

Interferometric investigation of the gas-state monolayer of monorhamnolipid adsorbing at an oil/water interface.

P. Brocca^{1*}, V. Rondelli¹, M. Corti², E. Del Favero¹, M. Deleu³, L. Cantu¹

¹*Department of Biotechnology and Translational Medicine, University of Milan, Italy*

²*CNR-IPCF, Viale F. Stagno d'Alcontres, 37, 98158 Messina, Italy*

³*Laboratoire de Biophysique Moléculaire aux Interfaces, Gembloux Agro-Bio Tech, Université de Liège, Gembloux, Belgium*

Corresponding author:

Paola Brocca

Dept. Medical Biotechnology and Translational Medicine.

University of Milano.

LITA. Via F.lli Cervi, 93, 20090 Segrate (MI), Italy.

tel: +39 02 503 30351 - Fax: +39 02 503 30365

paola.brocca@unimi.it

Declarations of interest: none.

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 5V/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.

keywords: Confocal Fabry-Perot interferometer, drop interferometer, capillary waves, monolayer gas state, monolayer gas phase, liquid/liquid interface, bio-surfactant, squalene.

Abbreviations

ACN- acetonitrile

FWHM -Full Width at Half Maximum

HPLC- High-Performance Liquid Chromatography

ELSD - Evaporative Light Scattering Detector

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 Figure 1) is a highly hydrophobic molecule, naturally produced by plants and animals, being the precursor of cholesterol. Its wide use in food and pharmaceuticals 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].

A recent pendant-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 Figure 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 [18,14,19]. 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 $\text{pH} > 5.9$ [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 -hydroxydecanoate (RL-1,210: 40%) and 2-O- α -L-rhamnopyranosyl- α -L-rhamnopyranosyl- β -hydroxydecanoate (RL-2,210: 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 μ m) on an Interchim Puriflash 4250 system. Before injection, the mix was solubilized in pure methanol and filtered through a 0.22 μ m 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 sec 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 1mM-solution with filtered milli-Q water (18.2 M Ω /cm). Small aliquots of this 1 mM-solution, in the order of 1 μ L, were progressively added to the bulk water, 0.5mL, in contact with the squalene drop (~1mm size, ~5 μ L). Error in weighting is estimated to be less than 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 1mm 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.

Due to the closed geometry of the drop, only discrete stationary modes, l , are excited. In fact, the oscillation frequencies deviate from the Rayleigh frequencies of a free drop [27] as the drop is bound to a constraint. This introduces a lower frequency mode, F_1 , associated to center-of-mass motion [28,29], connected to the same restoring force. In general, the frequencies of the higher-order modes, F_l , are blue-shifted with respect to free-bubble modes by the constrained geometry. The resonance frequencies depend on the drop radius, on the constraint size, and are related to the restoring force determined by the interfacial tension as reported in Equations 1a and 1b.

$$\omega_1^2 = \frac{A(a)}{\Omega^2} \frac{\sigma}{\rho R^3} \quad \text{for } l = 1 \quad (1a)$$

$$\omega_l^2 = A(l, a) \frac{\sigma(l-1)(l+1)(l+2)}{(l\rho_{water} + (l+1)\rho_{oil})R^3} \quad \text{for } l > 1 \quad (1b)$$

where σ is the surface tension, R is the radius of the drop, the coefficient $A(l, a)$ depends on the constraint size a determined by the geometry of attachment to the glass capillary [28,29], ρ_{oil} and ρ_{water} are the densities of oil and water, $\Omega = \rho_{oil} / \rho_{water}$

On the other hand, the amplitude of the excited fluctuations depends on the interfacial charge that, of course, is affected by absorption of any charged molecular species at the interface. Several characteristic modes can be observed for some liquid/liquid interfaces already studied [26], while only very few are seen in the case of SQ, as will be shown below. The typical lower frequency mode is of the order of 10^2 Hz, with an amplitude of oscillation in the nanometer range, well above the ultimate sensitivity of the instrument.

