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### ► To cite this version:

Rémy Bois, Bemba Abdellahi, Benjamin Mika, Séma Golonu, Pascale Vigneron, et al.. Physicochemical, foaming and biological properties of lowly irritant anionic sugar-based surfactants. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 2020, 607, pp.125525. 10.1016/j.colsurfa.2020.125525 . hal-02946872

**HAL Id: hal-02946872**

**<https://hal.science/hal-02946872>**

Submitted on 9 Sep 2022

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1 **Physicochemical, foaming and biological properties of lowly irritant anionic sugar-based**  
2 **surfactants**

3

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5

6 **Abstract**

7 Surface-active compounds derived from biomass, especially sugar-based amphiphiles, have  
8 received wide attention regarding their biodegradability, low toxicity and ecological  
9 acceptability. Compared to nonionic sugar-based surfactants, the anionic ones show  
10 significantly better solubility, higher surface activity and foaming performance. Thus they are  
11 largely used in personal care formulations and many technological applications. However,  
12 anionic surfactants are well known to induce skin and eye irritation.

13 In this study, three sugar-based anionic surfactants, bearing a lipidic chain grafted to the  
14 anomeric position of a monosaccharide (glucose or xylose) and a sulfate group on the primary  
15 hydroxyl, were synthesized: 6-*O*-sulfo-*N*-( $\beta$ -D-glucopyranosyl) dodecanamide (GlcNC12S),  
16 *N*-dodecyl-6-*O*-sulfo-D-gluconamide (GlcCC12S) and *N*-dodecyl-6-*O*-sulfo-D-xylonamide  
17 (XylCC12S). These molecules were investigated in details for their self-assembling behavior,  
18 foaming properties and biological effects. All their properties were compared to those of two  
19 commercially available anionic surfactants, sodium laureth sulfate (SLES) and sodium  
20 dodecylsulfate (SDS).

21 Results revealed that the three anionic glycolipids show surface properties and foaming  
22 behavior comparable to those of SDS. Furthermore, their cytotoxic and irritation potentials

23 are significantly lower compared to commercial molecules, which make these renewable  
24 molecules potential candidates for replacement of petroleum-based compounds.

25

26 **Keywords:** anionic surfactant, sugar-based surfactant, glycolipid, foaming properties,  
27 cytotoxic activity, irritation potential

28

## 29 **Introduction**

30 Surface-active compounds performing detergency, wetting, emulsifying, solubilizing,  
31 dispersing and foaming effects are among the most representative chemical products  
32 consumed on a world scale [1, 2]. Depending on the charge of the hydrophilic group,  
33 surfactants can be nonionic, anionic, cationic or amphoteric. Anionic surfactants are used in  
34 greater volume compared to other surfactants (about 60% of worldwide surfactant production)  
35 because of their excellent performance in cleaning processes and low cost manufacture [3].  
36 During the transition period from 1940 to 1970 due to the shift from the use of powder  
37 detergents to liquid ones, anionic amphiphiles became widely used in formulation of laundry  
38 detergents, domestic and industrial cleaning products, agricultural and pharmaceutical  
39 formulations, cosmetic and personal care products. Anionic surfactants are mainly constituted  
40 of a hydrophobic part (linear aliphatic hydrocarbon chain with length between C<sub>8</sub> and C<sub>18</sub>,  
41 alkylphenyl ethers, alkylbenzenes, etc) linked to a hydrophilic part containing a sulphate,  
42 sulfonate, carboxyl or phosphate group, associated to a counter ion (for example, Na<sup>+</sup>, K<sup>+</sup>,  
43 NH<sub>4</sub><sup>+</sup> or an alkanolamine cation) [4-6].

44 However, the use of these surfactants is somewhat limited by their irritation profiles. Anionic  
45 surfactants show an important skin and eye irritation potential [6]. Moreover, cytotoxicity  
46 studies showed that anionic surfactants were more toxic than nonionic ones [4]. The  
47 development of new anionic amphiphiles with tailored functionalities and showing at the  
48 same time skin tolerance, has become a priority in the field of surface and colloid science.

49 Over the past few decades, the pressure from legislation and growing consumer trend toward  
50 environmentally benign chemicals had a strong impact on surfactants development. There is a  
51 growing need for the substitution of anionic surfactants of particular concern in different  
52 applications. The biodegradability and biocompatibility of surfactants have become very  
53 important not only to the consumer but also to its functional performance. To increase the  
54 environmental sustainability of amphiphilic molecules, new surface-active compounds,  
55 commonly referred as “green surfactants”, have been intensively developed [7]. Different

56 biomass substrates such as peptides and amino acids [8, 9][8, 10], sugars [10], fatty acids and  
57 alcohols [11] [12, 13] have been extensively studied for the production of these surfactants.  
58 Sugar-based surfactants have increasingly become a viable bio-based alternative to  
59 petrochemical-based amphiphiles. Particularly, nonionic ones showed high performance,  
60 environmental compatibility, low toxicity and low irritancy towards living organisms [10, 12-  
61 15][11, 14-16, 18]. The raw material sources used are mainly sucrose from sugar beet or  
62 sugarcane, carbohydrate-based residues from hemicellulose and starch derivatives (glucose,  
63 galactose, mannose, xylose, sorbitol, lactose). Nowadays, most important sugar-based  
64 surfactants used for different applications are nonionic alkylpolyglucosides, sorbitan esters  
65 and sucrose esters. Numerous syntheses of such amphiphilic molecules have been described  
66 [10, 16-18]. Nevertheless, to the best of our knowledge, only few studies on anionic sugar-  
67 based surfactants have been reported. Among them, several articles dealt with the synthesis  
68 and properties characterization of anionic D-glucopyranoside derivatives; these molecules  
69 showed good adsorption properties similar to other ionic surfactants [19-21] and can also be  
70 applied for enantiomeric separation of racemic compounds [22, 23]. Otherwise, the analysis  
71 of literature data demonstrates a significant decrease in Krafft temperature ( $T_K$ ) for anionic  
72 sugar derived surfactants [20] compared to their nonionic homologous with the same chain  
73 length [24]. The micelle formation is also impacted by addition of anionic group, higher value  
74 of critical micelle concentration (CMC) is observed for sulfated alkyl glucoside [25]  
75 compared to nonionic one [26]. Besides, anionic sulfosucrose derivatives showed very  
76 promising surface-active properties with CMC values from two to three orders of magnitude  
77 lower than those of commercial anionic molecules [27]. Other studies demonstrated that  
78 anionic galactoside [28] and glucoside [17] derivatives had interesting wetting properties for,  
79 respectively, textile treatment applications and enhanced oil recovery.

80 Nevertheless, there is no data in the literature dealing with neither foaming behavior, which is  
81 an important property for various surfactants applications, nor dermal irritation potential of  
82 anionic sugar-based amphiphiles. Based on the facts mentioned above, studying the potential  
83 of eco-friendly anionic glycolipids for the substitution of petroleum-derived anionic  
84 surfactants is worthy of experimental study.

85 The present contribution reports the complete characterization of three anionic surfactants  
86 based on sugar-derived hydrophilic units. These molecules were chosen from among a  
87 number of glycolipids synthesized in our laboratory after pre-screening of physicochemical  
88 properties. The main objective of this work is to identify new surfactants which can  
89 potentially replace analogous petroleum-derived amphiphiles for specific applications. Thus,

90 all the properties analyzed were compared to commercial standard anionic surfactants with  
91 the same alkyl chain length. Surface tension was measured to study micellar and interfacial  
92 phenomena. Foaming behavior was studied using gas-sparging method. To evaluate the  
93 biological effects of surfactants, cellular and tissue models (mouse dermal fibroblasts and  
94 reconstituted human epidermis (RHE)) were treated with the molecules and the cell metabolic  
95 activity and IL-1 $\alpha$  release (skin irritation) were measured.

96

## 97 **2. Materials and methods**

98

### 99 *2.1. Synthesis of anionic glycolipids*

100 Three sugar-based surfactants with anionic sulfated polar head and alkyl chain, constituted of  
101 12 carbon atoms, were synthesized in this study. 6-*O*-Sulfo-*N*-( $\beta$ -D-glucopyranosyl)  
102 dodecanamide (GlcNC12S,  $M_w=463.5 \text{ g}\cdot\text{mol}^{-1}$ ) was prepared from commercial glucose  
103 following the procedure described in literature [29]. *N*-dodecyl-6-*O*-sulfo-D-gluconamide  
104 (GlcCC12S,  $M_w=465.5 \text{ g}\cdot\text{mol}^{-1}$ ) and *N*-dodecyl-6-*O*-sulfo-D-xylonamide (XylCC12S,  
105  $M_w=435.5 \text{ g}\cdot\text{mol}^{-1}$ ) were produced in three steps, by 1) oxidation of commercial glucose and  
106 xylose [30], 2) ring-opening aminolysis of the as-obtained glyconolactone and 3) sulfation  
107 using Pyr-SO<sub>3</sub> complex [25]. The monosulfated sugars were separated from disulfated  
108 compounds using reverse phase chromatography. Produced anionic surfactants were stored at  
109 -20°C.

