Titre / Title : Stability and Behavior of Thin Oil/Water/Oil Films with beta-Lactoglobulin

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Catégories / *Categories*

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Résumé / *Abstract* (1798 caractères / characters)

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By means of interference microscopy, we investigate emulsion films stabilized by beta-lactoglobulin (BLG). The films are subjected to capillary pressure in the range 10-50 Pa, which is of the same order of magnitude as the buoyancy in creams and concentrated emulsions. The film lifetime and the thickness are measured. Our aim is to explore in a systematic way how the thin film properties are influenced by pH (in the range 3-8), concentration of NaCl (up to 1 M), ageing of the surfaces before film formation. The film stability is discussed in view of the DLVO theory (interplay of van der Waals attraction and electrostatic repulsion). The electric properties of the BLG-laden o/w interfaces are characterized by measured zeta-potential; the data are used for calculation of the disjoining pressure barrier. With 0.15 M NaCl, the observed trends in the film stability cannot be explained by the DLVO model. When the protein is charged (at pH away from pI), and the aqueous phase contains significant amount of salt, local short-range repulsion between the molecules in lateral direction might be responsible for decreased film stability (e.g., because of hindrance to the formation of a stiff protein layer). The films at the isoelectric pH in the bulk solution, pI=5.2, are very stable. This may be attributed to the existence of mechanically reinforced layer (gel-like network), favored by high salt content. The interfaces adhere strongly, which is manifested as sticking (large contact angle hysteresis). In systems containing 1 M NaCl, wrinkles of a 'skin' are visible. Between pH values of 4 and 5.2, the interfaces are essentially uncharged; very thin Newton Black Films exist. So, on o/w boundary the isoelectric state of BLG occurs in a broader pH range compared to the bulk solution.

Mots clés / Keywords :

emulsion films
film thickness
globular protein
film stability
surface charge

1. Introduction

We study aqueous films immersed in soybean oil (SBO), stabilized by the globular milk protein beta-lactoglobulin (BLG). The films are made in a capillary cell, where the applied pressure is in the range 10-50 Pa. This is of the order of the pressure exerted on droplets in concentrated emulsions and creams; e.g., in a 5 cm column of a SBO dispersion in water with volume fraction 0.5, the pressure on the uppermost layer of drops is 19.6 Pa (the density difference is 0.08 g/cm^3). So, we expect the properties and stability of films under these conditions to be relevant to the behavior of the interdroplet contacts in real O/W dispersions.

We present results for film thickness and lifetime; the films have been observed in reflected monochromatic light (i.e., by interferometry). It is investigated how the film properties are influenced by pH and salt concentration. Previous studies from literature have shown qualitative agreement with the predictions of the DLVO theory (1-3), that is, the van der Waals and electrostatic interactions play predominant role for films which contain aqueous core. Clark (4) reported an equilibrium thickness of 35 nm for oil/ water/ oil films with 0.02 % BLG; the film surfaces were completely immobile.

In this work, the data for the film lifetime are discussed in view of the envisaged DLVO stabilization by force/ energy barriers. Zeta potentials of emulsion droplets are measured, and the corresponding surface charge is used to determine the disjoining pressure isotherm and the interaction energy vs. film thickness. The latter functions have maxima, serving as stabilization barriers. It turns out that the force barrier may be high, but still it can be overcome by fluctuations that require relatively little amount of energy (comparable to kT). Consequently, the film is destabilized. We also comment on the exceptionally high film stability at the isoelectric pH of BLG, where a mechanically reinforced gel-like network of protein molecules prevents the film from rupturing.

2. Experimental

The used protein was BLG from bovine milk (product of Sigma, Cat. No. L-0130, Lot No. 20K7043), at concentrations of 0.02 and 0.1 wt.%. Sodium azide was added to all solutions at a concentration of 0.1 g/l, to inhibit bacterial contamination. The pH, when different from the natural pH of ~6.2, was adjusted by NaOH or HCl. All solutions were prepared with deionized water, purified by a Milli-Q system (Millipore). In some cases we added inorganic electrolyte, 0.150 M NaCl, in the aqueous phase. The oil phase was always soybean oil, purified by passing through a glass column filled with Florisil adsorbent (activated magnesium silicate).

