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# A scaling analysis of β-casein monolayers at liquid–fluid interfaces

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**Abstract**

The experimental results recently provided by a novel application of the pendant drop technique to the formation of protein monolayers at liquid–fluid interfaces are further analysed on the basis of scaling arguments. Specifically, β-casein monolayers at the air–water and the tetradecane–water interface are directly compared and the structural differences inferred by the π–A isotherms are analysed in more detail in terms of the static elasticity modulus. In addition, the model developed by Leclerc et al. based on multiblock theory is applied to the experimental data at both interfaces [E. Leclerc, M. Daoud, Macromolecules 30 (1997) 293; V. Anguié-Béghin, E. Leclerc, M. Daoud, R.J. Douillard, Colloid Interface Sci. 214 (1999) 143]. In this manner, the experimental differences are corroborated and subsequently quantified with scaling arguments. Finally, the theoretical analysis is extended to the application to β-casein monolayers at various oil interfaces in order to shed light on the discrepancies reported on the structural configuration attained by this protein at different oil interfaces. As a result, a correlation between the interfacial tension of the liquid and the interfacial structure of the protein is probed.


*Keywords:* Monolayer; β-Casein; Oil phase; Scaling exponent; Interfacial tension

## 1. Introduction

Proteins play an extremely important role in the production and stabilization of many products. Their amphiphilic nature makes them surface active so that they adsorb at different kinds of interfaces [3]. Two types of interfaces may be considered, namely penetrable and impenetrable. The former is found when the molecule is attracted by the interface between two solvents, whereas the latter involves, for example a solid surface. This last type of interface has been extensively studied so far, and has important application in colloidal stabilization for instance. In relation to the penetrable interface, i.e. liquid–fluid interface, it has a large potential on emulsions and remains nowadays rather unknown. The structure attained by proteins at fluid interfaces is hence, a question of increasing relevance in order to understand the involved mechanisms with the aim of rationalising their technological use.

The monolayer technique appears as a useful tool in the clarification of these processes and has been extensively used in the study of proteins at the air–water interface [4,5]. However, the difficulty added by a liquid interface results in a certain lack of works regarding protein monolayers at oil interfaces. Even less studies in the literature, provide a quantitative analysis of the monolayer behaviour, by means, for example, of the application of a model.

The results presented by other authors appear in agreement regarding the behaviour of some model globular proteins. In particular, it seems to be accepted that a further unfolded state is attained by serum albumin at different oil interfaces with respect to that at the air–water interface [6–8]. Conversely, certain discrepancies appear regarding β-casein when changing the nature of the non-polar phase [7,9,10]. This is a model protein with important applications in the stabilisation of dairy products. It has a very flexible structure in solution and it looks more or less like a “naturally unfolded protein”.

In order to deepen the understanding of this protein’s interfacial behaviour, previous works were devoted to the adaptation of the Pendant Drop Technique to the formation of protein monolayers at both, the air–water and the oil–water interfaces [11,12]. Nonetheless, those results were shown independently and merely quantitatively analysed. At this point, two features must be highlighted. On one hand, taking into account that a

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direct comparison between the interfacial structure of β-casein at both interfaces was not shown before it was worth the performance of an explicit comparative analysis. On the other hand, in order to shed light on the discrepancies encountered in the literature, a theoretical model to further interpret the experimental data has been searched.

Since proteins are polymers, they should share some surface properties with other copolymers [10]. A polymer model has been developed recently to describe their surface properties as those of a multi-block copolymer, i.e. a polymer made of alternating hydrophobic and hydrophilic sequences [2]. In this frame, the modelling of the structure and properties of protein adsorbed layers are tackled using the scaling law approach for polymers. This model has been applied to many experimental systems and appears very useful in the interpretation of the data enlightening the interfacial structure.

Recently, Douillard et al. have reviewed the interfacial properties of many proteins on the basis of polymer thermodynamics [10]. In this work, the advantages of using scaling arguments in the analysis of protein layers are illustrated and important conclusions arise, once again, especially at the air–water interface.

