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ORIGINAL ARTICLE

Synthesis and Evaluation of Surface and Biological Properties of Some Lactic Acid-Based Anionic Surfactants

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Abstract In the present study, 11 lactic acid-based anionic surfactants were synthesized and evaluated for their surface and biological activities. The synthesis involved the esterification of lactic acid with a range of fatty alcohols differing in chain length as well as in branching and unsaturation. The resultant ester was sulfonated by treatment with chlorosulfonic acid followed by salt formation with aqueous NaOH solution. The surface properties of all the synthesized surfactants were determined using surface tensiometry. Synthesized surfactants showed low critical micelle concentration (CMC) values and a decreasing trend with an increase in the alkyl chain length. Alkyl branching also led to a mild change in CMC values when compared with linear counterparts having the same number of carbon atoms, though such decreases or increases were observed to be dependent on the position and number of the branching. Some of the synthesized surfactants exhibited good antimicrobial and anti-cancer activities against the tested microbial strains and cell lines.

Keywords Sulfated sodium salt of alkyl lactate · Micellization behavior · Antimicrobial activity · Cytotoxicity

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Introduction

Surfactants have widespread importance for applications in diverse fields such as the detergent, personal care, textile, petrochemical, pharmaceutical and electronic industries for performing the desired function of cleaning, wetting, softening, foaming, emulsifying, etc. [1–9]. In recent years, interest has been generated to explore new types of surfactants having antimicrobial properties [10–12]. Oleo chemicals and hydroxy acids, such as citric, malic, tartaric, and lactic acids, etc., derived from renewable substrates have gained interest for the synthesis of surfactants [13].

Alkyl lactates and their salts are biodegradable, have low toxicity and are synthesized from renewable resources such as fatty alcohols derived from fatty acids which in turn are produced from vegetable oils, while lactic acid is produced by the action of bacteria and fungi on carbohydrate-based feedstocks [14, 15]. Alkyl lactates and their derivatives are widely used as emulsifiers in the food industry and as moisturizers in cosmetic formulations. Alkyl lactates are synthesized either by esterification of fatty alcohol with lactic acid or by esterification of fatty acid with hydroxyl functionality of lactic acid. There are many reports on the synthesis of alkyl lactates using H_2SO_4 as a catalyst in which polymerization of lactic acid occurs; however, in enzyme-catalyzed reactions only poor yields are obtained [16–18].

Among the different classes of surfactants, anionic surfactants with sulfate head groups have assumed significance. In the present study, a series of anionic surfactants with sulfate head groups were synthesized from lactic acid and with fatty alcohols derived mostly from castor and palm oils. Synthesis of sulfated sodium salts of alkyl lactates of decyl and dodecyl alcohols (2-sulfoxy propionate sodium salts of decyl and dodecyl lactates) have been

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reported in a European patent; however, detailed investigations on the surface and biological properties were not carried out [19].

In the present investigation, a series of lactic acid-based sulfated sodium salts of alkyl lactates were synthesized using fatty alcohols of varying chain lengths (C8–C22) and branched fatty alcohols (2-octanol, 3,7-dimethyl-1-octanol and 2-pentyl-1-nonanol). 10-undecenol, a terminal unsaturated fatty alcohol obtained from castor oil was also included in the study. Characterization of these compounds was carried out by using different spectral methods such as NMR, FT-IR, ESI–MS and HRMS. Elemental analysis of the synthesized surfactants was also carried out. Surfactant properties such as surface tension and critical micelle concentration (CMC) as well as the biological activities such as antimicrobial activity and cytotoxicity were evaluated.

Experimental

Materials

The raw materials needed for the synthesis of sulfated sodium salts of alkyl lactates, such as fatty alcohols of straight chains with 8, 10, 12, 14, 16, 18, and 22 carbon atoms were purchased from S.D. Fine Chemicals, Mumbai, India. 2-octanol and 3,7-dimethyl 1-octanol were purchased from Sigma-Aldrich Chemicals (USA). 2-pentyl nonanol (Guerbet alcohol) derived from 1-heptanol and 10-undecenol derived from methyl undecenoate were synthesized in our laboratory by reported methods [20, 21]. Chlorosulfonic acid ($ClSO_3H$), sodium hydroxide (NaOH) and para-toluene sulfonic acid (PTSA) was purchased from S.D. Fine Chemicals. All solvents and chemicals were of reagent grade and used directly without further purification. Silica gel (60–120 mesh) for column chromatography was purchased from Acme Synthetic Chemicals, Mumbai, India. Precoated TLC plates were purchased from Merck, Darmstadt, Germany. All microbial strains were obtained from the Microbial Type Culture Collection and Gene Bank, CSIR-Institute of Microbial Technology, Chandigarh, India. Human tumor cell lines were obtained from the American Type Culture Collection, Manassas, VA, USA.