2.1. Capillary cell method

Emulsion films were formed by sucking out aqueous phase from a biconcave meniscus held in a glass capillary. The capillary was immersed in the oil phase. The films were observed in reflected monochromatic light (whose wavelength was 546 nm), by means of a microscope Jenavert. The film behavior was recorded by a CCD camera and VCR. The films in most experiments had diameters of about 150 μm. The local thickness, h, was measured with the help of a photomultiplier, which read the reflected light intensity from a given spot in the film. The relationship between the thickness and the light intensity is explained in Ref. (4). We work with h defined as the thickness of water layer in the film interior (the refractive index of the adsorbed protein is close to that of the oil).

The films were formed without ageing of the surfaces, i.e., just after obtaining a biconcave meniscus in the capillary cell. The film behavior was observed during at least 30 minutes. In order to check if sticking of the film surfaces existed, about 30 minutes after opening

a film we started to decrease the capillary pressure of the meniscus by infusing aqueous phase through the capillary. In absence of sticking, the two opposing film surfaces began to disjoin immediately. In some cases, at a certain film radius (smaller than the initial one), the contact line stopped moving and the advancing contact angle grew larger (the Newton fringes merged and became invisible). Such a behavior is designated as "sticking"; there is a direct physical contact of the two opposing protein layers, and adhesion takes place.

No.	Added NaCl	pH	Film thickness, h, nm Film appearance	Lifetime, s
1	none	3.0	50 nm	580
2		4.0	50 nm, spots NBF (5-6 nm)	120
3		5.2 (pI)	18 nm, plane-parallel, sticking	>1800
4		6.0 (~natural)	50 nm	150
5		7.0	38 nm, plane-parallel	112
6		8.0	23 nm, plane-parallel	>1800
7	0.150 M	3.0	<50 nm, uneven thickness	120
8		4.0	Uneven (grey), aggregates, sticking	>1800
9		5.2 (pI)	4.5 nm, NBF, sticking	>1800
10		6.0 (~natural)	<50 nm, uneven thickness	330
11		7.0	13 nm, thicker lens, sticking	>1800
12		8.0	30 nm, aggregates	>1800

Table 1. Results for emulsion films with 0.02 wt.% BLG.

2.2. Zeta potential measurements

We prepared O/W emulsions with 10% volume fraction of soybean oil. Systems without inorganic electrolyte and with added 0.150 M NaCl were studied. The dispersions were formed by intensive stirring of corresponding volumes of the protein solution and the soybean oil, by means of a rotor-stator homogenizer Ultra Turrax T25 (Janke & Kunkel GmbH, IKA-Labortechnik), operating at 13 500 rpm. The duration of stirring was fixed at 5 min for all emulsions. The mean diameter of the produced drops was about 40 μm. The electrophoretic mobility was measured on a Zetasizer II C equipment (Malvern Instruments, Ltd., England). The zeta-potential was determined according to the simple Smoluchowski formula.

3. Results and Discussion

Experimental data for the thickness and stability of films at different values of pH are collected in Table 1. For the sake of clarity, the measured lifetimes are presented graphically in Fig. 1.

The pH dependence of the film lifetime without added NaCl qualitatively corresponds to the predictions of the simple DLVO model: at the extreme pH values of 3 and 8 the stability is higher compared to the intermediate pHs (=4, 6, 7). The isoelectric pH of 5.2 is an exception, with very high stability (Table 1, Fig. 1), which is explained with formation of mechanically stiff layer (see below). At pH=3 and 8, the adsorbed protein layers are highly charged and the engendered electrostatic repulsion keeps the films stable. Weaker repulsion (at pH=4, 6, 7) results in film rupture, yet not immediately, but after a couple of minutes, which indicates a fluctuation mechanism. Such a view was verified by us through calculation of the disjoining

pressure isotherm. For that purpose, we used the results for zeta-potentials; the latter were recalculated as surface charge, according to the classical double layer theory:

[1] $$\Gamma_{ch} = \frac{4\sqrt{c_{el}}}{\kappa_c} \sinh\left(\frac{e\zeta}{2kT} \right)$$

where Γ_{ch} is the number of charges per unit area,
c_{el} is the concentration of (monovalent) electrolyte,
$\kappa_c = 0.001338$ cm$^{0.5}$, and e is the electronic charge.

