelasticity.

Since the drop area periodically oscillates, the dilational modulus exhibits two contributions: an elastic part accounting for the recoverable energy stored in the interface (storage modulus, *E'*), and the dissipative part, accounting for energy lost through relaxation processes (loss modulus, *E''*).

$$E^* = E' + iE'' = \frac{\Delta\gamma}{\Delta A/A_0}\cos(\delta) + i\frac{\Delta\gamma}{\Delta A/A_0}\sin(\delta) \tag{8}$$

Then the surface dilatational modulus, E*, as a measure of the total material resistance to dilatational deformation (elastic and viscous), is a complex quantity composed of real and imaginary part [Freer et al. (2004), Ravera et al. (2009), Myrvold and Hansen (1998)].

A perfectly elastic material stress and strain are found in phase $\delta = 0$ and the imaginary term is zero, on the contrary, for a perfectly viscous material $\delta = 90°$, where the real part is zero. The loss angle tangent can be defined by equation (9):

$$\tan\delta = E''/E' \tag{9}$$

In this work, we applied a periodic strain by differentially oscillating the drop area at a prefixed frequency value, and we measured the periodic stress response with time.

Then the dilational viscoelastic parameters of interface, the dilational complex modulus (E*), its elastic (E') and viscous (E'') components and the loss angle tangent were measured as a function of the adsorption time, *t*.

The *Time Sweep Tests* were carried out by using deformation amplitude ($\Delta A/A_0$) values of 3-5% and angular frequency ones varying in the 0.005 Hz-0.1 Hz range.

The percentage area change was determined before each time sweep test by performing *Amplitude Sweep Experiments* (data not shown) realized at the extreme frequency values of the investigated range. The latter experiments were useful to assure that system response was not influenced by perturbation amplitude (linear viscoelastic behavior) and to be in the linear region, so as to avoid oscillation amplitude that causes disruption of the supramolecular organization or provides adequate measurement sensitivity.

The duration of each test was established so as to register equilibrium values of the dilational moduli with time (maximum variation of 3% was accepted).

The E* *vs* ω curves were then obtained by using the latter values for the frequency range investigated.

3. Results and discussion

3.1 Emulsion short-time stability

3.1.1 Droplets size distribution

Average droplet diameter (d_s) and standard deviation (σ_s) of the O/W emulsions from the lognormal model were evaluated and compared in figures (1) and (2) respectively, at different storage times in order to investigate the effects of both the carrageenan type and concentration on them [McClements (1999), Ahmed at al. (1999)]. Specifically, the time dependence of these parameters was evaluated up to 24 hours to study the short-term stability of the emulsions investigated, which has often been accompanied by changes to the morphology of the oil droplets and thereby, by an increasing of either d_s or σ_s [McClements (1999), Tchlakova et al. (2004), Bylaite et al. (2001), Ahmed at al. (1999)].

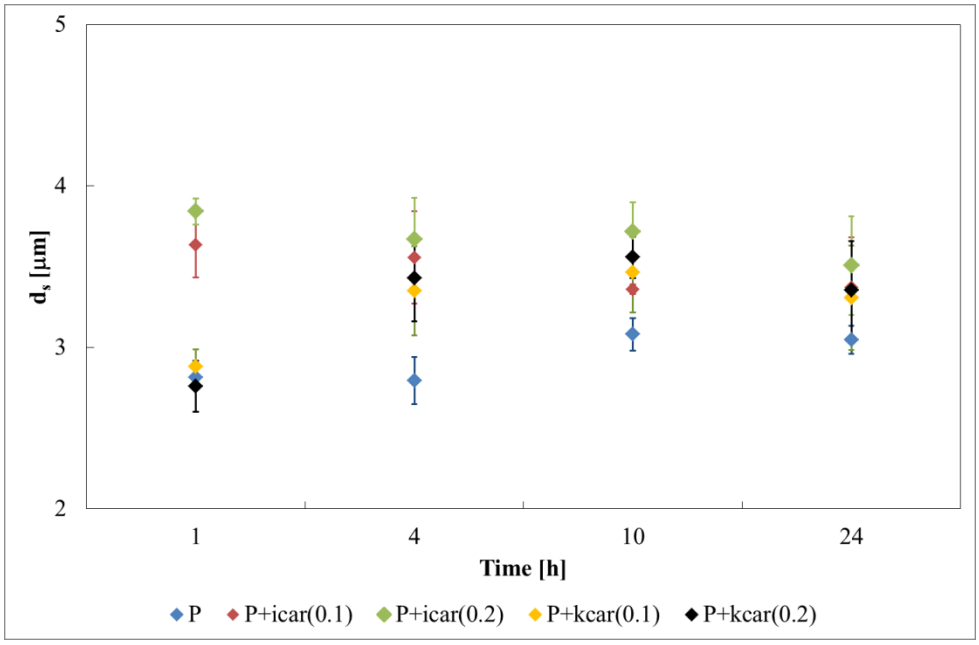


Figure 1. Effect of i-carrageenan and k-carragenan on average droplet diameter (d_s) with time of emulsions formed with 3 wt% milk whey proteins

From figure (1) it can be noticed that in the absence of polysaccharides (sample P) the d_s was smaller than in their presence at each storage time considered, except after 1 hour when its value deviated from those of the k-carrageenan emulsions. Then, the average droplet diameter of the emulsions investigated increased slightly for the P+kcar and P+icar systems, and this enhancement proved to be more relevant when increasing the bulk carrageenan concentration, especially in the case of the i-carrageenan system.

The d_s variation obtained is in accordance with the findings of many authors [Singh et al. (2003), Gu et al. (2004), Gu et al. (2005)] and it can be ascribed either to the thickening of the emulsion continuous phase (figure 5) made by polysaccharide molecules owing to their known gelation behavior or to some changes of the protein interfacial activity (figures 7-8) as a result of the steric and electrostatic interactions between protein and carrageenan molecules [Dickinson (2003), Rodrìguez Patino and Pilosof (2011), Perez et al. (2009)].

In fact, the presence in the bulk of high molecular weight molecules such as the carrageenans, which develop more viscous solutions, can hinder the protein adsorption at the interface or lead to the formation of complexes between these biopolymers mainly by electrostatic interactions or hydrogen bonding [Leroux et al (2003), Rodrìguez Patino and Pilosof (2011)]. Both these phenomena could be considered responsible for the increase in the average droplet diameter.

Regarding the time dependence of d_s, from the figure (1) it can be noticed that no significant changes in the mean droplet diameter was detected up to 24 hours for all the emulsions prepared, indicating no relevant evidence of droplet aggregation [Gu et al. (2005)].