110 In addition, two commercially available anionic surfactants were used: Sodium Laureth  
111 Sulfate (SLES,  $M_w=370 \text{ g}\cdot\text{mol}^{-1}$ ) provided from THOR Personal Care SAS (La Croix Saint-  
112 Ouen, France) as 27 wt% aqueous solution (Texapon® NSO UP) and Sodium Dodecylsulfate  
113 (SDS,  $M_w=288 \text{ g}\cdot\text{mol}^{-1}$ , purity>99%) was purchased from Sigma-Aldrich (Saint-Quentin  
114 Fallavier, France). The chemical structures of studied anionic surfactants are presented in  
115 Fig. 1.

116

### 117 *2.2. Preparation of surfactant solutions*

118 Water from Milli-Q Millipore system with a resistivity of 18.2 M $\Omega$ cm produced by a PureLab  
119 Classic purification chain (Elga/Veolia, Wissous, France) was used to prepare aqueous  
120 solutions of surfactants for surface tension and foaming properties evaluation. Cell-culture  
121 medium was prepared as described previously [31] and used for preparation of solutions for  
122 biological responses evaluation. Surfactant solutions with different concentrations were

123 prepared by dissolving a known quantity of pure surfactant in water or cell-culture aqueous  
124 medium. All surfactants were soluble in water at 25°C.

125

### 126 2.3. Surface tension measurement

#### 127 2.3.1. Equilibrium surface tension

128 Equilibrium surface tensions ( $\gamma_{eq}$ ) of aqueous surfactant solutions prepared in pure water and  
129 cell-culture medium were measured using K100 KRÜSS tensiometer (Hamburg, Germany) by  
130 the Wilhelmy plate method. The Wilhelmy plate has a length, width and thickness of  
131 19.9 mm, 10 mm and 0.2 mm, respectively and is made of platinum. The tensiometer operates  
132 by holding a plate in a fixed vertical position attached to a microbalance (resolution 10  $\mu$ g)  
133 measuring the force acting on the plate. The surface tension is directly determined from the  
134 force-balance measurement. The surfactant solution was put in a glass vessel surrounded by a  
135 circular thermostated system maintained at  $25\pm 1^\circ\text{C}$  throughout the duration of the test. Prior  
136 to each determination, the plate was thoroughly cleaned and burnt to red hot conditions in a  
137 blue flame to ensure perfect wetting (zero contact angle). The measurement of surface tension  
138 at the air/liquid interface was conducted over 12 000s. The surface tension values are reported  
139 as the mean of three repeat measurements. The Critical Micelle Concentration (CMC) of each  
140 surfactant was determined from the minimum point of the plot of the surface tension with  
141 respect to the logarithm of the concentration. The efficiency of surfactant adsorption ( $pC_{20}$ )  
142 was associated to the surfactant concentration required to reduce the surface tension of pure  
143 water by 20 mN/m. The surface tension of pure water measured at 25°C was equal to 72.8  
144  $\text{mN}\cdot\text{m}^{-1}$ .

145 The maximum surface excess of surfactant at the air/water interface,  $\Gamma_{max}$ , was calculated  
146 using the Gibbs adsorption isotherm equation [32]:

$$147 \Gamma_{max} = \frac{-1}{nRT} \frac{d\gamma}{d\ln(C)} \quad (1)$$

148 where  $n=2$  for a conventional ionic surfactant, assuming electrical neutrality of the interface  
149 [33],  $R$  is the universal gas constant ( $8.314 \text{ J}\cdot\text{mol}^{-1}\cdot\text{K}^{-1}$ ),  $T$  is the absolute Kelvin temperature  
150 and  $\left(\frac{d\gamma}{d\ln(C)}\right)$  is the slope of  $\gamma$  versus  $\ln(C)$  determined at the CMC. The minimum area  
151 occupied by a surfactant molecule,  $A_{min}$ , at the interface:

$$152 A_{min} = \frac{1}{\mathcal{N}\Gamma_{max}} \quad (2)$$

153 where  $\mathcal{N}$  is Avogadro's number.

154

### 155 2.3.2. *Dynamic surface tension*

156 Measurement of dynamic surface activity of the surfactant solutions were performed using the  
157 maximal bubble pressure method (BP2 Krüss tensiometer, Hamburg, Germany). This method  
158 is the most appropriate one for the determination the kinetics of surface tension decrease at  
159 very short times. Briefly, an inert gas (nitrogen) is slowly bubbled through a fine capillary,  
160 which is immersed in the test aqueous solution. Hydrophobically modified glass capillary  
161 with an inner diameter of 0.24 mm was used for the measurements. When the bubble area  
162 grows at the tip of the capillary, the pressure within the bubble increases and passes through  
163 a maximum. The surface tension can be calculated from the measured maximum capillary  
164 pressure using the Laplace equation [34]. The time associated with the interface formation  
165 (surface age) was varied from 30 ms to 50 s in every single experiment. The constant  
166 temperature of  $25\pm 1^\circ\text{C}$  during measurements was assured by the external thermostat.  
167 Analyses were performed in triplicate.

168

### 169 2.4. *Foaming properties*

170 The determination of foaming properties of surfactant solutions was performed using an  
171 automated foaming device (FoamScan, Teclis, France). This instrument produces foam by  
172 blowing gas through a porous frit at a controlled gas flow rate. The conductivity measurement  
173 and image analysis are used to monitor different foam properties such as foam ability, foam  
174 stability, liquid fractions, drainage rate, and bubble size distribution. The experimental setup  
175 consisted of a glass column (diameter 3.5 cm, height 30 cm) equipped with a porous glass frit  
176 at its bottom. The foam generated was observed with two CDD cameras: one camera allowing  
177 the measurement of total foam and liquid amounts and a second camera equipped with a  
178 macro objective used to record the variation of the air bubbles size. The experimental  
179 conditions for determination of foam-related properties using gas-sparging method were  
180 established in our previous work [35]. Low frit porosity, low concentration and high gas flow  
181 rate turned out to be the best choice for the screening of foaming performances of surfactants. In  
182 the present study, 40 mL of surfactant solution at a concentration of  $C=0.1\text{CMC}$  was  
183 introduced into the column equipped with frit porosity of grade P3 ( $\phi=16\text{-}40\ \mu\text{m}$ ), at ambient  
184 temperature ( $24 \pm 2^\circ\text{C}$ ). Foam was generated by injection of pressurized air (0.5 bar) at a flow  
185 rate of  $200\ \text{mL}\cdot\text{min}^{-1}$  during 60 s. The total duration of one experiment was set to 30 minutes  
186 for measurement of foam stability. All experiments were carried out in triplicate. Several  
187 foaming properties were examined, namely foaming capacity, foam stability, half-life time of  
188 foam and maximum foam density.

189 The foamability at the end of bubbling is defined by the foam capacity, FC:

$$190 \quad FC = \frac{V_{\text{foam},0}}{V_{\text{gas}}} \quad (3)$$

191 where  $V_{\text{foam},0}$  is the volume of foam at the end of gas injection and  $V_{\text{gas}}$  is the volume of the  
192 sparged gas.

193 Foam stability is a parameter taking into account the whole behaviour of the foam collapse  
194 process as a function of time. For evaluation of foam stability (S) the Experimental area under  
195 the plot  $V_{\text{foam}}=f(\text{time})$  starting at the end of gas-injection was calculated and compared to the  
196 Maximal area (ideal behaviour in which the foam would remain stable all along the  
197 experiment).

$$198 \quad S = \frac{\text{Experimental area}}{\text{Maximal area}} \quad (4)$$

199 Foam half-life time ( $t_{1/2}$ ) corresponds to the time (seconds) needed for the reduction of the  
200 foam volume to half of the initial volume value ( $V_{\text{foam},0}$ ).

201 Maximum foam density (MD), related to the amount of liquid in the foam, was measured at  
202 the end of gas bubbling. The density was determined by conductivity measurements within  
203 the bottom solution.

$$204 \quad MD (\%) = \frac{V_{\text{solution},0} - V_{\text{solution},f}}{V_{\text{foam},0}} \times 100 \quad (5)$$

205  $V_{\text{solution},0}$  is the initial volume of liquid,  $V_{\text{solution},f}$  is the volume of liquid at the end of gas-  
206 injection.

