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Colloids and Surfaces A: Physicochemical and Engineering Aspects

Surface properties of adsorption layers formed from triterpenoid and steroid saponins

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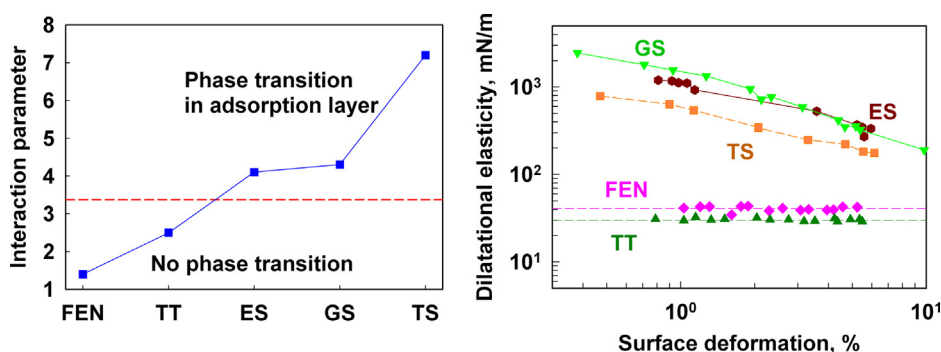[b] Unilever R&D, Vlaardingen, The Netherlands
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HIGHLIGHTS

- Layers of Tea Saponin, Escin and Berry saponins exhibit visco-elastic behavior.
- These layers have very high viscosities and elasticities under dilatational and shear.
- Visco-elastic behavior is due to the strong attraction between the saponin molecules.
- These saponins form surface condensed phases in their adsorption layers.
- Experimentally measured elasticities are close to the theoretically calculated ones.

GRAPHICAL ABSTRACT

ARTICLE INFO

ABSTRACT

Saponins are natural surfactants with non-trivial surface and aggregation properties which find numerous important applications in several areas (food, pharma, cosmetic and others). In the current paper we study the surface properties of ten saponin extracts, having different molecular structure with respect to the type of their hydrophobic fragment (triterpenoid or steroid aglycone) and the number of sugar chains (1 to 3). We found that the triterpenoid saponins Escin, Tea Saponin and Ginsenosides have area per molecule in the range between 0.5 and 0.7 nm^2, and the adsorbed molecules are orientated perpendicularly to the interface. The comparison of the experimentally measured surface elasticities with theoretically estimated ones shows that the saponins with very high dilatational and shear elasticities (up to 2000 mN/m) have molecular interaction parameter in the adsorption layers which is above the threshold value for two-dimensional phase transition. In other words, the highly elastic layers are in surface condensed state, due to strong attraction between the adsorbed molecules. Furthermore, these adsorption layers have non-linear rheological response upon expansion and contraction, even at relatively small deformation. Layers from the other studied saponins (steroids and crude mixtures of triterpenoid saponins), which are unable to form strong intermolecular bonds within the adsorption layer, have zero shear elasticity and viscosity and low dilatational elasticity and viscosity, comparable in magnitude to those reported in literature for protein adsorption layers. The comparison of the results, obtained by several independent experimental methods, allowed us to formulate the conditions under which the results

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from different interfacial rheology tests could be compared, despite the complex non-linear response of the saponin adsorption layers.

1. Introduction and aim of the study

Saponins are a class of natural surfactants found in more than 500 plant species [1–3]. Due to their amphiphilic nature saponins have strong surface activity. The adsorption layers of some saponins exhibit visco-elastic behavior in shear [4–6] or in dilatational deformation [7–9]. It is known that that foams with higher surface modulus are generally more stable to Ostwald ripening, [10,11] bubble coalescence [12,13] and foam drainage [10,14]. The magnitude of the surface modulus impacts also the rheological properties of bulk foams [15]. Saponins have a number of positive bio-effects [1,3,16,17]: anti-fungal, anti-bacterial, anti-cancer [18,19], cholesterol-lowering [20,21], anti-oxidant, anti-inflammatory [22], etc. Their unique surface and biological properties make them a very good choice for foam stabilizers in practical application: food, cosmetics, and pharmaceuticals.

In our previous study [7] we showed that triterpenoid saponins from *Quillaja saponaria* formed adsorption layer with area per molecule, $A \approx 1\,nm^2$ in which the molecules lay parallel to the air–water interface with the hydrophilic glucoside tails protruding into the aqueous phase. Upon small deformation, these adsorption layers exhibit very high surface dilatational elasticity (280 ± 30 mN/m), much lower shear elasticity (26 ± 15 mN/m), and negligible true dilatational surface viscosity. We showed that the measured dilatational elasticity is in a very good agreement with the theoretical predictions of Volmer adsorption model (260 mN/m).

In another study [6] we characterized the rheological behavior of adsorption layers of 11 different saponin extracts, subjected to creep-recovery and oscillatory shear deformations. These experiments showed that all steroid saponins exhibited no shear elasticity and had negligible surface viscosity. In contrast, most of the triterpenoid saponins showed complex visco-elastic behavior with extremely high shear elastic modulus (up to 1100 mN/m) and shear viscosity (130 N s/m). The saponin extracts, showing the highest elastic moduli, were those of Escin, Tea saponins and Berry saponins, all containing predominantly monodesmosidic triterpenoid saponins. Similarly high surface modulus was measured with Ginsenosides extract, containing bidesmosidic triterpenoid saponins with short sugar chains.

The current study is dedicated to the relation between the molecular characteristics of the adsorption layers, determined from surface tension isotherms, and the surface rheological behavior of the same systems, subjected to dilatational and shear deformations. To achieve our aim we measured the surface tension isotherms for different saponin extracts and analyzed them by van der Waals and Volmer model isotherms. The rheological characteristics of the adsorption layers, subject to shear deformation, are taken from our previous study [6], whereas the characteristics of adsorption layers under dilatational deformation are measured for the current study using two experimental techniques: Capillary pressure tensiometry (CPT) and Langmuir trough (LT). Experiments were performed in oscillatory (CPT, LT) or steady deformation (LT). There are relatively few publications on dilatational rheology of saponins [7–9], and they are focused exclusively on one particular system (Quillaja saponin). As far as we know, this is the first systematic study of the characteristic behavior in surface dilatation of different types of saponins.

A study of this kind is important in another aspect as well. As already mentioned, results published in the literature show specific trends about the relation between surface and bulk properties in foams. However, in some cases, rigorous explanations of these trends are still missing. It is not yet clear which characteristics of the adsorption layer, shear or dilatational (or both), are relevant to the dynamics of a particular dynamic process in foams (Ostwald ripening, bubble coalescence, viscous friction, etc.). Detailed information of the behavior of the systems both in dilatation and in shear deformation is essential for the understanding of these relations. Such knowledge would ultimately help in the selection and utilization of saponins for stabilization of foams and emulsions.