Despite this, it is possible to individuate small variations of d_s for the emulsions containing k-carrageenan and milk proteins whereas no changes were found for the i-carrageenan emulsions at each polysaccharide concentration investigated, which then can be considered the most stable. In fact, the increase of the mean droplet diameter with time has often been accompanied by the onset of creaming instability phenomena owing to the growth of drop size which reflects an ongoing coalescence process inside the emulsion [McClements (1999), Tchlakova et al. (2004)]. Thus, to examine this aspect also the emulsion polydisperity degree, in terms of the standard deviation values (σ_s), was considered in this analysis, being an important factor to evaluate the morphology change of the emulsion droplets as well as the mean droplet size.

The comparison of σ_s (figure 2) allowed the individuation of similar time dependences for all the emulsions investigated with the exception of the sample P+icar(0.2), which exhibited constant values of the standard deviation with time, and also the highest ones. the On the contrary, the other samples showed a slight increment of σ_s with time up to reaching of the same values of P+icar(0.2) sample after 24 hours. This growth confirmed the above considerations about the greater ability of i-carrageenan than k-carrageenan to reduce partial coalescence of oil droplets in the protein emulsions considered.

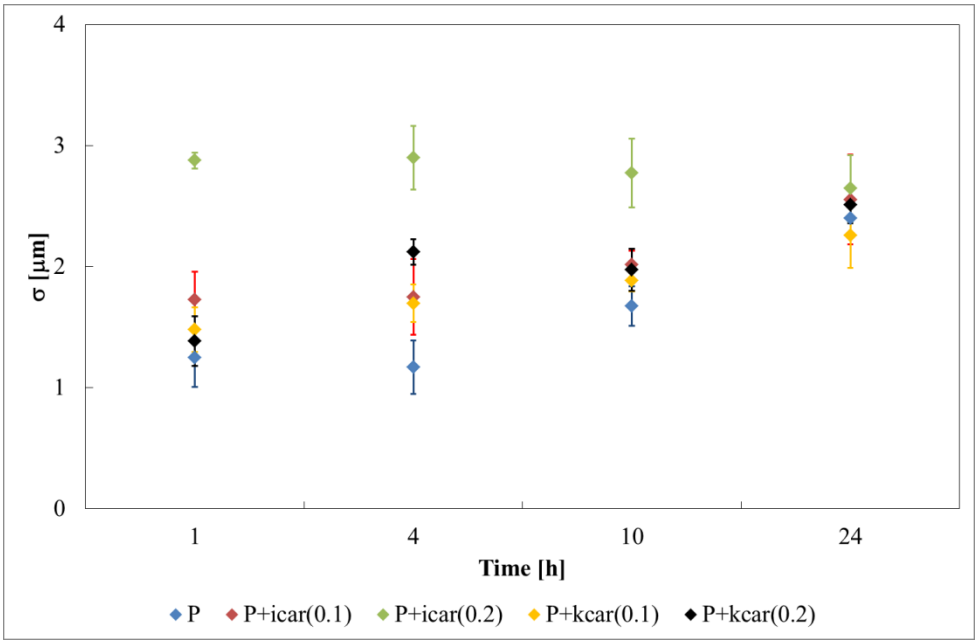


Figure 2. Effect of i-carrageenan and k-carrageenan on the standard deviation (σ_s) with time of emulsions formed with 3 wt% milk whey proteins

To support data obtained by analyzing drop size distributions (DSD), also microphotographs of emulsions after 1 hour and 24 hours storage time were reported for all the samples prepared in figures (3a) and (3b) respectively.

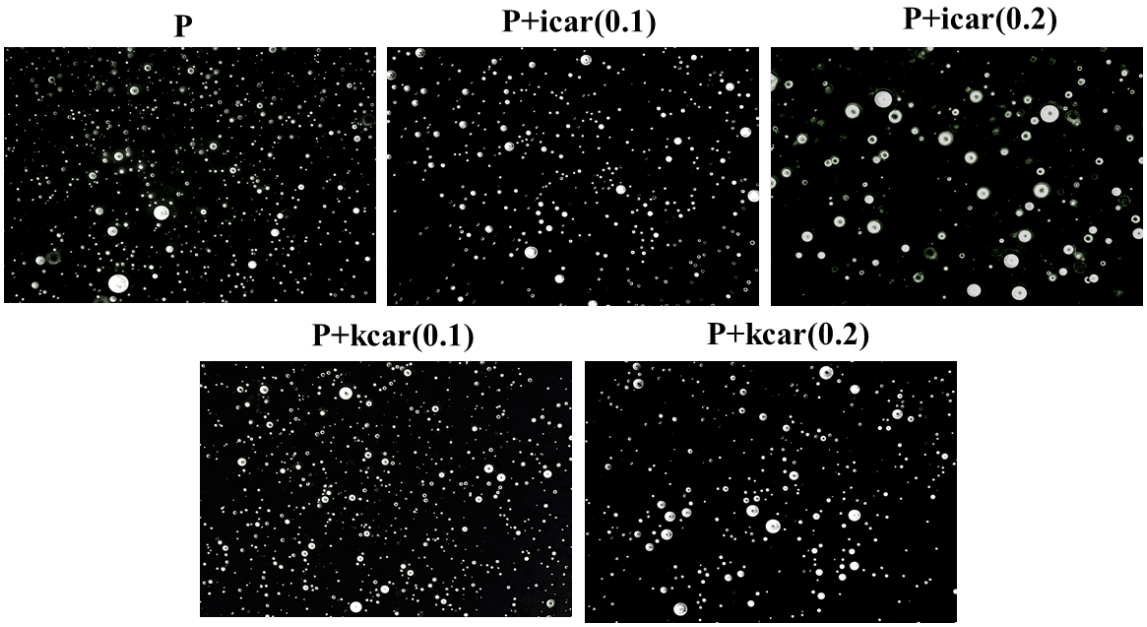


Figure 3a. Microphotographs of the samples (dilution 1:10) after 1 hour storage time (magnification 40X)