207

## 208 *2.5. Biological responses on cellular and tissue models*

### 209 *2.5.1. Cell and tissue models*

210 The mouse fibroblast L929 cell line was obtained from ATCC (ATCC-CCL-1, LGC  
211 Standards, France). Cells (passage 15-40) were cultured in 75 cm<sup>2</sup> Falcon® cell culture flasks  
212 with DMEM (Gibco, Thermofisher scientific, France) supplemented with fetal bovine serum  
213 (FBS, Gibco), 2 mM L-glutamine (Gibco), 100 U/mL penicillin (Gibco) and 100 µg/mL  
214 streptomycin (Gibco). Cells were maintained in a humid atmosphere at 37°C with 5% CO<sub>2</sub>.

215 Reconstituted Human Epidermis (RHE) model (EpiDerm™ EPI-200-SIT kit) was purchased  
216 from MATTEK (Bratislava, Slovakia). This model consists of normal human epithelial  
217 keratinocytes, which have been cultured at air/liquid interface to form a multilayered, highly  
218 skin-like structure on specially prepared permeable cell culture inserts. Upon reception, RHE  
219 were pre-conditioned by standard protocol following manufacturer's instructions.



220

### 221 2.5.2. Cell metabolic activity assay

222 The cytotoxic potential of surfactants was evaluated by measuring the cell metabolic activity  
223 of both fibroblasts L929 and RHE. Cells and RHE were cultured and treated by surfactants at  
224 different concentrations following the procedure described previously [31, 36].

225 Shortly, L929 cells (2600 cells/cm<sup>2</sup>) were seeded in Costar® culture plate with culture  
226 medium (2 mL). After 24h cell adhesion, surfactant dissolved in culture medium was added to  
227 the cells at different concentrations for 48h. The tetrazolium salt (200 µL), commonly referred  
228 as MTS (Cell titer 96® Aqueous One solution cell proliferation assay, Promega, France), was  
229 added to each well. After 4 h, 3 aliquots (100 µL) from each well were transferred into a 96  
230 well cell culture plate.

231 The effects of surfactants on RHE were evaluated according to the OECD N° 439 guideline  
232 for *in vitro* skin irritation (OECD 439, 2019). Surfactants dissolved in phosphate buffer saline  
233 (PBS) (30 µL, 1 mg/mL) or negative control (30 µL PBS) were applied for 1h (25 min at  
234 room temperature and 35 min at 37°C) on the top of each tissue with nylon meshes used to  
235 cover the tissue surface and to guarantee the spreading of liquids. Tissues in inserts were  
236 extensively washed to discard surfactants and RHE were incubated for 24h. Then the culture  
237 media were harvested and stored for a second end point, release of the cytokine IL1-α. Fresh  
238 culture medium was added on tissues, RHE were cultured for 18h and then were transferred  
239 into wells of Costar 24-well plate containing 270 µL of Hank's buffered salt solution (HBSS,  
240 Life Technologies) and 30 µL of MTS. After incubation period (3h at 37°C), 3 aliquots (100  
241 µL) from each well were transferred into a 96 well cell culture plate.

242 The amount of viable cells was evaluated by the measuring their dehydrogenase activity. The  
243 MTS was reduced by living cells into a formazan compound with characteristic adsorption  
244 band at 490 nm. The absorbance (A) of all samples was read at 490 nm using microplate  
245 reader (Bio-Rad Model 680, Bio-Rad, USA). Cell-less controls were used in each test to avoid  
246 any background absorbance due to possible interactions between MTS and tested molecules.  
247 Metabolic activity of L929 cells and RHE model related to the amount of living cells in  
248 culture was determined as: Cell metabolic activity = (A of sample treated with surfactant – A  
249 of culture medium with surfactants without sample)/(A untreated sample – A of culture  
250 medium). All experiments were performed six times.

251

### 252 2.5.2. Irritation test on RHE

253 The irritant effect of commercial and synthesized anionic surfactants on the RHE model was  
254 evaluated by measuring the secretion rate of human interleukin-1 $\alpha$  (IL-1 $\alpha$ ) in culture  
255 medium. IL-1 $\alpha$  cytokine concentrations were quantified after 1h surfactant exposure  
256 (followed by 24h post-incubation according to the RHE manufacturer's instructions) by  
257 ELISA test according to the kit manufacturer's instructions (Human IL-1 $\alpha$  Platinum ELISA  
258 kit, ThermoFisher Scientific, France). Six repetitions were performed for each surfactant  
259 solution.

260

### 261 *2.6. Statistical analysis*

262 Statistical analyses of foaming properties and biological responses were performed using  
263 GraphPad InStat software. Different variables are expressed as means  $\pm$  standard deviation.  
264 For foaming properties, experimental data was studied using one-way analysis of variance  
265 (ANOVA). Tukey's test was used for the multiple comparison procedure and the significance  
266 was accepted at the  $P < 0.05$  level.

267 For biological responses, non-parametric Dunn and Kruskal-Wallis tests were used for  
268 comparisons as most of the continuous values measured had a non-Gaussian distribution. A  
269 value of  $P < 0.05$  was taken as significant.

270

## 271 **3. Results**

272

### 273 *3.1. Surface tension reduction and CMC*

274 For comparison purposes, different surface properties of the three selected glycolipids were  
275 studied and the two classical anionic surfactants (SDS and SLES). The CMC values of  
276 anionic glycolipids were determined using equilibrium surface tension measurement at  
277 different concentrations and are taken in consideration at the minimum point of the decreasing  
278 surface tension curve. Plots of surface tension versus glycolipids and model anionic  
279 surfactants concentration are shown in Fig. 2A. Physicochemical properties of surfactants in  
280 water determined from these experimental curves are reported in Table 1.

281 When compared to commercial surfactants, sugar-based molecules have the same order of  
282 magnitude of CMC values as that of SDS. However, the CMC of SLES is significantly lower.  
283 For instance, the CMC of GlcNC12S is 20 times higher than that of SLES. The minimum  
284 observed for all curves indicated the presence of small amount of residual molecules with  
285 amphiphilic properties participating in the surface tension reduction. This particularity is

286 generally observed for SDS solutions due to the presence of dodecanol impurity [37, 38]. The  
287 surface tension values at CMC ( $\gamma_{\text{CMC}}$ ) indicate the effectiveness in surface tension reducing.  
288 By comparing the  $\gamma_{\text{CMC}}$  of five anionic surfactants, results indicate that highest value of 34.9  
289  $\text{mN}\cdot\text{m}^{-1}$  was obtained for SDS and the lowest of 27.3  $\text{mN}\cdot\text{m}^{-1}$  for SLES. The  $\gamma_{\text{CMC}}$  of sugar-  
290 based molecules were comprised between those of commercial surfactants. Similar tendencies  
291 can be observed for the results of calculated adsorption efficiency ( $pC_{20}$ ), maximum surface  
292 excess ( $\Gamma_{\text{max}}$ ) and corresponding minimal area per molecule ( $A_{\text{min}}$ ). For these physicochemical  
293 parameters, the values obtained for sugar-based molecules were comprised between those of  
294 SLES and SDS. Results reveal that GlcCC12S was characterized by highest  $pC_{20}$  value of  
295 3.92, followed by XylCC12S with  $pC_{20}$  of 3.43 and GlcNC12S with  $pC_{20}$  of 2.89. In regards  
296 to  $\Gamma_{\text{max}}$  and  $A_{\text{min}}$  parameters, estimated by applying Gibbs adsorption isotherm to the surface  
297 tension curves, the values obtained for anionic glycolipids were in the range of  $1.68\times 10^{-10}$ –  
298  $2.33\times 10^{-10}$   $\text{mol}\cdot\text{cm}^{-2}$  and 71.3–99.1  $\text{\AA}^2/\text{molecule}$  respectively.

299 The dynamic surface adsorption of surfactant was characterized using maximum bubble  
300 pressure method. This technique allows an accurate regulation of air/solution surface  
301 adsorption with time, ranges from 0.03 to 50 s. Fig. 2B presents the plots of dynamic surface  
302 tension of studied anionic surfactant as a function of bubble age at concentration equal to  
303 0.1CMC. For a new liquid/gas interface formation, the initial surface tension ( $\gamma_0$ ) corresponds  
304 to 72.8  $\text{mN}\cdot\text{m}^{-1}$ , which is a surface tension for pure water/nitrogen interface at room  
305 temperature and at atmospheric pressure. It can be observed that at short times, before surface  
306 age of 1 s, the dynamic surface tension of SLES and sugar-based surfactants changes very  
307 little and remains close to that of pure water, whereas SDS is characterized by lower surface  
308 tension of about 68–70  $\text{mN}\cdot\text{m}^{-1}$ , suggesting faster adsorption at the air/water interface. For  
309 higher surface ages, a little decrease in dynamic surface tension can be noticed for SLES after  
310 1 s and for XylCC12S after 20 s. In addition, results show that dynamic surface tension is not  
311 significantly affected by the increase in air bubble age for GlcCC12S and GlcNC12S.

