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Association of saponins in water and water–gelatine mixtures

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Abstract

The solution properties of a commercial saponin obtained from Quillaja Bark (QBS), have been investigated in a wide range of experimental conditions in water and in the presence of moderate amounts of porcine skin gelatine, GEL. Saponins are surface active and form micelles at very low concentration. Significant changes in the solution dielectric properties are concomitant to micelle formation. The combination of thermodynamic, spectroscopic, transport and dielectric methods characterises the micelle formation, giving information on interactions between the components. NMR relaxation times, NMR self-diffusion and dielectric measurements were used.

Micelle aggregation numbers, inferred from light scattering, indicate the formation of relatively large aggregates. No evidence for interactions between protein and surfactant was obtained. This is presumably due to the limited ionisation of acidic groups on the surfactant, which does not allow significant electrostatic binding with the protein.

Keywords: Gelatine; Saponins; Surface properties; Light scattering and NMR; Dielectric permittivity

1. Introduction

Interest in bio-compatible surfactants arises from the need to minimise the irritant properties that synthetic detergents exert on skin and tissues. Thus, mild surfactants having sugar, or betaine-like units as polar groups are being extensively used in formulations [1–3].

Studies oriented to optimise the behaviour of surfactants obtained from natural sources [4] show the saponins are extremely promising. Chemically, saponins are composed of steroids, or triterpenoids, linked to different saccharidic units [5] (Fig. 1). Depending on the nature the components polar groups can be ionised.

Saponins are extracted from the seeds and barks of many vegetables, for instance, from "Saponaria Officinalis" [6], legumes (soy and lentils [7]) and more exotic sources. Saponins find applications in food, agricultural, pharmaceutical and cosmetic industries. They exert mild antibacterial, anticoagulant, antimycotic and antiseptic activity [8,9], and reduce cholesterol adsorption [10]. It has been observed, for instance, that the regular uptake of dietary saponins inhibits hypercholesterolaemia in blood [11].

The results of a systematic investigation on the physico-chemical properties of a product obtained from Quillaja Bark are reported here. This work focuses on surface properties and related thermodynamics. The investigation was performed by combining thermodynamic data with transport and spectroscopic investigation. NMR relaxation and self-diffusion, dielectric properties, viscosity and quasi-elastic light scattering experiments have been performed.

Due to its potential as a bio-compatible surfactant, solution properties were also investigated in the presence of moderate amounts of a protein, i.e. commercial porcine gelatine.

2. Experimental

2.1. Materials

Porcine gelatine, type A 60 Bloom, referred to as GEL, was from Sigma. It was desiccated under vacuum, at room temperature, and used as such. Density, viscosity and ionic conductivity measurements on its solutions confirmed the product purity [12].

Technical Quillaja Bark saponin (QBS) was from Sigma. The product was purified by dissolution in hot water and extraction by butan-1-ol, filtration by Gooch funnels (to

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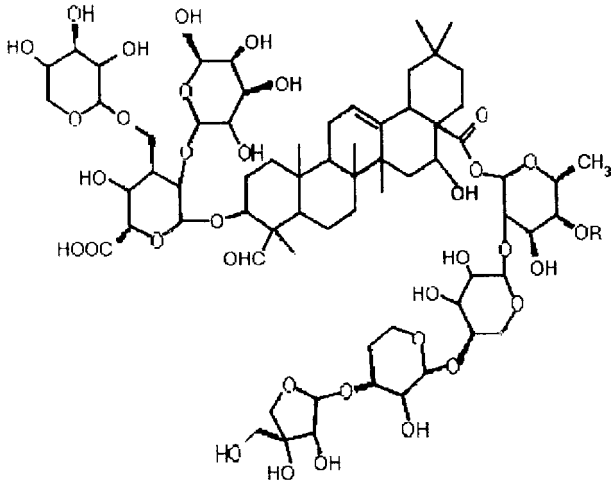

Fig. 1. The chemical structure of Quillaja Bark saponin (QBS).

remove dust and particles) and vacuum evaporation of the alcoholic solution. The waxy solid was recovered with methanol and the product vacuum dried at 70 °C for 2 days. Surface tension measurements confirmed the product purity. The agreement with available CMC values is ±3%.