2. Materials and methods

2.1. Materials

Table 1 provides information on the studied saponins: abbreviations used in the text, suppliers, concentration of saponins in each extract. Additional information for studied saponins such as part of the plant which was processed is given in Table S1 in Supporting information. Fig. S1 presents the basic molecular structure of the saponins, isolated from each extract.

2.2. Method for measurement of surface tension

The Wilhelmy plate method [23,24] was used to determine the equilibrium surface tension, σ_e, of the solutions. The measurements were performed on a K100 tensiometer (Kruss GmbH, Hamburg, Germany) by using a platinum plate. Before each measurement, the plate was cleaned by heating on a flame, followed by abundant rinsing with deionized water. The experiments were performed at constant temperature of 20 °C.

2.3. Experimental techniques for the characterization of surface rheological properties of adsorption layers

Two different experimental techniques were applied to characterize the surface rheological and adsorption/desorption behavior of the adsorption layers. Capillary pressure tensiometry (CPT) was performed via DSA 100 automated instrument (Kruss GmbH, Germany). The CPT method allows one to calculate the surface tension, σ, after measuring the capillary pressure difference, P_C, across the surface of a spherical drop with a radius of curvature of R [25–28]. The elastic and viscous moduli were determined from harmonic surface oscillations of spherical drops. First, a drop was formed, the surface was left to age for 30 min, and then the experiment was performed. A sinusoidal variation of the drop surface area, A, is applied, with a defined angular frequency, ω (the respective oscillation period is $T = \omega/2\pi$), and amplitude, ΔA. Simultaneously, one measures the resulting variations of the surface tension, σ. From the relation between $\sigma(t)$ and $A(t)$ we deteremined the complex surface modulus $E^* = E' + iE''$ (here i is the imaginary number) and E' and E'' are the surface storage (elastic) and loss (viscous) moduli, respectively. The values of E' and E'' were obtained using the linear regression procedure, described in Ref. [27]. All experiments were performed at constant frequency of oscillation (0.1 Hz) and constant temperature (20 °C).

Table 1
Studied saponins.

Trade name	Abbreviation used in text	Plant species	Supplier	Concentration of saponin, wt%
Escin	ES	*Aesculus hippocastanum*	Sigma	≥95
Tea Saponin	TS	*Camelia oleifera* Abel	Zhejiang Yuhong Import & Export Co. Ltd.	96.2
Berry Saponin concentrate	BSC	*Sapindus mukurossi*	Ecological Surfactants, LLC	53
Quillaja Dry 100, non-preserved	QS	*Quillaja saponaria* Molina	Desert King, Chile	27.8
Ginsenosides	GS	*Panax ginseng*	Xianyang Hua Yue Biological Engineering Co. Ltd.	80
Horse chestnut extract	HC	*Aesculus hippocastanum*	Xi'an Biof Bio-technology Co. Ltd.	20
Sapindin	SAP	*Sapindus triofilatus*	Sabinsa Corporation	50
Ayurvedic Saponin concentrate	ASC	*Acacia concina*	Ecological Surfactants, LLC	30
Tribulus terrestris extract	TT	*Tribulus terrestris*	Sabinsa Corporation	45
Foamation Dry 50	FD	*Yucca schidigera*	Desert King, Chilie	9
Fenusterols®	FEN	*Trigonella foenum graecum*	Sabinsa Corporation	50

Table 2
Dilatational elasticities for various saponin adsorption layers measured by two different methods at surface deformation of 5% and frequency of oscillation of 0.1 Hz.

Saponin	Dilatational elasticity at 5% deformation, mN/m		Shear elasticity at 5% deformation, mN/m	
	Langmuir trough	Oscillating drop method	Langmuir trough	DWR[a]
ES	165 ± 10	390 ± 10	57 ± 6	100 ± 25
QD	180 ± 10	180 ± 10	20 ± 1	44 ± 5
TS	115 ± 5	200 ± 10	32 ± 2	59 ± 5
BSC	133 ± 5	130 ± 5	46 ± 5	52 ± 5
ASC	75 ± 5	55 ± 5	0	0
FEN	55 ± 5	40 ± 5	0	0
TT	28 ± 2	28 ± 2	0	0
SAP	27 ± 2	24 ± 2	0	0
HC	26 ± 2	21 ± 2	0	0
FD	8 ± 1	10 ± 2	0	0

Data taken from Ref. [6].

[a] Experiments are performed at 1 Hz frequency of oscillations.

The rheological properties of the adsorption layers were also studied with a Langmuir trough (model 302LL/D1 Nima Technology Ltd., U.K.). The area of the trough was varied with two parallel barriers that moved symmetrically at a predefined linear speed. The surface pressure was measured with a Wilhelmy plate made of chromatographic paper. The plate was positioned in the middle between the barriers and oriented either perpendicularly or parallel to the barriers.

The adsorption layers were subjected to oscillatory deformation (triangular surface oscillations). Depending on the type of surface tension response, one can apply different procedures to analyze the experimental data and to extract information about the surface rheological properties [29,30]. For the visco-elastic saponin layers studied here, we found it appropriate to use the approach developed in Ref. [29] in which two types of measurements are made, to determine both the dilatational and shear rheological parameters of the layers. The stress measured at parallel orientation, τ_1, or perpendicular orientation of the plate, τ_2, are given by the expressions [29]:

$$\tau_1 = \tau_\parallel = (K + \mu)\alpha + (\zeta_S + \eta_S) \cdot \dot{\alpha} \quad (1a)$$

$$\tau_2 = \tau_\perp = (K - \mu)\alpha + (\zeta_S - \eta_S) \cdot \dot{\alpha} \quad (1b)$$

Here α and $\dot{\alpha}$ are the deformation and rate of deformation of the layer, respectively; K and μ are the dilatational and shear elastic moduli, respectively; ζ_S and η_S are the dilatational and shear viscous moduli, respectively. The experimental data for τ/α can be plotted as a function of $\dot{\alpha}/\alpha$:

$$\frac{\tau_\parallel}{\alpha} = (K + \mu) + (\zeta_s + \eta_s) \cdot \frac{\dot{\alpha}}{\alpha} \quad (2a)$$

$$\frac{\tau_\perp}{\alpha} = (K - \mu) + (\zeta_s - \eta_s) \cdot \frac{\dot{\alpha}}{\alpha} \quad (2b)$$

From the intercepts of the linear fits of the data one can determine the elastic moduli K and μ, and form the slopes—the

viscous moduli ζ_S and η_S (see Fig. S4 in Supporting information). In all experiments the amplitude of deformation was 5%, and the frequency—0.1 Hz.

The layers were also subjected to steady deformation in compression and expansion. The layers were deformed for ≈8 s with a given speed ($0.0164\,\mathrm{s}^{-1}$) up to deformation ≈12%. Afterwards the relaxation of the stress was recorded. The surface stress is positive on expansion (σ higher than the equilibrium value) and negative on compression (σ lower than the equilibrium value).