The conductance of the bulk solution is continuously monitored during the measurements, allowing a control on surfactant concentration in bulk.

3. Results and discussion

MR is a short-double-chain glycolipid bearing a negative charge in water solution at neutral pH. This makes MR a suitable molecule to perform absorption/desorption studies with the drop interferometer in the electric-field excitation mode.

Figure 1 shows the molecular structures of SQ and MR applied in this study.

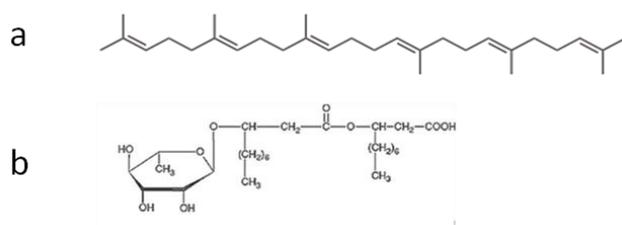


Figure 1. Molecular structures of a) squalene and b) mono-rhamnolipid.

In fact, upon surfactant uptake, the immiscible SQ drop interface progressively increases its charge, hence the amplitude of its electrically excited fluctuations. Confocal interferometry is then extremely sensitive to adsorption of the charged molecules.

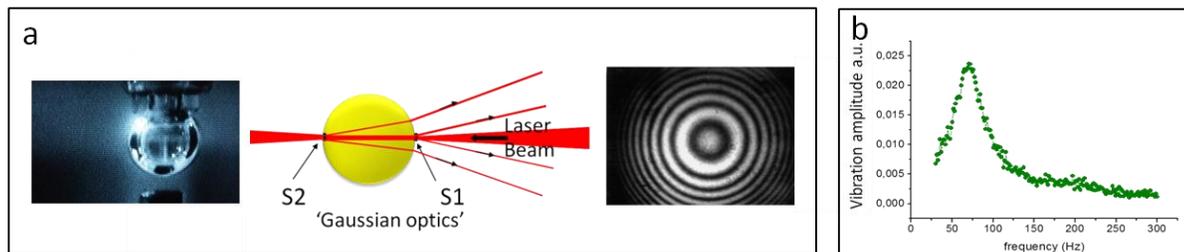


Figure 2. a) The picture shows a drop attached to the capillary protruding from the top electrode (left), a sketch of the interfering beams reflected from opposite interfaces of the drop (center) and the concentric fringes of the interference pattern (right). b) the resonance spectrum of a pure squalene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

Figure 2 shows the experimental model and an example of the lowest frequency resonance of a drop of SQ in water, the only one being easily excited for this oil. Thus, while several characteristic modes can be observed for some liquid/liquid interfaces already studied [26], much less are detectable in the case of SQ, due to strong dampening ($\eta = 12\text{mPas}$ at 25°C). As can be seen in Figure 2b the FWHM of the resonance peak is quite high, as for strong dissipation associated to a high interfacial viscosity of the SQ/water system.

A SQ drop is first created inside a $500\ \mu\text{l}$ cell filled with pure water and its resonance spectrum was collected. The production of a suitable drop is challenging, as any contact of SQ with the electrode has to be prevented, while providing the required proximity of the drop to the top electrode. This is obtained by a glass capillary protruding from the electrode (see Figure 2 left), then, in turn, requiring great care in order to overcome the tendency of SQ oil to wet the glass. The resonance spectrum of the drop in pure water is kept as a reference spectrum all along the absorption experiments. The typical amplitude of resonant oscillations is in the 1-10 nm range, involving an interfacial slab where the molecular layers contributing to the surface pressure are found [30]. Starting from pure water, the cell is progressively supplied with small aliquots of surfactant, hundred times lower than the CMC concentration, followed by continuously monitoring the conductance of the solution. The addition of MR was performed by adding aliquots, in the order of 1-10 μL , of the 1mM MR solution in a bottom corner of the cell. The diffusion time of the MR monomers towards the SQ drop surface was reasonably short, due to the small size of the cell, and in few minutes equilibrium was reached.

