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Synthesis and characterization of surface-active ionic liquids used in the disruption of *Escherichia coli* cells

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Abstract: Twelve surface-active ionic liquids (SAILs) and surface-active derivatives, based on imidazolium, ammonium and phosphonium cations and containing one, or more, long alkyl chains in the cation and/or the anion, were synthesized and characterized. The aggregation behavior of these SAILs in water, as well as their adsorption at solution/air interface, were studied using surface tension and conductivity. The CMC values obtained (0.03 - 6.0 mM) show the high capacity of these compounds to self-aggregate in aqueous media. Their thermal properties were also characterized, namely the melting point and decomposition temperature by DSC and TGA, respectively. Furthermore, the toxicity of these SAILs was evaluated using the marine bacteria *Aliivibrio fischeri* (Gram-negative). According to the EC₅₀ values obtained (0.3 - 2.7 mg·L⁻¹), the surface-active compounds tested should be considered % 63.4. 1.02 @ 1.02. Their ability to induce cell disruption of *Escherichia coli* cells (also Gram-negative), releasing the intracellular green fluorescent protein (GFP) produced, was investigated. The results here reported clearly evidence the capacity of these SAILs to act as cell disruption agents.

Introduction

Ionic liquids (ILs) are a class of low melting point ionic compounds, which have attracted increasing attention for multiple applications.^[1-5] These compounds possess high thermal stabilities and negligible vapor pressures at room temperature, making them a promising alternative to conventional solvents. Moreover, due to their amphiphilic nature they can dissolve a wide

range of compounds and can be structurally tailored for specific applications by choosing the appropriate anion-cation combination.^[6,7] A number of ILs containing long alkyl chain substituents (typically more than eight carbons - octyl chains) have been reported to be surface-active (or tensioactive). These ionic compounds are currently termed as surface-active ionic liquids (SAILs) and are attracting increased interest in classical colloid and surface chemistry research.^[8, 16] As the conventional surfactants, SAILs have the capacity to self-aggregate in aqueous media above their critical micelle concentration (CMC).^[17] Due to their tunable structure, these compounds allow to design many new families and types of surfactants. A number of analytical applications have emerged in separation science regarding the use of SAILs, not only as solubilisation media for proteins,^[18] and cell disruption,^[19,20] but also in analytical techniques, namely chromatography or electrophoresis,^[21, 24] in biocatalysis^[25] and also in extractive, pre-concentration and recovery techniques.^[11,26,27] The potential industrial application of SAILs, as well as their impact on the environment, is closely dependent on their self-assembly behavior and aggregate structure in aqueous solution.^[28] Thus, the study of the aggregation behavior of these compounds in water is of high importance. The amphiphilic structure of ILs in water began to be intensively studied in 2004.^[29, 33] A significant amount of research has focused on the study of the aggregation behavior of alkylimidazolium ILs. Sirieix-Plénet and co-authors^[29] described the self-aggregation of 1-decyl-3-methylimidazolium bromide ([C₁₀C₁Im]Br) at low concentrations, and the CMC value was estimated to be 0.04 M by using potentiometric and conductimetric studies. The authors also observed that at higher concentrations it adopts a fairly complex structure with interpenetrated domains of the water and electrolyte.^[29] Bowers and co-authors^[30] reported the aggregation behavior in aqueous solution of three ILs, namely 1-butyl-3-methylimidazolium tetrafluoroborate [C₄C₁Im][BF₄], 1-methyl-3-octylimidazolium chloride [C₈C₁Im]Cl and 1-methyl-3-octylimidazolium iodide [C₈C₁Im]I, by using surface tension, conductivity and small-angle neutron scattering (SANS) measurements. The CMC values were estimated for these three ILs, being approximately 0.1 M for [C₈C₁Im]-based ILs and 0.8 M for [C₄C₁Im][BF₄]. SANS data allowed for the proposition that the short chain [C₄C₁Im][BF₄] system can best be modeled as a dispersion of polydisperse spherical aggregates, whereas the [C₈C₁Im]I solutions can be considered as a system of regularly sized near-spherical charged micelles above the CMC.^[30] Miskolczy et al.^[31] studied aggregation of ILs with *n*-octyl alkyl chain by measuring conductivity, turbidity and using a solvatochromic probe. The 1-butyl-3-methylimidazolium octyl sulfate ([C₄C₁Im][C₈SO₄]) was found to form micelles above 0.031