Fig. 2 displays the plot of Γ_{ch} vs. pH. It is worthwhile to emphasize that the isoelectric point of the adsorbed protein is shifted to somewhat lower values of pH in comparison to the pI of 5.2 for BLG in a bulk solution. The most probable reason for this is the configurational change upon adsorption. Another feature, evident from Fig. 2, is the role of added NaCl, which increases the surface charge in the interval of pH above pI.
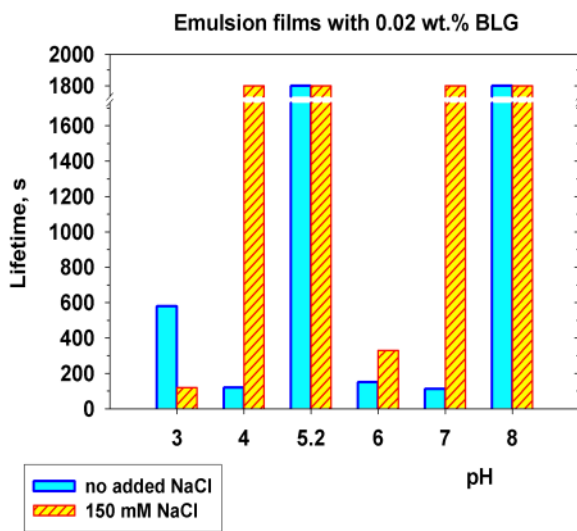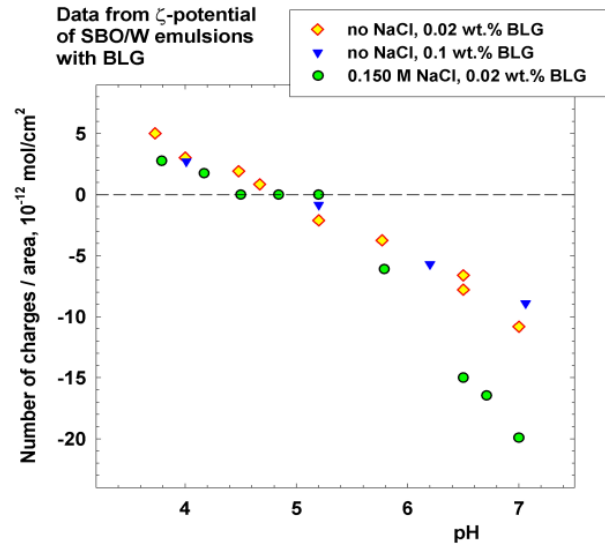

Fig. 1. Lifetime of films from Table 1.

Fig. 2. Surface charge, determined from the zeta potential of SBO droplets.

We utilized the data for Γ_{ch}, Fig. 2, to calculate the electrostatic component of disjoining pressure, based on the solution of the non-linear Poisson-Boltzmann equation under the assumption of constant surface potential (see Ref. (2) for details). In the frames of the DLVO theory, the total disjoining pressure, Π, includes also a contribution from the van der Waals attraction. The latter was calculated following the procedure from Refs. (2, 3). Fig. 3 shows a sample curve for $\Pi(h)$; the maximum plays a stabilizing role, since the capillary pressure applied to the film in the experimental cell ($P_c \sim 20$ Pa, dashed horizontal line) is much lower than the repulsive disjoining pressure around the barrier. Instability would occur if $P_c > \Pi$ (then, the film is forced to thin down), or if $d\Pi/dh > 0$ (then, fluctuation waves grow spontaneously, Refs. (5, 6)); in equilibrium $P_c = \Pi$.