Adsorption of anionic MR at the interface showed up evident by the increase of the amplitude of the electrically-excited fluctuations. Figure 3 shows the resonance peak of a SQ drop evolving towards the steady-state after addition of MR to the bulk water in 0.22 μ M concentration. It is evident that the expected increase in the fluctuation amplitude is not accompanied by a shift in the resonance frequency, indicating that in this range of concentration the surface tension is not affected by MR upload.

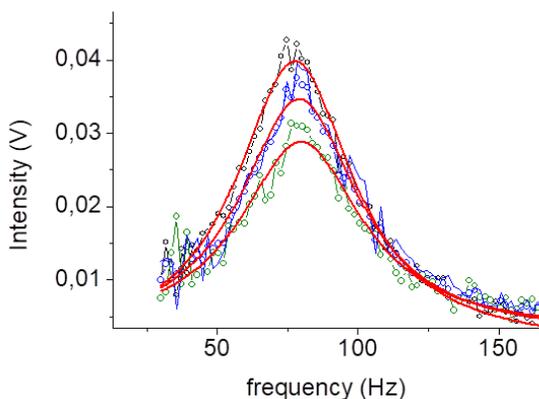


Figure 3 Resonance amplitude increasing during MR adsorption at the interface of a SQ drop ($R=0.6\text{mm}$) after addition of MR to the bulk, in the concentration range corresponding to the gas-state for the interfacial monolayer, surface pressure being unaffected. The three presented spectra are taken 5 , 7 and 11 minutes after administration. Red lines are the lorentzian fits.

The transition of the monolayer to the liquid-expanded phase is marked by a change in the resonance frequency, associated to a modification of the interfacial tension of the drop. Figure 4 reports the resonance frequency of a SQ drop in the entire concentration range investigated. The onset of the red-shift in the drop resonance was observed upon addition of 66 μ L of the MR source solution to the cell, corresponding to a MR bulk concentration of 7.7 μ M. Upon further MR addition, the peak kept on shifting to lower frequency, consistently with the expected reduction in surface tension. An absolute calibration is not feasible for squalene drops in our setup, yet applying eq. 1, we can evaluate the normalized surface tension decrease $(\sigma_0 - \sigma)/\sigma_0$, σ_0 being the squalene-water interfacial tension. At the highest investigated concentration the normalized surface tension decrease was 0.62, well reproducible in different experiments. However, theory is still missing to model the behavior of interfacial properties when active charged molecules are adsorbed on 100Hz oscillating

drop. In this condition, interactions among adsorbed molecules can play an important role.

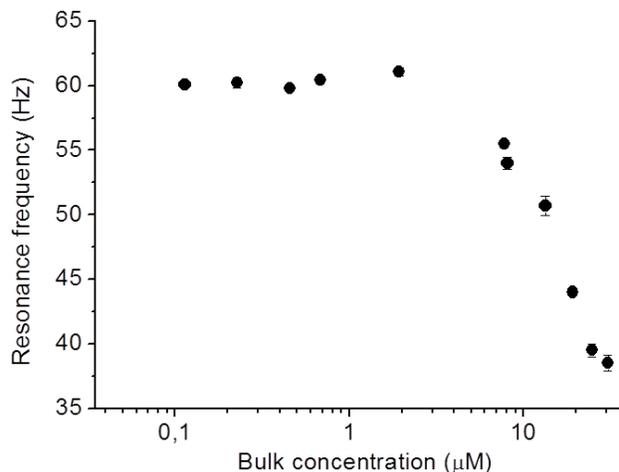


Figure 4. Resonance frequency versus MR bulk concentration of a SQ drop of radius 0.7mm. The two regimes corresponding to the gas-state and the liquid-expanded state for the surface monolayer of MR at the SQ/water interface can be identified. Standard errors from the fitting procedure are reported.