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M. In contrast, $[C_8C_{14}Im]Cl$ produced an inhomogeneous solution of larger aggregates.^[31] More recently, the self-assembly behavior of dicationic and tricationic imidazolium-based SAILs has been studied.^[16] Baltazar et al.^[32] investigated eighteen mono- and dicationic (or gemini) imidazolium bromide ILs using surface tension measurements. The results showed a correlation between substituted alkyl chain length and the CMC value for mono and dicationic ILs. Furthermore, dicationic ILs possessing hydrophilic and hydrophobic moieties analogous to two molecules of monocationic ILs exhibited nearly 2-fold lower CMC values, and longer alkyl linkage chain connecting the two imidazolium rings were found to slightly decrease the CMC values.^[32] Moreover, Nacham et al.^[34] investigated the interfacial and aggregation behavior of three dicationic and two tricationic imidazolium-based SAILs in aqueous solutions. The results showed the higher capacity of dicationic SAILs to self-aggregate by increasing the alkyl chain length, for the same linker. On the other hand, the tricationic ILs studied presented lower CMC values than analogous linear trimeric surfactants.

Besides the imidazolium family, other types of SAILs have also been investigated, namely ammonium-,^[35, 38] phosphonium-,^[23] morpholinium-^[38, 41] and guanidinium-based SAILs.^[42, 44] Brown et al.^[35] reported a series of IL based surfactants composed of tetraalkylammonium cations and common surfactant anions (derived from dodecyl sulfate and aerosol-OT) as alternatives to imidazolium-based systems, being claimed as cheaper and environmentally more benign. T [: ^ A ^ A } d ^ E o ^ A : a ^ A ^ A [] [A e j a A a B o m] a A a e { [] a { A a i { a ^ A C S ^ A ^ A ^ A ^ A } c @ a ^ A a A e j a A • c a a a A ^ A : a a A e a A e] -workers.^[36] The results showed that the absence of the spacer group may confer relatively low flexibility to the molecules, with potential implications on the interfacial properties, namely, on micellization.^[36] In 2017, Muslim and co-workers^[37] reported the synthesis and characterization of two groups of asymmetrical dicationic ILs based on ammonium cation. The first group consisted of SAILs with one hexyl pyrene tail and one alkyl chain, whereas the second group had two asymmetrical alkyl chains. With an increase of the dissymmetry ratio, the CMC decreased linearly. Furthermore, the addition of the bulky pyrenyl tail group resulted in much lower CMC values compared to their symmetrical counterparts, leading to high-ordered structures (lamellar and inverted micelles).^[37] The morpholinium- and guanidinium-based ILs have also been reported as a promising alternative to the commonly used imidazolium-based ILs due to their lower toxicity. A series of cationic amphiphilic compounds with the morpholinium moiety in the head group was synthesized and characterized by Mirgorodskaya and co-authors.^[45] These amphiphilic compounds showed a pronounced surface activity, presenting CMC values similar to the homologous series with trimethylammonium head group. Over the years, morpholinium gemini surfactants have also been the focus of deeper research, namely by analyzing the influence of both the hydrophobic chain length and spacer on the properties of surface and bulk phase.^[38, 40] Regarding the guanidinium cation, the alkyguanidinium chloride series was one of the most studied up to now.^[42, 44] Bouchal et al.^[43] studied the micellization behavior of alkyguanidinium chlorides containing 10, 12, 14 and 16 carbon atoms in the hydrophobic tail, being these compounds less

soluble in water and more surface-active than the analogous alkyltrimethylammonium bromides or chlorides.