312 From the results presented in Figure 2 and Table 1, it can be concluded that for the same alkyl  
313 chain length, the structure of the hydrophilic head group significantly affects adsorption at the  
314 air/water interface of anionic surfactants.

315

### 316 3.2. Foaming properties

317 Foaming behavior of the three synthesized anionic sugar-based surfactants and the two  
318 anionic reference surfactants was studied using gas-sparging method. Fig. 3 displays the foam

319 volume and liquid volume as a function of time for surfactant solutions and Table 2 presents  
320 the summary of experimental data on foaming properties. In order to compare foaming  
321 properties of different surfactant systems, it is more appropriate to consider these systems in  
322 the same thermodynamic state. Below CMC, surfactants are under a monomer form. Thus the  
323 flux of surfactant molecules to the interface and the foaming behavior of solution are not  
324 influenced by kinetic of micellization and break-time of micelles [39]. Moreover, the results  
325 of our previous study showed that low concentration conditions are favorable for the  
326 discrimination of foaming behavior of different surfactants [35]. Thus, solution concentration  
327 applied for all surfactants was 0.1CMC.

328 The results obtained demonstrated that the whole foam process consists of two stages: the  
329 generation of the foam (from 0s to 60s) and the destabilization of the foam (from 60s to  
330 1800s). In the first stage, i.e. gas sparging into the liquid, the liquid volume decreases and the  
331 foam volume increases gradually. In the decay stage, the foam volume decreases because of  
332 different destabilization processes, and the liquid volume increases due to the liquid drainage  
333 through foam.

334 As can be seen on Fig.3A, the volume of formed foam is close to 200 mL (maximal volume  
335 of produced foam at gas flow rate of 200 mL.min<sup>-1</sup> during 60 s) for all surfactants except  
336 GlcCC12S and GlcNC12S. Another information which can be observed from this figure is the  
337 collapsing rate or foam stability expressed by area under the curve (S, Table 2). This property  
338 is closely related to the half-life time of foam ( $t_{1/2}$ ). SLES solution showed significantly  
339 higher foam stability S of 0.79 compared to other surfactants. The stability of the foams  
340 produced by XylCC12S (0.36) and SDS (0.27) was intermediate, whilst in the case of  
341 GlcCC12S and GlcNC12S a significant reduction in the foam stability was observed.

342 The variation of liquid volume with time (Fig. 3B) showed that the liquid volume of  
343 GlcCC12S slightly decreased during bubbling and remained higher than that of other  
344 surfactants throughout the experience. The liquid drainage rate for foams produced with SDS,  
345 XylCC12S and GlcNC12S was similar. These observations are in agreement with maximum  
346 foam density values (MD, Table 2) representing the amount of liquid in the foam and  
347 measured at the end of bubbling. It can be seen that GlcCC12S formed very dry foam  
348 (MD=2.0%) whereas liquid content in foams produced by other anionic surfactants varied  
349 from 16.9 to 20.6%.

350

351 *3.3. Cell metabolic activity and irritation response of RHE after surfactant treatment*

352 The interaction of surfactants with L929 model cells was assessed to study surfactant-  
353 membrane interaction at low surfactant concentration (below CMC). The results reported on  
354 Fig.4 illustrates the metabolic activity of cells treated with anionic glycolipids at different  
355 concentrations. As can be seen, in the studied concentration range, the results show that cell  
356 viability decreased with increase in surfactant concentration, but for the concentrations below  
357 0.006 mM, the three molecules did not modify significantly the cell metabolic activity  
358 compared to untreated cells. A significant cytotoxic effect is observed for 0.06 mM of  
359 surfactants, with no difference observed between the three anionic glycolipids. The biological  
360 effects of anionic sugar-based surfactants were compared to responses of commercial anionic  
361 surfactants at the same molar concentration, 0.2 mM. This maximal concentration was fixed  
362 based on our previous studies, as at this concentration a significant cytotoxic effect of  
363 commercial surfactants on the cell metabolic activity was observed [36, 40]. The anionic  
364 glycolipids show the same cytotoxicity than SDS and SLES. However, at this concentration,  
365 the metabolic activity of cell treated with SLES seems to be lower compared to those of other  
366 molecules.

367 Table 3 summarizes the results obtained after treatment of RHE tissues with surfactant  
368 solutions, according to the OECD 439 guideline for the surfactant concentration and exposure  
369 conditions. These results show that the treatment with the anionic sugar-based surfactants did  
370 not modify significantly the cell metabolic activity of keratinocytes in RHE tissue compared  
371 to untreated RHE. Cells exposed to anionic glycolipids still maintain high metabolic activity  
372 (75.3-89%) even at high surfactant concentrations ( $1 \text{ mg.mL}^{-1}$ ). Metabolic activity of cells  
373 seems to be affected by SLES, but considerable variability of obtained results ( $51.1 \pm 34.6\%$ )  
374 leads to no significant statistical difference between SLES and glycolipids. In contrast, the  
375 commercial anionic surfactant SDS shows clear cytotoxic effect on cells.

376 Simultaneously, the irritation effect of surfactants on RHE tissue was evaluated by measuring  
377 the release of IL-1 $\alpha$  in culture medium after 24h of surfactant treatment. A significant  
378 increase of IL-1 $\alpha$  concentration in RHE models treated by SDS and SLES was observed,  
379 compared to non-treated control (PBS), indicating a strong irritant effect of both commercial  
380 surfactants on the *in vitro* skin models. Synthetized anionic glycolipids showed a lower  
381 release of IL-1 $\alpha$ , and were not considered different from negative control PBS.

382

#### 383 4. Discussion

384

#### 385 4.1. Surface active properties in water

386 To act as an efficient surfactant, an amphiphilic molecule is usually characterized by  
387 adsorption properties leading to decrease in surface tension and by formation of aggregates  
388 from a certain concentration. Since these properties are mainly affected by the structure, it is  
389 fundamental to characterize the potential on new surfactants from an amphiphilic point of  
390 view.

391 The particular low CMC value of SLES compared to anionic glycolipids can be attributed to  
392 intermolecular and intramolecular attractive ion-dipole interactions between the sulfate group  
393 ( $\text{SO}_4^-$ ) and the  $\text{O}\rightarrow\text{CH}_2$  dipole of oxyethylene group [41]. Such attractive interaction is very  
394 specific and arising only with oxyethylene group adjacent to sulfate ion. It is not occurring for  
395 SDS and anionic sugar-based surfactants micelles, where strong self-repulsion between  
396 sulfate groups is dominant. Consequently, the self-assembling for these molecules was  
397 observed at higher concentrations. A higher value of  $\text{pC}_{20}$  usually indicates a higher ability for  
398 the reduction of surface tension, assuming that lower energy is needed to bring the surfactant  
399 molecule from the bulk to the air/water interface [42].  $\text{pC}_{20}$  values of anionic glycolipids  
400 presented in Table 1 suggest a high surfactant efficiency in lowering the surface tension of  
401 water comparable to commercial anionic surfactants.

402 The size of hydrophilic head group is dominant factor to determine the adsorption quantity of  
403 surfactant molecules at the air/water interface,  $\Gamma_{\text{max}}$ , and corresponding area occupied by a  
404 single surfactant molecule,  $A_{\text{min}}$  [32].  $\Gamma_{\text{max}}$  and  $A_{\text{min}}$  values calculated for anionic surfactants  
405 can be correlated with their molecular structure. SDS with lowest size of polar moiety has  
406 high packing density at the interface air/water. The cross-sectional area of the hydrophilic  
407 group increases for SLES and glycolipids resulting in lower  $\Gamma_{\text{max}}$  value and thereby, larger  
408  $A_{\text{min}}$  value. The same tendency, where an adsorption quantity of surfactant molecules at the  
409 air/water interface decreases with the increasing of the polar part size, was reported for  
410 anionic polyethylene oxide derivatives [43, 44]. Moreover, for sugar-based surfactants the  
411 intermolecular interactions of the -OH groups and their steric hindrance can be responsible for  
412 higher distance between the molecules in the adsorption layer and increased  $A_{\text{min}}$  value. For  
413 the same length of hydrophobic chain, the surface adsorption properties of sugar-based  
414 surfactants depend not only on headgroup size, but also on the linker type and structure [45].  
415 Consequently, the differences in physiochemical properties of studied anionic glycolipids can  
416 be related to the nature of linker group. Similar results on surface adsorption properties for  
417 other families anionic surfactants have been reported in the recent literature [17, 42, 43, 46].