Methods

IR spectra were recorded on a Perkin Elmer Spectrum BX FT-IR spectrometer using $CHCl_3$ and KBr. All ^1H and ^{13}C spectra were recorded on 300 MHz (Brucker) and 500 MHz (Varian) spectrometers, respectively. HRMS data were recorded on a Thermo Scientific Exactive Orbitrap mass spectrometer (Germany) and are given in mass units (m/z). ESI–MS spectra were recorded on a Waters Q STAR XL mass spectrometer (Applied Biosystems, USA) equipped with an electrospray ionization source. Gas chromatography was performed on an Agilent 6850 gas chromatograph equipped with a flame ionization detector. The column used was a HP-1 column having a length of 30 m, 0.25 mm i.d and 0.25 μm film thickness. The carrier gas was nitrogen at a flow rate of 1 mL min^{-1}. The oven programming was as follows: 150 °C for 2 min, which rose to 300 °C at a rate of 10 °C min^{-1} and was held at 300 °C for 20 min. The injector and detector temperatures were maintained at 280 and 300 °C, respectively.

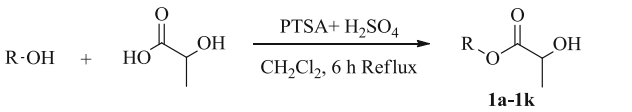
Elemental analysis was done by using Elementar Vario Micro Cube (Germany). Surface tension and CMC were measured using a Krüss K100 tensiometer equipped with a platinum ring having a mean circumference of 6 cm. All surface properties presented in this work are the mean of three independent measurements.

Synthesis

A series of lactic acid-based anionic surfactants were synthesized following the pathway as described in Fig. 1. The synthesis consists of two steps: step 1: alkyl lactates (1a–1k) were prepared by reacting lactic acid with a fatty alcohol differing in functionality in the alkyl chain (linear, branched and unsaturation); step 2: synthesis of sulfated sodium salts of alkyl lactates by sulfation of alkyl lactates with chlorosulfonic acid. This was followed by neutralization with aqueous NaOH solution to get the desired compounds, 2a–2k.

General Procedure for Synthesis of Alkyl Lactates

Fatty alcohol (1 equivalent) and lactic acid (4 equivalents) were dissolved in dichloromethane and stirred for



Compound	R = Alkyl group
1a	1-Octyl
1b	Decyl
1c	Dodecyl
1d	Tetradecyl
1e	Hexadecyl
1f	Octadecyl
1g	Docosanyl
1h	2-Octyl
1i	3,7- Dimethyl-1-octyl
1j	2-Pentanonyl
1k	10-Undecenyl

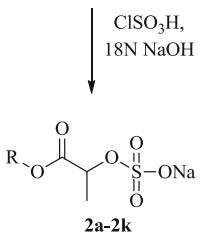


Fig. 1 Synthesis of alkyl lactates and sulfated sodium salts of alkyl lactates

15 min, followed by the addition of the PTSA catalyst (10 % by weight of alcohol) and refluxed for 6 h. After 6 h, one drop of concentrated H_2SO_4 was added and the reaction continued for a further 1 h for completion of the reaction. The pH of the reaction mixture was adjusted to pH 7 with the addition of aqueous $NaHCO_3$ solution, and the crude product was extracted with diethyl ether. Pure alkyl lactate was separated from the crude reaction product by column chromatography using stationary phase silica gel (60–120 mesh) with hexane and ethyl acetate (97:3, v/v) as eluent. The pure alkyl lactate was eluted first followed by fatty alcohol, as pure alkyl lactate (R_f −0.85) was less polar than the starting material of fatty alcohol (R_f −0.70). Column chromatography was monitored by TLC with the solvent system hexane and ethyl acetate (4:1, v/v) and identified by exposure to iodine vapor. Isolated yields were in the range of 81–95 %. Column-purified alkyl lactates (1a–1k) were checked for purity by GC.

General Procedure for Synthesis of Sulfated Sodium Salts of Alkyl Lactates (2a–2k)

Alkyl lactate (1.0 equivalent) dissolved in dichloromethane was charged to a round-bottom flask equipped with a spin bar. Chlorosulfonic acid (1.1 equivalents) was added to the reaction mixture dropwise at 0 °C over 10 min under N_2 atmosphere. The reaction was continued for 3 h at room temperature. Then, the reaction mixture was neutralized with aqueous NaOH (adjusted to pH 7). Dichloromethane and water were removed under vacuum by lyophilization [19, 22]. The crude product was washed with diethyl ether to remove unreacted alkyl lactate and the product was obtained as a white powder. The isolated yields were in the range of 81–96 % and the purity determined by elemental analysis wasis in the range of 96–98 %.

Surface Active Properties

Aqueous solutions of surfactants were prepared by dissolving appropriate amounts in Milli-Q water (deionized water). Surface tension was measured at 25 °C using a Krüss K100 Processor Tensiometer (Krüss Instruments, Germany). The critical micelle concentration (CMC) was determined from the plot of surface tension versus ln *C*, from the intersection of two lines after fitting linearly. The ability of surfactants to lower surface tension at the CMC (γ_{cmc}) was calculated from the surface properties of these surfactants, which were measured three times with an interval of 50 min between each reading to ensure equilibrium data.