Recently, a new family of biodegradable SAILs composed of a cation derived from prolinium esters and dodecylsulfate and dodecylbenzenesulfonate anions was proposed.^[11] These SAILs proved to be more effective in reducing the surface tension of water than the respective sodium salts, being promising candidates for enhanced oil recovery applications.^[11] Although the phenomena of IL self-organization in aqueous solutions are currently under investigation by a number of authors, their correlation with ILs structure is limited.^[36, 46, 48] This lack of information is limiting their study and compromises the development of potential industrial applications. The present work reports the synthesis and characterization of several families of ILs with a surfactant nature, namely imidazolium, quaternary ammonium and phosphonium, containing one or more long alkyl chains in the cation and/or anion. As a result, cationic, gemini, and catanionic ILs were prepared, as illustrated in Figure 1. The structures of these ILs were confirmed by ¹H and ¹³C NMR. The aggregation behavior of these SAILs in water, as well as their adsorption at solution/air interface, were studied using surface tension and conductivity, with the exception of $[N_{1,1,14,14}]Cl$, $[N_{1,1,10,14}]I$, $[C_{14}C_{14}Im]Br$ and $[N_{1,1,1,14}][C_{10}SO_4]$ due to their low solubility in water at 298.2 K. Their thermal properties were characterized, namely the melting point and decomposition temperature by differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA), respectively. Moreover, the ecotoxicity of the entire set of ILs prepared was evaluated in what concerns the effect of different structural features, in particular the cation core, anion nature, and cation and anion alkyl chain lengths. For that purpose, several Microtox® test assays were performed using the marine luminescent bacteria *Aliivibrio fischeri*.^[49] Finally, the ILs obtained were studied in order to understand their capacity to promote the disruption of *Escherichia coli* BL21 cells and release of green fluorescent protein (GFP), produced intracellularly. This protein is used as a biomarker in biomedical applications due to its unique spectral and fluorescence features. However, its extraction is still a challenge inducing high costs on the final product.^[50] Mechanical,^[51, 52] and non-mechanical^[50, 53] processes are the two categories of conventional methodologies recurrently described for the extraction of intracellular bioactive products from cells. To evaluate the effect of SAILs as cell disrupting agents over the *E. coli* BL21 membrane, the ionic structures here studied were investigated, and the results compared with the experimental data obtained for ultrasonic-assisted extraction and Tris-HCl buffer, as the mechanical and non-mechanical conventional methodologies adopted in this work.

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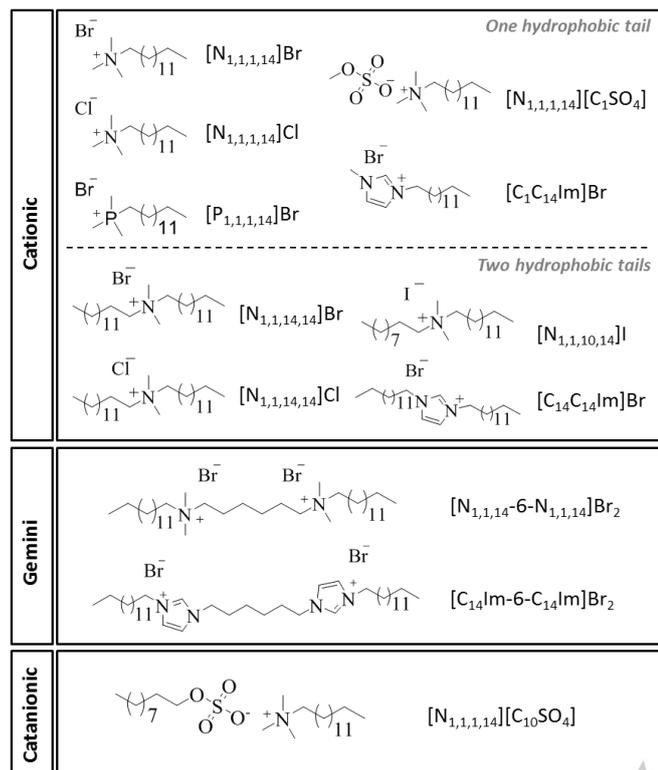


Figure 1. Chemical structures and acronyms of SAILs synthesized in this work.

Results and Discussion

Twelve SAILs, based on the imidazolium, ammonium and phosphonium cations and containing one, or more, long alkyl chains in the cation and/or anion, were synthesized and characterized. Their chemical structures and acronyms are depicted in Figure 1. These surface-active compounds were obtained with high purity levels and yield. Experimental Section. The self-organization of these SAILs in aqueous solution, as well as their adsorption behavior, was investigated using surface tension and conductivity measurements. The data obtained was thus employed to calculate the CMC, the surface tension at the CMC (γ_{CMC}), the concentration required to reduce the surface tension of the solvent by 20 mN m⁻¹ (C_{20}), CMC/ C_{20} ratio (a measure of the tendency to form aggregates relative to adsorb at the air/water interface), the minimum area *per* surfactant molecule at the air/water interface (A_{min}), the maximum surface excess concentration at the air/aqueous solution interface (Γ_{max}), as well as, the degree of ionization of the aggregates (U) and the standard Gibbs energy of micellization ($\Delta G_{\text{mic}}^{\circ}$). These parameters are presented in Tables 1 and 2. CMC is the concentration at which the solution property of a tensioactive compound shows an abrupt change, and constitutes a basic parameter of the surface chemistry and colloid science.^[54]

