

Dilatational and Shear Rheology of Protein Layers on Water/Air Interface

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ABSTRACT

We propose a simple method for simultaneous measurement of the surface dilatational and shear elasticities and viscosities of gel-like protein layers on air/water boundary. The stress response to compression/expansion of the interface in a Langmuir trough is measured at two different orientations of the Wilhelmy plate- collateral and perpendicular to the movable barrier. Experiments with several systems containing proteins have been carried out. Different rheological behaviour is observed. The stress/strain results are interpreted in terms of a simple model (Maxwell body), whose predictions are in a good agreement with the measurements. The fit of the stress as a function of time yields values for the material properties of the interface. Adsorbed layers of β -lactoglobulin at low concentration are subject to ageing: the dilatational elasticity increases and shear elasticity gradually develops in a time scale of several hours. At higher concentrations of BLG the interface becomes fully visco-elastic. Mixed layers of β -casein and nonionic surfactant (Tween 20) exhibit dilatational viscoelasticity (the quantities which characterise the shear response are small).

1 INTRODUCTION

The interfacial rheology has been recognised to play an important role for the stability of emulsions and thin liquid films. There are several aspects of this role, connected with different physical effects. The elasticity of the o/w surfaces was shown to be among the main factors which determine the rate of thinning of films or gaps between approaching drops [1, 2]. On the other hand, the mechanism for film rupture involves propagation and growth of interfacial fluctuation waves. The damping of these corrugation waves by adsorbed surfactants is due to the combined action of interfacial elasticity and viscosity [3-5]. The rheology of the liquid boundary is especially important during emulsification [4], when stability of drop contacts has to be ensured under the conditions of incomplete surfactant coverage on the newly created interfaces. As far as the long-term stability is concerned, the resistance of the adsorbed layers to shear deformation may be crucial for the occurrence of rupture and coalescence of the drops [6].

Proteins on liquid surfaces are known to form a strong visco-elastic network in which the molecules are essentially immobile [7]. This network opposes the film stretching, damps the interfacial fluctuations, and slows down the thinning [7]. Common methods for studying the surface shear rheology in protein-containing systems rely on measuring the rotational motion of a knife-edged bob, disc, or ring, which is placed in the plane of the interface [4, 8]. Different techniques are usually applied to explore the dilatational rheology: the interfacial tension (σ) is measured whilst the surface area is subjected to changes. One distinguishes "trough" and "drop" methods [4]. The investigation of a large liquid interface in a trough is often carried out by creating periodic lateral deformations which give rise to longitudinal waves. A complex visco-elastic modulus is then obtained from the analysis of the measured σ ; it may be resolved into a storage modulus (connected with the elasticity), and a loss part (giving information for the dissipation processes in the layer).

Here we propose a simple method for simultaneous determination of the dilatational and shear elasticity and viscosity of adsorbed protein layers on water/air interface. Flat liquid surface is deformed by a slowly moving barrier in a Langmuir trough. The fact that the surface tension of visco-elastic layers is a tensorial quantity is used for measure-

ments with Wilhelmy plate oriented in two different directions: collateral and perpendicular with respect to the barrier. The data interpretation is carried out according to the rheological model of Maxwell; this allows us to extract values of the relevant physical parameters.

2 THEORY

2.1 Stress Response of Visco-Elastic Layers

In general, the stresses which develop when a protein-laden interface is deformed may originate from the changes in the adsorption (the so-called Gibbs elasticity), from the elasticity of the (gel-like) network of entangled molecules, as well as from viscous dissipation. The latter, in its turn, can be due either to the friction when neighbouring molecules are laterally displaced with respect to each other, or to mass exchange with the bulk or sub-surface ("apparent" interfacial viscosity). A phenomenological treatment of the rheology of adsorbed protein layers is possible in the frames of a very simple model, known as "Maxwell body" (Fig. 1c). It will be shown below that the experimentally observed time dependence of the stress when the layer is subjected to periodic compression/expansion is in full agreement with the predictions of this model. The latter fact enables us to determine important material characteristics of the layers: dilatational and shear elasticities (denoted by K , μ) and viscosities (ζ_s , η_s , respectively).

Let us first write separately the expressions for the elastic and the viscous stresses which arise when an interface is stretched in the Langmuir trough. The geometry of the deformation is illustrated in Fig. 1b. According to Eqs. (3), (4) in Ref. [9], we have

$$\tau_{11,el} = (K + \mu)u_{11,el} ; \quad \tau_{22,el} = (K - \mu)u_{11,el} ; \quad (1)$$

$$\tau_{11,visc} = (\zeta_s + \eta_s)D_{11,visc} ; \quad \tau_{22,visc} = (\zeta_s - \eta_s)D_{11,visc} . \quad (2)$$