Biological Evaluation

Cytotoxicity Assay

Cytotoxicity of all the synthesized lactic acid-based anionic surfactants was determined on the basis of the measurement of in vitro growth inhibition of tumor cell lines in 96-well plates by cell-mediated reduction of tetrazolium salt to form water insoluble formazan crystals using doxorubicin as a standard. The cytotoxicity was assessed against a panel of four different human tumor cell lines obtained from the American Type Culture Collection, Manassas, VA, USA: A549 derived from human alveolar adenocarcinoma epithelial cells (ATCC No. CCL-185), HeLa derived from human cervical cancer cells (ATCC No. CCL-2), MDA-MB-231 derived from human breast adenocarcinoma cells (ATCC No. HTB-26), MCF7 derived from human breast adenocarcinoma cells (ATCC No. HTB-22), K-562 derived from human myelogenous leukemia cell line (ATCC No. CCL-243) and HEK-293 derived from human embryonic kidney cell line (normal cell line) (ATCC No. CRL-1573) using the MTT assay [23].

All tumor cell lines were maintained in a modified DMEM medium supplemented with 10 % fetal bovine serum, along with 1 % non-essential amino acids except L-glutamine, 0.2 % sodium hydrogen carbonate, 1 % sodium pyruvate and 1 % antibiotic mixture (10,000 units penicillin and 10 mg of streptomycin per mL). The cells were washed and re-suspended in the above medium and then 100 mL of this suspension was seeded into a 96-well bottom plate. The cells were kept at 37 °C in a humidified incubator (Model 2406 Shellab CO_2 incubator; Sheldon, Cornelius, OR, USA) under a 5 % CO_2 atmosphere. After incubation for 24 h, the cells were treated for 2 days with the test compounds at concentrations ranging from 0.1 to 100 mM in DMSO (1 % final concentration) and were assayed at the end of the second day. Each assay was performed with two internal controls: (1) an IC_0 with the cells only, and (2) an IC_{100} with the media only. After incubation for 48 h, the cells were subjected to an MTT colorimetric assay (5 mg mL^{-1}). The effects of the different test compounds on the viability of the tumor cell lines were measured at 540 nm using a multimode reader (Infinite® M200Pro; Tecan, Switzerland). Doxorubicin was used as a positive control for comparison purpose and 1 % DMSO as a vehicle control. In order to account for the toxicity of the DMSO, the values obtained for the DMSO control were subtracted from those of the test compounds. Dose–response curves were plotted for the test compounds and controls after correction by subtracting the background absorbance from that of the blanks. The IC_{50} values (50 % inhibitory concentration) were calculated from the plotted

absorbance data of the dose–response curves. The IC_{50} values (in μM) were expressed as the average of two independent experiments.

Antimicrobial Activity

Antimicrobial activity of lactic acid-based anionic surfactants was determined using the modified broth dilution method as described previously [24]. The target strains used for screening the antimicrobial activities were *Micrococcus luteus* MTCC 2470, *Staphylococcus aureus* MTCC 96, *S. aureus* MLS16 MTCC 2940, *Bacillus subtilis* MTCC 121, *Escherichia coli* MTCC 739, *Pseudomonas aeruginosa* MTCC 2453, *Klebsiella planticola* MTCC 530 and *Candida albicans* MTCC 3017. These strains were procured from the Microbial Type Culture Collection and Gene Bank (CSIR-Institute of Microbial Technology, Chandigarh, India).

The pathogenic reference strains were seeded on the surface of the media Petri plates, containing Muller–Hinton agar with 0.1 ml of previously prepared microbial suspensions individually containing 1.5×10^8 cfu mL^{-1} (equal to 0.5 McFarland standards). Wells of 6.0 mm diameter were prepared in the media plates using a cork borer and the synthesized compounds dissolved in 10 % DMSO at a dose range of 125–0.97 μg were added to each well under sterile conditions in a laminar air flow chamber. Standard antibiotic solutions of Neomycin at a dose range of 125–0.97 μg $well^{-1}$, served as positive controls, while the well containing DMSO served as a negative control. The plates were incubated for 24 h at 30 °C and the well containing the least concentration showing the inhibition zone is considered as the minimum inhibitory concentration. All experiments were carried out in duplicate and mean values are represented.

Results and Discussion

Synthesis

In the present study, a series of lactic acid-based anionic surfactants (Fig. 2) were synthesized by a two-step procedure. In the first step, alkyl lactates were synthesized by a simple and efficient chemical method using different straight chain, branched chain and unsaturated fatty alcohols and lactic acid employing PTSA as the catalyst. When 10 % PTSA was used as the catalyst, the yields were low (71–75 %). However, after 6 h, the addition of one drop of concentrated H_2SO_4 to the reaction mixture increased the product yields to 81–95 %. In the second step, sulfated sodium salts of alkyl lactates resulted from treatment of corresponding alkyl lactates with chlorosulfonic acid followed by neutralization with aqueous NaOH solution. The isolated yields were in the range of 81–96 %.