Main goal of the study was to highlight the adsorption process at low concentrations, where only the resonance amplitude is affected by the surfactant uptake at the interface. This behavior was observed over more than one decade in bulk concentration, from 0.1 to 2 μM (Figure 4), and is consistent with a partial coating of the surface, corresponding to the so-called 'gas state' in the π -A diagrams of 2D amphiphilic monolayers. This region is usually unaccessed by other methods, while it can be easily explored by the present interferometric technique in the electric field excitation mode, given the ionic nature of the adsorbing molecule. Figure 5a shows that the dependence of the measured equilibrium resonance amplitudes on the logarithm of MR bulk concentration is linear. In our measurements, the resonance amplitude evaluates the surface uptake of the surfactant, increasing with the surface density. Thus it is used as the significative parameter in investigating the behavior of the adsorption process. Among the different models describing absorption [31], this makes evident the agreement with the Temkin isotherm model [32], which predicts a linear dependence of the interface surfactant density versus the logarithm of the bulk

surfactant concentration. In fact, the Temkin isotherm model is suitable for describing the gas state, when tight packing and orientational order of the adsorbed molecules are not present. Testing the MR uptake with the Langmuir isotherm model shows good agreement except for the lowest concentration, as seen in Figure 5b. Error in concentration can be excluded, being linear the increase of bulk conductivity, online continuously checked. On the other hand, the deviation appears to be in the limit of the standard error evaluated for the amplitude fitting parameter. Nonetheless, this suggests that some adsorbate/adsorbate interaction may affect the charge dependent response of the oscillating drop in some concentration interval. Figure 5a also reports successive transient values of the fluctuation amplitude vertically arranged in the highlighted slice, in correspondence to the second addition of MR ($0.11 \rightarrow 0.22 \mu\text{M}$ bulk concentration). In fact, the evolution of the oscillation amplitude could be observed to follow the kinetics of uptake after addition of surfactant to the contacting aqueous solvent out of equilibrium, before attaining the steady state.