Here τ_{11} , τ_{22} are the eigenvalues of the stress tensor (along the x and y axes, Fig. 1b), and u_{11} , D_{11} are the components of the strain and the rate-of-strain tensor ($u_{22} = u_{12} = 0$, $D_{22} = D_{12} = 0$ for uni-axial deformation in the trough, Fig. 1b). The subscripts "el", "visc" in Eqs. (1), (2) designate the elastic and viscous contributions in the stress and strain. According to the rheological model adopted by us (Maxwell body), the following relations hold:

$$\tau_{11} = \tau_{11,el} = \tau_{11,visc} ; \quad \tau_{22} = \tau_{22,el} = \tau_{22,visc} ; \quad (3)$$

$$u_{11} = u_{11,el} + u_{11,visc} ; \quad D_{11} = D_{11,el} + D_{11,visc} , \quad (4)$$

where τ_{11} , τ_{22} , u_{11} , D_{11} are the total quantities. If $u = u_{el} + u_{visc}$ and $v = v_{el} + v_{visc}$ are the displacement and the velocity of the surface material points, then

$$u_{11} = \frac{\partial u}{\partial x} ; \quad D_{11} = \frac{\partial v}{\partial x} . \quad (5)$$

Besides, $v = du / dt$. In the limit of small deformations the material derivative d / dt may be approximated by $(\partial / \partial t)|_{x=const}$, so from Eqs. (5) it follows that

$$\frac{d}{dt} u_{11} = D_{11} , \quad \text{or} \quad \frac{d}{dt} u_{11,el} = D_{11,el} ; \quad \frac{d}{dt} u_{11,visc} = D_{11,visc} . \quad (6)$$

The balance of the linear momentum on the interface requires that

$$\nabla_{II} \cdot \tau = 0 ; \quad \frac{\partial \tau_{11}}{\partial x} = 0 , \quad (7)$$

which is equivalent to $\frac{\partial^2 u_{el}}{\partial x^2} = 0 ; \quad \frac{\partial^2 u_{visc}}{\partial x^2} = 0$ (cf. Eqs. (3), (1), (2), (5)). We come to

the conclusion that the displacement is a linear function of the x -coordinate, and u_{11} , D_{11} do not depend on x . In such a case the rate of total deformation is

$$D_{11} = \frac{d\alpha}{dt} , \quad \text{where} \quad \alpha = u_{11} = \ln \frac{A}{A_0} \quad (8)$$

is the relative area dilatation (cf. Eq. (7) in Ref. [9]).

Let us now differentiate Eqs. (1) with respect to time, and sum up with Eqs. (2). Taking into account Eqs. (3), (4), we derive the following differential equations for the stresses:

$$\frac{d\alpha}{dt} = \frac{1}{K + \mu} \frac{d\tau_{11}}{dt} + \frac{\tau_{11}}{\zeta_s + \eta_s} ; \quad \frac{d\alpha}{dt} = \frac{1}{K - \mu} \frac{d\tau_{22}}{dt} + \frac{\tau_{22}}{\zeta_s - \eta_s} . \quad (9)$$

Our Langmuir trough allows us to carry out deformation with constant rate (in other words, $d\alpha / dt$ is fixed). Then, it is easy to solve Eqs. (9), and the result is

$$\tau_{11}(t) = \left[\tau_{11}(0) - \frac{d\alpha}{dt}(\zeta_s + \eta_s) \right] \exp\left(-\frac{K + \mu}{\zeta_s + \eta_s} t\right) + \frac{d\alpha}{dt}(\zeta_s + \eta_s) ; \quad (10)$$

$$\tau_{22}(t) = \left[\tau_{22}(0) - \frac{d\alpha}{dt}(\zeta_s - \eta_s) \right] \exp\left(-\frac{K - \mu}{\zeta_s - \eta_s} t\right) + \frac{d\alpha}{dt}(\zeta_s - \eta_s) . \quad (11)$$

The stresses τ_{11} , τ_{22} are simply the respective deviations of the interfacial tensions σ_{11} , σ_{22} from the equilibrium value σ_{eq} (in the non-deformed state $\sigma_{11} = \sigma_{22} = \sigma_{\text{eq}}$). From Fig. 1b it becomes evident that σ_{11} is in fact the interfacial tension which one would measure if the Wilhelmy plate is oriented *parallel* to the barrier (σ_{\parallel}), and σ_{22} (or σ_{\perp}) corresponds to *perpendicular* orientation;

$$\tau_{11} = \sigma_{\parallel} - \sigma_{\text{eq}} ; \quad \tau_{22} = \sigma_{\perp} - \sigma_{\text{eq}} . \quad (12)$$

In our experimental setup the deformation is periodic- we apply compression-expansion cycles with low frequency (less than 0.03 Hz). It is worthwhile to point out that the speed with which the deformation propagates along the layer is rather high- from the data provided by Lucassen & van den Tempel [10] one can estimate this velocity to be about 10 cm/s. Given the dimensions of our trough (~ 20 cm), we may conclude that the time required for the deformation induced by the barrier to reach any point is much shorter than the period of oscillations. Therefore, it seems reasonable to accept that the deformation establishes almost instantaneously throughout the layer, i.e., the material follows the motion of the barrier.