In Fig. 4, the maxima of the disjoining pressure isotherms are plotted vs. pH. In systems without added NaCl, the film stability should increase as pH departs from the isoelectric point;

this is qualitatively true, as judged from Fig. 1. However, it remains to be explained why rupture happens easily at pH=6, 7 (Fig. 1), with thick films (Table 1), while P_c is much smaller than the pressure barrier (Fig. 4). Here we need to invoke the change in the interaction energy, as a function of the thickness h, see Fig. 3. Passing over the barrier to reach an unstable thickness (e.g., $\Pi < P_c$), would require an increase of ~0.1 erg/cm^2 in the energy per unit area.

The smallest piece of surface area capable of undergoing fluctuations should be of the order of the area per one protein molecule; that is ~20 nm^2 (7). Hence, the concomitant energy barrier is about 2×10^{-14} erg, which is half of kT. In other words, it is quite easy to have a small spot in the film that jumps spontaneously into instability.
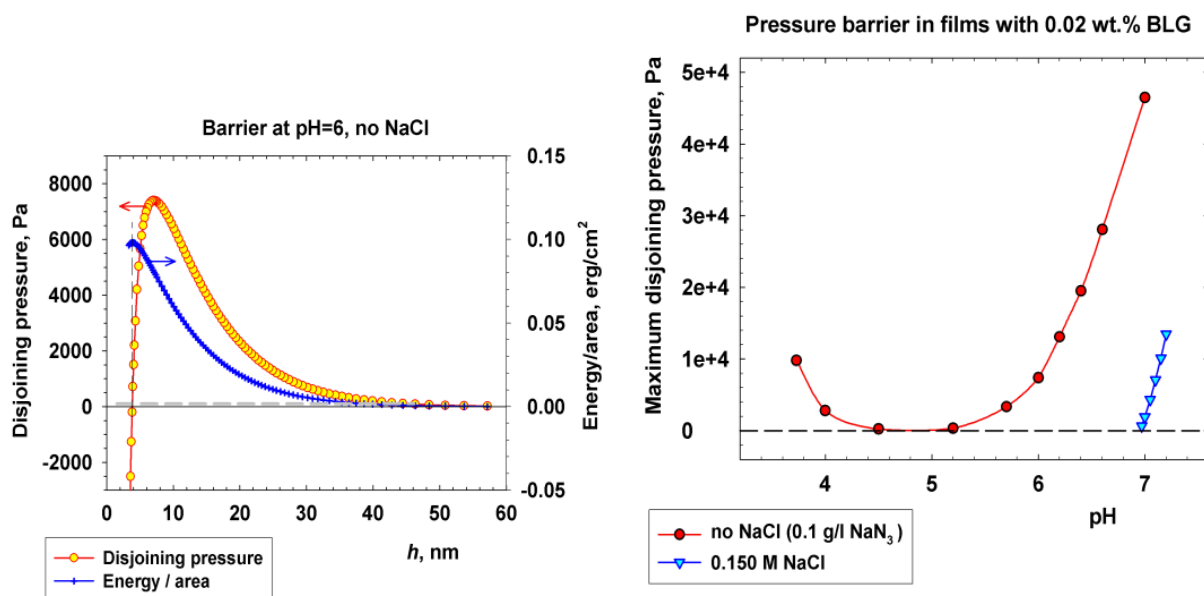

Fig. 3. Calculated disjoining pressure and interaction energy.

Fig. 4. Maxima in the $\Pi(h)$ isotherms.

Of course, this is a simplified consideration, not taking into account important effects such as, e.g., the extension of the adsorbed layers during fluctuation. Still, the low barrier of interaction energy gives a clue as to why thick films at low capillary pressure are unstable.

Let us now pay attention to the case of pH= 5.2 (no added NaCl), where the film stability is exceptionally high (Fig. 1). Our observations comply with the results in Ref. (8), reporting greatly improved stability around the pI. The fact that the film surfaces adhere strongly (which we call "sticking", Table 1), indicates formation of a skin-like entangled network of protein molecules. Despite the absence of electrostatic stabilization in the films, they cannot rupture because of the mechanical strength of the skin, which endures even upon a direct physical contact of the two layers.