418 Dynamic surface tension measurements have been performed in order to get information on  
419 the instantaneous decrease of surface tension at very short times. After creation of the fresh  
420 surface, the dynamic tension decreases with time as amphiphilic molecules diffuse and adsorb  
421 at the interface. The results demonstrate that SLES exhibits faster adsorption kinetics  
422 compared to other surfactants. Surprisingly, the dynamic surface tension of three sugar-based  
423 surfactants was not changing significantly over time period considered, indicating the absence  
424 of surfactant adsorption at short times. In this case, it can be suggested that the time required  
425 to attain the equilibrium surface tension is higher for glycolipids compared to commercial  
426 molecules, and the adsorption of sugar-based surfactants is controlled not only by the  
427 diffusion of molecules. The repulsive electrostatic interactions of anionic groups might  
428 explain the higher potential barrier of glycolipids adsorption and slower adsorption rate at the  
429 gas/liquid interface for these surfactants [33, 47]. Additionally, this low surfactant diffusivity  
430 and slow reduction in dynamic surface tension can be linked to the low concentration of  
431 analyzed solutions ( $C = 0.1\text{CMC}$ ).

432

#### 433 *4.2. Evaluation of foaming behavior*

434 Aqueous foams are thermodynamically unstable disperse systems. They consisted of gas  
435 bubbles separated by liquid films where gas/liquid interface is generally stabilized by  
436 surfactants. Different self-destructive processes take place simultaneously in foam during  
437 ageing. The main processes limiting the foam stability are drainage of the liquid under the  
438 action of gravity, and foam ageing phenomena like coalescence (film rupture) and ripening  
439 (exchange of gas between bubbles due to differences in Laplace pressure) [48, 49]. These  
440 factors cause the transformation of foams to their thermodynamically most stable state –  
441 complete destabilization to initial liquid.

442 Foaming capacity describes the ability of a surfactant solution to produce foam. It depends on  
443 both the rate of transport of the surfactant to the gas/liquid interface and the total adsorbed  
444 amount of surfactant [50]. High foam capacity of SLES and SDS could be related to the  
445 decrease of dynamic surface tension for these solutions. This result indicates that the diffusion  
446 of the surfactant to the surface is fast enough for the formation of interfacial layer in the foam  
447 generation process. However, despite low adsorption rate and dynamic surface tension  
448 reduction, anionic glycolipids XylCC12S and GlcNC12S showed high foaming capacity close  
449 to those of commercial molecules. Significant differences in foam properties, such as foam  
450 stability (S), maximum foam density (MD) and foam half-life time ( $t_{1/2}$ ) can be attributed to  
451 the variation of head group structure, since all surfactants studied have the same length of

452 hydrophobic chain. The organization of the hydration water around an ethylene oxide and  
453 sugar group under similar conditions, were found to be completely different [51, 52].  
454 Moreover, flexible hydrophilic part of negatively charged surfactants can cause the adoption  
455 of various molecular configurations on the air/water interface. The compactness of adsorbed  
456 molecules on the two air/water interfaces of the foam lamellae affects both foamability and  
457 foam stability [42]. While SDS and SLES have short and flexible hydrophilic units,  
458 XylCC12S and GlcCC12S have more voluminous open chain sugar part, and the cyclic sugar  
459 GlcNC12S head group behaves like a hard disk with reduced conformational flexibility.  
460 These mentioned differences influence the behavior of surfactants in solution as well as their  
461 adsorption and foaming properties. It is important to indicate that the viscosity of aqueous  
462 surfactant solution could significantly affect its foaming properties. Typically, the foam  
463 stability increased for bulk solutions with higher viscosity [53]. In this study, very diluted  
464 solutions were analyzed in foaming experiments ( $C = 0.1$  CMC), the viscosity remained  
465 constant and very close to that of pure water.

466 To sum up, foaming properties of two of the three synthesized anionic glycolipids (XylCC12S  
467 and GlcNC12S) are close to those of commercial anionic surfactants. They form the similar  
468 amount of foam that SDS and SLES, and exhibit foaming stability comparable to that of SDS.  
469 Since foaming performances of anionic surfactants are of great importance in formulation of  
470 personal care products, an interesting approach is to study the biological activity of new  
471 glycolipids and to evaluate their potential use in such applications.

472

#### 473 4.3. *Biological responses*

474 The potential use of surfactants in several cosmetic and pharmaceutical applications depends  
475 on their cytotoxicity, biocompatibility and toxic effects. Our previous studies revealed a  
476 significant difference in biological responses, such as metabolic activity, cytotoxicity and  
477 irritancy, between commercial model surfactant and bio-based nonionic glycolipids [31, 36].  
478 Biological effects of anionic glycolipids have not been investigated before. In the present  
479 study, the metabolic activity related to the amount of viable cells was studied on L929 model  
480 cells and on RHE tissue. As expected, cell metabolic activity decreases with the rise of  
481 surfactant concentration. While at fixed concentration, cells treated with sugar-based  
482 surfactants showed similar metabolic activity. We used an *in vitro* RHE EpiDerm system and  
483 the OECD guidelines for the testing of chemicals and cosmetic formulations (irritant effect)  
484 were strictly applied to characterize the surfactant's effect on a human skin model [54]. The  
485 significant differences in keratinocyte metabolic activity observed in RHE models treated



486 with commercial or synthesized anionic glycolipids revealed that glycolipids are mild and  
487 tolerant towards epidermal cells. Notably no significant difference between the three  
488 glycolipids was found, suggesting that the structure of polar part (cyclic or acyclic) of  
489 surfactant cannot be directly correlated to biological responses. In contrast, significant loss in  
490 metabolic activity was found after the exposure of human keratinocytes to SDS and SLES  
491 solutions suggesting an important amount of damaged cells. The irritancy potential  
492 measurements showed clear difference in IL-1 $\alpha$  release between the three anionic glycolipids  
493 and model anionic surfactants, highlighting that SDS and SLES had significantly higher  
494 irritation potential. Previous study reported that skin irritation profile of SDS was significantly  
495 higher compared with 15 other nonionic, amphoteric and anionic surfactants [55].  
496 Overall, all obtained results confirmed that in the concentration range studied, GlcCC12S,  
497 GlcNC12S and XylCC12S are significantly less toxic than SLES or SDS. *In vitro* test systems  
498 used here were particularly interesting for pre-screening of new surfactants for novel  
499 formulation development in cosmetics, avoiding the use of animals for testing. Obviously,  
500 surfactant combinations are used in formulation, and it is necessary to evaluate biological  
501 responses of fully formulated products.

502

## 503 **5. Conclusion**

504 A multidisciplinary approach taking into account surface properties, foaming behavior and  
505 biological responses of three anionic surfactants (GlcNC12S, GlcCC12S, XylCC12S),  
506 obtained from renewable raw materials (carbohydrates) comparatively to model commercial  
507 anionic surfactants (SDS and SLES) has been chosen. Sugar-based surfactants were found to  
508 exhibit good surface activities including low surface tension and CMC values close to those  
509 of commercial surfactant (SDS). Foam capacity and foam stability of XylCC12S and  
510 GlcNC12S were found to be similar to those of SDS at the same C/CMC concentration. The  
511 analysis of biological responses on model cells and RHE tissues indicated significantly lower  
512 toxic effects of the three anionic glycolipids compared to those of commercial anionic  
513 surfactants. The lowest irritant effect, evaluated on a human skin model, was also observed  
514 for the new synthesized surfactants, GlcNC12S and XylCC12S. The analysis of obtained data  
515 demonstrates that the three sugar-derived anionic surfactants can be categorized as bio-based  
516 compounds and considered as excellent potential candidates for the substitution of petroleum-  
517 based surfactants for cosmetic and detergence applications. These promising results contribute  
518 to the knowledge about sugar-based surfactants and encourage us to explore other anionic  
519 sugar-based structures for future work.

520

## 521 **Acknowledgements**

522 This work has been performed, in partnership with the SAS PIVERT, within the frame of the  
523 French Institute for the Energy Transition P.I.V.E.R.T. ([www.institut-pivert.com](http://www.institut-pivert.com)) selected as  
524 an Investments for the Future. This work was supported, as part of the Investments for the  
525 Future, by the French Government under the reference ANR-001-01.