All experiments in Langmuir trough were performed at room temperature (≈23 ± 3 °C). Before each experiment the layer was left to equilibrate until the surface tension reached a constant value, and did not change further over time. The average of at least 3 experiments is presented.

3. Experimental results and discussion

This section presents experimental results obtained from measurements of equilibrium surface tension as a function of saponin concentration, and the specified characteristics of the layers after interpretation with appropriate adsorption isotherms (Section 3.1). Section 3.2 presents the results from measurements with CPT method. Section 3.3 describes the experimental data from experiments in Langmuir trough, and Section 3.4 presents a comparison of the elasticities determined by different experimental methods and the theoretically calculated Gibbs elasticities from the model adsorption isotherms.

3.1. Characteristics of the equilibrium saponin adsorption layers

As shown in our previous study, the dependence $\sigma(t)$ for a layer formed from saponin solution of Supersap is well described when using the equation with two exponents [7]. For the experimental data, obtained for the current study with different saponin extracts,

we also found that the equation with two exponents describes very well the experimental data for various saponins:

$$\sigma(t) = \sigma_e + \Delta\sigma_{1C} \exp\left(-\frac{t}{t_{1C}}\right) + \Delta\sigma_{2C} \exp\left(-\frac{t}{t_{2C}}\right)$$

where σ_e is the equilibrium surface tension, t_{1C} and t_{2C} are the characteristic relaxation times, whereas $\Delta\sigma_k$ are the related amplitudes of the relaxing stresses. Illustrative results for the surface tension as a function of time along with the best fit of experimental data are shown in Fig. S2 in Supporting information. From the best fit of experimental data we determined the values of equilibrium surface tension, which are used to construct the adsorption isotherms, see Fig. 1.

To extract molecular information from the surface tension isotherms, we fitted the experimental data below CMC with several adsorption isotherms:

(i) Gibbs adsorption isotherm [24,31–33]

$$\frac{d\sigma_e}{d\ln C_S} = -k_B T\Gamma \tag{3}$$

where k_B is Boltzmann constant, T is absolute temperature, C_S is surfactant concentration in the bulk solution, and Γ is surfactant adsorption (surface concentration). From the slope of $\sigma(\ln C_S)$ we determine the surfactant adsorption around the CMC.

(ii) Volmer adsorption and surface tension isotherms [24,31–33]

$$K_d C_S = \frac{\Gamma}{\Gamma_\infty - \Gamma} \exp\left(\frac{\Gamma}{\Gamma_\infty - \Gamma}\right) \tag{4a}$$

$$\sigma_e = \sigma_0 - kT\Gamma_\infty \left(\frac{\Gamma}{\Gamma_\infty - \Gamma}\right) \tag{4b}$$

where K_d is the adsorption constant, σ_0 is the surface tension of the pure solvent ($\sigma_0 = 72.8$ mN/m for air–water at 20 °C), and Γ_∞ is the maximum adsorption in the respective dense adsorption monolayer. From the values of K_d and Γ_∞ we calculated the adsorption at CMC by using Eqs. (4a) and (4b). From the value of K_d we determined the adsorption energy of the molecule by using:

$$K_d = \frac{\delta}{\Gamma_\infty} e^{\frac{\Delta\mu}{k_B T}} \tag{5}$$

Here δ is thickness of the adsorption layer which was approximated with the length of the adsorbed molecules; $\Delta\mu$ is standard free energy of adsorption (the energy gain of bringing a molecule from the bulk of the solution to the surface).

(iii) van der Waals adsorption and surface tension isotherms [24,31–33]

$$K_d C_S = \frac{\Gamma}{\Gamma_\infty - \Gamma} \exp\left(\frac{\Gamma}{\Gamma_\infty - \Gamma} - \frac{2\beta\Gamma}{k_B T}\right) \tag{6a}$$

$$\sigma = \sigma_0 - k_B T\Gamma_\infty \frac{\Gamma}{\Gamma_\infty - \Gamma} + \beta\Gamma^2 \tag{6b}$$

Here β is the parameter which accounts for the interactions between two adsorbed molecules. $\beta > 0$ corresponds to attraction between adsorbed molecules, whereas $\beta < 0$ corresponds to repulsion. Phase transition occurs in the adsorption layer when the dimensionless parameter $\overset{\approx}{\beta} = \beta\Gamma_\infty/k_B T > 3.375$.

To use the above equations we have to define the main component which is adsorbed on the air–water interface from the different saponin solutions. We analyzed the information published in the literature about the content of the studied extracts, and for each extract we chose the component with predominant concentration. The surface tension isotherms for each system were processed by using the molecular mass of those predominant components, as presented in Table S2.

Illustrative results for the best fits to the data with three isotherms (Gibbs, Volmer and van der Waals) are shown in Fig. 1.

It is seen that the main difference in the description of the experimental data by Volmer and van der Waals isotherms is at very low saponin concentrations, where the description is better with van der Waals isotherm which has one more adjustable parameter.

The results for the characteristics of saponin adsorption layers, determined from the best fits to the experimental data by the model adsorption isotherms are shown in Table S3. The results for the area per molecule in the adsorption layer at concentrations around the CMC and the interaction parameters for various studied saponins are shown in Fig. 2.

The values of the area per molecule in a dense adsorption layer, determined by Volmer and van der Waals isotherms, have the meaning of geometrical cross section of the molecule on the interface and vary between 0.32 nm^2 (for GS and TT) to 0.98 nm^2 for Supersap. For most of the remaining saponin layers this value varies between 0.4 and 0.6 nm^2. This area is smaller than the area of the triterpenoid aglycone, if it lays parallel to the surface. For such a configuration, from the molecular modeling we determined that the area is ≈0.75 nm^2, which means that, unlike the adsorption layers of Supersap where the molecules lay parallel to the surface, for the adsorption layers of Escin, TS and GS aglycone must be located approximately perpendicular to the surface—see Fig. 2A for a schematic representation of the two types of adsorption layers. For layers of TT and FEN we cannot do such an analysis, since no similar data from computer modeling of the steroid aglycone are available.

The values of the adsorption energy determined using Volmer equation, range from 12 to 18 $k_B T$ (see Table S3 in Supporting information), the highest value is determined for Supersap ≈ 18 $k_B T$, whereas for other saponins the value is between 12 and 14 $k_B T$. This relatively high energy of adsorption for Supersap compared to the other saponins is related to the additional energy gain, arising from the transfer of the extra hydrophobic tail (present in this saponin only) from the water into the air, whereas the energy of adsorption for all other saponins is the same, because it is due to the transfer of the similar aglycones only.