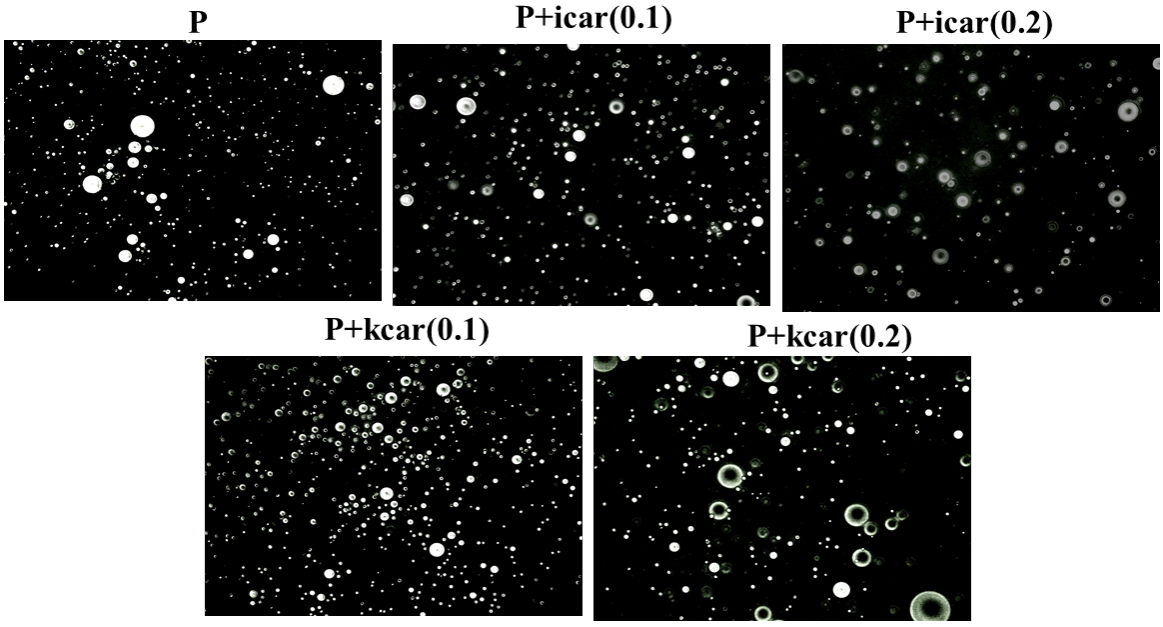


Figure 3a. Microphotographs of the samples (dilution 1:10) after 24 hour storage time (magnification 40X)

In fact, the microphotographs reported can show a wide size range of oil droplets for all emulsions and their substantial increasing with the storage time, except for the P+icar(0.2) sample, thus supporting average droplet size and polydispersity characteristics discussed above.

3.1.2 Creaming stability

The creaming stability of the emulsions prepared and stored for 1 day, expressed in terms of the creaming index , *% Cream.*, (paragraph *2.5*) is here discussed. Data obtained measuring the height of the boundaries separating the different layers formed with time were reported in figure (4). It showed the *% Cream.* of the O/W emulsions investigated proved to be strongly affected by the type and bulk concentration of carrageenan used in the system.

Specifically, the emulsions formed with carrageenan proved to be more stable compared with that stabilized with milk whey proteins alone. In fact, the *% Cream* index recorded for the P emulsion was higher than those of all the other samples at each time investigated, in accordance with the results of previous studies [Gu et al. (2005), Singh et al.(2003)].

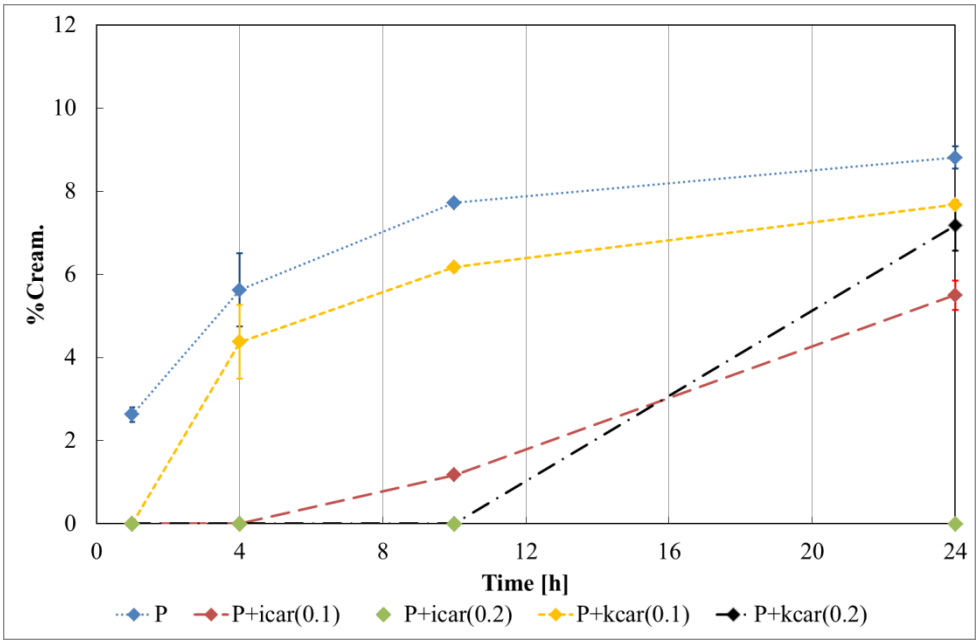


Figure 4. Effect of i-carrageenan and k-carrageenan on creaming stability (% Cream.) with time of emulsions formed with 3 wt% milk whey proteins

Thus, it can be noticed that the addition of the i-carrageenan and the k-carrageenan caused a decreasing of both the rate of cream layer formation and the *% Cream*, which became more relevant when increasing the polysaccharide bulk concentration.

However, it is important to emphasize that this effect was strongly dependent on the polysaccharide type used, because the emulsion stability proved to improve by adding i-carrageenan rather k-carrageenan [Gu et al. (2005)].

In fact, the emulsions stabilized with k-carrageenan exhibited *% Cream* not very different from that of the P sample after 24 hours and were also more unstable than those formed with i-carrageenan, which then showed the best behavior against the creaming phenomena.
This behavior can be attributed to the structure characteristic of i-carrageenan, which has been demonstrated to be a more densely charged than that of k-carrageenan at pH 6 values, and then more effective at creating highly charged interfacial membranes, reducing the tendency to flocculation depletion and to coalescence [Gu et al. (2005)].
Thus, at the pH conditions above the IEP of the protein, just the presence of electrostatic interactions between carrageenan and milk whey proteins coated droplets, which are strongly dependant on the carrageenan type present in the system, can be responsible for the different emulsion stability observed.
From a comparison of the droplet size distribution and the creaming data it appears clear that the emulsion stability was independent of their droplet size distribution for all emulsions studied. In fact, no increase of emulsion stability was found with decreasing droplet size and the P+icar(0.2) sample, despite its larger droplet size and standard deviation, proved to have the best behavior.
This suggests that the stability of the O/W emulsion investigated could be dependent on their bulk and interfacial properties, which are both the result of the addition of the carrageenan molecules to the protein system. Polysaccharides are in fact thickener agents used to increase the external phase viscosity reducing the movement and aggregation of the droplets [McClements (1999), Singh et al. (2003), Nor Hayati at al. (2009)]. Moreover, they can interact with protein adsorbed at the O/W interface, modifying the adsorption rate and the protein network structure.
In this regard the importance of considering the bulk viscosity and the interfacial properties of the investigated systems is clear. These aspects are discussed below.