Characterization of Alkyl Lactates

Spectral Data of Some Important Alkyl Lactates

1-Octyl lactate (1a): Colorless liquid; FT-IR (cm-1, CHCl3): 3480 (br.m, –O–H symmetric mode), 2927, 2858 (s, alkyl chain –C–H symmetric mode), 1738 (s, –C = O symmetric mode), 1460 (m, –C–H scissoring mode), 1212 (m, –C–O symmetric mode), 1131 (s, –O–C = O asymmetric mode); ^{1}H-NMR ($CDCl_3$, 500 MHz, δ in ppm): 4.26 (1H, q, $J = 6.9$ Hz), 4.18 (2H, m), 2.99 (brs, OH), 1.66 (2H, m), 1.41 (3H, d, $J = 6.9$ Hz), 1.39–1.22 (10H, m), 0.88 (3H, brt); ^{13}C-NMR ($CDCl_3$, 75 MHz, δ in ppm): 175.8, 66.7, 65.7, 31.7, 29.1 ($2 \times CH_2$), 28.5, 25.7, 22.5, 20.4, 13.9; M.W: 202.29. ESI–MS *m/z*: 225.21 ($M^+ + Na^+$). HRMS (*m/z*) calculated for $C_{11}H_{22}O_3Na$ is 225.1461, found at 225.1461 ($M^+ + Na$).

2-Octyl lactate (1 h): Colorless liquid; FT-IR (cm^{-1}, $CHCl_3$): 3491 (br.m, –O–H symmetric mode), 2930, 2868 (s, alkyl chain –C–H symmetric mode), 1742 (s, –C = O symmetric mode), 1464 (m, –C–H scissoring mode), 1253 (m, –C–O symmetric mode), 1130 (s, –O–C = O asymmetric mode); ^{1}H-NMR ($CDCl_3$, 500 MHz, δ in ppm): 5.02–4.94 (1H, m), 4.26–4.19 (1H, m), 2.99 (brs, OH), 1.67–1.47 (2H, m), 1.40 (3H, d, $J = 6.1$ Hz), 1.36–1.20 (11H, m), 0.88 (3H, brt); ^{13}C-NMR ($CDCl_3$, 75 MHz, δ in ppm): 175.4, 72.7, 66.8, 35.7, 31.6, 28.9, 25.2, 22.5, 20.4, 19.8, 13.9; M.W: 202.29. ESI–MS *m/z*: 225.46 ($M^+ + Na$). HRMS (*m/z*) calculated for $C_{11}H_{22}O_3Na$ is 225.1461, found at 225.1457 ($M^+ + Na$).

3,7-Dimethyl 1-octyl lactate (1i): Colorless Liquid; FT-IR (cm^{-1}, $CHCl_3$): 3467 (br.m, –O–H symmetric mode), 2955, 2927 (s, alkyl chain –C–H symmetric mode), 1738 (s, –C = O symmetric mode), 1471 (m, –C–H scissoring mode), 1245 (m, –C–O symmetric mode), 1133 (s, –O–C = O asymmetric mode); ^{1}H-NMR ($CDCl_3$, 500 MHz, δ in ppm): 4.29–4.24 (1H, m), 4.23–4.16 (2H,m), 2.89 (brs, OH), 1.74–1.64 (1H, m), 1.58–1.47 (3H, m), 1.41 (3H, d, $J = 6.9$ Hz), 1.35–1.21 (3H, m), 1.18–1.09 (3H, m), 0.91 (3H, d, $J = 5.999$ Hz), 0.87 (6H, d, $J = 6.9$ Hz); ^{13}C-NMR ($CDCl_3$, 75 MHz, δ in ppm): 175.8, 66.7, 64.2, 39.1, 37.1, 35.4, 29.7, 27.9, 24.5, 22.6, 22.5, 20.4, 19.4; M.W: 230.34, ESI–MS *m/z*: 253.43 ($M^+ + Na^+$). HRMS (*m/z*) calculated for $C_{13}H_{26}O_3Na$ is 253.1774, found at 253.1772 ($M^+ + Na$).

2-Penta nonyl lactate (1j): Colorless Liquid; FT-IR (cm^{-1}, $CHCl_3$): 3467 (br.m, -O–H symmetric mode), 2962, 2874 (s, –C–H symmetric mode), 1736 (s, –C = O symmetric mode), 1463 (m, –C–H scissoring mode), 1212 (m, –C–O symmetric mode), 1131 (s, –O–C = O asymmetric

mode); ^{1}H-NMR ($CDCl_3$, 500 MHz, δ in ppm): 4.26 (1H, q, $J = 6.9$), 4.15–4.01 (2H, m), 1.69–1.59 (1H, m), 1.40 (3H, d, $J = 6.9$ Hz), 1.34–1.18 (20H, m), 0.87 (brt, 6H); ^{13}C-NMR ($CDCl_3$, 75 MHz, δ in ppm): 175.2, 68.3, 37.2, 32.1, 31.8, 31.1, 31.0, 29.8, 29.2, 26.6, 26.3, 22.6, 20.4, 14.0; M.W: 286.45. ESI–MS *m/z*: 309.51 ($M^+ + Na^+$). HRMS (*m/z*) calculated for $C_{17}H_{34}O_3Na$ is 309.2400, found at 309.2397 ($M^+ + Na$).

