Available online at www.sciencedirect.com

SCIENCE DIRECT®

Colloids and Surfaces A: Physicochem. Eng. Aspects 282–283 (2006) 99–108

COLLOIDS AND SURFACES A

www.elsevier.com/locate/colsurfa

Interactions in oil/water/oil films stabilized by β-lactoglobulin; role of the surface charge

Elka S. Basheva[a], Theodor D. Gurkov[a,*], Nikolay C. Christov[a], Bruce Campbell[b]

[a] *Laboratory of Chemical Physics & Engineering, Faculty of Chemistry, University of Sofia, James Bourchier Avenue 1, Sofia 1164, Bulgaria*
[b] *Kraft Foods, Inc., 801 Waukegan Road, Glenview, IL 60025, USA*

Received 7 November 2005; received in revised form 14 January 2006; accepted 16 January 2006
Available online 21 February 2006

Dedicated to Professor Ivan B. Ivanov (LCPE, University of Sofia) on the occasion of his 70th birthday.

Abstract

In this work we investigate emulsion films (oil/water/oil), stabilized by β-lactoglobulin (BLG). Isotherms of disjoining pressure versus the film thickness are measured experimentally, at different pH (4.0, 5.2, and 6.5), and ionic strength. The data are fitted successfully with the classic theory of DLVO (assuming superposition of electrostatic repulsion and van der Waals attraction). One adjustable parameter, the surface potential, is determined from the best fit; the results are used to calculate the surface charge density in the films. At the pH of 5.2 (which is the isoelectric point, p*I*, in the bulk solution), the interface is charged. Possible reason is the conformational change, which the protein undergoes upon adsorption. At bulk pH of 4.0, the BLG-laden oil/water interface is close to isoelectric state (the surface charge density is very low). Under these conditions, there is evidence for long-range steric repulsion, possibly due to favored aggregation at the interface. In some cases, after eventual collapse of the repulsion, we observe formation of spots of very thin Newton Black Films (NBF). Addition of inorganic salt, NaCl, leads to increase of surface charge (up to a certain limit). This effect is derived both from results with thin liquid films, and from zeta-potentials of emulsion drops. At the "natural" pH of 6.5, with 150 mM ionic strength, the extent of charging of adsorbed BLG is considerably lower than that in the bulk aqueous solution. Thus, also at pH 6.5, the charge state of the protein molecules residing on the oil/water interface is significantly influenced by the conformational transformations that accompany the adsorption. The emulsion films with BLG are less stable than the foam films, and rupture without overcoming a barrier (maximum) in the disjoining pressure isotherm. The latter fact implies that certain weakness of the interfacial layer is brought about by the contact with the oil phase (hydrophobization).

Keywords: Thin liquid films; β-Lactoglobulin; Surface forces in emulsion films; Charge of adsorbed protein; Disjoining pressure; Stability of protein films

1. Introduction

Thin liquid films of the type oil/water/oil are important for the stability of O/W emulsions, because such films form between drops which are pressed against each other either by some external force (for example, buoyancy), or due to restricted volume. The conditions for film formation, and its consequences, are discussed in [1]. Emulsion- and foam-type films are convenient as a model system that allows quantitative determination of the surface forces (or the "disjoining pressure", Π), as a function of the thickness, h. Molecular interactions of different origin, van der Waals, electrostatic, steric, structural, hydration, protrusion, etc. [2] contribute to Π and influence to a large extent the film behavior (thinning and stability). One of the preferred experimental methods for precise measurement of Π versus h involves film formation in a porous glass cell (also called Mysels cell, or Exerowa-Scheludko cell); it is suitable both for foam-type films [3] and for emulsion films [4]. Depending on the pore size and the interfacial tension, one can reach capillary and disjoining pressures as high as thousands of Pascals [4].

β-Lactoglobulin (BLG) is the major whey protein in the milk; its stabilizing role in films and emulsions is a topic of practical interest in view of the numerous food applications. Air/water/air films with BLG were investigated in [5]. The measured surface force at larger thicknesses ($h > \sim 24$ nm) was well represented as a superposition of van der Waals attraction and electrostatic

---

[*] Corresponding author. Tel.: +359 2 8161 688.
E-mail address: tg@lcpe.uni-sofia.bg (T.D. Gurkov).

0927-7757/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.colsurfa.2006.01.023

repulsion, while in thinner foam films a steep steric repulsion arose [5]. In an earlier work, Clark [6] reported equilibrium thicknesses of about 25 nm for foam films and ∼35 nm for emulsion films with 0.02 wt.% BLG. Some other results with thin aqueous films containing proteins (not specifically disjoining pressure data, $\Pi(h)$) are reviewed by van Aken et al. [7]. Isotherms $\Pi(h)$ for foam films in the presence of β-casein and BSA are reported in [8].