From the processing of the experimental data with van der Waals equation we obtain slightly lower values for the adsorption energy—it decreases from 12 to 14 $k_B T$ down to 10–13 $k_B T$. The greatest reduction is determined for TS layers, where there is a 50% reduction of adsorption energy, which is compensated by the presence of strong interaction energy between the adsorbed molecules, which is so great that it leads to a phase transition in the adsorption layer. The interaction between the saponin molecules, as determined from the van der Waals isotherm, is shown in Fig. 2B and one sees that the interaction parameter is greater than 3.375 for Escin, TS and GS, which means that phase transition occurs in these adsorption layers. This phase transition is most probably caused by hydrophobic attraction between aglycon groups and formation of hydrogen bonds between the sugar residues in the hydrophilic aqueous phase.

For some of the saponin solutions we were not able to determine the parameters of the adsorption layers, because the experimental curves of $\sigma(\ln C_S)$ have a declining slope, which, according to Gibbs isotherm would mean that there is a decrease of adsorption with increasing of saponin concentration in the solution. Illustrative examples for $\sigma(\ln C_S)$ for two of the studied saponins are presented in Fig. S3. The most probable explanation for this behavior is the formation of aggregates in solutions, at concentrations lower than CMC. Our attempts to break the aggregates using an ultrasonic bath and stirring were unsuccessful. In all experiments we obtained similar curves. The scattering of the data depends on what procedure we used to prepare solutions. Therefore, for these saponins we cannot determine the characteristics of the adsorption layers.
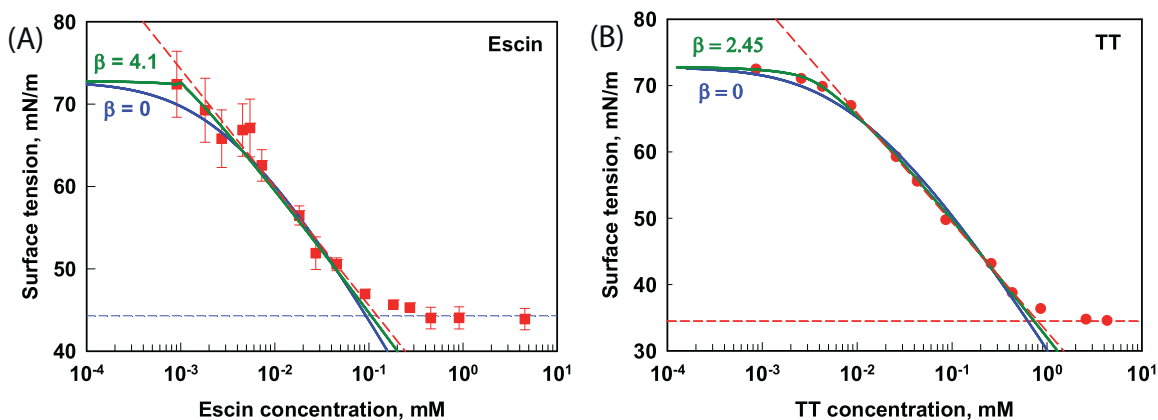
Fig. 1. Equilibrium surface tension isotherms, measured by the Wilhelmy plate method at 20 °C, for two saponin samples—(A) Escin; and (B) *Tribul terestis*. The lines below CMC correspond to the best fits of the experimental data by Gibbs (dashed red lines), Volmer (continuous blue curves), and van der Waals (green curves) adsorption models. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).
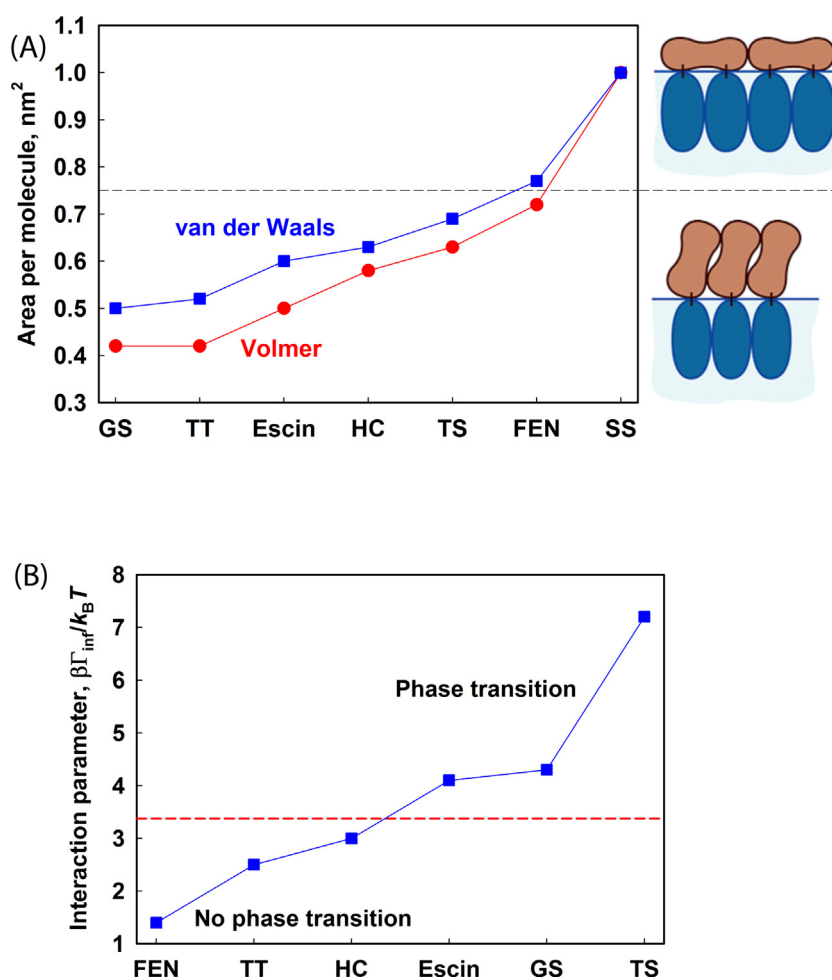

Fig. 2. (A) Area per molecule in adsorption layer formed from solution with concentration equal to CMC as determined from the best fit of experimental data with Volmer (red symbols) and van der Waals (blue symbols) isotherms and (B) interaction parameter between adsorbed molecules. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

From this series of experiments we can conclude that the adsorption layers formed from Escin (ES), Ginsenosides (GS) and Tea saponins (TS) are in condensed state, due to strong attraction between the molecules inside the adsorption layers, which is facilitated from the perpendicular orientation of the molecules with respect to the air–water interface.

