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for the article

Shear rheology of hydrophobin adsorption layers at oil/water interfaces and data interpretation in terms of a viscoelastic thixotropic model

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Appendix A. Applications of the VT model

1 Effect of enzyme on the rigidity of β-casein layers

The addition of small amounts of the enzyme transglutaminase increases the rigidity of β-casein adsorption layers as a result of the cross-linking of the β-casein molecules by the enzyme. The application of the viscoelastic-thixotropic (VT) model enables one to determine how the enzyme affects the surface shear elasticity and viscosity. For this goal, we processed data for G' and G'' obtained in ref. [1] for β-casein layers at the interface between water and tetradecane (C14) in the presence of 0.001 wt% transglutaminase. Three sets of experimental data were processed corresponding to 0.001, 0.005 and 0.01 wt% β-casein concentrations. They correspond to weight fractions x_{TG} = 0.50, 0.17 and 0.09 of the transglutaminase in its mixture with the β-casein. The aging time of the adsorption layers was 8 h; pH = 7 was maintained by 10 mM phosphate buffer. G' and G'' have been measured in the frequency sweep oscillatory regime, i.e. ω was varied at a fixed amplitude, $\gamma_a = 2$ % [1].

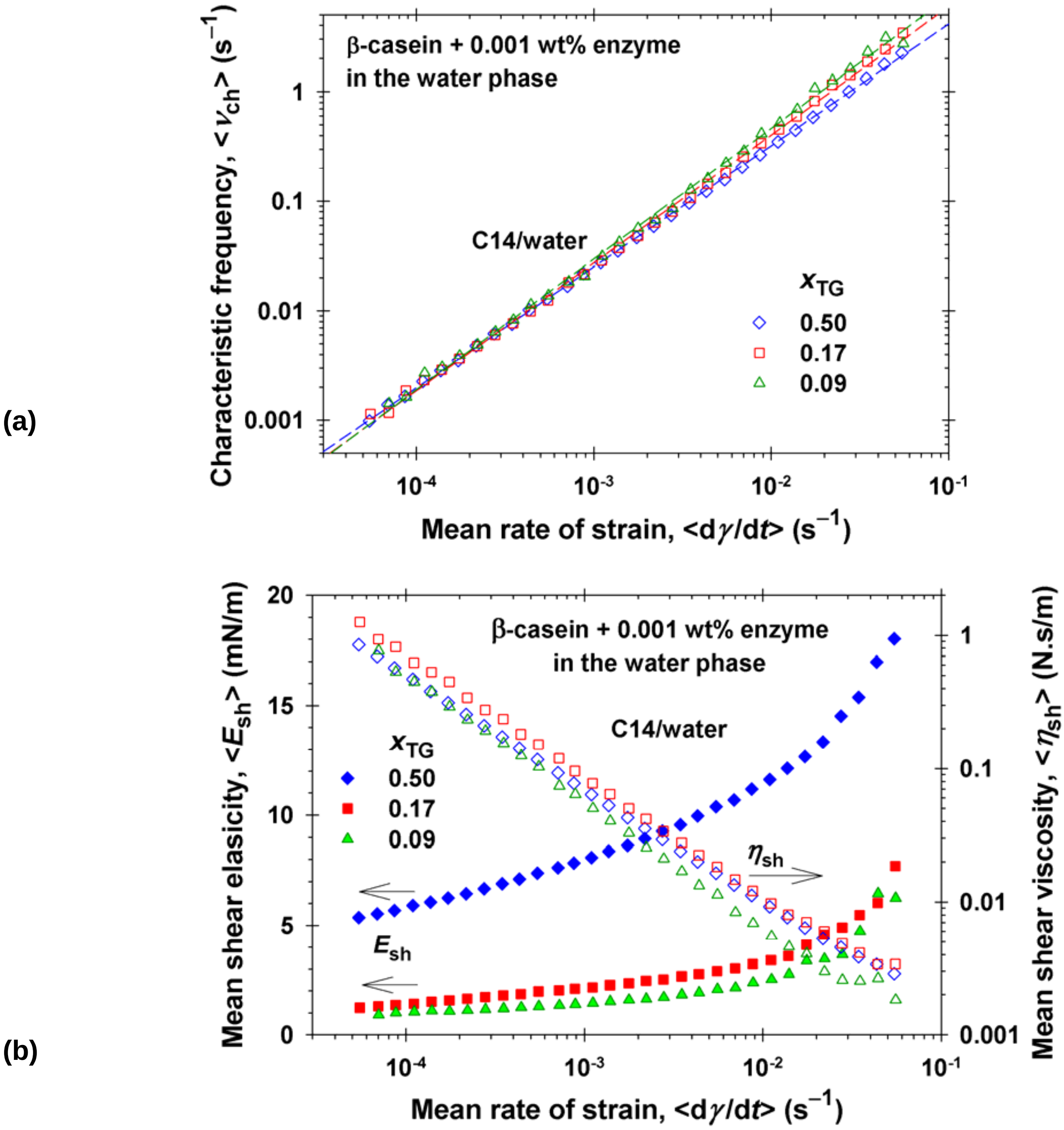

Fig. A1. Rheological parameters of adsorption layers from β-casein + transglutaminase enzyme at the C14/water interface determined from data [1] for G' and G'' at three different enzyme weight fractions, x_{TG}. (a) Plot of the characteristic frequency $\langle \nu_{ch} \rangle = G''\omega/G'$ vs. the rate of strain $\langle \dot{\gamma} \rangle$ in accordance with eqn (9). (b) Plots of the surface shear elasticity $\langle E_{sh} \rangle$ and viscosity $\langle \eta_{sh} \rangle$ vs. $\langle \dot{\gamma} \rangle$ calculated from eqn (11) and (12).