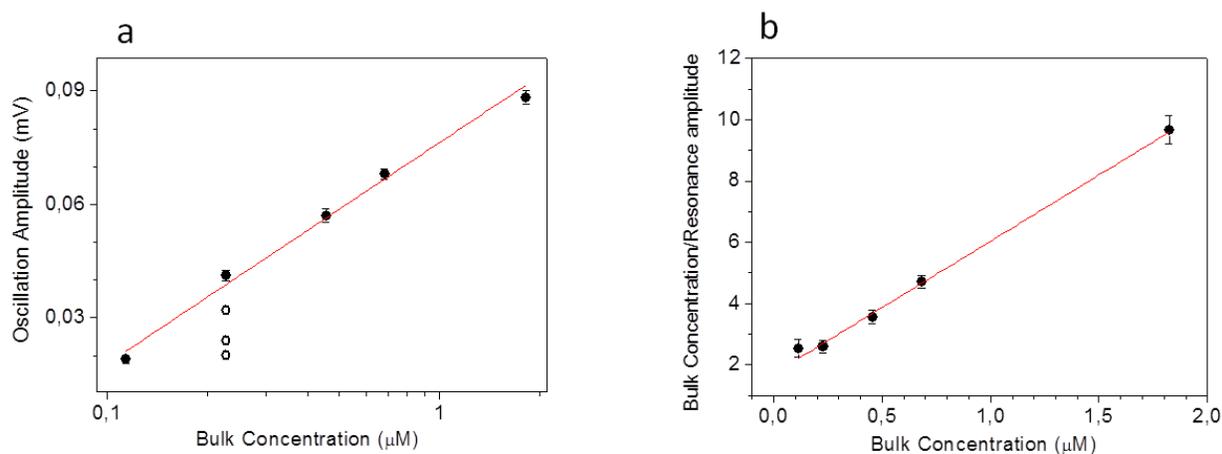


Figure 5. *a)* Resonance amplitude versus MR bulk concentration, in the regime corresponding to the gas state for the interfacial monolayer. The highlighted slice shows successive transient values of the resonance amplitude (empty dots) after administration of the second surfactant dose ($0.11 \rightarrow 0.22 \mu\text{M}$ bulk concentration), soon after, 3 and 6 minutes after administration. The line corresponds to the fit to Temkin isotherm model of the steady state experimental values (full dots). *b)* The same

experimental data are reported for testing the Langmuir isotherm model. Errors on the parameter of the fitting procedure are reported.

Surfactant desorption from the drop interface could also be obtained by gentle dilution of the bulk solution with pure water, without moving the drop. Online check of the solution conductivity allowed following the rinsing progression until conductivity went back to the typical value for pure water, with a contemporary up-shift of the resonance frequency back to that of a squalene drop in pure water.

Conclusions

We applied the recently developed drop-interferometry technique to study the properties of a squalene-oil/water interface upon uptake of the anionic monorhamnolipid bio-surfactant.

The adsorption process could be followed at extremely low surfactant concentration, because the incremental uptake of molecules at the oil/water interface produces an increase in the amplitude of the drop response to the exciting electric field without affecting the resonance frequency, i.e. the interfacial tension. The evolution of the oscillation amplitude could be observed in two features, namely, in the kinetics of uptake following each step of surfactant addition to the contacting aqueous solvent, and in the increase of the steady-state value as a function of surfactant concentration. The latter is well reproduced by the Temkin isotherm model, adapted to describing the gas state, when tight packing and orientational order at the surface are not present. This monolayer gas state evolves towards the monolayer liquid-expanded state that is detected by a shift of the drop resonance towards lower frequency, associated to a decrease of the surface tension. Evaluating the initial steps of the adsorption process at the interface is of relevance for applications requiring optimization of microemulsion features.

Aknowledgements

We thank the Fonds National de la Recherche Scientifique (FRS-FNRS) (CDR grant RHAMEMB - J.0086.18) and The University of Liège (Fonds Spéciaux de la Recherche, Action de Recherche Concertée-Project FIELD) for financial support. M.D. thanks the FRS-FNRS for her position as Senior Research Associate.

References

- [1] Stauffer C.E., The Measurement of Surface Tension by the Pendant Drop Technique, *J. Phys. Chem.*, 1965, 69 (6), 1933–1938; doi: 10.1021/j100890a024.
- [2] Sommerling J-H., de Matos M.B.C., Hildebrandt E., Dessy A., Kok R.J., Nirschl H., Leneweit G., Instability Mechanisms of Water-in-Oil Nanoemulsions with Phospholipids: Temporal and Morphological Structures, *Langmuir*, 2018, 34(2), 572-584. doi: 10.1021/acs.langmuir.7b02852. Epub 2018 Jan 3.
- [3] Fox C.B. Squalene Emulsions for Parenteral Vaccine and Drug Delivery *Molecules* 2009 (14) 3286-3312; doi: 10.3390/molecules14093286.
- [4] Hildebrandt E., Vrânceanu M., Nirschl H., Leneweit G., Phospholipids as emulsifiers for micro/nano droplets suitable for biotechnological systems integration, *La Houille Blanche*, 2013, 2, 68-73; doi: 10.1051/lhb/2013018
- [5] Richens J.L., Lane J.S., Mather M.L., O'Shea P., The interactions of squalene, alkanes and other mineral oils with model membranes; effects on membrane heterogeneity and function, *J Colloid Interface Sci.* 2015, 457, 225-231. doi: 10.1016/j.jcis.2015.06.052.
- [6] Hildebrandt E., Dessy A., Sommerling J-H., Guthausen G., Nirschl H., Leneweit G., Interactions between Phospholipids and Organic Phases: Insights into Lipoproteins and Nanoemulsions, *Langmuir* 2016, 32, 5821–5829 DOI:10.1021/acs.langmuir.6b00978
- [7] Hildebrandt E., Sommerling J-H., Guthausen G., Zick K., Stürmer J., Nirschl H., Leneweit G., Phospholipid adsorption at oil in water versus water in oil interfaces: Implications for interfacial densities and bulk solubilities *Coll Surf A*, 2016, 505, 56-63. doi: 10.1016/j.colsurfa.2015.12.024.
- [8] Abdel-Mawgoud A.M., Lépine F., Déziel E., Rhamnolipids: diversity of structures, microbial origins and roles, *Appl. Microbiol. Biotechnol.*, 2010, 86, 1323–36. doi: 10.1007/s00253-010-2498-2
- [9] Rikalović M.G., Vrvic M.M., Karadžić I.M., Rhamnolipid biosurfactant from *Pseudomonas aeruginosa* – from discovery to application in contemporary technology, *J. Serb. Chem. Soc*, 2015, 80441, 279–304.
- [10] Irfan-Maqsood M., Seddiq-Shams M., Rhamnolipids: Well-Characterized Glycolipids with Potential Broad Applicability as Biosurfactants. *Ind. Biotechnol.*, 2014, 10, 1–7. doi: 10.1089/ind.2014.0003

