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Interferometric investigation of the gas-state monolayer of mono-rhamnolipid adsorbing at an oil/water interface☆

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ABSTRACT

We present a study on biosurfactant uptake at the interface of a millimeter-sized drop of squalene in water, by a recently developed differential interferometric technique. The technique allows detecting capillary waves, with amplitudes of the order of 10^{-9} m, excited on the surface of the drop by an electric field in the order of 5 V/cm. By studying the resonant surface modes of the drop it is possible to assess the interfacial properties as a function of the surfactant concentration in the water bulk. The technique allows to follow the adsorption process at extremely low surfactant concentration, not accessible by other methods, investigating the gas state in the π-A diagrams of 2D amphiphilic monolayers.

1. Introduction

The determination of the physico-chemical properties of a liquid/liquid interface in the presence of surface-active molecules is important in many applied fields. Low-molecular-weight surfactants are commonly used in pharmaceuticals, food and cosmetics production. In the last decades, properties of interfaces and their response to the uptake of surface-active molecules have been studied by the optical observation of the shape of pending drops [1], as it depends on the surface tension, modulated by molecule uptake at the interface. The interest in oil/water emulsion properties has refocussed on the process of loading of molecules at the interface in the very low concentration limit, well below the cmc. In fact, this appears to be a key factor in the production of emulsions with the desired features, that is, the optimization of the initial active surfactant concentration, both in traditional and innovative applications [2].

At low surface concentration of adsorbed molecules the interface monolayer is in the gas state where adsorbed molecules do not affect surface tension (or surface pressure). In this regime the system is out of the pending-drop technique applicability. Recently, a differential interferometric technique has been developed, which is able to explore the properties of the oil/water interface of oil drops at extremely low surfactant concentration. The interferometer applied, exploits the analysis of stationary modes of a resonating drop under small perturbations induced by an external forcing field, namely an electric field. Upon adsorption, the amplitude, the frequency and the width of the resonant response can be followed, depending on the charge properties of the surface, on the restoring force and on the dissipation at the interface, and allow studying the surface properties with extremely high sensitivity.

We present a study of the evolution of the squalene/water interface properties upon addition of very low amounts of a natural anionic biosurfactant, namely a rhamnolipid, that elucidates the potentiality of the technique.

Squalene (SQ, see Fig. 1) is a highly hydrophobic molecule, naturally produced by plants and animals, being the precursor of cholesterol. Its wide use in food and pharmaceutics technology is connected to its very low miscibility in water (solubility = 0.124 mg/L) and the low SQ/water interfacial tension that, together with its large availability, makes it suitable for high-performance microemulsions, [3–5]. SQ is liquid at room temperature. In the literature, extremely variable values for interfacial tension with water can be found, ranging from 17 to 47 mN/m, depending on aging and degree of purification [6].

Abbreviations: ACN, Acetonitrile; FWHM, Full Width at Half Maximum; HPLC, High-Performance Liquid Chromatography; ELSD, Evaporative Light Scattering Detector.

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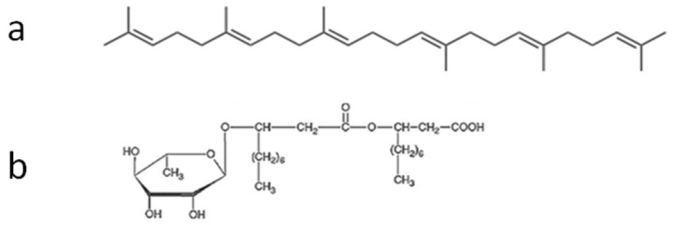

Fig. 1. Molecular structures of a) squalene and b) mono-rhamnolipid.

A recent pending-drop study has investigated SQ/water interface upon addition of a phospholipid at the interface [7]. A drop of the same oil is here investigated in a diluted water solution of mono-rhamnolipid, well below the cmc.

In fact, an interesting family of surface-active natural surfactants is that of rhamnolipids. Rhamnolipids are amphiphilic glycolipids produced by bacteria of the genera *Pseudomonas* and *Burkholderia* and are involved in the formation of biofilms, cell motility and access to hydrophobic substrates [8, 9]. There is a great diversity of rhamnolipid structures. Among those produced by *P. aeruginosa*, the mono-rhamnolipid (MR) studied in this paper is shown in Fig. 1. Its polar head comprises one L-rhamnose, linked by a glycosidic bond with a pair of fatty acids, 10 carbons each, hydroxylated in position 3, connected by an ester bond, and presenting a carboxylic group [8]. Their bio-surfactant properties offer possibilities of use in many fields, in particular in the formulation of cosmetic and pharmaceutical compounds, as well as for the decontamination of sites polluted by metals or hydrocarbons [10] . They are also looked at as antimicrobial for a large panel of micro-organisms [11] and for their property of stimulating plant defenses [12], then interesting for the plant biocontrol. The physico-chemical properties of rhamnolipids and in particular their behavior in water have been the subject of numerous studies [13–18] . Due to their amphiphilic structure, rhamnolipids aggregate beyond a concentration of critical aggregation (CMC) ranging from 10 to 180 μM depending on the medium conditions and on the structure of rhamnolipids [14, 18, 19]. For MR with β-hydroxydecanoic acids, CMC values in water solution have been reported in the range 100–180 μM, for pH's in the range 6.5–7.0, lowering the surface tension from 72 mN m^{-1} to around 30 mN m^{-1} [19–21]. Due to the presence of a carboxylic function, MR is negatively charged at pH > 5.9 [18] .

