Application Note 30

Determination of the critical micelle concentration of *Quillaja* saponin

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Determination of the critical micelle concentration (CMC) of *Quillaja* saponin at the air/water and the oil/water interface via pendant drop measurements with the OCA contour analysis system from DataPhysics

Problem

With respect to product labelling and increased awareness of consumers towards food additives suitable food-grade "natural" surfactants gain more and more importance.

An extract from the soap bark tree *Quillaja saponaria* Molina is an approved ingredient for beverages in the EU and is generally regarded as safe according to the FDA. Main compounds of this extract are **saponins**, which are surface-active components. Their interfacial properties are based on the hydrophobic triterpene moiety (aglycone) and one or more hydrophilic side chains of sugars.

A knowledge of the **critical micelle concentration (CMC)** of the *Quillaja* saponin (QS) is very important in the context of *Quillaja* extract applications such as foam and emulsion stabilisation, as well as the incorporation of lipophilic compounds into micelles, i.e. nutritional oils, fat-soluble vitamins and food colorants.

At the air/water or oil/water interface the hydrophilic side chains of saponins are directed towards the aqueous phase. The hydrophobic aglycone faces towards the nonpolar air or oil phase. This results in a decrease of the water's surface tension or the interfacial tension between water and oil, respectively, which proceeds until the CMC is reached. At the CMC the interface is saturated with surfactant molecules. Further increase of the saponin content leads to self-aggregation of the surfactant molecules in the aqueous phase, resulting in the formation of micelles. Thus, no further decrease in surface (or interfacial) tension can be observed. The main reason for micelle formation is the attainment of a minimum free energy state.

To determine the CMC of QS one measures the surface or interfacial tension of aqueous saponin solutions with different concentrations. This can be performed fast and easily using the pendant drop technique on an OCA contour analysis system.

Note that the comparability of interfacial tension measurements for CMC determination with other more involved techniques like dye solubilisation

and light scattering methods has long been shown (Wan & Lee, 1974).

Method

A buffered and an unbuffered aqueous QS stock solution are prepared and diluted to appropriate concentrations. To determine the CMC the surface tension of the dilutions as well as the interfacial tension between the dilutions and purified MCT (medium chain triglycerides, all surface-active compounds have been removed using an active magnesium silicate) oil are measured employing the optical contour analysis system OCA 20. For this purpose the QS solutions are filled into syringes which are successively fixed in the automated direct dosing unit SD-DM.

In the SCA software the parameters needed for pendant drop measurements are set, i.e. the diameter of the needle and the densities of the phases used within the experiment which are predetermined using an appropriate hydrometer or chosen from the internal databank, respectively.

For a measurement a small drop of a QS sample solution is created at the tip of a needle that is attached to the syringe. For experiments at the oil/water interface the drop is generated into a quartz glass cuvette filled with the MCT oil. For measurements at the air/water-interface the drop is created into the headspace of a closed plastic cuvette filled to one third with distilled water in order to avoid shrinkage of the drop due to evaporation. All cuvettes are placed in a temperature-controlled chamber (see Figure 1).

Then the change in drop shape is monitored until equilibrium interfacial tension is reached (about 30 min). The calculation of the interfacial tension is based on the shape analysis of a pendant drop according to the Young-Laplace equation

$$\Delta P = \gamma \left(\frac{1}{R_1} + \frac{1}{R_2} \right).$$

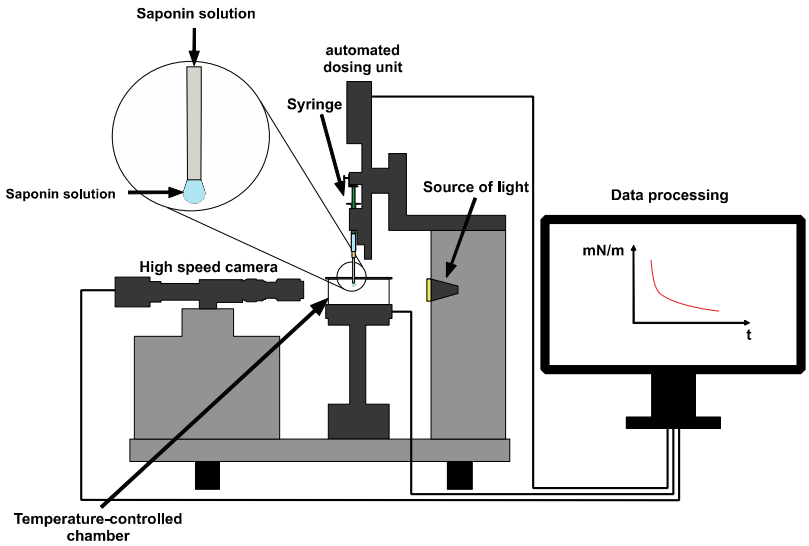

Figure 1: Optical contour analysis system OCA with an automated dosing unit (Tamm, Sauer, Scampicchio, & Drusch, 2012).

Following this equation γ, the interfacial tension, can be determined via the Laplace pressure ΔP, i.e. the pressure difference across the interface with R_1 and R_2 as the two principal radii of curvature (Runke, Song, & Springer, 1994). However, it must be ensured that the drop has the characteristic shape of a pendant drop, i.e. it must not be spherical.