Fig. A1a shows the plots of $\langle \nu_{\mathrm{ch}} \rangle$ vs. $\langle \dot{\gamma} \rangle$ for the three β-casein concentrations. In double-log scale, the three sets of data excellently comply with linear dependences and consequently, the respective viscoelastic layers obey the VT model. The values of the parameters *m* and *Q*, determined from the slopes and intercepts of the three lines, are listed in Table A1. Both *m* and *Q* increase with the rise of the β-casein concentration.

Table A1. Parameters of the VT model determined by fits of data for $\langle \nu_{\mathrm{ch}} \rangle = G''\omega/G'$ in accordance with eqn (9) in the main text.

System	m	Q (s^{m-1})	μ	Reference**
lysozyme at C16/water	1.04	34	0.428	[2]
β-casein at air/water	0.90	885	0.415	[3]
β-casein at C16/water	0.856	74	0.411	[2]
β-casein + TG*, $x_{\mathrm{TG}} = 0.50$ at C14/water	1.11	53	0.434	[1]
β-casein + TG*, $x_{\mathrm{TG}} = 0.17$ at C14/water	1.17	88	0.439	[1]
β-casein + TG*, $x_{\mathrm{TG}} = 0.09$ at C14/water	1.20	114	0.442	[1]
5 g/L asphaltene at toluene-heptane/brine	1.08	1890	0.432	[4]
7.5 g/L asphaltene at toluene-heptane/brine	1.01	1184	0.425	[4]
10 g/L asphaltene at toluene-heptane/brine	1.06	1561	0.429	[4]

* TG = transglutaminase enzyme

** Source of data for *G′* and *G″*

Substituting the determined *m* and the experimental *G′* and *G″* values in eqn (11) and (12), we calculated $\langle E_{\mathrm{sh}} \rangle$ and $\langle \eta_{\mathrm{sh}} \rangle$ vs. the rate of strain; see Fig. A1b. At $x_{\mathrm{TG}} = 0.09$ and 0.17, the surface shear elasticity $\langle E_{\mathrm{sh}} \rangle$ is only slightly greater than that of β-casein alone, see Fig. 10b in the main text. The effect of the transglutaminase on $\langle E_{\mathrm{sh}} \rangle$ becomes significant at $x_{\mathrm{TG}} = 0.50$.