2. Material and methods

2.1. Materials

Squalene (SQ) was purchased from Sigma Aldrich s.r.l.

Rhamnolipids from *P. aeruginosa* were purchased from AGAE Technologies (AGAE Technologies, LLC, Corvallis, OR, USA) and consist of a mix of two 90% pure rhamnolipid species: α-L-rhamnopyranosyl-β-hydroxydecanoyl-β-hydroxydecanoate (RL-1210: 40%) and 2-O-α-L-rhamnopyranosyl-α-L-rhamnopyranosyl-β-hydroxydecanoyl-β-hydroxydecanoate (RL-2210: 60%). Mono- and di-rhamnolipids were isolated from this commercial mixture by preparative High-performance liquid chromatography coupled to an evaporative light scattering detector (HPLC–ELSD) on an Interchim Uptisphere Strategy C18–2 column (21.2 mm, 15 μm) on an Interchim Puriflash 4250 system. Before injection, the mix was solubilized in pure methanol and filtered through a 0.22 μm PTFE membrane. Distilled water (0.1%, vol/vol, of formic acid) and ACN (0.1%, vol/vol, of formic acid) were used as mobile phase. For the first 8 min, the percentage of ACN was increased from 60% to 100%. Pure ACN was then used for 8 min. The percentage of ACN was decreased to 60% in 30 s and the column was cleaned during 3 min. The flow was 20 mL/min. The purity of the collected fractions was checked by HPLC-ELSD. The ELSD parameters were: 35 °C and 2.5 bar. The pure fractions were then pooled and dried with a speed vac apparatus (10 mbar, 40 °C). An amount of 3 mg of the mono-rhamnolipid pool was diluted in milli-Q water, lyophilized and conserved in nitrogen environment until weighting and dilution to the final 1 mM-solution with filtered milli-Q water (18.2 MΩ/cm). Small aliquots of this 1 mM-solution, in the order of 1 μL, were progressively added to the bulk water, 0.5 mL, in contact with the squalene drop (~1mm size, ~5 μL). Error in weighting is estimated to be <10%.

2.2. The drop interferometry technique

The technique here applied to study interface properties is based on the study of capillary waves rising at the oil/water interface of a 1 mm sized drop when perturbed by an external field. In particular an oscillating electric field is applied in this study. The interferometric method recently developed [22–26], is briefly summarized in the following.

A plexiglass cubic measuring cell, 0.5 ml total volume, is equipped with a couple of stainless-steel electrodes, one protruding from the bottom and the second, a mobile one, entering the cell from the top. The top electrode is hollow and hosts a calibrated glass capillary, 0.8 mm inner diameter, provided with a piston that is used to create the desired drop in the center of the cell. The geometry of the measuring system is that of a drop constrained to the edge of a glass capillary. Drop oscillations are excited by a periodic electric field, and are due to the effective net charge existing at the interface, of negative sign for oil in water [22, 23].

The amplitude of the exciting field is kept low enough in order to produce oscillations of the interface in the range of few nanometers, without affecting the bulk. Drop surface deformation is probed by a differential interferometric technique [23–26] that analyzes the interference of the beams reflected from the drop, once laser light crosses it on its diameter. In fact, when traversed by a Gaussian laser beam, the drop oil/water interfaces act as the mirrors of a confocal Fabry—Perot interferometer, due to the difference in refractive indexes. A set of concentric fringes are formed in the backward direction, the pattern being sensitive to the variations in the optical path inside the drop while deformed by the forcing field.

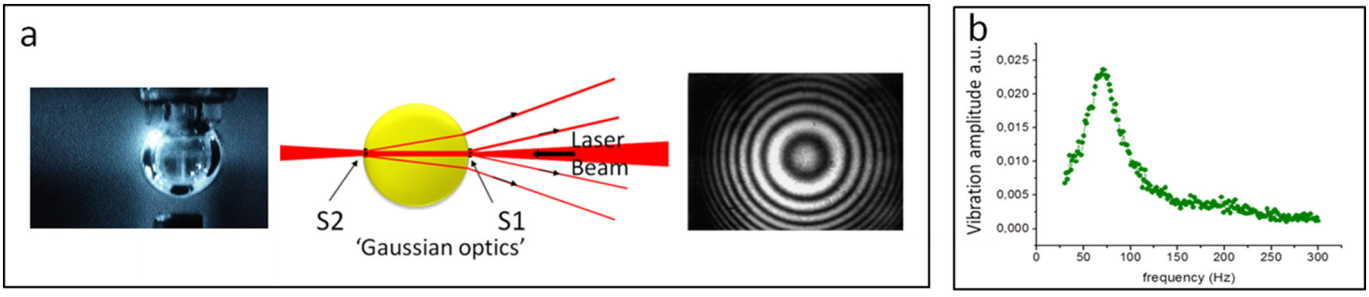

Fig. 2. a) The picture shows a drop attached to the capillary protruding from the top electrode (left), a sketch of the interfering beams reflected from opposite interfaces of the drop (center) and the concentric fringes of the interference pattern (right). b) the resonance spectrum of a pure squalene drop in water.