526

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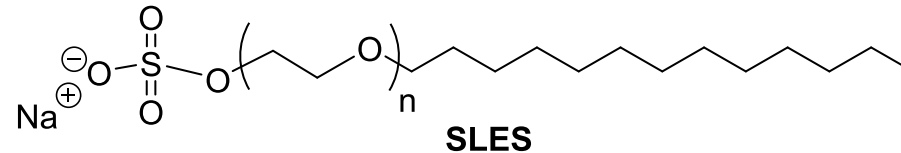
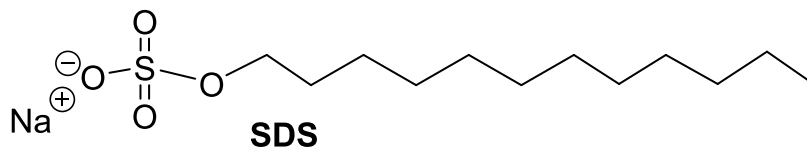
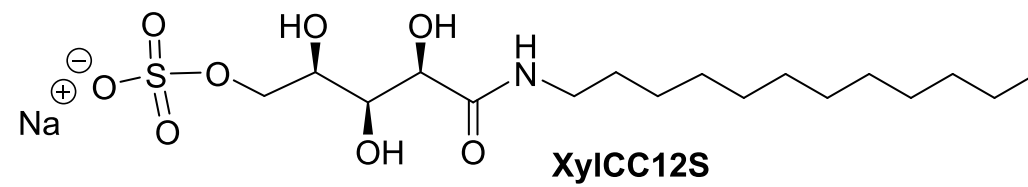
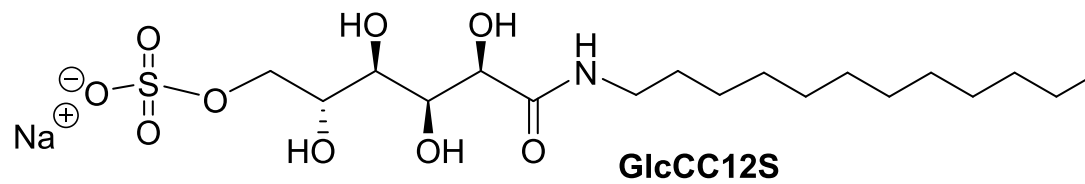
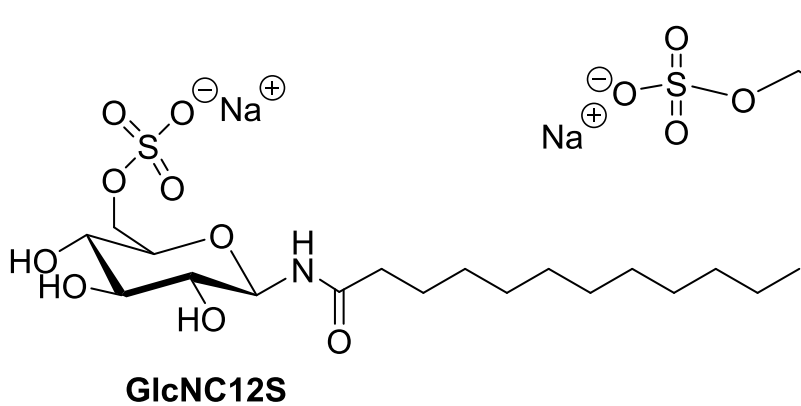
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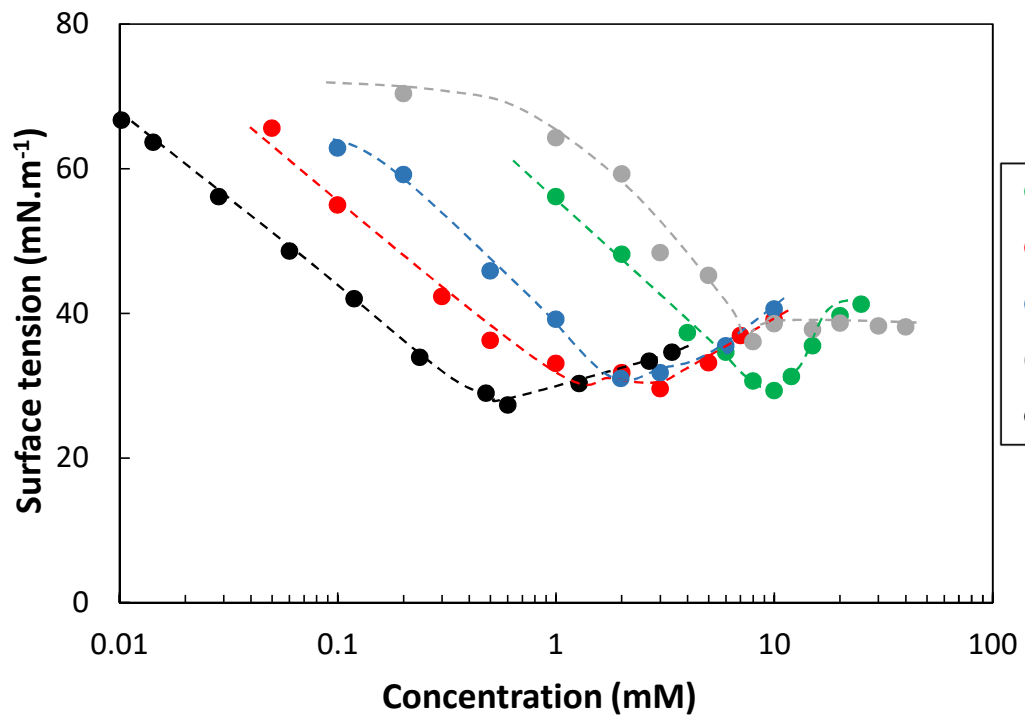
**Fig. 1.** Anionic surfactants studied for their surface-active properties, foaming behavior and biological effects.

**Fig. 2.** (A) Plots of surface tension versus concentration of anionic surfactants. Dotted lines are displayed as a visual guide. (B) Dynamic surface tension behavior of anionic surfactants at  $C=0.1\text{CMC}$ ; standard deviation bars are smaller than used symbols ( $n=3$ ).

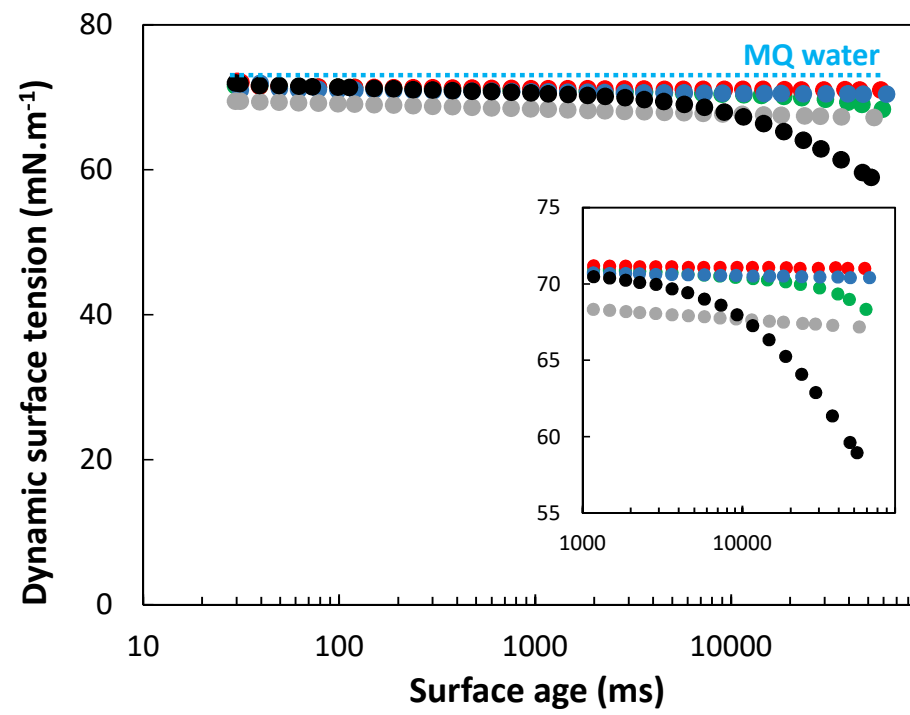
**Fig. 3.** Variation of the foam volume (A) and liquid volume (B) with time for different surfactants at  $C=0.1\text{CMC}$ . The bubbling rate is  $200\text{ mL}\cdot\text{min}^{-1}$ .

**Fig. 4.** Metabolic activity of L929 cells after surfactant treatment.  $n=6$  per group, data are presented as mean value with standard deviation. \*  $P<0.05$  and \*\*\*  $P<0.001$  indicate significant difference *versus* untreated cells.