2.2 Purely Elastic Behaviour

In gel-like protein layers viscous dissipation might be absent (or may not manifest itself under certain conditions of deformation). Such will be the case when the two viscosities ζ_s , η_s are high, or the deformation is relatively fast. Then, only the elastic element in the Maxwell rheological model (Fig. 1c) will be active. This case can be analysed by expanding the exponents in Eqs. (10), (11). The result for the stresses reads

$$\tau_{11}(t) - \tau_{11}(0) = (K + \mu)[\alpha(t) - \alpha(0)] , \quad (13)$$

$$\tau_{22}(t) - \tau_{22}(0) = (K - \mu)[\alpha(t) - \alpha(0)] \quad (14)$$

(here we have assumed that $d\alpha / dt = \text{const}$, which holds in our experiments). In Section 4 below we will give examples of protein-containing systems where the water/air interface exhibits elastic behaviour.

3 EXPERIMENT

3.1 Materials and Method

β -lactoglobulin (Catalogue No. L-0130, Lot No. 114H7055), β -casein (Cat. No. C-6905, Lot No. 108H7812), and Tween 20 were purchased from Sigma (St. Louis, MO, USA), and were used as received. Sodium chloride (NaCl, p.a.) was purchased from Aldrich Co. In order to avoid enzyme contamination, which can destroy the native protein structure, we added sodium azide (NaN_3 , p.a.)- 0.1 g/l in the aqueous solutions. The water used throughout all experiments was obtained from a Milli-Q purification system (Millipore, USA).

Figure 1a shows our experimental setup. It comprises a home-made Langmuir trough (manufactured from Teflon), with the following dimensions: width 211 mm, length 202 mm, depth 6 mm. The surface tension, σ , was measured by means of a sand-blasted glass plate (15×15 mm)- Wilhelmy method. The signals obtained from the surface tension sensor were passed to an Analogue-Digital Converter (ADC), and were recorded on a PC (Intel 486) for subsequent processing. The Langmuir trough only allows compression/expansion deformation at a constant rate of barrier translation in a given run. This rate may be changed in different runs.

3.2 Experimental procedure

Before each experiment the Langmuir trough was thoroughly cleaned with chromic acid, and was rinsed abundantly with Milli-Q-quality water. The cleanness of the trough was additionally checked, by compression of the bare air-water interface and measurement of the surface pressure. If the surface pressure at maximum compression did not exceed 0.1 dyn/cm, the trough was assumed clean enough. After that we removed the water and poured the respective protein solution in. All solutions were freshly prepared (within no more than 24 hours). A quasi-equilibrium value of the surface tension had been reached in all cases before starting the deformations of the adsorbed protein

layer. Bearing in mind the tendency of globular proteins to change very slowly their conformation on the interface, the term "quasi-equilibrium" should be understood to mean reaching a more or less constant value of σ (absence of change for 15-20 minutes). This is achieved approximately one hour after loading the aqueous solution in the trough.

The deformation experiments were performed as follows: Firstly, an area A_0 (Fig. 1b) was chosen in such a way that the deformation in both directions, $\pm\Delta A$, did not exceed 5%. Secondly, we chose the barrier rate and carried out a compression/expansion cycle. Thirdly, after completing a deformation cycle (usually 2-3 compressions and 2-3 expansions) at a given rate with collaterally positioned Wilhelmy plate, we carefully changed the plate orientation (from collinear to perpendicular with respect to the moving barrier, see also Figure 1), and repeated the same compression/expansion cycle.

4 RESULTS AND DISCUSSION

We chose to investigate several systems with protein-laden interfaces, whose rheological behaviour is different. The analysis of the experimental data for the stress-strain relationship allows us to find the surface dilatational and shear elasticities and viscosities. The results are summarised in Table 1.

At very low concentration of β -lactoglobulin (cases 1, 2 in the Table) the layer is elastic. We apply triangular-shaped deformation, i.e. periodic expansions/compressions with constant rate (α is a linear function of time during each stage of dilatation/compression). The stresses (σ_{\parallel} , σ_{\perp}) also turn out to be perfectly linear with time, which means that Eqs. (13), (14) are followed. In addition, when the layer is fresh there is no difference between σ_{\parallel} and σ_{\perp} (case 1 in Table 1). Therefore, the shear elasticity in this system is vanishingly small (cf. Eqs. (12)-(14)), and K is the only relevant physical parameter. The absence of dissipation effects could be attributed to the low surface coverage Γ , and to slow adsorption at this protein concentration: the deformation is relatively fast and the process of adsorption from the bulk cannot follow the periodic changes in Γ .