10-Undecenyl lactate (1 k): Colorless liquid; FT-IR (cm^{-1}, $CHCl_3$): 3468 (br.m, –O–H symmetric mode), 3077 (s, = C–H symmetric mode), 2928, 2855 (s, alkyl chain –C–H symmetric mode), 1737 (s, –C = O symmetric mode), 1641 (s, –C = C symmetric mode)), 1462 (m, –C–H scissoring mode), 1239 (m, –C–O symmetric mode), 1132 (s, –O–C = O asymmetric mode); ^{1}H-NMR ($CDCl_3$, 300 MHz, δ in ppm): 5.89–5.74 (dd,1H), 5.07–4.89 (m, 2H), 4.27 (1H, q, $J = 6.8$ Hz), 4.23–4.13 (m, 2H), 2.78 (brs, OH), 2.11–1.99 (2H, m), 1.42 (3H, d, $J = 6.8$ Hz), 1.39–1.22 (12H, m); ^{13}C-NMR ($CDCl_3$, 75 MH, δ in ppm z): 175.6, 138.9, 113.9, 66.6, 65.5, 33.6, 29.3, 29.2, 29.0, 28.9, 28.4, 25.6, 20.2; M.W: 242.35. ESI–MS *m/z*: 265.40 ($M^+ + Na^+$). HRMS (*m/z*) calculated for $C_{14}H_{26}O_3Na$ is 265.1774, found at 265.1772 ($M^+ + Na$).

Characterization of Sulfated Sodium Salts of Alkyl Lactates (2a–2k)

The characterization of all synthesized sulfated sodium salts of alkyl lactates was carried out based on FT-IR, ESI–MS and ^{1}H-NMR spectra. The IR spectrum of all the products showed some important features: (1) two strong bands in the region between 1143–1125 cm^{-1} and 1252–1212 cm^{-1} due to asymmetric –S = O stretching mode, (2) two additional bands in the region between 622 and 595 cm^{-1} resulting from the bending and wagging modes of the –O–SO_3 group, (3) alkyl –C–H stretching frequencies at 2916–2960 cm^{-1} and 2850–2875 cm^{-1}, and (4) the disappearance of the –O–H stretching frequency in the region of 3550–3650 cm^{-1} indicates the formation of the sulfated sodium salt of alkyl lactate. In the ^{1}H-NMR (D_2O, 500 MHz, δ in ppm) spectra of all sulfated sodium salts, the –OH signal was absent, which confirms the conversion of alkyl lactate into sulfated sodium salt. The proton signal of the –CH group attached to the –OSO_3 unit was found to be shifted to the region of δ ≈ 4.80–5.05 ppm because of the electron-withdrawing effect of the –OSO_3 group.

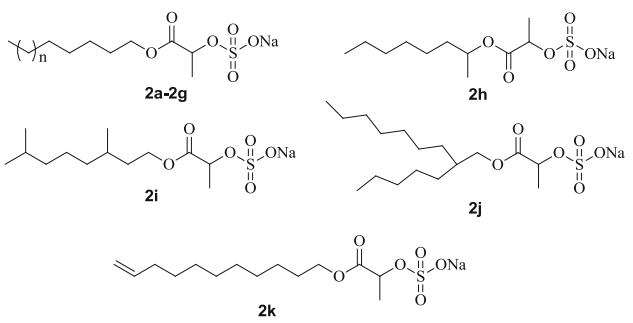


Fig. 2 Molecular structures of sulfated sodium salts of alkyl lactates: $n = 2$ (2a), 4 (2b), 6 (2c), 8 (2d), 10 (2e), 12 (2f) and 16 (2g) followed by 2h, 2i, 2j, and 2k

Elemental Analysis of Synthesized Sulfated Sodium Salts of Alkyl Lactates (2a–2k)