Note that prior to sample measurements one should proof the needle cleanliness. The absence of surface-active components is checked by an interfacial tension measurement of distilled water which has to be constant for 60 minutes (Wüstneck, Moser, & Muschiolik, 1999).

Results

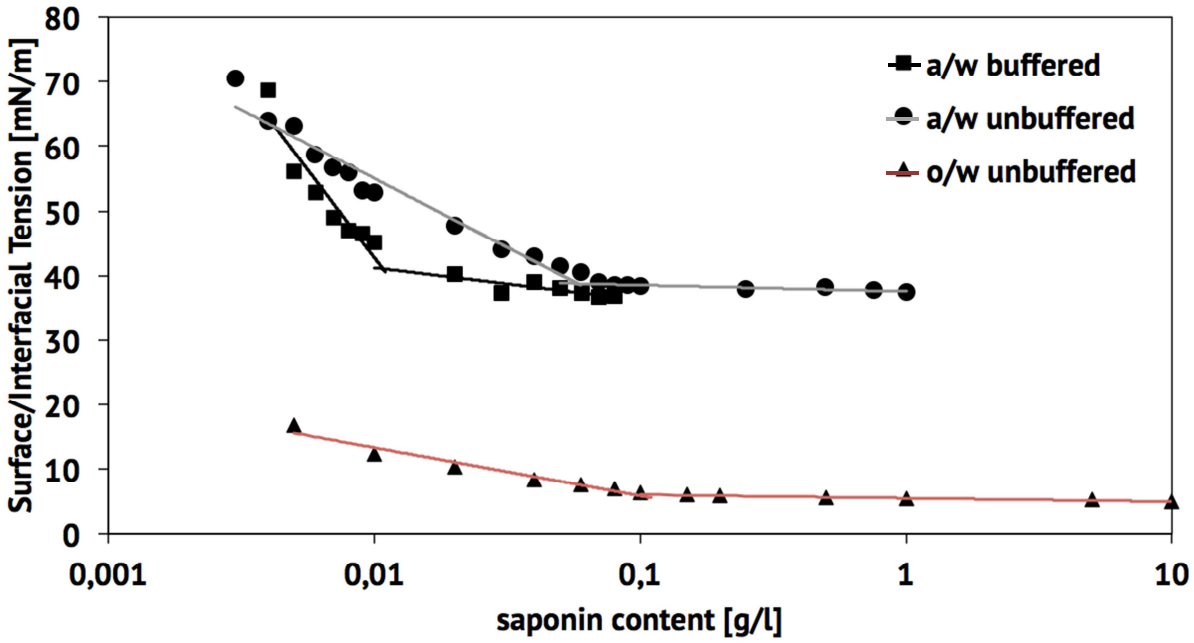

Figure 2: Surface and interfacial tension of buffered and unbuffered *Quillaja* saponin solution at the air/water (a/w) and oil/water (o/w) interface. Linear regression curves are shown. Their intercepts indicate the respective CMC values (black: a/w buffered; grey: a/w unbuffered; red: o/w unbuffered) (Tippel et al., manuscript submitted).

Even at low concentrations QS causes a distinct decrease of the surface or interfacial tension (see Figure 2).

The CMC is defined as the slope break in the equilibrium interfacial tension versus saponin concentration curve. Thus, one determines two linear regression curves, e.g. in Microsoft Excel. The first one is in the range of high surfactant concentrations with a constant equilibrium interfacial tension. The second linear regression curve contains the decreasing interfacial tensions in the low QS concentration range. The CMC is calculated from the intersection of both linear regression curves.

One can see that the CMC of QS varies depending on the medium (see Fig-

ure 2). At the air/water interface the CMC of a buffered QS solution (0.01 g/l) is lower compared to an unbuffered solution (0.05 g/l). Electrolytes present in the buffered QS solution lead to a screening of the charged carboxyl group, thus the self-assembly of molecules into micelles is facilitated. At the oil/water interface a higher CMC (0.09 g/l) and a lower total interfacial tension are observed compared to the air/water interface. This CMC indicates a higher amount of QS incorporated into the interfacial film at the oil/water interface.

Summary

The pendant drop method of the contour analysis system OCA 20 from DataPhysics Instruments, equipped with an automated dosing unit, provides a simple, convenient and reliable way to determine the critical micelle concentration of *Quillaja* saponin both at air/water and oil/water interfaces.

References

Runke, T., Song, B., & Springer, J. (1994). Surface- and interfacial tensions of liquid crystalline polymers.pdf. *Berichte Der Bunsengesellschaft Für Physikalische Chemie*, *98*(3), 508–511.

Tamm, F., Sauer, G., Scampicchio, M., & Drusch, S. (2012). Pendant drop tensiometry for the evaluation of the foaming properties of milk-derived proteins. *Food Hydrocolloids*, *27*(2), 371–377.

Wan, L. S. C., & Lee, P. F. S. (1974). CMC of polysorbates. *Journal of Pharmaceutical Sciences*, *63*(1), 136–137.

Wüstneck, R., Moser, B., & Muschiolik, G. (1999). Interfacial dilational behaviour of adsorbed β-lactoglobulin layers at the different fluid interfaces. *Colloids and Surfaces B: Biointerfaces*, *15*(3-4), 263–273.