When the system with 1×10^{-4} wt% BLG is left to age for one night, we notice appearance of shear elasticity- row 2 in Table 1. Figure 2 shows two curves for the stress response at the two different orientations of the measuring Wilhelmy plate: collinear,

$|\sigma_{\parallel}(\alpha) - \sigma_{\text{eq}}|$, and perpendicular, $|\sigma_{\perp}(\alpha) - \sigma_{\text{eq}}|$. (At $t=0$ $\alpha=0$ and $\sigma=\sigma_{\text{eq}}$.) From the two slopes of the straight lines in Fig. 2 Eqs. (13), (14) yield $K=70.5$ dyn/cm, $\mu=7.5$ dyn/cm. Evidently, the ageing of the BLG solution is accompanied by strengthening of the protein network on the a/w boundary. The layer acquires solid-like properties (shear elasticity), and the dilatational elasticity K increases in comparison with the fresh system.

Another type of rheological behaviour is observed with a mixture of β -casein and Tween 20- row 3 in Table 1. In Fig. 3a we present the measured time dependence of the stress, which is highly non-linear (compare with the area changes, represented by the dashed lines). The latter fact is an indication for significant dissipation effects. In this system we apply Eqs. (10), (11) for data interpretation (the expansion branch is only considered). Fig. 3b demonstrates that the fit of $\sigma_{\text{eq}} - \sigma(t)$ according to the Maxwell model is quite good. The experiment reveals that $\sigma_{\parallel} = \sigma_{\perp}$, which is possible if $\mu \ll K$ and $\eta_s \ll \zeta_s$ — cf. Eqs. (10), (11). Thus, the two parameters necessary to draw the fit in Fig. 3b are sufficient for us to obtain the following results: $K=42.6$ dyn/cm, $\zeta_s = 127.9$ dyn.s/cm.

The absence of detectable shear response is not surprising in view of the properties of the interfacial layer. β -casein is a disordered protein which is known to form layers with vanishingly small surface shear viscosity [11]. On the other hand, the low molecular weight surfactant (Tween 20) renders the a/w boundary completely fluid [4, 12] (and that is why the shear elasticity μ is zero, as was found also in Ref. [9]). The dilatational viscosity ζ_s (row 3, Table 1) is probably connected with mass exchange with the bulk solution, but of course a contribution from a "true" viscous dissipation within the layer (due to the protein) cannot be excluded.

Our last example is with a system containing 0.01 wt% β -lactoglobulin and 0.15 M NaCl- row 4 in Table 1. Figure 4 shows the respective curves for the time dependence of the two stress components, τ_{11} and τ_{22} (during expansion). We fit these data with the full set of Eqs. (10), (11). Thus, the theoretical curve for $\tau_{11} = \sigma_{\parallel} - \sigma_{\text{eq}}$ was calculated from Eq. (10) with $(\zeta_s + \eta_s) d\alpha / dt = 7.22$ dyn/cm, $(\zeta_s + \eta_s) / (K + \mu) = 17.8$ sec, and $\tau_{22} = \sigma_{\perp} - \sigma_{\text{eq}}$ was calculated from Eq. (11) with $(\zeta_s - \eta_s) d\alpha / dt = 6.29$ dyn/cm, $(\zeta_s - \eta_s) / (K - \mu) = 23.0$ sec. The four adjustable parameters, K , μ , ζ_s , η_s , are now eas-

ily found (see Table 1). We notice that $\mu < K$ and $\eta_s < \zeta_s$ (similarly to the case 3 discussed above). Still, the system containing 0.01 wt% BLG exhibits fully developed viscoelasticity. Comparison with the diluted solution of BLG (row 1, Table 1) reveals that increasing protein content leads to higher elasticity, and brings about viscous dissipation. The latter effect is probably due to appreciable friction in the more dense layer, manifested especially in the *shear* viscosity, η_s . On the other hand, the high *dilatational* viscosity, ζ_s (row 4 in Table 1), is likely to stem from the faster exchange between the surface and the bulk phase (which may give rise to what is called "apparent" viscosity).

5 CONCLUSIONS

In this work we propose a method for determination of four physical parameters characterising the interfacial rheological properties of protein layers. A simple experiment in a Langmuir trough is carried out, with the surface tension being measured at two orientations of the Wilhelmy plate. Data for the time dependence of the stresses are well fitted by the Maxwell rheological model, which allows us to gain information for the elasticities and viscosities that refer to dilatation and shear. Results with particular systems indicate that increasing protein concentration leads to layer reinforcement. This is manifested as increased values of the elasticities and the shear viscosity. The dilatational viscosity is probably affected by mass exchange between the interface and the bulk phase.