- [11] Deepika K.V., Ramu Sridhar P., Bramhachari P., Characterization and antifungal properties of rhamnolipids produced by mangrove sediment bacterium *Pseudomonas aeruginosa* strain KVD-HM52, *Biocatal. Agric. Biotechnol.*, 2015, 4, 608-615. doi: 10.1016/j.bcab.2015.09.009
- [12] Varnier A-L., Sanchez L., Vatsa P., Boudesocque L., Garcia-Brugger A., Rabenoelina F., Sorokin A., Renault J.-H., Kauffmann S., Pugin A., Clément C., Baillieux F., Dorey S. Bacterial rhamnolipids are novel MAMPs conferring resistance to *Botrytis cinerea* in grapevine., *Plant. Cell Environ.*, 2009, 32, 178–193. doi: 10.1111/j.1365-3040.2008.01911.x
- [13] Champion J.T., Gilkey J., Lemparski H., Retterer J., Miller R.M., Electron Microscopy of Rhamnolipid (Biosurfactant) Morphology: Effects of pH, Cadmium, and Octadecane. *J. Colloid Interface Sci.*, 1995, 170, 569–574. doi: 10.1006/jcis.1995.1136
- [14] Sánchez M., Aranda, F.J. Espuny, M.J. Marqués, A. Teruel J., Manresa A., Ortiz A., Aggregation behaviour of a dirhamnolipid biosurfactant secreted by *Pseudomonas aeruginosa* in aqueous media, *J. Colloid Interface Sci.*, 2007, 307, 246–53. doi: 10.1016/j.jcis.2006.11.041
- [15] Pornsunthorntawe O., Chavadej S., Rujiravanit R., Solution properties and vesicle formation of rhamnolipid biosurfactants produced by *Pseudomonas aeruginosa* SP4. *Colloids Surf. B. Biointerfaces*, 2009, 72, 6–15. doi: 10.1016/j.colsurfb.2009.03.006
- [16] Khoshdast H., Abbasi H., Sam A., Noghabi K.A., Frothability and surface behavior of a rhamnolipid biosurfactant produced by *Pseudomonas aeruginosa* MA01. *Biochem. Eng. J.*, 2012, 60, 127–134. doi: 10.1016/j.bej.2011.10.015
- [17] Wang H., Coss C.S., Mudalige A., Polt R.L., Pemberton J.E., A PM-IRRAS investigation of monorhamnolipid orientation at the air-water interface. *Langmuir*, 2013, 29, 4441–4450. doi: 10.1021/la3051725
- [18] Abbasi H., Noghabi K.A., Hamedi M.M., Zahiri H.S., Moosavi-Movahedi A.A., Amanlou M., Teruel J., Ortiz A., Physicochemical characterization of a monorhamnolipid secreted by *Pseudomonas aeruginosa* MA01 in aqueous media. An experimental and molecular dynamics study., *Colloids Surf. B. Biointerfaces*, 2013, 101, 256– 65. doi: 10.1016/j.colsurfb.2012.06.035
- [19] Chen M.L., Penfold J., Thomas R.K., Smyth T.J.P., Perfumo A., Marchant R., Banat I.M., Stevenson P., Parry A., Tucker I., Grillo I., Solution self-assembly and