Water was distilled twice over alkaline $KMnO_4$. Its ionic conductivity, at 20 °C, is 1 μS cm^{-1}. Solutions were prepared by weight and allowed to equilibrate before use.

All other solvents and reactants were Sigma products of synthetic grade.

2.2. Methods

2.2.1. Surface tension

A Kruss unit, model K10T, was used to measure the surface tension, σ, of the solutions to ±0.2 mN m^{-1}. Temperature is controlled at ±0.1 °C by a water circulation jacket. To avoid drifts due to interfacial adsorption kinetics, the solutions were equilibrated before running the experiments. Each datum is the mean value of at least five independent measurements. Details of the apparatus are given elsewhere [13].

2.2.2. NMR

A pulsed NMR spectrometer, working at 16.0 MHz, was used to measure the longitudinal relaxation times of water protons, τ_1, and water self-diffusion coefficients, D. The unit is equipped with a home-made pulsed field gradient unit and a temperature control system. The thermostatic part was a copper furnace equipped with a sample holder. The temperature was controlled at ±0.2 °C by a thermocouple. More details of the apparatus are given elsewhere [14].

Relaxation times of water were measured by the inversion recovery method, according to the sequence 180°–τ–90°–τ-acquisition. The rf pulse width, at 90°, is 6.7 μs long, when the delay time between two rf pulses goes from 0.04 to 40.96 s. The relaxation times were determined by a non-linear fitting of the longitudinal magnetisation recovery [15].

The PFG–SE method was used to measure self-diffusion. The gradient field pulses were 50 G cm^{-1}, the gradient pulse was between 0.5 and 2 ms, and the time between gradients was 20 ms. The system electronics control an automatic balance of the two gradient pulses. D values were inferred by a logarithmic fitting procedure of the spin echo attenuation [16]. In the limits set by experimental accuracy, the uncertainty on apparent D values is ±2%.

The trend of water relaxation time versus QBS wt.% is reported in Fig. 2 and the water self-diffusion versus concentration fit is reported in Fig. 3.

2.2.3. Viscosity

Relative viscosity measurements (with respect to the solvent) were performed by Ubbelohde-type viscometers, having flow times for the solvent close to 200 s. Data were

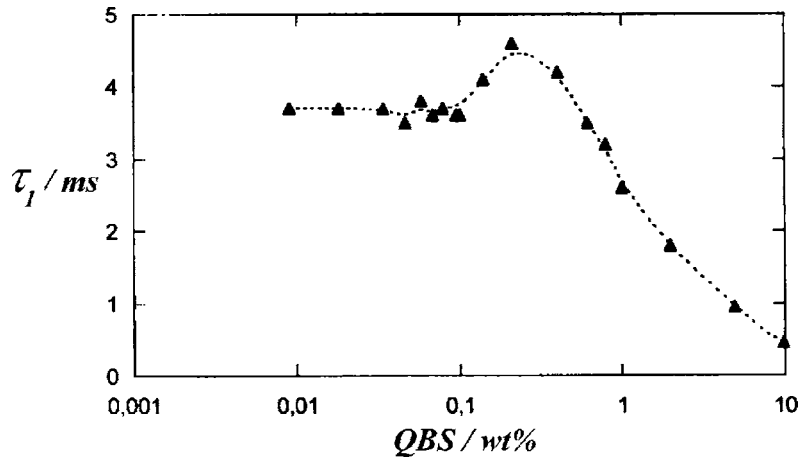

Fig. 2. Dependence of water relaxation time, τ_1, in ms, on the wt.% of QBS, at 25 °C. Data are plotted on a logarithmic scale to show the pre-micellar region. The line is simply a guide to the eye.