3.2. Surface rheological properties determined by capillary pressure tensiometry

Fig. 3 presents the storage and loss viscous modulus for the saponin adsorption layers studied, as a function of the surface deformation measured by CPT at 0.1 Hz frequency of oscillation. Saponin adsorption layers of ES, TS and GS have very high dilata-
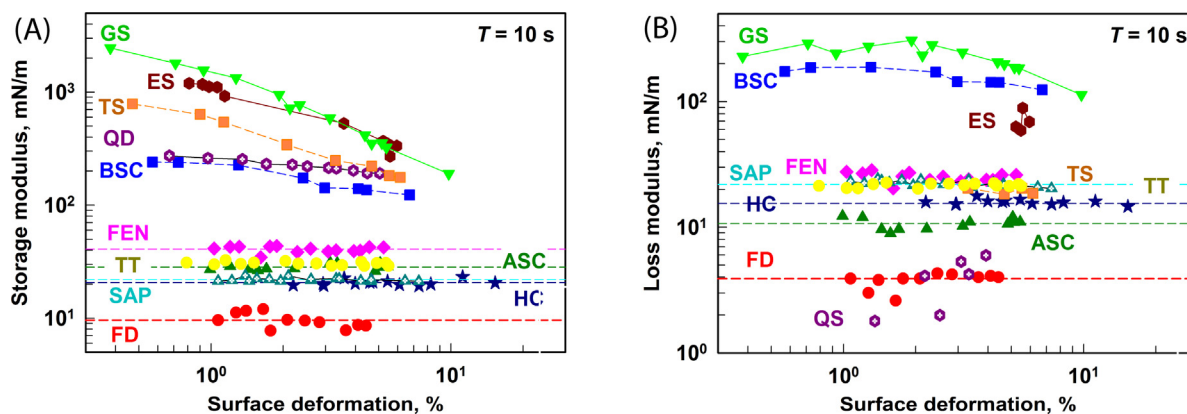
Fig. 3. Surface moduli for saponin adsorption layers as a function of surface deformation determined by CPT: (A) Storage modulus; (B) Loss modulus.

tional modulus up to 2500 mN/m, which is due to the strong interactions between molecules inside the layer. The condensed adsorption layers formed from ES, TS and GS solutions are characterized not only with very high surface dilatational elasticities, but also with very high shear elasticities (up to 1100 mN/m) as shown in our previous study [6]. Therefore, the interactions between the molecules affects significantly the rheological response in both type of deformations-dilatational and shear. The properties of these layers depend very strongly on the applied surface deformation. The increase of surface deformation leads to significant reduction in the elastic modulus for these layers, which means that they have non-linear rheological response. The most sensitive to the applied deformation is the GS—the dilatational storage modulus reduces more than 6 times, from 2500 to ≈400 mN/m, with the increase of the amplitude of surface deformation from 0.4 to 5%. Note that the elastic shear modulus for this saponin was also found to be the most sensitive to shear deformation—see Fig. 6 in Ref. [6]. Therefore, the interactions between the molecules are very sensitive to the applied deformation and the layer structure is lost, while the the modulus decreases significantly above given deformation.

The surface moduli of QS and BSC are ≈270 mN/m and all other saponins have the surface modulus below 50 mN/m. The surface modulus of QS is relatively close to the surface modulus of Supersap adsorption layers studied in our previous study, where we measured surface modulus ≈310 mN/m with the same method—see Fig. 7 in Ref. [7]. QS is a crude saponin extract from *Q. saponaria*, which along with the saponins present in the Supersap, contains also polyphenols, calcium, etc., [34]. The fact that the both solutions formed adsoption layers with similar dilatational modulus shows that the main surface active components in QS are the saponins. On the other hand, HC is a crude extract which contains Escin as a main saponin, but the adsorption layer of HC behaves very differently from Escin. The latter comparison shows that the other components in HC extract are also surface active and they prevent the formation of condense adsorption layer of Escin molecules–as a consequence, the elastic modulus for HC is much lower as compared to ES layers.

Saponins with low elastic modulus ($E' < 60$ mN/m) are steroidal saponins (FEN, TT, FD) as well as the crude extracts of triterpenoid saponins—SAP, HC, ASC. Note that the triterpenoid saponins which show very high surface modulus in both dilatational and shear (ES, TS and GS) contain more than 80% saponins, whereas the saponins with relatively low surface modulus (SAP, HC, ASC) contains less than 50% saponins which makes possible that the other surface active species adsorb on the interface and prevent the formation of condensed adsoption layer, due to disturbed packing of the saponin molecules inside the layer. The surface modulus for steroid saponins is in the range of 10–55 mN/m, and the highest is for FEN, while the lowest is for FD. For these saponins there is no

dependence of surface modules on the surface deformation, which means that they have linear rheological response. Fig. S9 presents the results for E' and E'' for these saponin layers, averaged from measurements at different deformations. For the majority of the saponins we observe $E' > E''$, the ratio E'/E'' for various saponin varies from 1.3 to 3.3. The only exception is SAP, for which $E'/E'' \approx 1$.

From this series of experiments we can conclude that condensed adsorption layers formed from GS, ES and TS have non-linear rheological response under dilatational deformation with very high surface storage modulus (up to 2500 mN/m), which decreases more than 3-times with increasing the deformation from 0.5 to 5%. The adsorption layers formed from crude extract of *Q. saponaria* (QS) behaves similarly to adsorption layers of Supersap, which is purified saponin extract with intermediate surface modulus of 260 mN/m. The surface modulus for this saponin depends very slightly on surface deformation—it decreases from 270 mN/m to 190 mN/m with increasing the deformation from 0.5% to 5%. The crude extracts of triterpenoid saponins (HC, SAP and ASC) and steroid saponins (TT, FD and FEN) formed adsorption layers with linear rheological response and surface dilatational modulus below 50 mN/m. For all saponins the storage modulus are much higher than the loss modulus (exception is SAP, where these two moduli are equal).

3.3. Surface rheological properties determined in experiments with Langmuir trough

To characterize the behavior of saponin adsorption layers under mixed shear and dilatational deformations, we performed two types of experiments in Langmuir trough: (1) Fast triangular oscillations and (2) Fast step-change deformation of the layer with subsequent relaxation of the surface stress. In Section 3.3.1 we presented the results from the fast triangular oscillations, whereas in Section 3.3.2 the results from the step- relaxation experiments are described.