Remarkably, the effect of the transglutaminase on the surface viscosity $\langle \eta_{\mathrm{sh}} \rangle$ is considerable at all three values of x_{TG}. Thus, at $\langle \dot{\gamma} \rangle = 10^{-4}$ s^{-1} the surface viscosity in Fig. A1b becomes about 100 times greater than that for β-casein alone shown in Fig. 10b in the main text. Hence, the cross-linking of β-casein by the transglutaminase enzyme increases both $\langle E_{\mathrm{sh}} \rangle$ and $\langle \eta_{\mathrm{sh}} \rangle$, the effect on $\langle \eta_{\mathrm{sh}} \rangle$ being stronger.

2 Rheology of asphaltene films at toluene-heptane/brine interfaces

The rheology of asphaltene films has been studied in relation to their importance for the stability of emulsions in petroleum industry. Asphaltenes are defined as as the fraction of petroleum insoluble in n-heptane (n-alkane) but soluble in toluene (aromatic solvents). The formation of a viscoelastic, physically cross-linked network of asphaltene aggregates at the oil/water interface has been recognized to be mainly responsible for building a physical barrier to droplet–droplet coalescence [4].

Here, we apply the VT model to determine the surface shear elasticity and viscosity, $\langle E_{\mathrm{sh}} \rangle$ and $\langle \eta_{\mathrm{sh}} \rangle$, by processing of data for G' and G'' from ref. [4] for asphaltene films at the toluene-heptane/brine interface. The oil phase is a 6:4 mixture of toluene and heptane, which contains dispersed asphaltene particles at concentrations 5, 7.5 and 10 g/L. The water phase is a solution of 3.5 wt% NaCl (brine). G' and G'' have been measured in frequency sweep regime at a fixed strain amplitude, $\gamma_{\mathrm{a}} = 0.1\%$, after aging for 20 hours.

Fig. A2a shows the plots of $\langle \nu_{\mathrm{ch}} \rangle$ vs. $\langle \dot{\gamma} \rangle$ for the three asphaltene concentrations. In double-log scale, the three sets of data perfectly comply with linear dependences and consequently, the respective viscoelastic layers obey the VT model. The values of the parameters m and Q, determined from the slopes and intercepts of the three lines, are listed in Table A1. In the investigated concentration range, m and Q are not so sensitive to the asphaltene concentration.

Substituting the determined m and the experimental G' and G'' values in eqn (11) and (12), we calculated $\langle E_{\mathrm{sh}} \rangle$ and $\langle \eta_{\mathrm{sh}} \rangle$ vs. the rate of strain; see Fig. A2b. At 5 and 7.5 wt% asphaltene, the surface shear elasticities, $\langle E_{\mathrm{sh}} \rangle$, are close to each other. At 10 g/L asphaltene, $\langle E_{\mathrm{sh}} \rangle$ markedly increases. The values of $\langle E_{\mathrm{sh}} \rangle$ for the cross-linked asphaltene layers (Fig. A2b) are somewhat greater than for the cross-linked β-casein layers (Fig. A1b) at the same $\langle \dot{\gamma} \rangle$. However, for both cross-linked β-casein and asphaltene $\langle E_{\mathrm{sh}}\rangle$ and $\langle \eta_{\mathrm{sh}}\rangle$ are considerably smaller than those for HFBII adsorption layers at the same $\langle \dot{\gamma} \rangle$; compare Fig. 9 in the main text with Figs. A1 and A2.