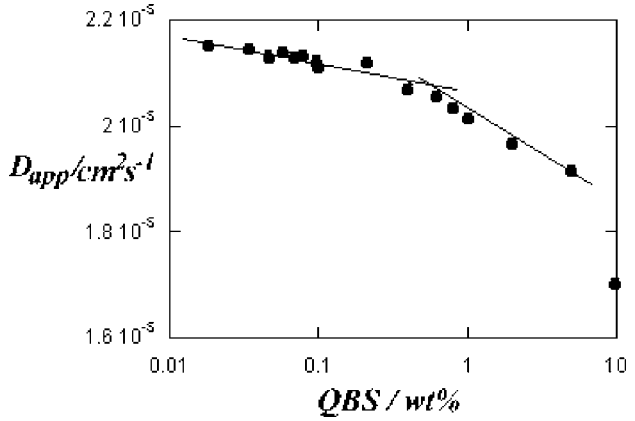

Fig. 3. Water self-diffusion, D_{app}, as a function of the wt.% of QBS, at 25 °C, on a semi-logarithmic plot. The point at the extreme right of the figure indicates the water self-diffusion value at high QBS content.

analysed in terms of the following relation:

$$\eta_{rel} = \frac{\eta}{\eta^0} = \frac{\rho t}{\rho^0 t^0} \quad (1)$$

where ρ and ρ^0 are the densities of the solution and of the solvent, respectively, and t or t^0 are the corresponding flow times. Measurements were performed in a thermostatic bath, keeping the temperature constant at ±0.01 °C. The accuracy on flow times, which were repeated five times, is to 0.2 s. Before measurements, the solutions were passed through 0.25 μm Nucleopore filters, to avoid dust or other particles.

Densities, ρ, were determined by a DMA 60 Anton Paar vibration densimeter, thermostated at ±0.005 °C by a Heto unit. A digital thermometer, ASL model F 25, measured the temperature, T. Density values were obtained by

$$\Delta\rho = \rho - \rho^0 = \left(\frac{1}{A}\right)(\tau^2 - \tau^{0^2}) \quad (2)$$

where τ^0 and τ are the vibration frequencies of the solvent and the solution, respectively. The constant A was obtained by fitting Eq. (2) for liquid of known density, i.e. water [17], acetone, ethanol and ethylene glycol [18]. The accuracy of ρ values was better than 5×10^{-6} g cm^{-3}.

2.2.4. Dielectric permittivity

A Bontoon electronic bridge, model 75D, working at 1.00 MHz, measured the relative (with respect to air) solution permittivity ε. The home built cell consists of two coaxial steel electrodes separated by a Teflon holder. The cell was thermostated by circulating water to 0.05 °C. Before running the experiment, measurements on the permittivity of water–DMSO mixtures were performed [19]. The accuracy on the resulting ε values is to 0.1 units. Details on the apparatus, on the measuring procedures and on data analysis are reported elsewhere [20].

2.2.5. Light scattering

A Brookhaven BI-2030 AT unit, equipped with a 136 channel readout, was used. The goniometer was a Brookhaven BI-200 SM. An argon ion laser (operating at 514.5 nm) was used as light source. Details of the apparatus, measuring procedures and data elaboration are given elsewhere [21,22].

3. Results

3.1. Micelle formation

The CMC values were obtained from the intersection point of σ values versus $\ln m_2$ plots. Relevant data are given in Table 1. Both the Gibbs energy of adsorption, ΔG°_{ads}, and the association contributions, i.e. the Gibbs energy due to micelle formation, can be analysed as a function of temperature. The former quantity is related to the latter by the equality [23]:

$$\Delta G^\circ_{ads} = \Delta G^\circ_{mic} - \frac{\Pi_{CMC}}{\Gamma_{2,max}} \quad (3)$$

where ΔG°_{mic} is the Gibbs energy of micelle formation ($\Delta G^\circ_{mic} = RT \ln \mathrm{CMC}$), Π_{CMC} the surface pressure at the CMC ($\Pi_{CMC} = \sigma^0 - \sigma_{CMC}$) and $\Gamma_{2,max}$ is obtained through the Gibbs adsorption isotherm, according to

$$\delta\sigma = -\Gamma_2(RT\, \mathrm{d} \ln m_2) \quad (4)$$

where R is the gas constant, m_2 the solute molality and T is the absolute temperature.

The entropic and enthalpic contributions to micelle formation and to adsorption can be evaluated from proper arrangements of Eqs. (3) and (4). Entropy of micelle formation is 40 ± 2 J K^{-1} mol^{-1} and the corresponding enthalpy is -7.4 ± 0.2 kJ mol^{-1}. The Gibbs energy of adsorption, ΔG°_{ads}, is not very sensitive to T, even if the area per molecule changed significantly.