Our purpose in this work is to carry out a systematic study of the surface forces in emulsion films stabilized by BLG. We measured disjoining pressure isotherms, $\Pi(h)$, at three different values of pH (4.0, 5.2, which is the isoelectric point in the bulk solution, and 6.5, the "natural" pH when protein is just dissolved in water). The concentration of inorganic salt, NaCl, was also varied.

Let us estimate, as an order of magnitude, the typical pressures exerted on the drops by buoyancy, in an O/W emulsion with oil volume fraction, φ, of approximately 0.7, and mass density difference $\Delta\rho \approx 0.1\ \mathrm{g/cm^3}$ between the two phases. An emulsion column of 10 cm height (H) will develop pressure $\Delta\rho g \varphi H \approx 70$ Pa (the gravity acceleration, g, is 981.55 cm/s^2). The disjoining pressures, Π, measured by us, are of this order (see Section 4 below). Hence, such data would bear relevance, for example, to stabilization of concentrated emulsions during shelf storage.

We focus on the following particular tasks related to the $\Pi(h)$ data obtained for films with 0.02 wt.% BLG:

- To reveal what interactions are important in the films. For this purpose, we fit the disjoining pressure curves with the known theory of electrostatic and van der Waals forces. In most cases the agreement is good; the surface potential and the surface charge in the film are deduced from one adjustable parameter in the fits.
- To analyze how the electric charge state of the adsorbed protein at the oil/water interfaces is influenced by the pH and the salt; to explore if it is different from the charge state in the bulk solution. Specifically, we have to clarify whether the surface isoelectric point is the same as the pI in the aqueous phase (the latter is equal to 5.2).
- To compare the behavior and stability of emulsion and foam films, and to see whether their rupture is associated with passing over a barrier (maximum) in the curve $\Pi(h)$.

Determination of surface charge through fitting of force versus distance data (obtained by means of surface force apparatus) was accomplished by Sivasankar et al. [9], who investigated the pH-dependent electrostatic properties of a single binding face of streptavidin. Thin film balance (porous plate method) was used in [10], where the surface charge was found by fitting of disjoining pressure in films stabilized by alkyl glucosides. We basically follow the data management procedures implemented by those authors.

The question about the charge state of adsorbed protein, and how it differs from the protein in bulk solution, bears significance for many biomolecular interactions. Such are, for instance, the directed ligand associations, kinetic binding rates, bio-fouling, etc. [9]. All these phenomena are sensitive to electrostatic interactions, since the energy is affected; they underlie applications related to molecular recognition and detection, sensor design, etc. The methods employed here may be helpful for determination of protein's surface charge under various conditions (pH, electrolytes, mixed adsorption layers), and in presence of specific ligands.

2. Materials and methods

2.1. Materials

The used protein was β-lactoglobulin from bovine milk, product of Sigma, lyophilized, Cat. No. L-0130, Lot No. 20K7043, mixture of A and B variants. Aqueous solutions with concentration of 0.02 wt.% were made. The "natural" pH of the solution, without additives for controlling it, was measured to be 6.5. We needed also solutions with pH lower than the natural pH; those were prepared by addition of small portions of diluted solution of HCl by means of a micropipette. Thus, the required pH was not surpassed. The ionic strength that corresponds to the set pH is taken into account as a contribution to the total ionic strength (Section 3.1).

All solutions were prepared with deionized water, purified by a Milli-Q system (Millipore). In some cases we added inorganic electrolyte, NaCl (Merck), in the aqueous phase, with concentrations 0.001, 0.002, 0.005, and 0.010 M. All solutions were used immediately after preparation. The pH was measured both before and after the experiments, and exhibited insignificant variation.

The oil phase was always soybean oil (from a local supplier). It was purified by passing through a glass column filled with alumina adsorbent (Florisil F101), as described by Gaonkar [11].

2.2. Methods

2.2.1. Liquid films in a porous glass cell

Equilibrium forces in thin liquid films are gauged by the disjoining pressure, Π, which is a function of the distance of separation, or film thickness, h. At equilibrium, and in the flat portion of the film, the disjoining pressure, Π, is equal to the capillary pressure, P_c. The latter is a measurable quantity, and represents the difference between the pressure in the outer phase that surrounds the film (in our case, oil), and the bulk liquid pressure in the Plateau border, i.e., in the aqueous phase from which the film is made.

The force law (the disjoining pressure isotherm, $\Pi(h)$) was measured with a thin-film balance, using a modified version of the porous-plate Mysels cell [4]. The experimental cell is shown schematically in Fig. 1. The film is formed by sucking out the inner liquid phase (aqueous solution). The cell is directly connected to a pressure transducer (Omega PC 136G01), the reference pressure being the atmospheric one. This configuration provides the opportunity to investigate both emulsion-type and foam-type films. The measured value of the pressure, P_m, should be corrected for the hydrostatic pressure difference between the plane of the film and the level of the measuring membrane of the