The CMC values were obtained from both conductivity and surface tension. The results acquired with both methodologies are consistent with each other, as shown in Tables 1 and 2. Furthermore, the CMC values obtained show the high capacity of these tensioactive ILs to form microaggregates by their self-aggregation. For $[\text{N}_{1,1,14,14}]\text{Cl}$, $[\text{N}_{1,1,10,14}]\text{I}$, $[\text{C}_{14}\text{Im}]\text{Br}$ and $[\text{N}_{1,1,1,14}][\text{C}_{10}\text{SO}_4]$, it was not possible to accurately determine the CMC values due to their low solubility in water.

Considering the $[\text{N}_{1,1,1,14}]$ -based SAILs, the CMC values do not seem to be significantly influenced by changing the anion (Br^- , Cl^- and $[\text{C}_1\text{SO}_4]$). The introduction of the second alkyl chain on the ammonium cation plays a dominant role in CMC values of ILs, as observed for $[\text{N}_{1,1,1,14}]\text{Br}$ (3.4 - 3.9 mM) and $[\text{N}_{1,1,14,14}]\text{Br}$ ((3.5 - 3.6) × 10⁻² mM). Furthermore, the CMC values of $[\text{N}_{1,1,1,14}]\text{Br}$ and $[\text{P}_{1,1,1,14}]\text{Br}$ suggest that changes in the cation's central atom have an impact on their aggregation behavior. According to the results obtained, phosphonium-based SAIL presents lower CMC than the respective ammonium SAIL. Moreover, the mono cationic ammonium IL $[\text{N}_{1,1,14,14}]\text{Br}$ ((3.5 - 3.6) × 10⁻² mM) presents lower CMC value than the dicationic ammonium IL $[\text{N}_{1,1,14-6-\text{N}_{1,1,14}}]\text{Br}_2$ (0.18 - 0.19 mM). The CMC value obtained for $[\text{N}_{1,1,1,14}][\text{C}_{10}\text{SO}_4]$ is 3.47 mM. The CMC values of $[\text{N}_{2,2,12,12}]\text{Br}$ and $[\text{N}_{2,2,16,16}]\text{Br}$ decreases with the increase in the alkyl chain length, from 0.10 mM to 0.015 mM, respectively.^[36] Finally, when the alkyl chain increases from 12 to 14 carbons in the series $[\text{C}_n\text{Im}-6-\text{C}_n\text{Im}]\text{Br}_2$, the CMC decreases from 0.78 mM^[55] to 0.11 mM.

Table 1. Parameters derived from conductivity data for SAILs synthesized in this work at 298.2 K and atmospheric pressure, namely the critical micelle concentration (CMC), the degree of ionization (U) and the standard Gibbs energy of micellization

	CMC ± sd / mM	$U \pm \text{sd}$	$\Delta G_{\text{mic}}^{\circ} / \text{kJ mol}^{-1}$
$[\text{N}_{1,1,1,14}]\text{Br}$	3.9 ± 0.2 (3.9 ^[a])	0.27 ± 0.02 (0.27 ^[a])	- 41 ± 3
$[\text{N}_{1,1,1,14}]\text{Cl}$	6.01 ± 0.07	0.35 ± 0.01	- 37.4 ± 0.5
$[\text{P}_{1,1,1,14}]\text{Br}$	3.00 ± 0.05	0.23 ± 0.01	- 43.1 ± 0.7
$[\text{C}_{14}\text{Im}]\text{Br}$	2.4 ± 0.1 (2.5 ^[b])	0.27 ± 0.02 (0.24 ^[b])	- 43 ± 3
$[\text{N}_{1,1,1,14}][\text{C}_1\text{SO}_4]$	3.47 ± 0.06	0.30 ± 0.01	- 41 ± 1
$[\text{N}_{1,1,14,14}]\text{Br}$	(3.5 ± 0.3) × 10 ⁻²	0.21 ± 0.03	- 63 ± 6
$[\text{N}_{1,1,14-6-\text{N}_{1,1,14}}]\text{Br}_2$	(1.95 ± 0.08) × 10 ⁻¹ (0.170 ^[c])	0.38 ± 0.01	- 70 ± 2
$[\text{C}_{14}\text{Im}-6-\text{C}_{14}\text{Im}]\text{Br}_2$	(1.09 ± 0.07) × 10 ⁻¹	0.59 ± 0.02	- 59 ± 2