3.2 Bulk viscosity

The flow behavior of the O/W emulsions after 1 hour storage was analyzed as described in *2.5* and the results obtained, expressed in terms of viscosity vs shear rate were compared in figure (5).

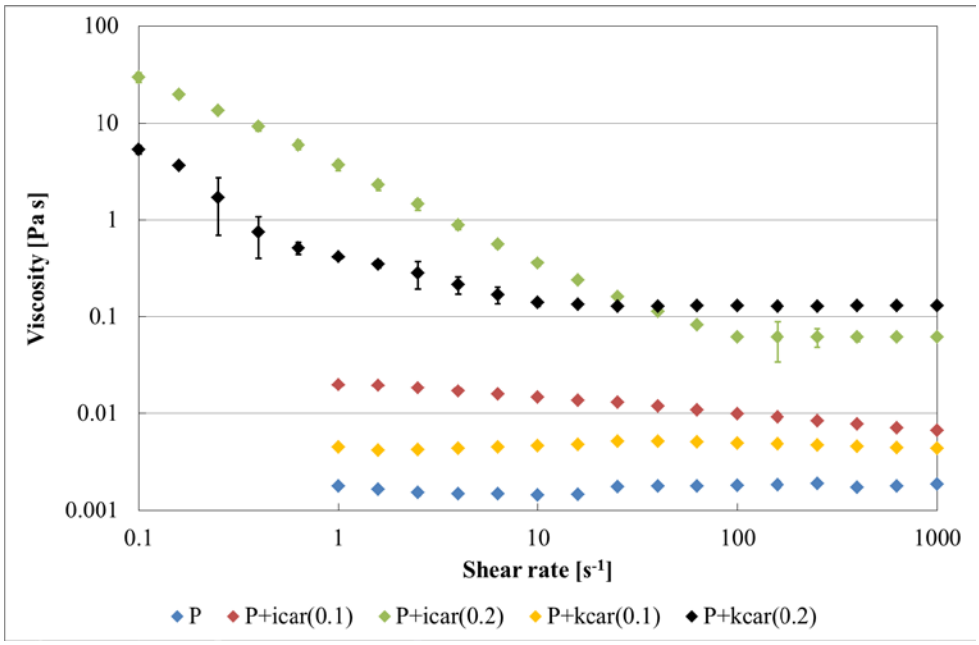


Figure 5. Effect of i-carrageenan and k-carragenan on the viscosity of emulsions formed with 3 wt% milk whey proteins after 1hour storage time with varying shear rate

Figure (5) showed that the emulsion formed with milk whey proteins alone (P) was Newtonian with a low viscosity (≈ 2 mPa·s). In the presence of the i-carrageenan and k-carrageenan the viscosity values strongly increased and the emulsions showed a flow behavior which turned from Newtonian for the P+kcar(0.1) and P+icar(0.1) samples, to substantially pseudoplastic or shear thinning by increasing the amount of carrageenan in the systems (P+kcar(0.2) and P+icar(0.2))

Then, the addition of the carrageenan to protein emulsion affected both the flow behavior and the viscosity absolute value characteristic of the emulsions investigated in accordance with its known thickening and gelation effects of the continuous phase.

Although the increase in the emulsion viscosity became more relevant when raising the bulk polysaccharide concentration in the system for both types of carrageenan, some important differences were found between them, which could be correlated to the different creaming behavior: thus confirming the above considerations.

In fact, it can be noticed in figure (5) that the higher viscosity values were found for P+icar emulsions rather than P+kcar ones, at each carrageenan concentration used. Specifically,

viscosities of 13 mPa·s and 5 mPa·s were recorded for P+icar(0.1) and P+kcar(0.1) respectively, and P+icar(0.2) exhibited higher values than those of P+kcar(0.2) up to shear rate of about 63 s^{-1}.

Finally, it is interesting to emphasize that the bulk viscosity values obtained for the samples investigated are in perfect accordance with their average droplet size and polydispersity characteristics. In fact, the latter were as great as the viscosity of the emulsion is large, indicating that high emulsion bulk phase consistency are an impediment to a formation of small droplets and with similar dimension. Then, the same mixing power being used in the emulsification step for all the samples, P and P+icar(02), which exhibited the smallest and the highest viscosity respectively, showed in turn after 1 hour the highest and smallest average droplet size and polydispersity.

To verify the stability of the systems investigated and to support the results obtained from the creaming analysis (figure 4), the time dependence of the emulsion viscosity was evaluated by subjecting the samples viscosity measurements also after 24 hours storage time.

The results are reported in figure (6) for the P+icar emulsions alone, because no consistent data were obtained for all the other emulsions. In fact, their consistent heterogeneity caused a substantial irreproducibility of the experiment data, which, thus, confirmed the relevant onset of coalescence and creaming phenomena for the emulsions stabilized with milk whey protein alone and also with k-carrageenan, as first discussed.