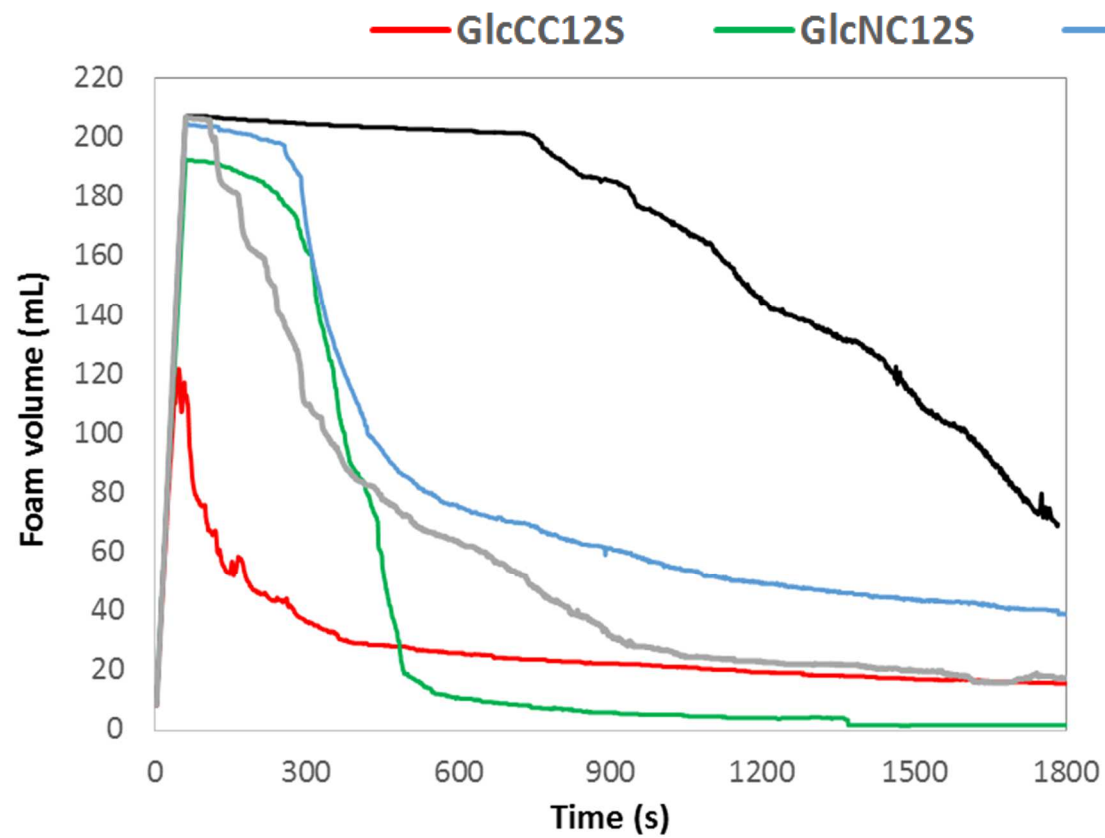


**A**

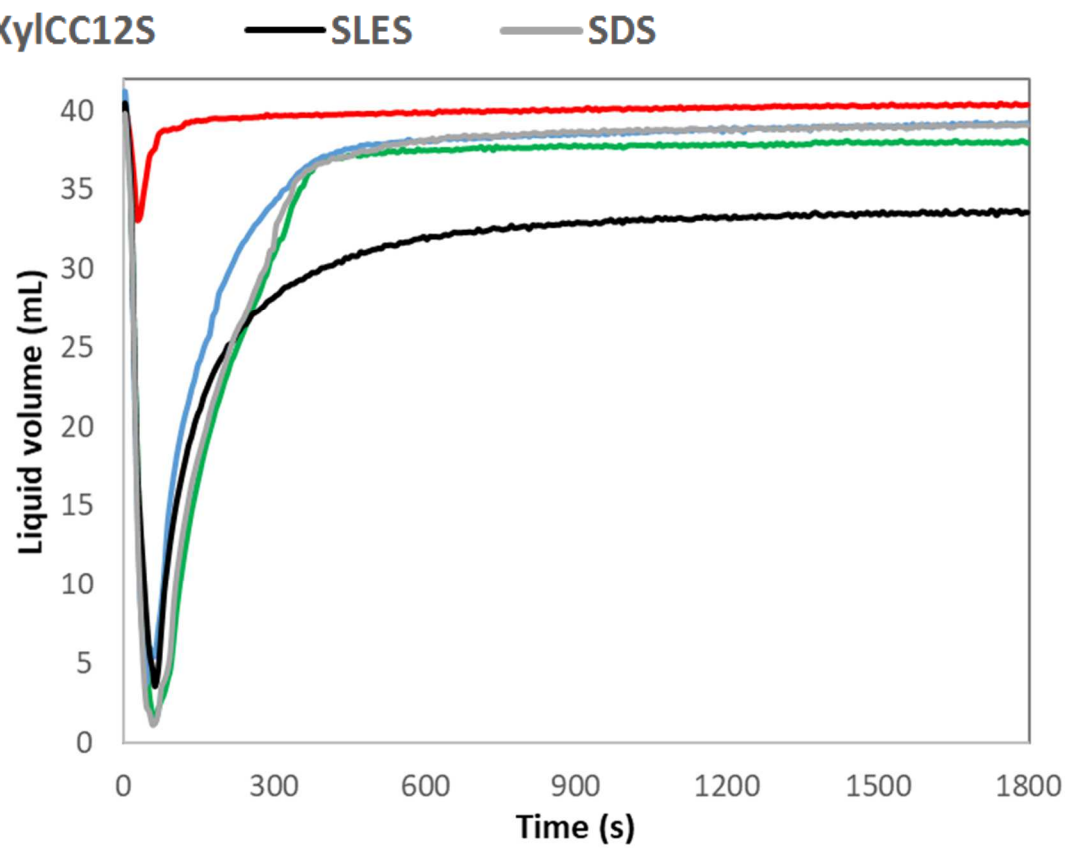


**B**

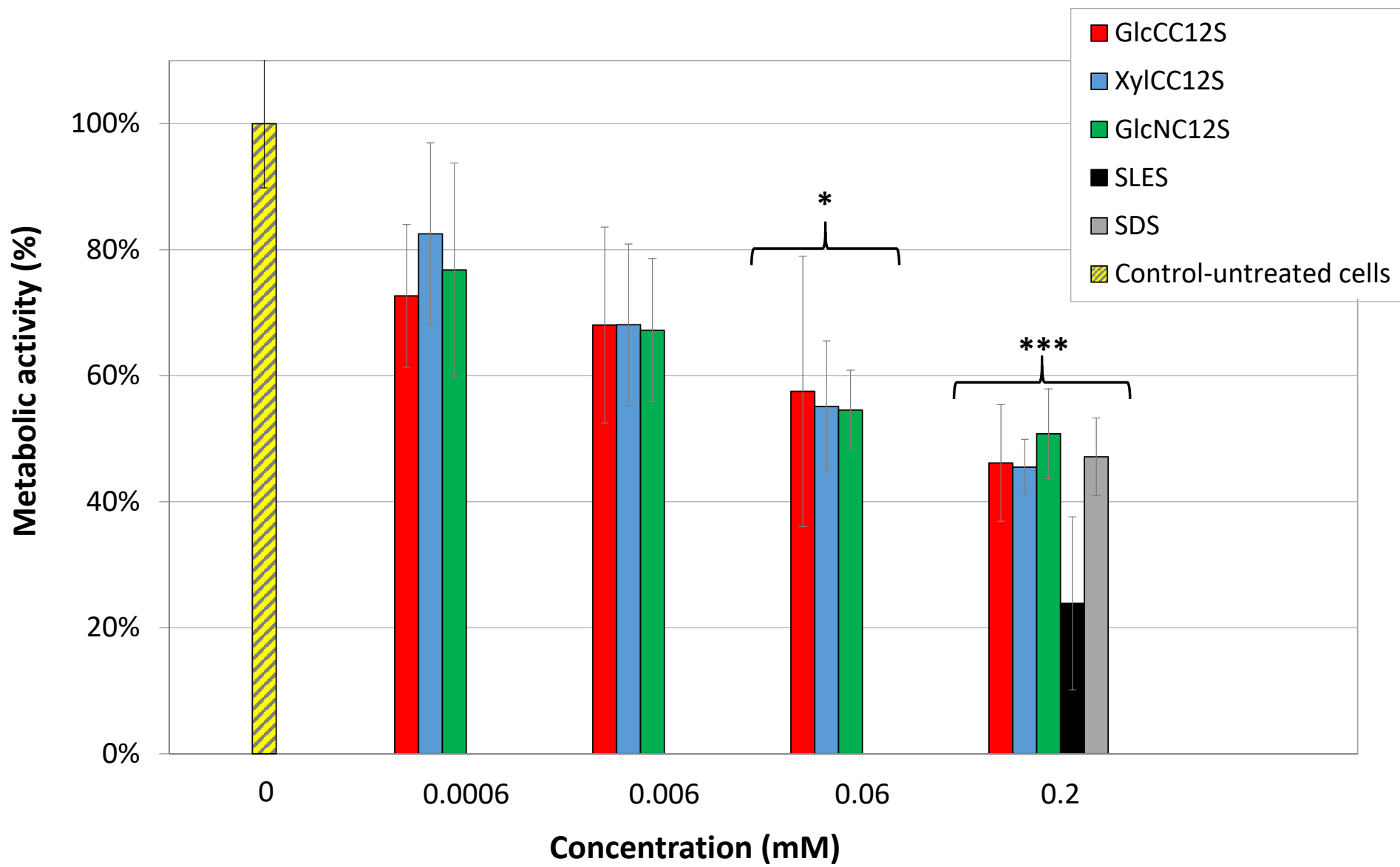




**A**



**B**



<b>Table 1.</b>	CMC (mM)	$\gamma_{\text{CMC}}$ (mN.m <sup>-1</sup> )	pC <sub>20</sub>	10 <sup>10</sup> . $\Gamma_{\text{max}}$ (mol.cm <sup>-2</sup> )	$A_{\text{min}}$ (Å <sup>2</sup> )
<p>Summary of surface properties and foaming properties of surfactants : the critical micelle concentration (CMC), the equilibrium surface tensions at CMC (<math>\gamma_{\text{CMC}}</math>), the adsorption efficiency (pC<sub>20</sub>), the maximum surface excess (<math>\Gamma_{\text{max}}</math>) and corresponding minimal area per molecule (<math>A_{\text{min}}</math>)</p>					

determined from plots in Fig.2A.Surfactant					
SDS	8.0	34.9	2.52	3.86	42.9
SLES	0.5	27.3	4.40	1.31	127.0
GlcCC12S	3.0	31.5	3.92	1.68	99.1
XylCC12S	2.0	31.0	3.43	2.33	71.3
GlcNC12S	10.0	28.7	2.89	1.76	94.2

**Table 2.** Summary of foaming properties of surfactants: the foaming capacity (FC), the foaming stability (S), the maximum foam density (MD) and the half-life time of foam ( $t_{1/2}$ ) determined from curves in Fig.3.

Surfactant	Foaming properties*			
	FC	S	MD (%)	$t_{1/2}$ (s)
SDS	1.05±0.01 <sup>a</sup>	0.28±0.01 <sup>c</sup>	18.8±0.2 <sup>b</sup>	271±46 <sup>bc</sup>
SLES	1.05±0.01 <sup>a</sup>	0.79±0.01 <sup>a</sup>	17.8±0.2 <sup>c</sup>	1502±85 <sup>a</sup>
GlcCC12S	0.57±0.05 <sup>c</sup>	0.23±0.01 <sup>cd</sup>	2.0±0.4 <sup>d</sup>	133±2 <sup>c</sup>