At pH=4 (no NaCl), we observed formation of black spots in the films (see Fig. 5). The measured thickness at the spots was about 5-6 nm (Table 1). Therefore, existence of a protein bilayer is likely; it is usually referred to as "Newton Black Film" (NBF). Since the diameter of the BLG molecule (in the bulk) is 3.58 nm (7), the very thin NBF most probably consists of two layers of molecules in contact, without any intervening aqueous phase. On the other hand, for foam films (in air), thickness of 11.3 nm was measured (8); that was said to correspond to a triple-layer structure (8).
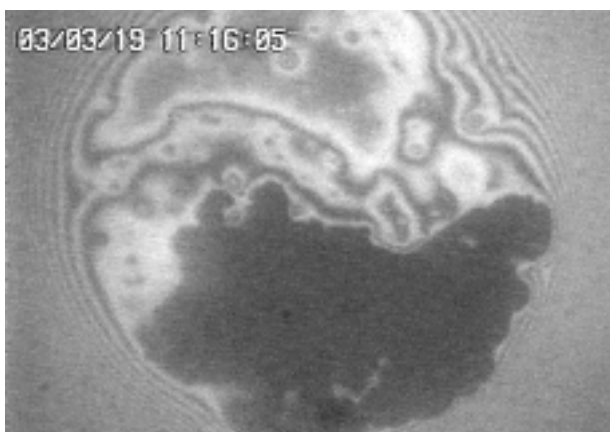
Fig. 5. Spot of NBF at pH=4.

In the presence of NaCl, we do not observe film behavior compatible with the simple DLVO predictions. Indeed, Fig. 4 shows that disjoining pressure barrier due to electrostatic repulsion is absent at all pH values below 7, if the system contains 0.150 M NaCl. In reality, the films with 0.150 M NaCl are very stable at pH=4, 5.2 and 7 (Table 1). The reason for this may be related to the well pronounced sticking (adhesion) in the thin films. Increased aggregation is also noticed (Table 1), as protein lumps on the film surfaces are seen in the microscope. The latter observations suggest that NaCl augments to a considerable extent the ability of the BLG molecules to form links with their neighbors. Consequently, gel-like layers (skin) build up easily. The electrostatic repulsion between the two o/w boundaries is screened by the salt; nevertheless, the adsorbed protein layers stay stable, even when they are in direct contact. The inorganic salt (NaCl) evidently causes changes in the protein configuration and properties; effects related to that were discussed in Ref. (2). In Ref. (9) it was found that addition of NaCl (1 M) led to increase of surface compressional modulus of spread BLG layers on a/w boundary at pH=7.

Another aspect of the molecular interaction pertains to the case when the protein is charged (at pH away from pI), and the aqueous phase contains significant amount of salt. For example, in the presence of 0.150 M NaCl and at pH=3, the BLG molecules carry charges, and at the same time the electric double layer is highly compressed: κ^{-1}= 7.86 Å,
which is smaller than the size of the protein molecule. In this situation, the surface charge can no longer be regarded as uniformly distributed (smeared out) along the plane of the interface. Therefore, DLVO treatment is unable to incorporate the corresponding forces. Instead, one should consider local interaction between the particles; that will be very short-ranged and will operate both laterally within the adsorbed layers and across the film (similarly to the steric repulsion). Such a short-range interaction might be responsible for decreased film stability at pH=3.0 and high salt (Fig. 1); due to local tangential repulsion, formation of stiff protein layers is eventually suppressed.

4. Conclusions

We have studied emulsion-type films stabilized by beta-lactoglobulin. If no salt is added, the film stability qualitatively complies with the DLVO theory (for dominance of the electrostatic and van der Waals forces). We discuss a possible explanation of the observed instability in thick films (~50 nm), at low capillary pressure, P_c. Despite the fact that the disjoining pressure barrier is much higher than P_c, it turns out that only a small amount of

energy would be sufficient for emergence of an unstable fluctuation. At the isoelectric pH, the film stability increases considerably; the effect is probably due to layer reinforcement that follows from the lack of tangential repulsion. In a similar manner, the inorganic electrolyte (NaCl) brings about increased film stability; it enhances the ability of the protein molecules to entangle and aggregate with their neighbors; formation of a skin-like layer is favored.

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