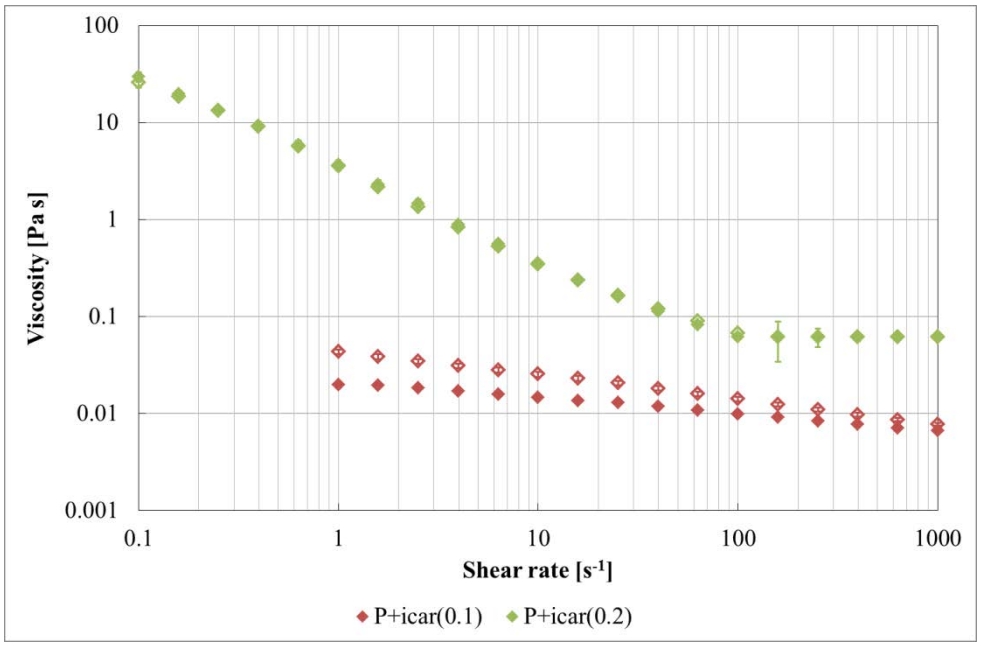


Figure 6. Effect of time storage on viscosity of emulsions formed with milk whey proteins and i-carrageenan after 1hour (full symbol) and 24 hours (empty symbol) with varying shear rate

The high stability of the P+icar(0.2) emulsion was confirmed by the data in figure (6) which allow us to note that no changes of the viscosity were found in the period of time considered. After 24 hours some variations can be noticed for the P+icar(0.1) which, however, becoming less important with increasing the shear arte probably owing to a mixing effect caused by the measuring conditions. Therefore , these variations can be explained by the creaming data registered after the same storage time.

3.3 Interfacial properties

In order to consider the interactions between protein and i/k-caraagenans at the droplet interface and, therefore, their eventual consequences for the emulsion stability, the interfacial behavior of the protein-carrageenan aqueous solutions in contact with sunflower oil was investigated by pendant drop method as described in *2.7*.

Specifically, the effects of milk whey proteins-carrageenan interactions on the adsorption phenomena were analyzed by measuring transient interfacial tensions for all samples for a time period of 180 minutes (figure 7).

No own interfacial activity was found for the carrageenan molecules, as expected, which reduced overall the interfacial activity of the milk whey protein alone (P), in a way dependent on the carrageenan type added to the aqueous solution. Specifically, the addition of the k-carrageenan in the bulk phase slightly affected the protein adsorption behavior at each polysaccharide concentration investigated, whereas the i-carrageenan presence caused a substantial growth of the interfacial tension, becoming more relevant when increasing the i-carrageenan amount in the solution.

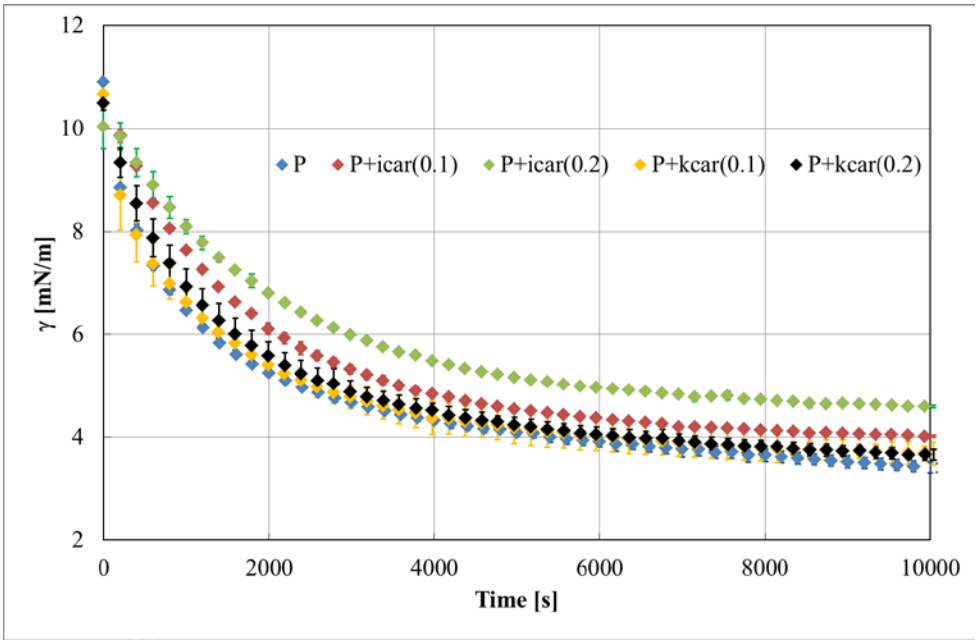


Figure 7. Time dependent interfacial tension, γ(t), of freshly aqueous solutions containing milk whey proteins (3wt%) and carrageenan at the interface with sunflower oil as a function i/k-carrageenan amount

In this regard and in order to compare the effect of carrageenan addition on the interfacial tension equilibrium/plateau values, the latter were also reported in table (1) for all systems considered. They confirmed the average droplet size characteristics obtained for the relative emulsions (*3.1.1*)

Sample	γ_{eq} [mN/m]
P	3.46 ± 0.08
P+icar(0.1)	4.06 ± 0.04
P+icar(0.2)	4.64 ± 0.04
P+kcar(0.1)	3.72 ± 0.03
P+kcar(0.2)	3.72 ± 0.06

Table 1. Plateau values of interfacial tension of freshly aqueous solutions containing milk whey proteins (3wt%) and carrageenan at the interface with sunflower oil as a function i/k-carrageenan amount

Then, also by considering data in table (1), it appears clearly that k-carrageenan caused a slight increase of γ plateau value or equilibrium value which any way remained unchanged at each k-carrageenan concentration considered.

On the contrary, very different values were obtained for the P+icar systems, probably owing to strong interaction between these biopolymers and to the possible presence of a significant fraction of i-carrageenan also at the interface [Gu et al. (2005)].

This behavior allows us to identify some differences in the interactions of i-carrageenan and k-carrageenan with the milk whey proteins adsorbed at O/W interface, which were probably linked to dissimilar charges of polysaccharide molecules studied, and to different degree of incompatibility between these molecules [Gu et al. (2005)].

It is important to emphasize that these differences may be linked to the dissimilar macroscopic behavior obtained for the O/W emulsions prepared by using both these protein-carrageenan solutions, confirming that the emulsion stability improved by adding i-carrageenan rather k-carrageenan [Gu et al. (2005)].