3.3.1. Fast triangular oscillations

Fig. S4 in Supporting information shows the results obtained by fast oscillations of Escin layer which has a typical visco-elastic behavior. The dependence of τ/α, as a function of $\dot{\alpha}/\alpha$, obtained by two different orientations of the plate to the moving barriers, is shown. From the best fit of the experimental data we can determine the four rheological parameters—dilatational and shear elasticities from the intercepts and dilatational and shear viscosities from the slopes, see Eqs. (2a) and (2b) above. The determined rheological parameters for different saponin extracts are presented in Fig. 4. It is seen that the adsorption layers of the various saponin extracts have
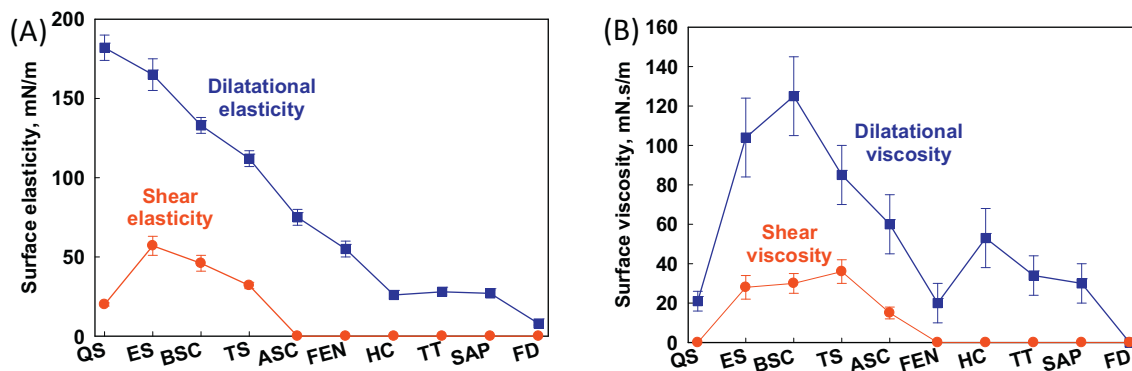
Fig. 4. Surface rheological properties of saponin adsorption layers determined from fast surface oscillations in Langmuir trough at amplitude of deformation (5%) and constant frequency of oscillation (0.1 Hz): (A) Surface elasticities; (B) Surface viscosities.

very different characteristics, as compared to the characteristics determined by the CPT method.

The layers obtained from the solutions of the QS, have high dilatational elasticity, $K \approx 180$ mN/m, non-zero shear elasticity, $\mu \approx 20$ mN/m, low dilatational viscosity $\zeta_S \approx 20$ mN s/m, and a zero shear viscosity. In other words, these layers show a pronounced elastic behavior with very high dilatational elasticity. We note that the value of K measured in the current study is lower than the value of $K = 260 \pm 30$ mN/m, determined in our previous work [7] with the purified Quillaja extract Supersap. On the other hand, the value of the shear elasticity determined in the current study is in a good agreement with the results in the work by Stanimirova et al. [7]. This means that the presence of other substances in the QS extract affects the adsorption–desorption processes to a greater extent than the shear elasticity of the layers.

The layers obtained from the solutions of Escin, TS and BSC have high dilatational elasticity, in the range of 100–160 mN/m; about 3 times lower shear elasticity—in the range of 30–55 mN/m; very high dilatational viscosity—in the range of 80–120 mN s/m; approximately 3-fold lower shear viscosity—from 28 to 35 mN s/m. In other words, these adsorption layers have distinct visco-elastic behavior. The measured surface viscosities are very high—if they are converted to bulk viscosities of the adsorption layers, they would correspond to $\approx 10^7$ Pa s, which is the typical viscosity of visco-elastic bulk materials and polymer melts. In other words, the saponin molecules of Escin, TS and BSC are able to form an adsorption layer which has a behavior of visco-elastic material with a very high viscosity and elasticity.

The layers obtained from the solutions of the ASC, FEN, HC, TT, Sap and FD, are characterized by a relatively low dilatational elasticity and viscosity, and zero shear elasticity and viscosity. The lack of shear elasticity and viscosity means that the interaction between the molecules in the adsorption layer is not very strong, as determined from the surface tension isotherms as well.

A comparison of the experimental results for HC and Escin indicates that the presence of other substances in the crude extract of HC strongly influences the properties of the adsorption layers of Escin. In other words, the impurities are able to displace (partially) Escin molecules from the surface layer, or at least prevent them from packing well and from forming a visco-elastic layer.

The significantly lower values of K for ES and TS adsorption layers measured in this method, as compared to the values for surface modulus measured by CPT method, is due to the higher surface deformation applied in the current method and the very strong dependence of the surface elasticity on the amplitude of surface deformation, as shown in Fig. 3. The quantitative comparison of the data, obtained by the various experimental methods, is presented in Section 3.4 below.

From this series of experiments we can conclude that the condensed adsorption layers of TS and ES molecules have high dilatational and shear viscosities, and high shear and dilatational viscosities. The adsorption layers of SS with small interaction parameter have high dilatational elasticity, low shear elasticity, low dilatational viscosity and zero shear viscosity. The adsorption layers from steroid saponins (TT, FEN and FD) as well as from crude extracts of triterpenoid saponins (HC, ASC, SAP) have zero shear elasticities and viscosities and very low dilatational elasticities and viscosities.

3.3.2. Experimental results obtained in experiments with fast deformation and subsequent relaxation

In this type of experiment we have two stages—the first stage we applied fast surface deformation for a relatively short period of about 7 s, increasing or decreasing the area of the layer, while in the second stage the barriers are fixed and we measure the surface stress as a function of time. From these series of experiments, the saponin adsorption layers can be divided into three groups (see Fig. 5):

(1) Saponin layers for which a substantial difference between the stresses measured during compression and expansion are observed, but in both directions (compression and expansion) the magnitude of the stress increases with the amplitude of deformation. To this group belong the adsorption layers of QS, TS, GS—see Fig. 5A;

(2) Saponins layers for which a significant difference between the stresses measured during compression and expansion, but the stress remains constant after a given deformation upon expansion. To this group belong layers of Escin and BSC—see Fig. 5B;

(3) Saponin layers, wherein the curves for compression and expansion coincide. To this group belong the layers of the TT, ACS, TT, FEN and HC—see Fig. 5C.

It should be mentioned that in the adsorption layers of the first two groups, the stress reached after expansion of the surface is much lower than the stress reached after compression. The fact that the stress after a certain deformation of the surface remains constant, despite the further layer expansion, is probably related to the presence of aggregates on the surface, which can rapidly provide molecules to the newly formed surface. In other words, the constant stress, after a small surface deformation, is most likely associated with surface diffusion, rather than by bulk diffusion in these layers.