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TABLE 1. Summary of results for the studied systems

No.	System	Type of rheological behaviour	Determined parameters (expansion)
1	BLG 1×10^{-4} wt% + 0.15 M NaCl Freshly prepared	Dilatational elasticity only No shear elasticity No viscous dissipation	$K = 53.0$ dyn/cm $\mu = 0$
2	BLG 1×10^{-4} wt% + 0.15 M NaCl Aged overnight	Dilatational and shear elasticity No viscous dissipation	$K = 70.5$ dyn/cm $\mu = 7.5$ dyn/cm
3	β -casein 0.001 wt% + 1×10^{-5} M Tween 20	Dilatational viscoelasticity Negligible shear elasticity and viscosity	$K = 42.6$ dyn/cm $\mu \ll K$ $\zeta_s = 127.9$ dyn.s/cm $\eta_s \ll \zeta_s$
4	BLG 0.01 wt% + 0.15 M NaCl	Full dilatational and shear visco-elasticity	$K = 83.2$ dyn/cm $\mu = 16.2$ dyn/cm $\zeta_s = 1654.8$ dyn.s/cm $\eta_s = 113.9$ dyn.s/cm

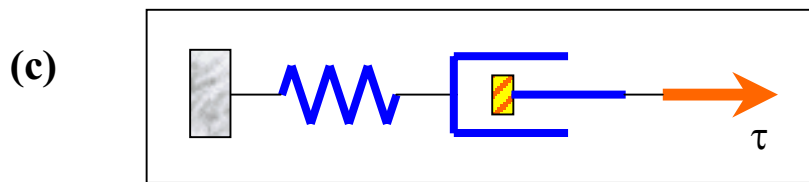
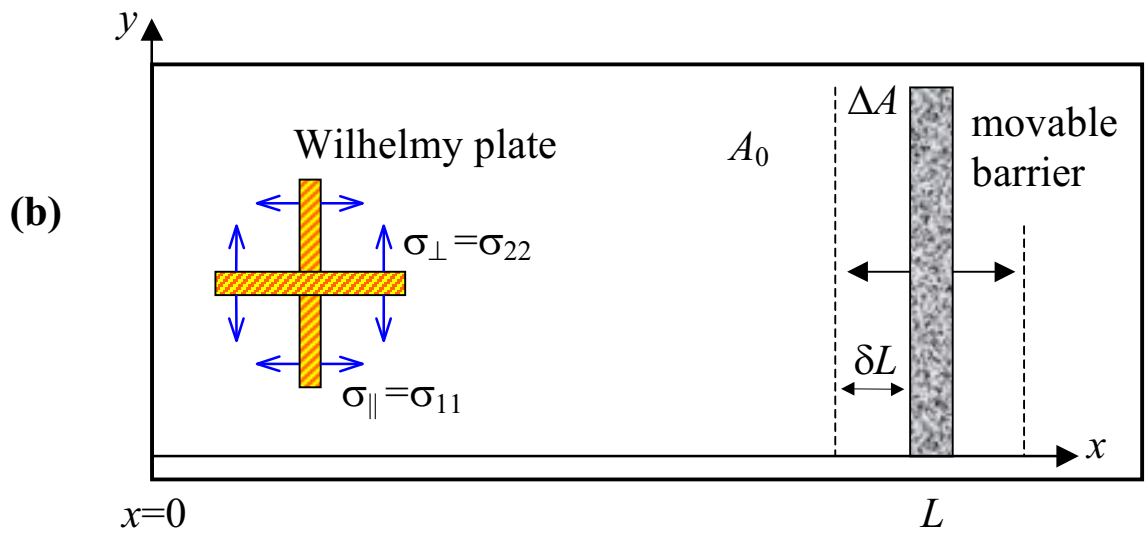
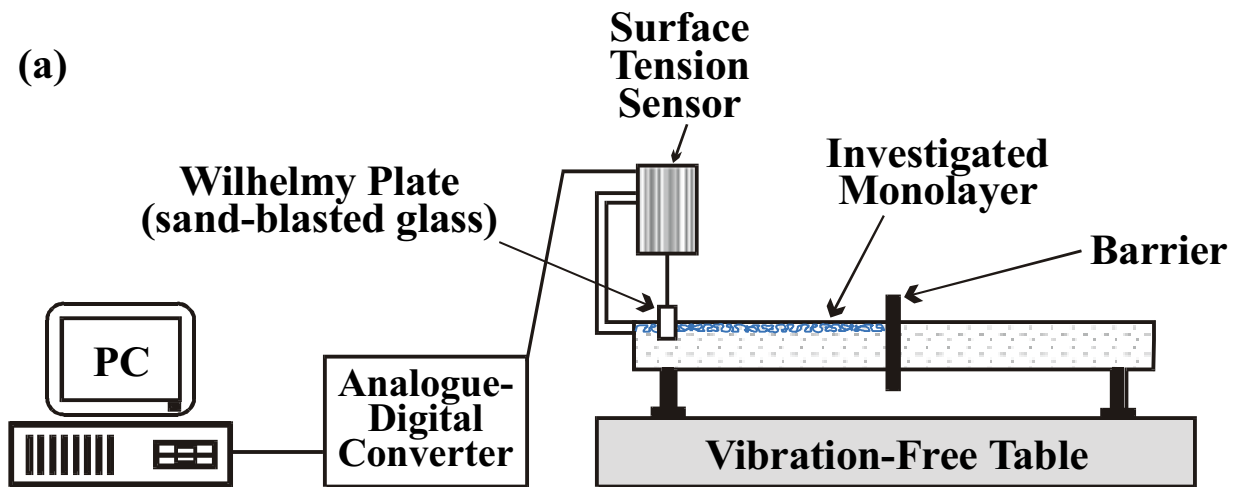
FIGURE CAPTIONS