In fact, k-carrageenan emulsions proved to be unstable to creaming probably because the droplets were mainly coated by only the protein membrane, and so the repulsive interactions between the droplets (electrostatic and steric) were insufficient to overcome the attractive interactions (van der Waals and depletion) [Gu et al. (2005)]. Conversely, the emulsions containing i-carrageenan were stable to creaming because the droplets were coated by a highly charged protein/i-carrageenan membrane, and hence the repulsive interactions between the droplets (electrostatic and steric) were sufficient to overcome the attractive interactions.

Although the interfacial activity is certainly an important attribute, the lowering of interfacial tension does not by itself explain the stability of protein-based emulsions. The essential stabilizing function of proteins is that they enable the fluid interface to resist tangential stresses from the adjoin flowing liquids and area change [Dickinson (1998)].

For this reason, rheological dilational dynamic tests, performed by pendant drop method, which are typically used to study protein capacity to form viscoelastic interfaces, were carried out on the samples investigated, except for the P+icar(0.2), for which the high viscosity of the solution made impossible to run the test. For them the dependence of the dilational rheological properties, E* and tanδ, on the angular frequency were evaluated and reported in figure (8)

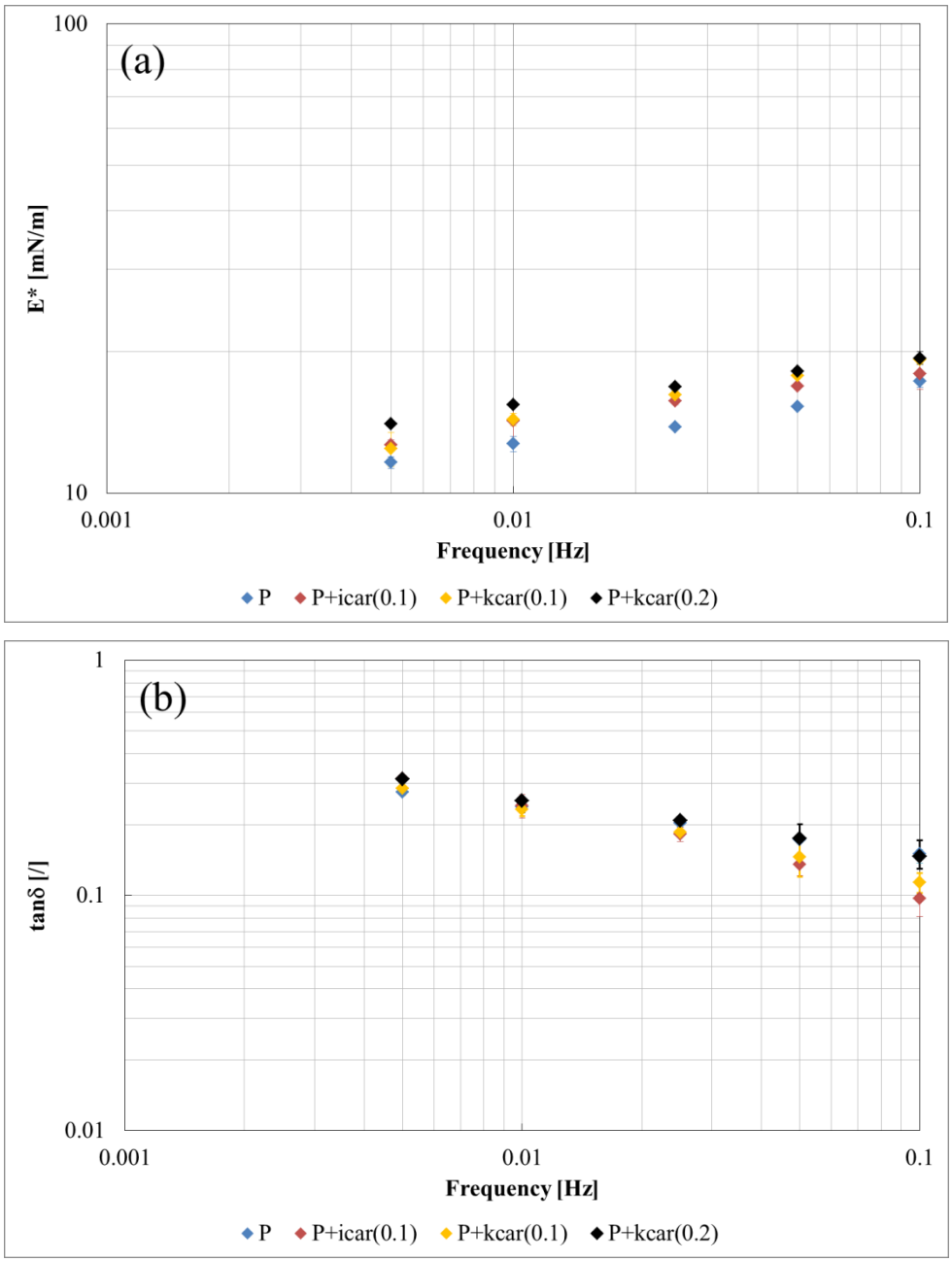


Figure 8. (a) Interfacial dilational modulus, E*, and **(b)** phase angle tangent, tan δ, as a function of drop oscillation frequency for the samples at O/W interface

Figure (8), for each system considered, allows us to note an almost independence of E* on the oscillation frequency and very small values of the loss tangent in the frequency range considered, which indicates a substantial viscoelastic solid-like behavior [Miquelin et al. (2010)].

Furthermore, the effect of the carrageenan addition to the solution proved to be little dependent on both the type and the bulk concentration used. In fact, from a comparison with the rheological data of the P system, both i-carrageenan and k-carrageenan caused only a slight increase of the E* modulus and no relevant changes of the loss tangent (data

in deviation), indicating a reinforcement of the protein network at the interface in accordance with the formation of protein-polysaccharide complexes in the aqueous phase, which is generally accompanied by an increase in the elasticity of the interfacial films compared with those of the protein alone [Dickinson (2003), Rodrìguez Patino and Pilosof (2011), Miquelim et al. (2010)].

In the case of P+kcar system the E* modulus slightly increased when increasing the polysaccharide concentration, confirming this reinforcement effect which, then, could make the fluid interfaces more able to resist stresses from the adjoining flowing liquids, determining also an increase in the stability of the emulsion systems formed with this solutions.