Table 1
The critical micellar concentration, CMC, the Gibbs energy of micelle formation, ΔG_{mic}, of adsorption at interfaces, ΔG_{ads}, and the area per molecule, A, as a function of temperature

T (°C)	CMC (mol kg^{-1})	ΔG_{mic} (kJ mol^{-1})	ΔG_{ads} (kJ mol^{-1})	A (Å^2)
25.0	4.8×10^{-4}	−28.7	−40.6	79.1
30.0	5.5×10^{-4}	−29.1	−40.5	70.4
35.0	5.8×10^{-4}	−29.4	−40.2	66.4
40.0	6.1×10^{-4}	−29.7	−38.4	53.6

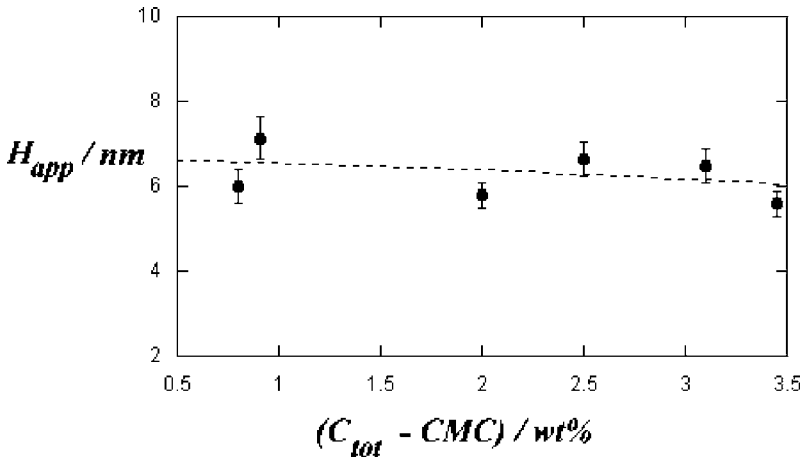

Fig. 4. Dependence of the apparent hydrodynamic diameter of QBS micelles, H_{app}, on the amount of surfactant in micellar form, $C_{tot} - CMC$. Data refer to 25 °C: the bars indicate the uncertainty on H_{app} values, due to micellar polydispersivity.

In the presence of gelatine, adsorption decreases significantly, since the protein is also surface active. No evidence of interactions between GEL and QBS are found from surface tension data.

3.2. Micelle aggregation numbers

In Fig. 4 is reported the dependence of the apparent hydrodynamic radius of QBS (nm) as a function of the amount of surfactant in micellar form, $C_{tot} - CMC$. As can be seen, the average hydrodynamic radius changes little in the concentration range investigated.

Considering the small polydispersivity, aggregation numbers were obtained. In such experimental conditions, we assume the validity of the following correlation function [24]:

$$C(k\Delta t) = B\langle 1 + b\ \exp(-2\Gamma k\ \Delta t)\rangle \tag{5a}$$

or, in logarithmic form:

$$\ln\left\langle \frac{C(k\Delta t)}{B} - 1 \right\rangle = \ln b - 2\Gamma k\ \Delta t \tag{5b}$$

From the above equation Γ is obtained by a linear fit of $\ln\langle[C(k\,\Delta t)/B] - 1\rangle$ versus $k\Delta t$. In Eqs. (5a) and (5b) C depends on the number of channels, k, and on the observation time, Δt. B is the instrumental baseline and b is a constant.

According to the theory:

$$\Gamma = D_{app}q^2 \tag{5c}$$

where the first term on the right-hand side of the equation is the apparent self-diffusion coefficient and q is the scattering vector. The hydrodynamic radius of the micelle, R_{app}, is obtained by the Stokes–Einstein equation as [25]

$$D_{app} = \frac{6\pi\eta^0 R}{kT} \tag{6}$$

After proper arrangement of data from Eq. (6), and taking into account the density of the solid (1.26 g cm^{-3}), we get aggregation numbers, $\langle n\rangle$, in the range 65 ± 5. This value is in very good agreement with the corresponding quantity reported by Oakenfull [26].