[a] From Ray et al., determined at 303 K^[56]

[b] From Vanyúr et al.^[57]

[c] From Azum et al., determined at 303 K^[58]

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Table 2. Parameters derived from surface tension data for SAILs synthesized in this work at 298.2 K and atmospheric pressure, namely the critical micelle concentration (CMC), the surface tension at the CMC (γ_{CMC}), the concentration required to reduce the surface tension of the solvent by 20 mN m⁻¹ (C_{20}), the CMC/ C_{20} ratio, the efficiency of surface tension reduction (ρC_{20}), the surface excess concentration and the area per molecule at the air/water interface.

	CMC \pm sd / mM	$\gamma_{\text{CMC}} \pm$ sd / mN m ⁻¹	$C_{20} \pm$ sd / mM	CMC/ C_{20}	$\rho C_{20} \pm$ sd	$\Gamma \pm$ sd / $\mu\text{mol m}^{-2}$	$A \pm$ sd / \AA^2
[N _{1,1,1,14}]Br	3 \pm 1 (4 ^[a])	38 \pm 2	1.30 \pm 0.06	2.6 \pm 0.3	2.89 \pm 0.02	1.31 \pm 0.08	127 \pm 7
[N _{1,1,1,14}]Cl	3.7 \pm 0.2	35 \pm 5	1.9 \pm 0.5	1.9 \pm 0.7	2.7 \pm 0.1	2.3 \pm 0.4	73 \pm 11
[P _{1,1,1,14}]Br	2.5 \pm 0.2	36 \pm 2	(8.5 \pm 0.3) $\times 10^{-1}$	2.9 \pm 0.3	3.07 \pm 0.02	1.30 \pm 0.09	128 \pm 9
[C ₁ C ₁₄ Im]Br	2.6 \pm 0.2 (2.7 ^[b])	37 \pm 1 (39 ^[b])	(9.3 \pm 0.3) $\times 10^{-1}$	2.8 \pm 0.2	3.03 \pm 0.01	1.26 \pm 0.07	132 \pm 7
[N _{1,1,1,14}][C ₁ SO ₄]	3.3 \pm 0.3	37 \pm 1	(9.7 \pm 0.3) $\times 10^{-1}$	3.4 \pm 0.3	3.01 \pm 0.01	1.04 \pm 0.04	159 \pm 6
[N _{1,1,14,14}]Br	(3.6 \pm 0.6) $\times 10^{-2}$	25 \pm 3	(1.4 \pm 0.8) $\times 10^{-2}$	3 \pm 1	4.9 \pm 0.2	2.5 \pm 0.3	57 \pm 6
[N _{1,1,14-6-N} _{1,1,14}]Br ₂	(1.8 \pm 0.5) $\times 10^{-1}$ (0.16 ^[c])	40 \pm 3	(3 \pm 1) $\times 10^{-2}$	7 \pm 3	4.6 \pm 0.2	(3.7 \pm 0.4) $\times 10^{-1}$ (0.33 ^[c])	448 \pm 42 (498 ^[c])
[C ₁₄ Im-6-C ₁₄ Im]Br ₂	(6 \pm 1) $\times 10^{-2}$	32 \pm 8	(1.3 \pm 0.7) $\times 10^{-2}$	5 \pm 3	4.9 \pm 0.2	(7.0 \pm 0.8) $\times 10^{-1}$	236 \pm 25

[a] From Ray et al., determined at 303 K^[56], [b] From Wey et al.^[59], [c] From Kabir-ud-Din et al., determined at 303 K^[60]