In this regard, it is important to emphasize that the interfacial rheological data obtained proved to be useful to support the results obtained about the better stability of the O/W emulsion stabilized with i/k-carrageenan than that of the emulsion formed with the milk proteins alone, but they did not allow us to confirm the differences found between the two types of carrageenan investigated.

4. Conclusions

The influence of the i-carrageenan and k-carrageenan concentration on the properties of oil-in-water (O/W) emulsions stabilized by milk whey protein was successfully investigated by measuring the particle size distribution, the creaming stability and the bulk and interfacial rheological properties.

The emulsions formed with carrageenan proved to be more stable compared with those stabilized with milk whey proteins alone but the stability improved considerably by adding i-carrageenan (icar) rather than k-carrageenan (kcar). The excellent stability towards phase separation found for the stored i-carrageenan emulsion, and their bulk and interfacial properties, suggests the ability of the i-carrageenan and the inability of the k-carrageenan to reduce partial coalescence either by providing a sufficiently thick continuous phase or by acting as a protective coating for oil droplets. The behavior against the creaming phenomena of these systems proved to be independent of their droplet size distribution for all emulsions studied but strongly affected by their bulk and interfacial properties. These were, in turn, highly influenced by the type and concentration of the carrageenan present in the continuous phase .

REFERENCES

1) McClements D.J. (1999). Food emulsion: Principles, Practice and Techniques. CRC Press: Boca raton, FL., chapter 7
2) Bos M.V. and van Vliet T. (2001), Interfacial rheological properties of adsorbed protein layers and surfactants: a review, *Advances in Colloid and Interface Science* (91), 437-471
3) McClements, D. J. (2005). Food emulsions (2nd ed.). Boca Raton, FL: CRC Press.
4) Dickinson E. (2003), Hydrocolloids at interfaces and the influence on the properties of dispersed systems Review. *Food Hydrocolloids* (17), 25-39
5) Murray S.B., (1998) Interfacial rheology of mixed food protein and surfactant adsorption layers with respect to emulsion and foam stability, in M*ö*bius D. and Miller R., Studies in interface science 7, Proteins at Liquid Interfaces, Elsevier editor, Amsterdam (The Netherlands), chapter 5
6) Dickinson E. (1998), Proteins at interfaces and in emulsions. Stability, rheology and interactions. *Journal of the Chemical Society, Faraday Transactions* (94), 1657-1669
7) Rouimi S., Schrorsch C., Valentini C., Vaslin S., (2005) Foam stability and interfacial properties of milk protein-surfactant systems. *Food Hydrocolloids* (19), 467-478
8) Dickinson E. (1999), Caseins in emulsions: interfacial properties and interactions. *International Dairy Journal* (9), 305-312
9) Dickinson E. (2001), Milk protein interfacial layers and the relationship to emulsion stability and rheology Review. *Colloids and Surfaces B: Biointerfaces* (20), 197-210
10) Raikos V. (2010), Effect of heat treatment on milk protein functionality at emulsion interfaces. A review. *Food Hydrocolloids* (24), 259-265
11) Williams A. and Prins A. (1996), Comparison of the dilational behavior of adsorbed milk proteins at the air-water and oil-water interfaces, *Colloids and Surfaces A: Physicochemical and Engineering Aspects* (114), 267-275
12) Cornec M., Mackie A.R., Wilde P.J., Clark D.C. (1996), Competitive adsorption of β-lactoglobulin and β-casein with Span80 at the oil-water interface and the effect on emulsion behavior. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* (114), 237-244

13) Gu S.Y., Decker E.A., McClements D.J. (2004). Influence of pH and ι-Carrageenan Concentration on Physicochemical Properties and Stability of β-Lactoglobulin-Stabilized Oil-in-Water Emulsions. *Journal of Agricultural Food Chemistry* (52),3626-3621

14) Rodríguez Patino J. M. and Pilosof A.M.R. (2011). Protein-polysaccharide interactions at fluid interfaces. *Food Hydrocolloids* (25), 1925-1937

15) Benichou A., Aserin A., Garti N. (2002). Protein-Polysaccharide Interactions for Stabilization of Food Emulsions. *Journal of Dispersion Science and Technology* (23), 1-3, 93-123

16) Syrbe A., Bauer W. J. and Klostermeyer H. (1998). Polymer Science Concepts in Dairy Systems-An Overview of Milk protein and food hydrocolloid interaction. *Dairy Journal* (8) 179-193

17) Singh H., Tamehana M., Hemar Y., Munro P. A.(2003). Interfacial compositions, microstuctures and properties of oil-in-water emulsions formed with mixtures of milk proteins and k-carrageenan: 1. Sodium caseinate. *Food Hydrocolloids* (17), 539-548

18) Piculell, L. (1995). Gelling carrageenans. In A. M. Stephen (Ed.), Food polysaccharides and their applications (pp. 205–243). New York: Dekker (Marcel).

19) Jensen R.G.(1995), Handbook of Milk Composition, Academic Press, New York (U.S.A), chapter 5

20) Hollingsworth K.G., Johns M.L. (2003). Measurement of emulsion droplet sizes using PFG NMR and regularization methods. *Journal of Colloid and Interface Science* (258), 383-389

21) Lupi F.R. (2009). Rheology of highly-concentrated-in-oil emulsions. PhD thesis

22) Melle S., Lask M., Fuller G.G. (2005). Pickering Emulsions with Controllable Stability. Langmuir (21), 2158-2162

23) Egger H. and McGrath K.M. (2006). Aging of oil-in-water emulsions: The role of the oil. *Journal of Colloid and Interface Science* (299), 890-899

24) Walpole R.E, Myers R.H., Myers S.L., Ye K. (2007). Probability & Statistics for Engineers & Scientists – 8th Edition. Pearson Education International, Prentice Hall, USA

25) Ye A. and Singh H. (2006). Heat stability of oil-in-water emulsions formed with intact or hydrolised whey proteins: influence of polysaccharides. *Food Hydrocolloids* (20), 569-576

26) Biresaw G., Liu Z. S., Erhan S.Z.(2008). Investigation of the Surface Properties of Polymeric Soaps Obtained by Ring-Opening Polymerization of Epoxidized Soybean Oil, Wiley InterScience (www.interscience.wiley.com)

27) Lin S.Y., Wang W.J., Lin L.W., Chen L.J. (1996). Systematic effects of bubble volume on the surface tension measured by pendat bubble profiles *Colloids and Surfaces A: Physicochemical and Engineering Aspects* (114), 31-39