3.3. Dielectric permittivity

As a consequence of QBS addition to water, and to the gelatine-containing pseudo-solvent, a significant decrease of the static dielectric permittivity, ε, is observed. The occurrence of a minimum in the very close proximity of the CMC can be inferred from data in Fig. 5. To the best of our knowledge, a few permittivity data on micelle forming systems have been reported [27–29].

The concentration at which the above effect is observed is within the experimental accuracy of the critical micellar concentration obtained from other methods. Hence, we assume the above effect is due to the release of hydrophobic-hydration water accompanying micelle formation. Above the CMC the dielectric behaviour conforms to that expected for large polysaccharidic solutes [30] and ε regularly decreases with concentration.

3.4. Solution viscosity

Measurements were performed at 25, 30, 35 and 40 °C. In very dilute concentration regimes, i.e. below the CMC, flow times can be lower than those of pure water, presumably because of the adsorption of the surfactant at the capillary surface [31]. Above the CMC, conversely, the viscosity linearly increases with the amount of surfactant in micellar form. This behaviour is in line with available literature data [32]. Fig. 6 reports the dependence of relative viscosity, η_{rel}, on the amount of surfactant in micellar form, $C_{tot} - CMC$. Viscosity data were rationalised in terms of the following equation [32,33]:

$$\frac{\ln \eta_{rel}}{C_{tot} - CMC} = A + B \ln \eta_{rel} \tag{7}$$

where A is the related to the hydrodynamic volume of the molecule in micellar form and B is the accounts for

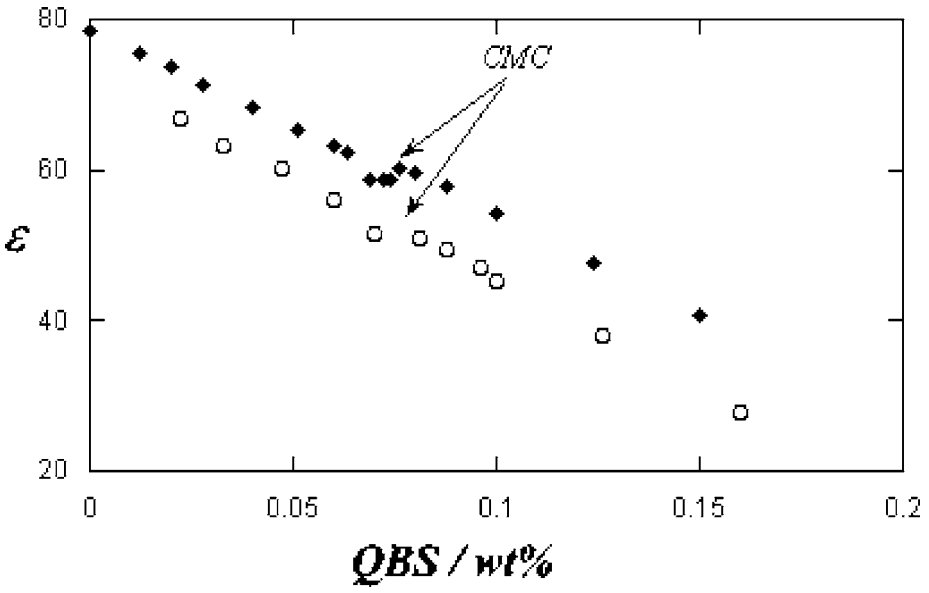

Fig. 5. Dependence of the dielectric permittivity, ε, on QBS wt.%, at 25 °C. Empty symbols refer to the behaviour observed in aqueous 1 wt.% GEL. CMCs are indicated by arrows.