Conductivity measurements are commonly used in the study of ionic micellar solutions due to a substantial change of slope at the onset of aggregation. The ionization degree (U) of micelles of the ILs under study was estimated from the ratio of the slopes above and below CMC. The high ionization degree of [N_{1,1,14-6-N}_{1,1,14}]Br₂ and [C₁₄Im-6-C₁₄Im]Br₂ is related to its low degree of compactness, which implies that less counter ions are attracted to the layer of the micelles.^[37] The high A_{min} of [N_{1,1,14-6-N}_{1,1,14}]Br₂ and [C₁₄Im-6-C₁₄Im]Br₂ reflects the low packing of its monomers at the interface, which is in clear agreement with literature.^[16,46] The standard Gibbs energy of micellization, ΔG° , for the SAILs with 1 or 2 counterions was calculated through equations 1 and 2, respectively:^[61]

$$(1)$$

$$(2)$$

where R is the gas constant, T is the temperature and C_{CMC} is the CMC expressed by mole fraction. The calculated values of ΔG° are also listed in Table 1. The micellization process occurs easier for [N_{1,1,14,14}]Br and two gemini SAILs, which is confirmed by a lower CMC as well as more negative free Gibbs energy. Furthermore, these SAILs proved to be much more efficient than the corresponding monomeric SAIL at decreasing the surface tension of water, as shown by the concentration C_{20} presented in Table 1.^[62]

The maximum surface excess concentration (Γ) and the area per molecule (A) at the water/air interface can be calculated by equation:^[61]

$$(3)$$

$$(4)$$

where γ is the surface tension, n is the number of particles per molecule of SAIL (its value is taken as 2 or 3 for monomeric and dimeric SAIL, respectively), K is the gas constant, C is the temperature, C is the surfactant concentration and N_A is the Avogadro constant. The derivative in equation (3) is the slope of the γ vs C curve at the pre-micellar region. As shown in Table 1, the replacement of chloride by bromide anion results in a lower effectiveness of SAIL adsorption at water-air interface, which is indicated by its lower Γ and higher A . On the other hand, the introduction of the second alkyl chain on the ammonium cation, increases the SAIL adsorption at that interface.

The thermal properties of the SAILs here studied, namely melting point and decomposition temperature, were additionally addressed. The melting temperatures (T_{fus}), and thermal transition temperatures (T_{tr}) were measured by DSC data and are presented in Table 3. The onset temperatures of decomposition (T_d) were further evaluated by TGA, and are also reported in Table 3. From the TGA profiles of these tensoactive compounds (shown in Supporting Information, Figure S3), as well as from the T_d values reported in Table 3, it is possible to conclude that all compounds studied present a high thermal stability. At least up to 453.2 K.

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different intracellular bioactive compounds, in particular in this work, the GFP.

Experimental Section

Materials: Twelve SAILs were here synthesized, namely [N_{1,1,1,14}]Br, *N,N,N*-trimethyl-*N*-tetradecylammonium bromide; [N_{1,1,1,14}]Cl, *N,N,N*-trimethyl-*N*-tetradecylammonium chloride; [P_{1,1,1,14}]Br, trimethyltetradecylphosphonium bromide; [N_{1,1,1,14}][C₇SO₄], *N,N,N*-trimethyl-*N*-tetradecylammonium methylsulfate; [C₁C₁₄Im]Br, 1-methyl-3-tetradecylimidazolium bromide; [N_{1,1,14,14}]Br, *N,N*-dimethyl-*N,N*-ditetradecylammonium bromide; [N_{1,1,14,14}]Cl, *N,N*-dimethyl-*N,N*-ditetradecylammonium chloride; [N_{1,1,10,14}]I, *N*-decyl-*N,N*-dimethyl-*N*-tetradecylammonium iodide; [C₁₄C₁₄Im]Br, 1,3-ditetradecylimidazolium bromide; [N_{1,1,14-6-N},1,1,14]Br₂, 1,6-bis(*N,N*-dimethyl-*N*-tetradecylammonium-*N*-yl)hexane dibromide; [C₁₄Im-6-C₁₄Im]Br₂, 1,6-*bis*(1-tetradecylimidazolium-*H*yl)hexane dibromide and [N_{1,1,1,14}][C₁₀SO₄], *N,N,N*-trimethyl-*N*-tetradecylammonium decylsulfate. Their acronym and chemical structure are depicted in Figure 1. Imidazole (99 wt % of purity), 1-bromotetradecane (97 wt % of purity), 1-methylimidazole (99 wt % of purity), 1,6-dibromohexane (96 wt % of purity), 1-chlorotetradecane (98 wt % of purity), trimethylamine (4.2 M in ethanol), 1-iododecane (98 wt % of purity), dimethylsulfate (99.8 wt % of purity), 1-decanol (99 wt % of purity), trimethylphosphine (1.0 M in toluene), sodium sulfate (99.9 wt % of purity), methanesulfonic acid (99.5 wt % of purity) and potassium carbonate (99 wt % of purity) were acquired from Sigma-Aldrich®. All organic solvents used (HPLC grade), namely methanol, ethyl acetate, hexane, dichloromethane, toluene and diethyl ether were from VWR. *Escherichia coli* (*E. coli*) BL21 (DE3) pLysS carrying the pET-28(a) plasmid encoding the GFP gene were kindly provided by the Molecular and Cellular Biology Laboratory of the School of Pharmaceutical Sciences from the University Estadual Paulista "Júlio de Mesquita Filho". The components of Luria-Bertani (LB) culture media for the *E. coli* growth, tryptone and yeast extract were purchased from Oxoid, while sodium chloride (99.5 wt % of purity) was acquired from Panreac. The antibiotic used in cell culture, tetracycline (Tet), as well as the components of the Tris-HCl buffer, tris(hydroxymethyl) aminomethane (99.8 wt % of purity) and chloridric acid (37 wt % in aqueous solution), were purchased from Sigma-Aldrich®. The water used was double distilled, passed by a reverse osmosis system and further treated with a Milli-Q plus 185 water purification apparatus.