Accordingly, the main goal of this work is to analyse the experimental data of β-casein at the air–water and the tetradecane–water interfaces formerly obtained [11,12], by using the polymer approach [1,2]. Furthermore, the theoretical analysis is subsequently applied to the experimental data obtained by other authors who performed studies with the same protein but at different oil interfaces. In particular, we have analysed the isotherms of β-casein at the toluene–water interface [7] and at the triolein–water interface [10,13] in which discrepancies in the behaviour at the oil interface were found. Finally, the role of the nature of the interface on the interfacial structure attained by the protein is discussed in detail in view of the theoretical considerations and an explanation to the discrepancies is proposed.

## 2. Theoretical background

### 2.1. Static elasticity modulus

The interfacial pressure, $\pi$, of a protein layer is the difference between the surface energy per unit area of the liquid–liquid interface, $\gamma_0$ and the surface energy measured with the layer in place, $\gamma$. The response of the adsorbed molecules to compression and expansion of the interface is represented by a change in area and measured due to a variation of the interfacial pressure. This change reflects, therefore, the magnitude of lateral forces presents in the monolayer and can be used to provide structural information. The surface elasticity modulus is defined as the increase in interfacial tension for an increase in area of an interface element [14,15]:

$$|\varepsilon| = \left| \left( \frac{\mathrm{d}\gamma}{\mathrm{d}\ \ln A} \right)_T \right| = \sqrt{\varepsilon_0^2 + (\eta_\mathrm{d}\omega)^2} \tag{1}$$

The elasticity modulus is a complex quantity with a storage part (elastic) and a loss part (viscous) resulting from a phase difference that occurs between $\mathrm{d}A$ and $\mathrm{d}\pi$. When there is no exchange of material with the adjoining bulk solution, i.e. when $\Gamma \times A$ is constant and the time of deformation is very large or very short compared to the time of rearrangements within the layer, $\varepsilon$ is called static elasticity modulus and it can be deduced from the equilibrium relationship between interfacial pressure and interfacial area [16]:

$$\varepsilon = \left( \frac{\mathrm{d}\gamma}{\mathrm{d}\ \ln A} \right)_{T_\mathrm{eq}} \tag{2}$$

This relationship provides structural information of the interface and has been widely used in the study of protein monolayers [4,5].

### 2.2. Polymer adsorption

The model, based on multi-block theory, is described in detail in [1], subsequently extended to proteins at fluid interfaces in [2] and further applied in very different experimental conditions in [10,17,18]. Hence, merely some important features will be briefly pointed out below.

In the current theory of adsorption of proteins at liquid–fluid interfaces, the polymer chain is assumed to be made of $N$ diblocks of two sequences, A and B consisting of $Z_\mathrm{A}$ and $Z_\mathrm{B}$ monomers, respectively. The monomers of the A and B sequences have different chemical nature, namely A is hydrophobic while B is hydrophilic. The chemical structure of the polymer is represented by the ratio $\alpha = Z_\mathrm{A}/Z_\mathrm{B}$. In particular, for β-casein a very good agreement has been found between the experimental pattern and the theoretical predictions in the case of a polymer where the hydrophobic and the hydrophilic blocks have the same order of length [2,17,18]. Let us assume an interface between two immiscible solvents 1 and 2, which are different for both kinds of monomers. Specifically, they are selectively better for A and B, respectively. Thus, each sequence of the diblock has a tendency to place itself in its better solvent, and this is the reason why the polymer locates at the interface. At this point, one can distinguish between gas–liquid and liquid–liquid interfaces.

On one hand, at the liquid–liquid interface we are dealing with a penetrable interface. When the diblock is in an isotropic state, the centres of masses of each sequence coincide with the centre of gravity of the macromolecule. Due to the difference between the qualities of the solvents, as the polymer becomes adsorbed, this is no longer true and the centres of gravity of the sequences are on different sides on the interface. Owing to the polymeric nature, a restoring force tends to bring them back to each other. Localization at the interface occurs either when the number of sequences ($N$) or the number of monomers ($Z$) is large, or else when selectivity is high [1,10].

On the other hand, at the air–water interface, it is assumed that the liquid is a good solvent for the B sequence; air is not a good solvent for any of the sequences. In this manner, the junctions between the hydrophobic and the hydrophilic blocks are supposed to stick strictly to the interface and the blocks do not cross the interface [2]. Once adsorbed, the copolymer adopts a