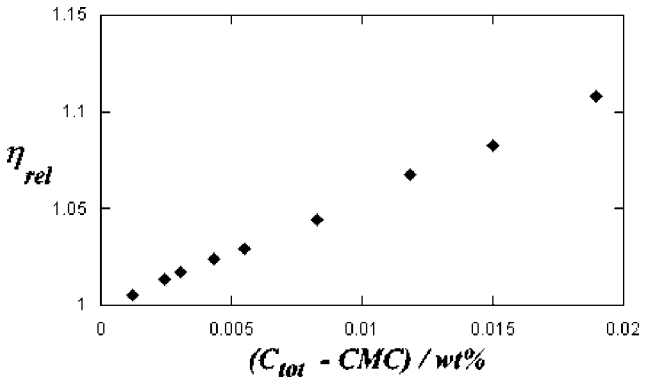

Fig. 6. Dependence of relative viscosity, η_{rel}, on the amount of QBS in micellar form, $C_{tot} - CMC$, at 25 °C.

particle–particle interactions, mediated by Brownian motion. By Eq. (7) the limiting hydrodynamic volume of the molecule in the micelle is inferred, from which the hydration per molecule, p, can be obtained. The latter quantity is related to the difference in the hydrodynamic volume, V_{hydr}, and the corresponding partial molal quantity, V_2. It can be demonstrated that $(V_{hydr} - V_2/18)$, where in the limits of spherical particles, $V_{hydr} = 2.303A/2.5$. From the data, the volume variation associated with a temperature increase can be evaluated. The limiting hydrodynamic volumes obtained by elaboration of Eq. (7) are reported in Table 2.

3.5. Self-diffusion and relaxation times

In low resolution NMR it is not possible to get unequivocal information on the dynamics of QBS and on the changes accompanying micelle formation. Thus, the focus was on the dynamic properties of the solvent since the water

Table 2
The limiting hydrodynamic volume of QBS, V_{hydr}, as a function of temperature, T, in °C

T (°C)	V_{hydr} (l mol^{-1})
25.00	2.21 ± 0.05
30.00	2.12 ± 0.04
35.00	2.06 ± 0.03
40.00	1.99 ± 0.04

self-diffusion trends contain information on the obstruction that micelles exert on solvent motion [34]. Micelles act as a barrier to the free motion of water and reduce the self-diffusion coefficient [35]. The decrease is proportional to the volume fraction of the disperse phase and is modulated by the amount of hydration water [36]. The following equality holds:

$$\frac{D_{app}}{D^0} = f(1 - P_b)\Phi_f + P_b\Phi_b \quad (8)$$

where D_{app} and D^0 are the solution and solvent self-diffusion, P_b the amount of irrotationally bound water (moving as a whole kinetic entity with micelles) and Φ is the volume fraction of the disperse phase, either free to move, Φ_f, or bound to micelles, Φ_b. The model in Eq. (8) is a "two-site approximation" to water self-diffusion [37]. Proper use of the above equation allows estimation of micelle size.

In NMR relaxation the experimental longitudinal relaxation times are weighted average values containing contributions due to water in both the free and bound states. The increase in relaxation times observed in close proximity of the CMC is reasonably ascribed to the release of water molecules involved in hydrophobic-hydration.

4. Discussion

The present findings indicate the formation of relatively large aggregates of about 65 molecules. According to viscosity and light scattering, the micelles are nearly spherical, up to moderate concentration limits (about 50 times the CMC).

The polar head groups of saponins are strongly hydrated. Estimates based on Eq. (8) indicate that about 30 water molecules per QBS unit are involved in hydration. Also water self-diffusion findings indicate the occurrence of significant micelle hydration. This behaviour is not surprising if we consider the large number of sugar units facing toward the bulk solvent. It must be pointed out, however, that hydrodynamic methods do not allow discrimination between firmly bound molecules and geometrically trapped water molecules not involved in hydration. In any case, dehydration regularly increases with temperature. Micelles become more compact at high temperatures, as can also be inferred from the decrease in area per molecule on increasing temperature.

The effect of gelatine on micelle formation is moderate. For instance, addition of 1% gelatine to the solvent increases the CMC from 0.08 to 0.10 wt.%. This is an indication that the interactions between the two components are weak. This hypothesis is confirmed by the absence of two changes in slope in the surface tension plots and, also, by dielectric permittivity findings. When interactions between the components occur, significant changes in slope in permittivity should be observed since the dipole moment of the protein would be significantly affected by interactions with the surfactant.

The above behaviour may imply a significant role for saponins in the preparation of mild and bio-compatible surfactants to be used, for instance, in topical applications.

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This paper is dedicated to the memory of Prof. Bianca Sesta (04-01-1932/19-01-2003).

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