Figure 1. (a) Sketch of the experimental setup (Langmuir trough); (b) Geometric configuration of the system: flat interface in the trough. Measurements were performed at two orientations of the Wilhelmy plate; (c) Rheological model for visco-elastic protein layers.

Figure 2. Elastic stress response of the surface, as a function of the relative area deformation during expansion for two different orientations of the Wilhelmy plate: collinear (squares), and perpendicular (circles). The system contains 1×10^{-4} wt% BLG and 0.15 M NaCl (at the "natural" pH, 6.2). The interface was aged overnight.

Figure 3. (a) Measured stress for periodic expansion/compression, at relative dilatation rate $d\alpha / dt = 6.49 \times 10^{-3} \text{ s}^{-1}$, for a system with 0.001 wt% β -casein and 1×10^{-5} M Tween 20. The symbols represent experimental data for the stress, while the dashed lines correspond to the area deformation (see the right-hand ordinate axis); (b) Fit of the data for expansion in the system from case (a). The solid line is drawn according to the Maxwell model (Eqs. (10), (11)). The shear elasticity and viscosity are negligible.

Figure 4. Fit of the experimentally measured stress at expansion of a visco-elastic layer formed from aqueous solution of 0.01 wt% BLG and 0.15 M NaCl. The dilatation rate was $4.08 \times 10^{-3} \text{ s}^{-1}$. From the two curves (at collinear and perpendicular orientation of the Wilhelmy plate with respect to the barrier) one determines the four material constants: K , μ , ζ_s and η_s .



Maxwell body

Figure 1. T. Gurkov *et al.*, "Dilatational and Shear Rheology of Protein Layers ..."