The increase of the stress with the increase of the deformation upon compression of the saponins from the first two groups, most likely is due to rearrangement of the molecules in the adsorption
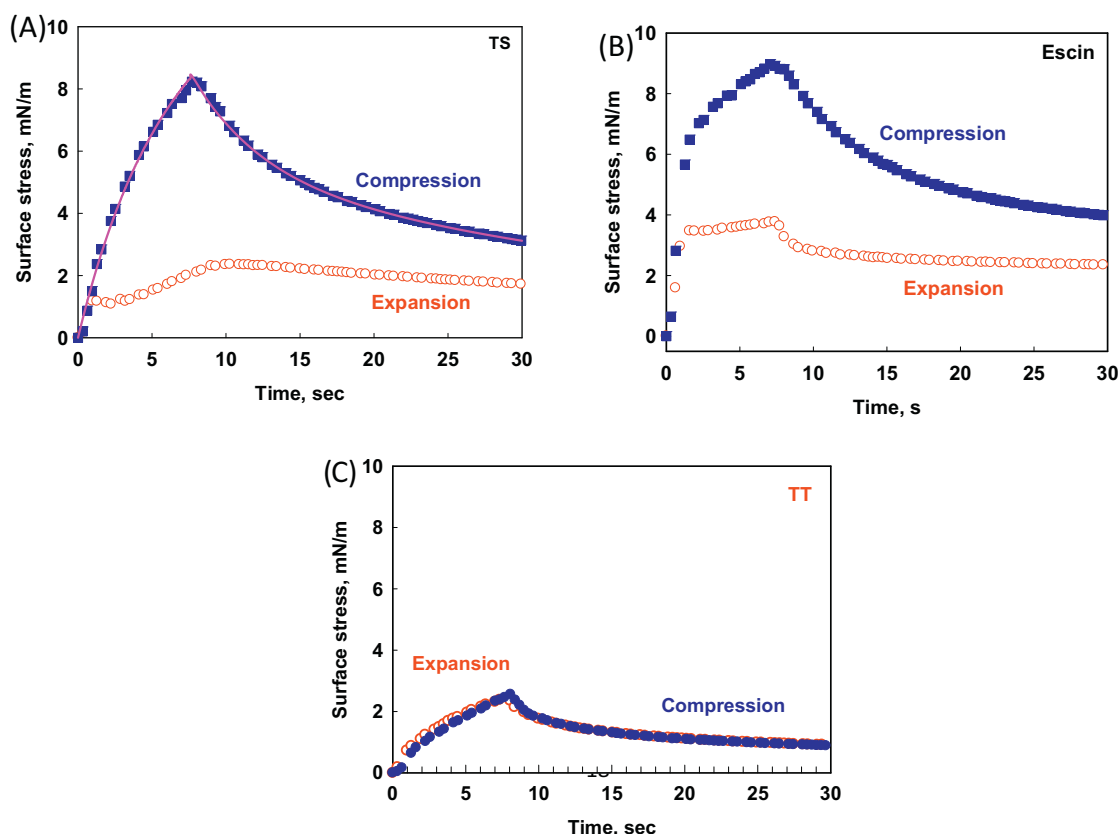
Fig. 5. Surface stress as a function of time for period of compression (blue points) or expansion (red points) and subsequent relaxation for adsorption layers of (A) TS; (B) Escin and (C) TT. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

layer and the very difficult desorption of the molecules from the layer in the solution.

The difference in the stresses measured upon compression and expansion of the layers from the first two groups can be attributed to the formation of hydrogen bonds between the sugar residues between neighbouring molecules—these bonds favor the formation of visco-elastic adsorption layer. The desorption of a saponin molecule from the surface back into the solution requires breakage of the hydrogen bonds which explains the significantly higher surface stress, measured upon compression compared to expansion at the same deformation.

For saponin layers in the third group, such visco-elastic structure is not observed or it is very weak. That is why there is no difference between the surface stress upon expansion and compression of the adsorption layers in this group.

By using Burgers model we described the rheological data for layers of ASC, TT, FEN, HC and QS. For layers of BSC and TS we could fit by this model the experimental data obtained after compression only. Illustrative results about the description of experimental data with the Burgers model are shown in Fig. S6. To determine the values of the dilatational and shear elasticities for the springs in the Maxwell and Kelvin elements of the Burgers model [35,36], we used the following procedure. From the experimental data obtained with each of the orientations of the plate (relative to the barriers), we determined the values of E_1 and E_2. Assuming that the determined values with the parallel orientation of the plate are the sum of dilatational and shear elasticities, while the values determined with perpendicular orientation of the plate are the difference between dilatational and shear elasticities, we determined the values of K_1, μ_1, K_2 and μ_2, where K denotes the dilatational elasticity and μ denotes the shear elasticity. Subscript 1 indicates

the elasticity of the spring in the Maxwell element, while subscript 2 is for a spring in the Kelvin element.

The determined values for both elements are compared with the experimentally determined values of K and μ from oscillation experiments, presented in Section 3.3.1 and Fig. S7 in Supporting information. It is seen that the dilatational elasticity of the spring in Kelvin element is substantially lower than the elasticity of the spring in Maxwell element. Similar trend is determined for shear elasticities. The values of K and μ, determined by oscillation experiments, are intermediate as compared to those for the two springs in the Maxwell and Kelvin elements.

We compared the values of K_1, K_2, obtained from the experimental results under compression and under expansion (Fig. 6). The values of K_1 and K_2 for a layer of QS, obtained after compres-
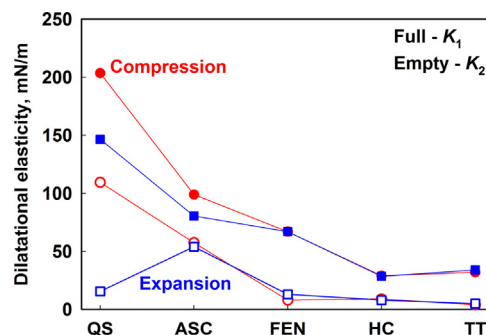

Fig. 6. Elasticities obtained after fitting the experimental data with Burgers model—K_1 (filled symbols), K_2 (open symbols) for various saponins. With red circles are values obtained after compression, and blue squares represent the values obtained after expansion. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

Table 3

Comparison of calculated Gibbs elasticties by Volmer (second column) and van der Waals (third column) equations and dilatational elasticity measured by OSDM at 3% amplitude of deformation.

Saponin	Gibbs elasticity, mN/m		E, mN/m
	Volmer	Van der Waals	
Escin	110	525	578
HC	180	595	21
TS	210	1030	264
GS	180	770	626
Supersap	240		260
TT	195	490	29
FEN	225	340	41

sion, are significantly higher than those obtained after expansion of the layer, and this difference is significantly greater for K_2. For the other saponins, the values are practically the same (FEN, HC, TT) or very similar (ASC).

3.4. Comparison of the experimental results obtained by the two different methods (Langmuir through and oscillating spherical drop)

Table 2 presents the results for the dilatational elasticity, measured by two different methods. In all experiments the surface deformation is 5% and the period of oscillation is 10 s. The table shows a very good agreement of the elasticity values, obtained by the two methods, with the exception of two saponins—ES and TS. For these two saponins the modulus measured by Langmuir trough are ≈2 times lower than the modulus measured by oscillating drop. As already mentioned, these two saponins exhibit an extreme sensitivity of the surface elasticity on the amplitude of surface deformation. These differences may be due to the different geometry of the layers tested in the two methods. The results obtained with oscilating drop are more reliable, as far as the drop is subject to pure dilatational deformation (no shear deformation).