The values were found for CHNS of synthesized sulfated sodium salts of alkyl lactates using elemental analysis (%). The values are as follows: 2a ($C_{11}H_{21}NaO_6S$) Calculated: C, 43.41; H, 6.96; S, 10.54. Found: C, 43.15; H, 6.78; S, 10.61; 2b ($C_{13}H_{25}NaO_6S$) Calculated: C, 46.97; H, 7.58; S, 9.65. Found: C, 46.75; H, 7.45; S, 9.70; 2c ($C_{15}H_{29}NaO_6S$) Calculated: C, 49.98; H, 8.11; S, 8.90. Found: C, 49.67; H, 8.02; S, 8.91; 2d ($C_{17}H_{33}NaO_6S$) Calculated: C, 52.56; H, 8.56; S, 8.25. Found: C, 52.28; H, 8.43; S, 8.34; 2e ($C_{19}H_{37}NaO_6S$) Calculated: C, 54.78; H, 8.95; S, 7.70. Found: C, 54.29; H, 8.72; S, 7.74; 2f ($C_{21}H_{41}NaO_6S$) Calculated: C, 56.73; H, 9.29; S, 7.21. Found: C, 56.55; H, 9.18; S, 7.31; 2 g ($C_{23}H_{45}NaO_6S$) Calculated: C, 58.45; H, 9.60; S, 6.78. Found: C, 58.11; H, 9.46; S, 6.89; 2 h ($C_{11}H_{21}NaO_6S$) Calculated: C, 43.41; H, 6.96; S, 10.54. Found: C, 42.02; H, 6.24; S, 10.68; 2i ($C_{13}H_{25}NaO_6S$) Calculated: C, 46.97; H, 7.58; S, 9.65. Found: C, 46.88; H, 7.51; S, 9.69; 2j ($C_{17}H_{33}NaO_6S$) Calculated: C, 52.56; H, 8.56; S, 8.25. Found: C, 52.39; H, 8.33; S, 8.35; 2 k ($C_{14}H_{25}NaO_6S$) Calculated: C, 48.82; H, 7.32; S, 9.31. Found: C, 48.50; H, 7.17; S, 9.35.

Surface Active Properties

The variation of surface tension as a function of logarithm of surfactant concentration was measured and the results are depicted in Figs. 3 and 4.

The CMC values and surface tension at the CMC (γ_{cmc}) are derived from the surface tension isotherms. Accordingly, surface excess (Γ_{max}), the amount of surfactant adsorbed per unit area at the air/water interface, the minimum surface area per molecule (A_{min}) and the C20 value, which gives the necessary amount of surfactant required to decrease the surface tension by 20 mN/m units, were determined from the same plot of ln C versus surface tension using the Gibb's adsorption equation [27].

$$\Gamma_{max} = -(1/RT)\,(d\gamma/d\ln C)$$

$$A_{min} = 10^{20}/N\,\Gamma_{max}$$

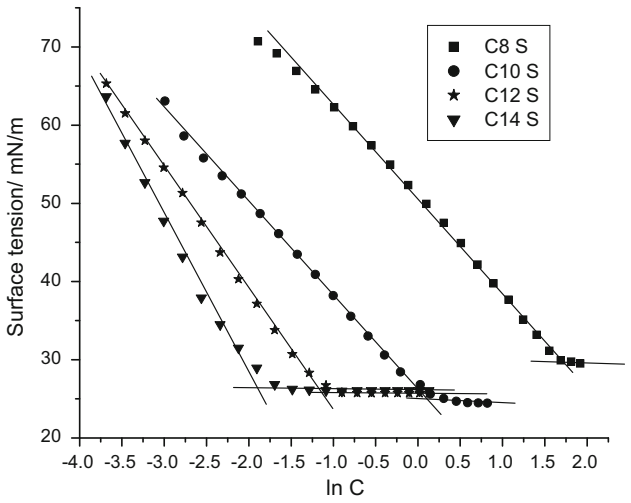


Fig. 3 Variation of surface tension as a function of the logarithm of surfactant concentration of (C8 S: 2a; C10 S: 2b; C12 S: 2c; C14 S: 2d) the straight chain fatty alcohol analogues

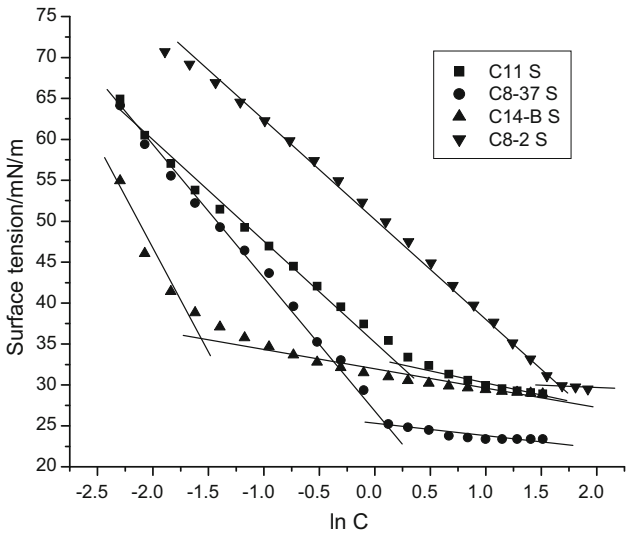


Fig. 4 Variation of surface tension as a function of the logarithm of surfactant concentration of (C11 S: 2 k; C8-37 S: 2i; C14–B S: 2j; C8-2 S: 2 h) branched chain and unsaturated fatty alcohol analogues

where R is the gas constant, T the temperature, *C* the surfactant concentration and *N* is the Avogadro number. The surface properties of the synthesized surfactants measured using surface tensiometry are shown in Table 1. Sulfated sodium salts of hexadecyl lactate (2e), octadecyl lactate (2f) and docosanyl lactate (2 g) were found to be insoluble in water at 27 °C. Thus, their surface active properties could not be evaluated.