28) Camino N.A., Pèrez O.E., Carrera Sanchez C., Rodriguez Patino J.M., Pilosof A.M.R. (2009). Hydroxypropylmethylcellulose surface activity at equilibrium and adsorption dynamics at the air-water and oil-water interfaces. *Food Hydrocolloids* (23) 2359-2368

29) Lucassen J. and van den Tempel M. (1972). Dynamic measurements of dilational properties of a liquid interface, *Chemical Engineering Science* (27) 1283-1291

30) Freer E. M., Sub Yim K., Fuller G. G., Radke C. J. (2004). Shear and Dilatational Relaxation Mechanisms of Globular and Flexible Proteins at the Hexadecane/Water Interface. *Langmuir (20),*10159-10167

31) Ravera F., Liggieri L., Loglio G. Dilational rheology of adsorbed layers by oscillating drops and bubbles. In: R. Miller, L. Liggieri Interfacial Rheology Volume 1 , Brill Academic Pub, Leiden Boston 2009, 138-177

32) Myrvold R. and Knut Hansen F. (1998). Surface Elasticity and Viscosity from Oscillating Bubbles Measured by Automatic Axisymmetric Drop Shape Analysis, *Journal of Colloid and Interface Science* (207) 97–105

33) Tcholakova S., Denkov N., Ibanov I.B.m Marinov R. (2004). Evaluation of Short-Term and Long-Term Stability of Emulsions by Centrifugation and NMR, *Bulg. J. Phys.* (31), 96-110

34) Bylaite E., Nylander T., Venskutonis R., Jonsson J. (2001). Emulsification of caraway essential oil in water by lecithin and b-lactoglobulin: emulsion stability and properties of the formed oil–aqueous interface, *Colloids and Surfaces B: Biointerfaces* (20), 327–340

35) Gu Y.S., Decker E.A., McClemets D.J. (2005). Influence of pH nad carrageenan type on properties of β-lactoglobulin stabilized oil-in-water emulsions. *Food Hydrocolloids* (19), 83-91

36) Perez A.A., Carrara C.R., Carrera Sánchez C., Rodríguez Patino J. M., Santiago L. G. (2009). Interactions between milk whey protein and polysaccharide in solution. *Food Chemistry* (116) 104–113

37) Leroux J., Langendorff V., Schick G., Vaishnav V., Mazoyer J. (2003). Emulsion stabilizing properties of pectin. *Food Hydrocolloids* (17) 455-462

38) Ahmed N.S., Nassar A.M., Zaki N.N., Gharieb H.K. (1999). Formation of fluid heavy oil-in-water emulsions for pipeline transportation. *Fuel* (78), 593-600

39) Dickinson E. (1998), Proteins at interfaces and in emulsions. Stability, rheology and interactions. *Journal of the Chemical Society, Faraday Transactions* (94), 1657-1669

40) Nor Hayatia I., Che Mana, Y.B., Tana C.P., Nor Ainib I. (2009). Droplet characterization and stability of soybean oil/palm kernel olein O/W emulsions with the presence of selected polysaccharides. *Food Hydrocolloids* (23), 233-243

41) Miquelim J.N., Lannes S.C.S, Mezzenga R. (2010). pH Influence on the stability of foams with protein-polysaccharide complexes at their interfaces. *Food Hydrocolloids* (24), 398-405

Conclusions

In the present PhD thesis the functionalities of protein surfactants typically used as basic ingredients for the formation and stabilization of food multhiphase systems have been investigated at the air-water (A/W) and oil-water (O/W) interfaces in order to obtain systematic information on the importance of mechanical and kinetic aspects linked to the formation of a viscoelastic protein network at these interfaces. This allows the understanding of the interfacial structure and behavior and, thereby, the possible link between them and the macroscopic system behavior.

To this purpose "model systems" (water-oil/air-surfactant) relevant for industrial applications and containing up to two types of surfactants have been characterized by transient interfacial tension measurements and dilational dynamic tests. They have proved to be very useful to evaluate the interactions between the single protein and surfactant at the interface and, at the same time, to study the evolution of synergic or competitive effects to be ascribed to the simultaneous presence in the system of other components (such as low molecular weight surfactants and polysaccharides). Both of these factors have been analyzed successfully by using kinetic models useful to describe the molecular diffusion and penetration phenomena of the adsorbed molecules at the interface and by investigating the frequency behavior of the interfacial layer structure.

Interfacial dilatational moduli have evidenced for all the proteins investigated, Ovalbumin (chapter 3), β-Casein and β-Lactoglobulin (chapter 4) the potential formation of a 2D critical gel at the oil/water interface and they were analyzed assuming the validity at the interface of rheological model used for the 3D critical gel.

This rheological approach has proved to be very useful to evaluate and to differentiate the protein network behavior also in the presence of the low molecular weight surfactants, Tween 60 and Admul Datem (chapter 3, 5), and various polysaccharides (chapter 6). The former agents caused a relevant weakening of the protein network in every system considered, whereas the latter, although they did not exhibit their own interfacial activity proved to affect the protein adsorption and network at the interface through different interactions with protein molecules resulting from blending these biopolymers. Important differences between A/W and O/W interfaces have been also individuated for the Ovalbumin-emulsifier systems investigated, probably because of dissimilar competitive phenomena among protein and tested emulsifiers (chapter 3).

Finally, the interfacial rheological properties of biphasic systems, based on milk whey protein, iota-carrageenan and kappa-carrageenan biopolymers, have been linked to the bulk properties and they have proved to be very useful to analyze the short term stability of the investigated systems.

International Journal

Baldino N., **Seta L**., Migliori M., Gabriele D. , De Cindio B. , Chidichimo G. (2010), " Rheological modelling of plaster deposition for painting restoration". Applied Rheology, Vol. 20 (2), n. 23310, pp. 1-9.

Proceedings of International Conferences

N. Baldino, **L. Seta**, F.R. Lupi, D. Gabriele, B. de Cindio (2011),"Modeling of drying process of candies obtained with starch molding technique". MAS 2011, Roma, 12-14 September

F.R. Lupi, N. Baldino, **L. Seta**, D. Gabriele, B. de Cindio (2011), "Sheeting process modelling nad rheological analysis of an olive-oil-emulsion-based puff pastry" . MAS 2011, Roma, 12-14 September.

Proceedings of Italian Conferences

Seta L. , Baldino N. , Migliori M. , Gabriele D. , De Cindio B.(2010) , " Influenza di additivi ispessenti sulle proprietà reologiche di sospensioni per restauro". XI Convegno di Reologia, Trieste, 23-26 May