Table 3 compares the values at a small surface deformation (3%) obtained by OSDM, with the values for Gibbs elasticity, calculated from the surface tension isotherms by Volmer and van der Waals models. The table shows that the Gibbs elasticity, calculated from van der Waals equation, is closer in value to the elasticity, measured at 3% strain of Escin and GS. A better agreement between Gibbs elasticity, determined by Volmer equation and that obtained by the OSDM, is determined for TS adsorption layers. For HC, TT and FEN layers, very large discrepancy in the values is observed, probably related to the faster processes of adsorption and desorption for these saponins. We note that the experimentally determined values for Escin and GS are higher than the theoretically calculated by the van der Waals equation, which means that there are even stronger interactions between the molecules in the layer than those determined using model adsorption isotherms.

4. Comparison of the rheological properties of saponins and other systems exhibiting surface visco-elasticity

A number of surface-active species exhibit surface visco-elasticity: proteins [37–40], pulmonary surfactants [41], low-molecular-mass surfactants (LMS) [42] etc. In general these systems are characterized well in the literature. In most cases their adsorption layers exhibit complex rheological behavior which is affected by a variety of factors. These include: pre-treatment (mechanical or thermal), aging of the layer, parameters of the test (frequency and amplitude of oscillation, rate of compression/expansion) etc. Due to these complexities, it is difficult to make an accurate comparison between the results for different systems. A rigorous approach to this task would require the per-

forming of experiments in a standardized test under carefully controlled conditions (experimental technique, type of deformation, test parameters, pre-treatment of the layer, etc.).

Such an analysis is outside the scope of the current article. Here we give some illustrative results for the response in dilatation of several types of systems, as published in the literature. Even without taking into consideration the factors described above, it can be seen that saponins stand out in comparison to all other surfactants. The experiments in the cited articles were performed either via the same method as in the current work (CPT) or via the oscillating drop method (ODM). Table 4 presents the elastic, E', viscous, E'', modulus and the complex modulus, $|E| = (E'^2 + E''^2)^{1/2}$. The results in Table 4 show that saponins with high surface modulus have remarkable properties in two aspects. First, they have exceptionally high elastic modulus. The modulus of some saonins (TS, ES, GS) is distinctly higher than the modulus of all of other systems discussed here. Secondly, the saponins have typically strongly pronounced elasticity, $E' \gg E''$, and ES, TS and QS behave like purely elastic bodies ($E'' \approx 0$) at low deformation (<1%). The specific features of these systems can be attributed to the very strong lateral interactions between the molecules in the adsorption layer.

The moduli of saponins from the group with low surface modulus are significantly lower, compared to group with high surface modulus, but are still substantial and close to the values for proteins.

5. Main experimental results and conclusions

Systematic experiments were performed to determine the main characteristics of saponin adsorption layers in terms of area per molecule, adsorption energy, energy of interaction between the adsorbed molecules, surface dilatational and shear elasticities and viscosities. Various saponin extracts were studied, having different types of aglycone (triterpenoids or steroids) and a different number of sugar chains (1–3). The main results can be summarized as follows:

- The experimentally determined dependencies of σ vs $\ln C_S$ for saponin solutions of TS, Escin, GS, TT and FEN can be described well by Volmer and Gibbs adsorption isotherms. From the best fit of the experimental data by Volmer isotherm, it is shown that the energy of adsorption is ≈12–13 $k_B T$ for most saponins.
- From the experimentally determined values of the area per molecule in dense adsorption layers, one can conclude that the molecules of TS, Escin and GS are orientated perpendicularly to the surface.
- The experimental data for σ vs $\ln C_S$ for TS, Escin and GS are described better by van der Waals' adsorption isotherm, accounting for the interaction between the adsorbed molecules. The determined values of the intermolecular interaction parameter are higher than the critical one, above which a phase transition occurs for van der Waals isotherm. This result is a strong indication that TS, Escin and GS saponins form surface condensed phases in their adsorption layers.
- QS layers have distinct elastic behavior with very high dilatational elasticity and noticeable shear elasticity but low viscosities. These layers are characterized by marked difference between the stresses measured upon large expansion and compression of the surface, which is explained by the difficult desorption of saponin molecules from the surface layer.
- Layers of TS, Escin and BSC exhibit visco-elastic behavior with very high surface viscosities and very high elasticities under both type of deformations (shear and dilatational). This visco-elastic behavior is explained by the strong attraction between the saponin molecules inside the adsorption layer. These layers

Table 4
Typical values of the surface elastic, E', viscous, E'', and complex modulus, $|E|$ for several systems exhibiting surface visco-elasticity (air–water interface).

Type of system	System	E', mN/m	E'', mN/m	$|E|$, mN/m	References
Saponins	High surface modulus (GS, ES, BSC and QS)	240/2400	0/300	250/2400	Current work
	Low surface modulus (ASC, FEN, TT, SAP, HC, FD)	10/40	3/30	10/50	Current work
Proteins	Hydrophobin	≈120	≈10	≈120	[39]
	BSA[a]	≈60	≈20	≈63	[37,40]
	β-Caseine	–	–	≈20	[40]
	Ovalbumine	–	–	≈80	[40]
	β-LG[b]	–	–	≈80	[40]
Pulmonary surfactants	DPPC[c] + 0.5 mol% SP-B[d]	≈300[e]	–	–	[41]
Low molecular surfactants	Mixture of zwiterionic and anionic surfactant and a fatty acid	200	330	360	[42]
Polysaccharide	Hydroxypropyl cellulose	–	–	60	[43]
Sugar-based surfactants	Oligofructose monoester (length of fatty acid chain–16 carbon atoms)	–	–	145	[44]

[a] Bovine serum albumin.
[b] β-Lactoglobulin.
[c] Dipalmitoyl phosphatidylcholine.
[d] Surfactant protein B.
[e] Data at 0.1 Hz, 20 °C, negligible dilatational viscosity.

also show a significant difference between the stresses, measured upon compression and expansion of the surface. The experimentally measured elasticities are close to the theoretically calculated ones, based on the van der Waals equation, which implies that there are strong attractive interactions between the adsorbed saponin molecules. These layers have exceptionally high surface elasticity—much higher than those measured with adsorption layers of other molecular systems (proteins, lipids, surfactants, etc.).

- Layers from all other studied saponins (FD, FEN, ASC, TT, HC, SAP) have zero shear elasticity and viscosity, and relatively low dilatational elasticity and viscosity. At fast deformation and subsequent relaxation, stress-vs-time curves obtained under expansion and compression of the surfaces, match. In experiments with an oscillating spherical drop, the elasticities do not depend on the surface deformation, which implies linear rheological response. The elasticities measured for these saponins are comparable in magnitude to those reported for proteins in the literature.

- For all tested saponins, the elasticities determined from different types of experiments are close to each other, if the conditions of the experiment (rate and amplitude of deformation) were also similar.

The obtained experimental results and the formulated conclusions may serve as a starting point for deep analysis of the complex relations between the various properties of adsorption layers and the different dynamic properties of foams, stabilized by such layers.

Acknowledgments

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.colsurfa.2015.12.001.

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