From Table 1, it can be seen that the CMC values of surfactants synthesized from straight chain, i.e., C8–C14 alcohols (2a–2d), decreased with an increase in the chain length of the molecule which increased the hydrophobicity, favoring aggregation [25–27]. A comparison of CMC values of surfactants prepared from primary and secondary alcohols, i.e., 1-octanol (2a) and 2-octanol (2 h), showed a decrease in CMC value, which may be attributed to branching at the C_1-position which appears to favor formation of micelles at a lower concentration. However, slight variation in the CMC values was observed for surfactants synthesized from 1-decanol (2b) and the corresponding alcohol with methyl branching at C_3 and C_7 position, i.e., 3,7-dimethyl-1-octanol (2i). In the case of surfactants synthesized from tetradecanol (2d) and corresponding alcohol with β-branching i.e., 2-penta nonanol (Guerbet alcohol) (2j), a threefold increase in CMC was observed. The branching at the β-position in the alkyl chain appeared to reduce the effective chain length and increase the steric hindrance to micellization. Such a type of effect has been reported in the literature for anionic surfactants synthesized from alcohols with β-branching [28].

In our study, we did not observe statistically significant differences in CMC values between the two pairs of molecules, 2a versus 2 h and 2b versus 2i. This may be due to the presence of methyl branching rather than alkyl branching (as in compound 2j) on the hydrophobic part of the molecule. The surfactant synthesized from a terminal unsaturated alcohol, i.e., 10-undecenol (2k) showed a CMC value comparable to the surfactant prepared from 1-decanol (2b), indicating that unsaturation in the alkyl chain delays the onset of micelle formation. Such an effect of unsaturation in the hydrophobic chain of anionic surfactants on its CMC has been previously reported in the literature [29]. There is no specific pattern of surface excess (Γ_{max}) value observed for all synthesized surfactants prepared from straight, branched and unsaturated chain fatty alcohols, which are calculated from the slope of the ln *C* versus surface tension. Similarly, in the case of minimum surface area per molecule (A_{min}), this is estimated from the corresponding value of the Γ_{max}. C20 values for all synthesized surfactant molecules decreased with an increase in the hydrophobicity of the molecules (2a–2d); however, no specific pattern of C20 value was observed for branched and unsaturated molecules.

Cytotoxicity Assay

The cytotoxicity of the synthesized sulfated sodium salts of alkyl lactates was assessed on the basis of the measurement of the in vitro growth in 96-well plates by cell-mediated reduction of tetrazolium salt to form water-insoluble formazan crystals according to the literature procedure [23]. These compounds were tested for cytotoxicity against four cancer cell lines (HeLa, MDA-MB 231, MCF-7 and K-562) and one normal cell line (HEK-293) up to a concentration of 100 μM in comparison to doxorubicin, a drug

Table 1 Surface active properties of the synthesized sulfated sodium salts of alkyl lactates

Compound (carbon chain)	CMC (mM/L)	Surface tension at CMC, γ_{cmc}(mN/m)	Surface excess, Γ_{max} (mol/mm^2) 1×10^{-12}	Surface area per molecule, A_{min} (Å^2)	C20 (mM)
2a (C8)	5.40 ± 0.02	29.89 ± 0.25	7.42 ± 0.56	22.35 ± 1.33	0.70 ± 0.06
2b (C10)	1.44 ± 0.06	25.15 ± 0.04	5.32 ± 0.49	31.29 ± 2.88	0.32 ± 0.01
2c (C12)	0.64 ± 0.01	25.38 ± 0.01	5.43 ± 1.15	31.27 ± 6.64	0.06 ± 0.01
2d (C14)	0.29 ± 0.01	25.99 ± 0.12	7.90 ± 0.27	21.02 ± 0.72	0.04 ± 0.01
2 h (C8–B*)	4.13 ± 0.22	29.09 ± 0.16	5.87 ± 0.37	28.29 ± 0.75	1.04 ± 0.15
2i (C10–B*)	1.2 ± 0.05	24.40 ± 0.25	14.72 ± 0.66	26.13 ± 1.39	0.23 ± 0.93
2j (C14–B*)	0.93 ± 0.14	30.28 ± 0.32	2.05 ± 0.30	80.61 ± 0.41	0.11 ± 0.06
2 k (C11–U**)	1.48 ± 0.21	32.17 ± 0.97	4.72 ± 0.25	35.22 ± 2.16	0.31 ± 0.01

*B** branched chain compound; *U*** unsaturated chain compound

Table 2 Cytotoxicity results of the synthesized sulfated sodium salts of alkyl lactates

Test compound (carbon chain)	IC_{50} values (μM)				
	HeLa	MDA-MB-231	MCF-7	K-562	HEK-293
2b (C10)	18.2	7.79	–[a]	99.89	–
2c (C12)	6.54	6.28	8.00	–	–
2d (C14)	17.29	17.44	10.58	–	–
2j (C14–B*)	9.69	7.99	–	–	–
2 k (C11–U**)	6.9	5.54	19.92	–	–
SDS	–	–	–	–	–
Doxorubicin (control)	0.45	0.50	1.05	1.21	–

*B** branched chain compound; *U*** unsaturated chain compound

[a] Not active up to 100 μM

used in cancer chemotherapy, and standard sodium dodecyl sulfate (SDS). The concentration of the compound at which 50 % of the cell growth was inhibited (IC_{50}) was calculated and is shown in Table 2.