Synthesis of surface-active ILs: In general these ILs (with surfactant nature) were synthesized via quaternization reaction. Additionally, a transesterification reaction was performed in order to prepare the [N_{1,1,1,14}][C₁₀SO₄].^[76, 80] More details about the synthesis of these ILs are reported in the Supporting Information, being divided into four major topics: I) synthesis of 1-tetradecylimidazole; II) synthesis of imidazolium-based ILs; III) synthesis of ammonium-based ILs and IV) synthesis of phosphonium-based ILs. The structure of all compounds synthesized was confirmed by ¹H and ¹³C NMR, showing the high purity level of all ionic structures after their synthesis, as reported in the Supporting Information.

Electric conductivity measurements: Conductivity measurements were carried out using a conductivity meter (Mettler Toledo) calibrated with a standard solution of KCl with known conductivity (1000 µS cm⁻¹). The conductivity meter was calibrated with a standard solution of KCl with known conductivity (1000 µS cm⁻¹). The conductivity measurements of the SAILs studied were carried out by continuous dilution of a concentrated solution of IL into water. After any addition of the IL solution, the solution was stirred and equilibrated for 10

min, and then three successive measurements of conductivity were performed (Supporting Information, Figure S1). Duplicated measurements were carried out.

Surface tension: The surface tension of a range of diluted aqueous solutions of each IL was determined by the analysis of the shape of a pendant drop and measured using a Dataphysics (model OCA-20) contact angle system (Supporting Information, Figure S2). For that, a Hamilton DS 500/GT syringe was used, being connected to a Teflon coated needle placed inside an aluminum air chamber able to maintain the temperature of interest within ± 0.1 K. The surface tension measurements were performed at 298.2 K. The temperature inside the aluminum chamber in which the surface tensions were determined was measured with a Pt100 within ± 0.1 K. After reaching a specific temperature inside the aluminum chamber, the measurements were carried out after 10 min to guarantee the thermal stabilization. Silica gel was kept inside the air chamber aiming at keeping a dry environment. For the surface tensions determination at each sample, at least 3 drops were formed and analyzed. For each drop, an average of 250 images was captured. The analysis of the drop shape was done with the software modules SCA 20 where the gravitational acceleration (g = 9.81 m s⁻²) and latitude (lat = 40°) were used according to the location of the assay. Further details on the equipment and its validity to measure surface tensions of ILs were previously addressed.^[81]

Thermogravimetric analysis: The decomposition temperature was determined by TGA. TGA was conducted on a Setsys Evolution 1750 (SETARAM) instrument. The sample was heated in an alumina pan, under a nitrogen atmosphere, over a temperature range of 300 - 900 K, and with a heating rate of 5 K min⁻¹.