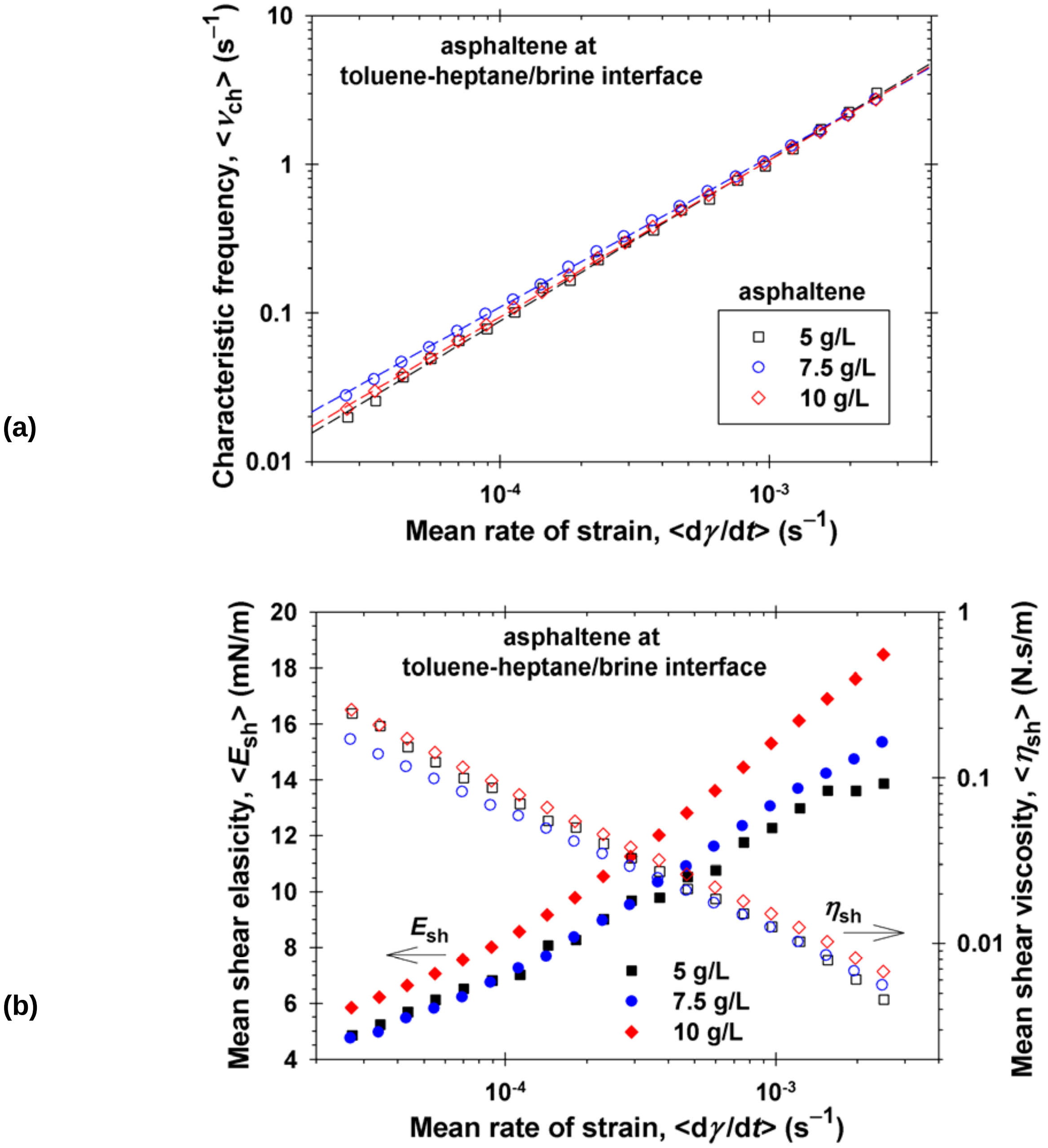

Fig. A2. Rheological parameters of asphaltene films at the toluene-heptane/brine interface determined from data [4] for for G' and G'' at three different asphaltene concentrations. (a) Plot of the characteristic frequency $\langle \nu_{\mathrm{ch}}\rangle = G''\omega/G'$ vs. the rate of strain $\langle \dot{\gamma} \rangle$ in accordance with eqn (9) in the main text. (b) Plots of the surface shear elasticity $\langle E_{\mathrm{sh}}\rangle$ and viscosity $\langle \eta_{\mathrm{sh}}\rangle$ vs. $\langle \dot{\gamma} \rangle$ calculated from eqn (11) and (12) in the main text.

Appendix B. Solidification of an HFBII layer at the o/w interface

Fig. B1 shows the relaxation of the interfacial tension, σ, of a 0.005 wt% HFBII solution. The $\sigma(t)$ dependence is obtained by using the drop shape analysis (DSA) method with a buoyant drop of SBO in the aqueous HFBII solution. This method determines σ by using a fit of the drop profile with the Laplace equation of capillarity. The error of this fit is also shown in Fig. B1.

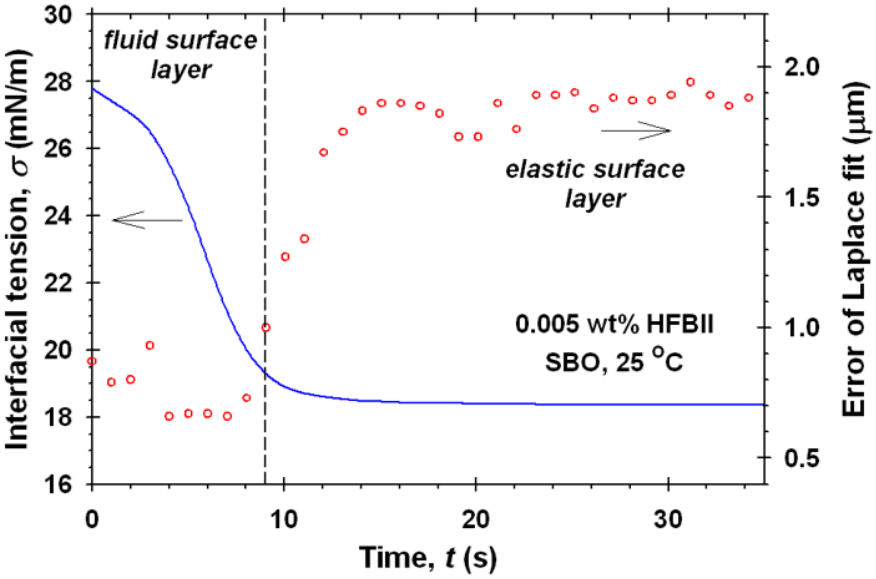

Fig. B1. Time dependencies of the interfacial tension and of the error of the fit of the drop profile by means of the Laplace equation: pendant drop method + DSA at 0.005 wt% HFBII in the water phase; the oil phase is soybean oil (SBO).

The data in Fig. B1 show that σ levels off at 18.4 mN/m for $t > 10$ s, where the error of the Laplace fit sharply increases. The latter indicates deviation of the drop profile from the Laplace profile, which can be interpreted as “solidification” of the interfacial layer, i.e. formation of a layer with non-uniform and non-isotropic (tensorial) surface tension [5]. In such a case, the Laplace equation, which holds for interfaces with uniform and isotropic surface tension, cannot describe the drop profile. The solidification of the interfacial layer indicates also the formation of a *dense* HFBII adsorption layer that spans the whole interface. Our measurements of surface rheology have been carried out with such dense hydrophobin layers, which are formed on the surface of 0.005 wt% HFBII solutions. At lower HFBII concentrations, the interfacial layers do not solidify, and then the rheological response of the system to *shear* deformation dramatically decreases, so that it can be registered only by highly sensitive surface rheometers.

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