Based on the cytotoxicity evaluation in Table 2, it was noticed that the surfactants prepared from medium chain alcohols including 2b, 2c, 2d, and 2j (from branched chain alcohol, Guerbet alcohol) and 2k (from unsaturated alcohol) showed cytotoxicity specifically to HeLa and MDA-MB-231 cell lines, while the remaining surfactants, 2a, 2e, 2f, 2g, 2h, 2i and standard surfactant sodium dodecyl sulfate (SDS), did not show any activity against any the tested cancer cell lines up to 100 μM concentrations. None of the synthesized surfactants and SDS showed any cytotoxicity towards the normal cell line, HEK-293, even up to 100 μM concentration.

Antimicrobial Properties

All the synthesized surfactants were tested against different Gram-positive and Gram-negative bacterial strains in comparison to Neomycin, a broad-spectrum antibiotic for antimicrobial activity and the results are shown in Table 3.

Some of the surfactants, 2a, 2b, 2e, 2g and 2i, were active against the studied strains, while the surfactants 2c, 2d, 2f, 2h, 2j, 2k and the standard surfactant sodium dodecyl sulfate (SDS) did not show any activity towards any of the tested Gram-positive and Gram-negative bacterial strains or *Candida albicans* MTCC 3017. Moreover, none of the tested surfactants showed any activity against *Micrococcus luteus* MTCC 2470 and *Candida albicans* MTCC 3017. The surfactants 2a, 2e and 2i showed promising activity specifically against *Bacillus subtilis* MTCC 121 with a MIC value of 9.37 μg/mL. Surfactant 2b showed promising activity with a MIC value of 9.37 μg/mL specifically towards the Gram-negative bacterial strain, *Klebsiella planticola* MTCC 530. From the perpective of the structure–activity relationship, it was observed that compound 2b has a straight chain and exhibited antimicrobial activity against *Klebsiella planticola* (Gram −ve), while 2i has a branched chain and exhibited antimicrobial activity against *Bacillus subtilis* (Gram +ve); however, these compounds exhibited nearly *B** branched chain compound; *U*** unsaturated chain compoundsame CMC values of 1.4 and 1.2 mN/m. The surfactant 2e showed promising activity specifically towards two Gram-positive bacterial strains, *Bacillus*

Table 3 Antimicrobial activities of the synthesized sulfated sodium salts of alkyl lactates

Bacterial strains	Minimum inhibitory concentration (MIC) (μg/mL) of test compound (carbon chain)						
	2a (C8)	2b (C10)	2e (C16)	2 g (C22)	2i (C8-37 B*)	SDS	Neomycin (control)
Staphylococcus aureus MTCC 96	>125	18.75	37.5	>125	>125	>125	18.75
Bacillus subtilis MTCC 121	9.37	>125	9.37	>125	9.37	>125	18.75
Staphylococcus aureus MLS16 MTCC 2470	>125	>125	9.37	>125	37.5	>125	18.75
Klebsiella planticola MTCC 530	>125	9.37	>125	>125	>125	>125	18.75
Escherichia coli MTCC 739	37.5	>125	>125	37.5	>125	>125	18.75
Pseudomonas aeruginosa MTCC 2453	>125	>125	>125	37.5	>125	>125	18.75
Micrococus luteus MTCC 2470	>125	>125	>125	>125	>125	>125	>125
Candida albicans MTCC 3017	>125	>125	>125	>125	>125	>125	>125

*B** indicates branched chain compound

subtils MTCC 121 and *Staphylococcus aureus* MLS16 MTCC 2470 with a MIC value of 9.37 μg/mL and the antimicrobial activity was superior as compared to the standard neomycin (MIC value of 18.75 μg/mL). However, the surfactant 2e showed no activity towards the tested Gram-negative bacterial strains, even up to the highest tested concentration of 125 μg/mL. The promising surfactants exhibiting antimicrobial activity may be causing an increase in the cell membrane permeability which resulted in the destabilization and disruption of the bacterial cell membrane and its lysis.

Conclusions

This work describes the physicochemical and biological properties of the sulfated sodium salts of alkyl lactates. Synthesis of the surfactants using lactic acid and different fatty alcohols by a two-step procedure was carried out with good yields. The surfactants showed good CMC and surface tension properties. Some of the surfactants exhibited good to moderate antimicrobial properties against the tested bacterial strains, while promising cytotoxicity was observed against the four tested cancer cell lines and no cytotoxicity was observed against the normal cell line, HEK-293. Based on these properties, some of the synthesized lactic acid-based anionic surfactants are promising candidates with biological functionality.

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