Differential Scanning Calorimetry: Thermal transition temperature and the melting temperature were measured in a DSC, PERKIN ELMER model Pyris Diamond DSC, using hermetically sealed aluminum crucibles with a constant flow of nitrogen (50 mL min⁻¹). Samples of about 15 mg were used in each experiment. The temperature and heat flux scales of the power compensation DSC were calibrated by measuring the temperature and the enthalpy of fusion of reference materials, at the scanning rate of 5 K min⁻¹ and flow of nitrogen of 50 mL min⁻¹. Temperatures of the thermal transitions and melting temperature were taken as the onset temperatures.

Microtox Assay: In order to evaluate the ecotoxicity of the SAILs synthesized, the Standard Microtox liquid-phase assay was carried out. Microtox® is a bioluminescence inhibition test based on the bacterium *Aliivibrio fischeri* (strain NRRL B-11177) luminescence after its exposure to each sample solution. In this work, the standard 81.9% test protocol was used. Thus, the bacteria were exposed to a range of diluted aqueous solutions of each compound (0 - 81.9%), where 100% corresponds to a previously prepared stock solution, with a known concentration. After 5, 15, and 30 minutes of exposure to each IL aqueous solution, the bacterial bioluminescence emission of *Aliivibrio fischeri* was measured and compared with the bioluminescence emission of a blank control sample. The corresponding 5 min-, 15min- and 30 min-EC₅₀ values (estimated concentration yielding a 50% of inhibition effect) were evaluated through the Microtox® Omni™ software version 4.1. Finally, the EC₅₀ values, as well as the corresponding 95% confidence intervals, were estimated for each SAIL tested by non-linear regression, using the least-squares method to fit the data to the logistic equation.

Cell disruption of *Escherichia coli* BL21: To evaluate their application as disrupting agents, the ILs here synthesized were tested in the disruption of *Escherichia coli* (*E. coli*) cells, and consequent release of GFP, produced intracellularly. The GFP extraction from *E. coli* BL21 cells by using tensioactive compounds was recently proposed by our group as an alternative method to conventional ultrasonic-assisted extraction. The

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preparation of the cell culture and the GFP release (cell disruption) was performed according to previous work.^[19] Briefly, the *E. coli* strain was grown at 310.2 K in LB medium with kanamycin and chloramphenicol (50 $\mu\text{g mL}^{-1}$) with a constant stirring (150 rpm). Six hours later, IPTG was added to induce the GFP expression aiming a final concentration of 0.25 mM. After 17h of protein induction, the culture medium was centrifuged at 5000 rpm for 30 minutes at 4 °C and the weight of wet cells was calculated. Cell pellets containing GFP were resuspended in 50 mM of Tris-HCl buffer at pH 8 in a concentration of approximately 0.025 wt% wet cells. The cell disruption by ultrasonication was done following the protocol described elsewhere.^[19] To evaluate the ability of the SAILs here synthesized to solubilize the cell membrane, a concentration of 100 mM was used. Moreover, it was studied the effect of the IL concentration between 50 and 500 mM. The cell suspensions were centrifuged at 25 rpm during 30 min, and the supernatant was analyzed in terms of GFP content by its fluorescence (emission at 530 nm and excitation at 485 nm).

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Keywords: surface-active ionic liquids ~ aggregation behaviour ~ ecotoxicity ~ cells disruption

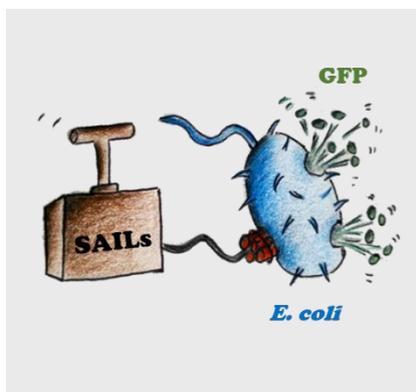
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Twelve surface-active ionic liquids (SAILs) were synthesized and characterized in terms of their aggregation behavior, thermal properties and ecotoxicity. Additionally, due to their high surface activity, these ionic compounds proved to be capable of promoting cell disruption of *Escherichia coli* and release of green fluorescent protein (GFP), produced intracellularly.



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Synthesis and characterization of surface-active ionic liquids used in the disruption of *Escherichia coli* cells