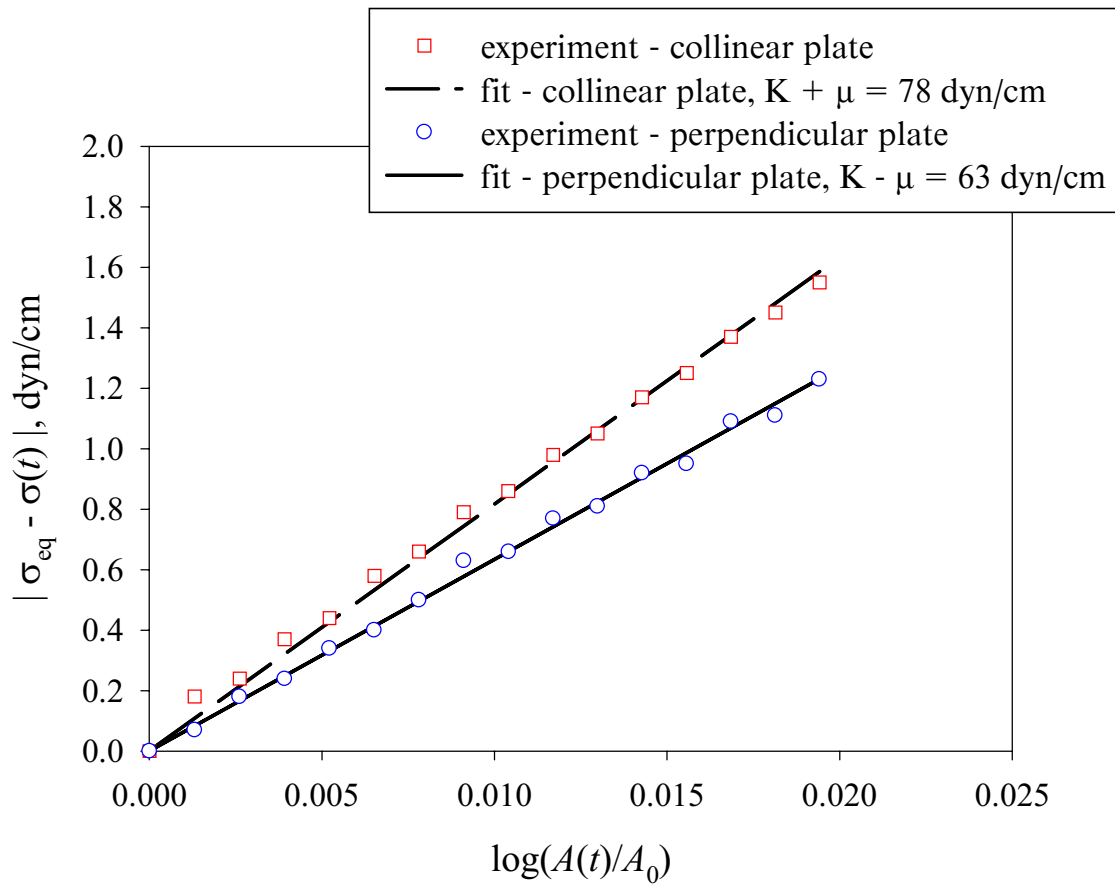


Figure 2. T. Gurkov *et al.*, "Dilatational and Shear Rheology of Protein Layers ..."

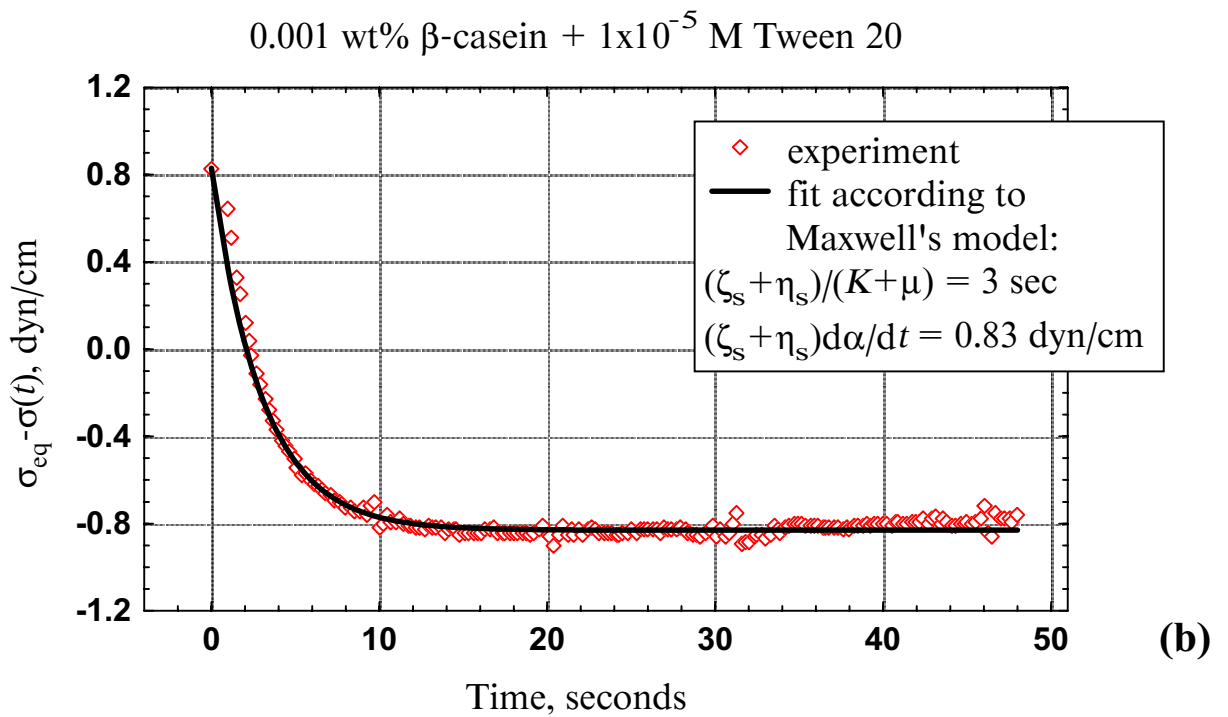
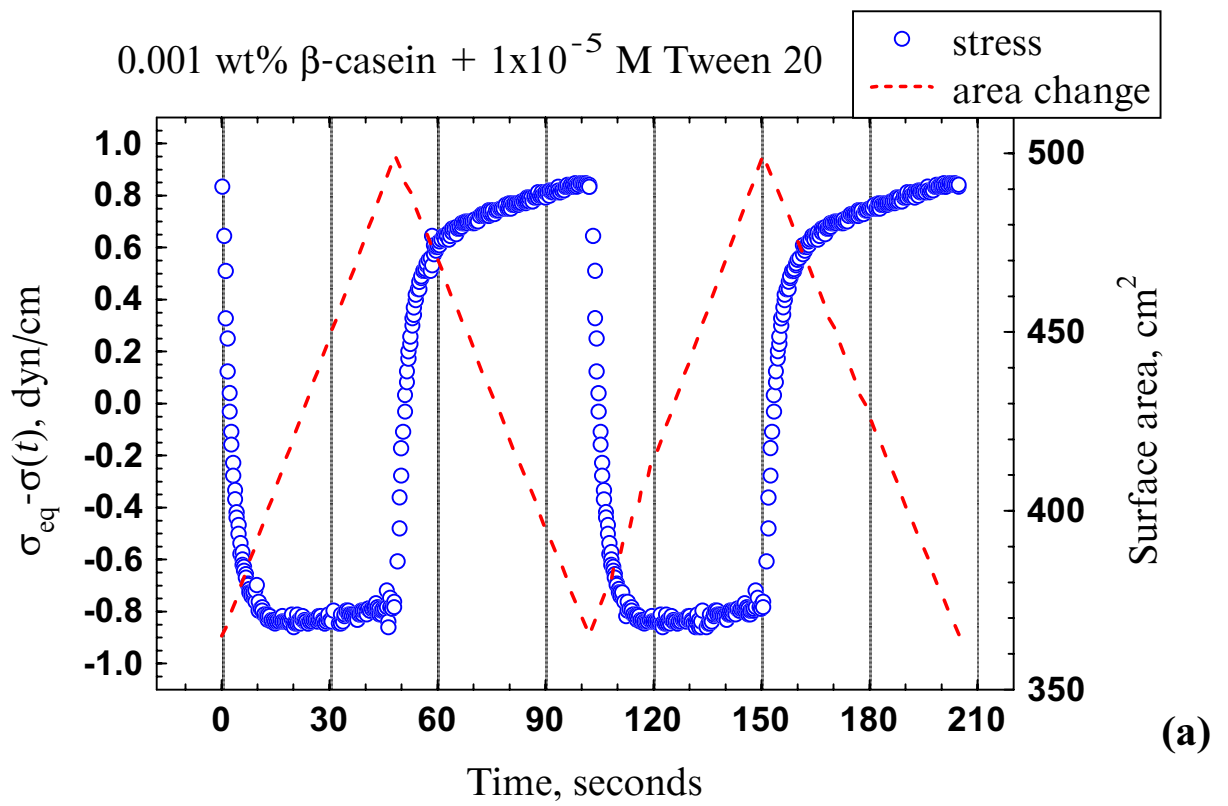