- adsorption at the air–water interface of the monorhamnose and dirhamnose rhamnolipids and their mixtures, *Langmuir*, 2010, 26, 18281-18292. doi: 10.1021/la1031812
- [20] Peker S., Helvacı S., Özdemir G., Interface-subphase interactions of rhamnolipids in aqueous rhamnose solutions, *Langmuir*, 2003, 19, 5838-5845. doi: 10.1021/la0269964
- [21] Özdemir G., Peker S., Helvacı S.S., Effect of pH on the surface and interfacial behavior of rhamnolipids R1 and R2. *Colloids Surf. A*, 2004, 234, 135-143. doi: 10.1016/j.colsurfa.2003.10.024
- [22] Hanni-Ciunel K., Schelero N., von Klitzing R., Negative charges at the air/water interface and their consequences for aqueous wetting films containing surfactants. *Faraday Discuss. Chem. Soc.*, 2009, 141, 41–53. doi: 10.1039/B809149H
- [23] Corti M., Bonomo M., Raudino A., New interferometric technique to evaluate the electric charges of gas bubbles in liquids, *Langmuir*, 2012, 28, 6060–6066. doi: 10.1021/la3003542
- [24] Corti M., Pannuzzo M., Raudino A., Out of Equilibrium Divergence of Dissipation in an Oscillating Bubble Coated by Surfactants, *Langmuir*, 2014, 30, 477–487. doi: 10.1021/la4040062
- [25] Corti M., Pannuzzo M., Raudino A., Trapping of Sodium Dodecyl Sulfate at the Air-Water Interface of Oscillating Bubbles, *Langmuir*, 2015, 31, 6277–6281. doi: 10.1021/la504677a
- [26] Cantu' L., Raudino A., Corti M., An interferometric technique to study capillary waves, *Adv. Colloid Int. Sci.*, 2017. doi: 10.1016/j.cis.2017.06.0
- [27] Lamb H., *Hydrodynamics*, 6th ed. (Cambridge University Press, Cambridge, England, 1932); reprinted by Dover, New York, 1993.
- [28] Bostwick J. B., Steen P. H., Capillary oscillations of a constrained liquid drop, *Phys. Fluids*, 2009, 21, 032108. doi: 10.1063/1.3103344
- [29] Prosperetti A., Linear oscillations of constrained drops, bubbles, and plane liquid surfaces, *Phys Fluids* 2012, 24, 032109, doi: 10.1063/1.3697796.
- [30] Segá M., Fábíán B., Horvai G., Jedlovský P., How Is the Surface Tension of Various Liquids *Distributed* along the Interface Normal?, *J. Phys. Chem. C*, 2016, 120, 27468–27477. doi: 10.1021/acs.jpcc.6b09880
- [31] Foo K.Y., Hameed B.H., Insights into the modeling of adsorption isotherm systems. *Chem. Eng. J.*, 2010, 156, 2–10. doi: 10.1016/j.cej.2009.09.013

- [32] Tempkin M.I., Pyzhev V., Kinetics of ammonia synthesis on promoted iron catalyst, *Acta Phys. Chim. USSR*, 1940, 12, 327–356.

squalene drop in water