XylCC12S	1.04±0.01 <sup>a</sup>	0.36±0.06 <sup>b</sup>	16.9±0.5 <sup>c</sup>	356±58 <sup>b</sup>
GlcNC12S	0.98±0.02 <sup>b</sup>	0.20±0.02 <sup>d</sup>	20.6±0.5 <sup>a</sup>	324±50 <sup>b</sup>

<sup>a-c</sup> Mean values in the same column followed by different letters indicate a statistical difference ( $P < 0.05$ ,  $n=3$ )

\*Measurement of foaming properties was realized at  $C=0.1\text{CMC}$

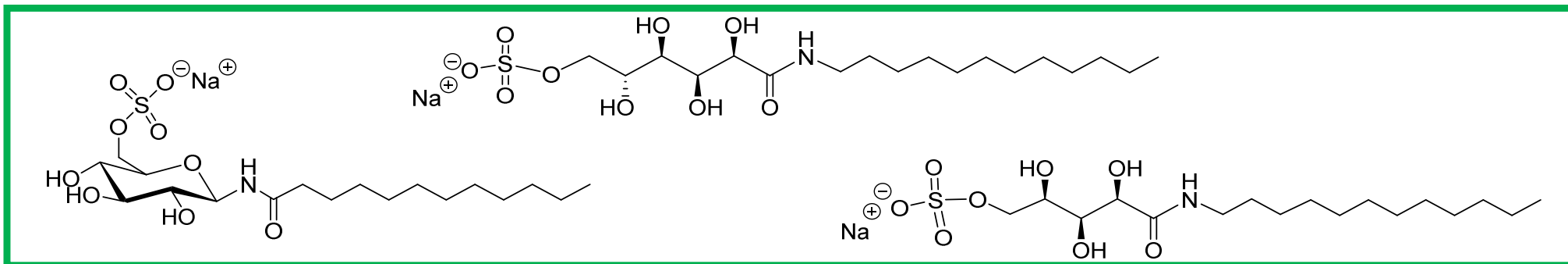
**Table 3.** Metabolic activity of cells and IL-1 $\alpha$  concentrations detected in culture medium of RHE after surfactant treatment.

Surfactant	PBS (negative control)	GlcCC12S	GlcNC12S	XylCC12S	SDS	SLES
Metabolic activity (%) <sup>a</sup>	100±4.9	89.0±12.3	85.4±10.0	75.3±19.6	2.9±2.7 <sup>***</sup>	51.1±34.6
IL-1 $\alpha$ concentration (pg.mL <sup>-1</sup> ) <sup>b</sup>	5.3±2.7	68.7±9.0	35.1±9.0	54.8±8.4	157.4±23.0 <sup>***</sup>	155.5±26.7 <sup>***</sup>

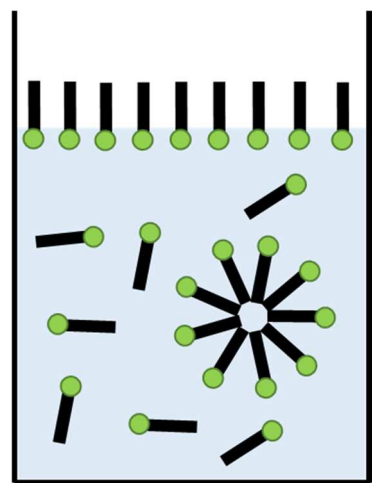
<sup>a</sup> Metabolic activity was measured by absorbance of cells in RHE models at 490 nm

<sup>b</sup> IL-1 $\alpha$  concentration of RHE model was assessed by ELISA test after 1h surfactant exposure

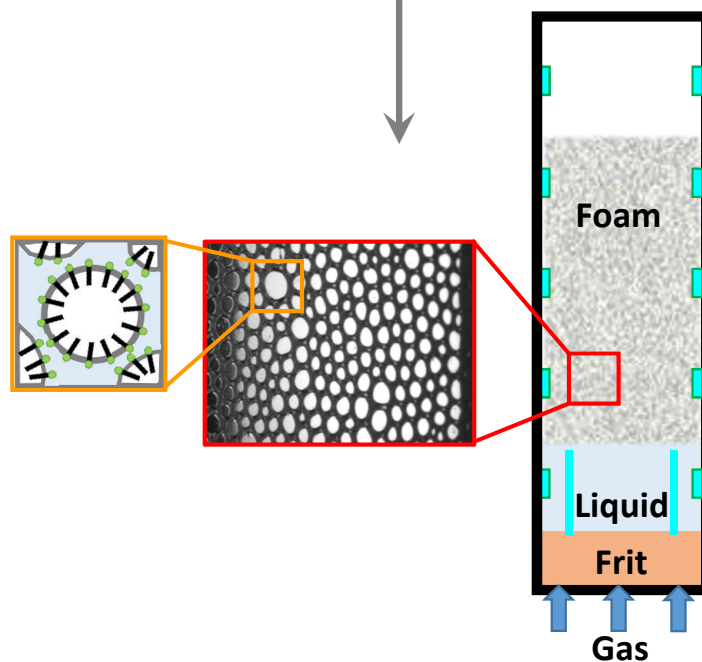
<sup>\*\*\*</sup>  $P < 0.001$  indicate significant difference versus untreated cells



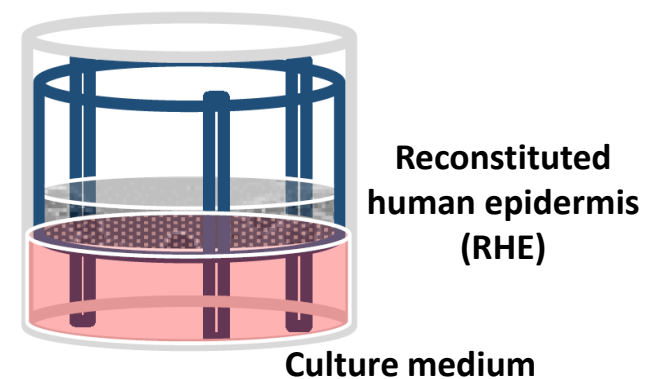
**anionic sugar-based surfactants**



**surface properties**



**foaming behavior**



**biological responses**