Figure 3. T. Gurkov *et al.*, "Dilatational and Shear Rheology of Protein Layers ..."

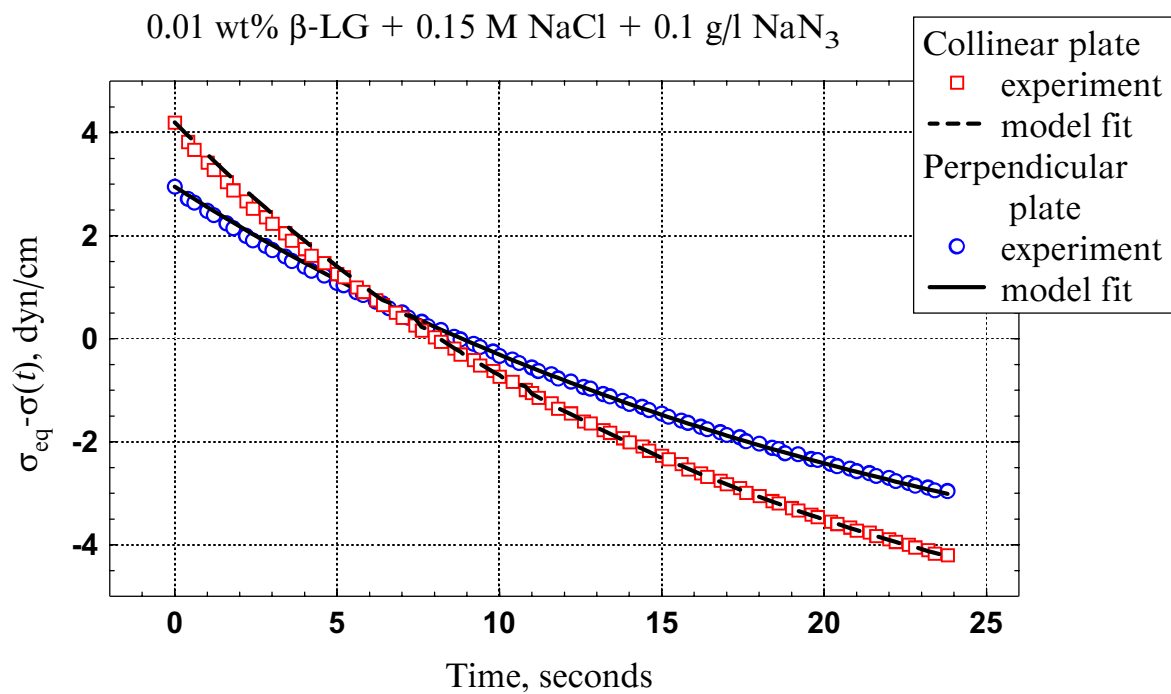


Figure 4. T. Gurkov *et al.*, "Dilatational and Shear Rheology of Protein Layers ..."