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Aggregation Behavior and Antimicrobial Activity of Ester-Functionalized Imidazolium- and Pyridinium-Based Ionic Liquids in Aqueous Solution

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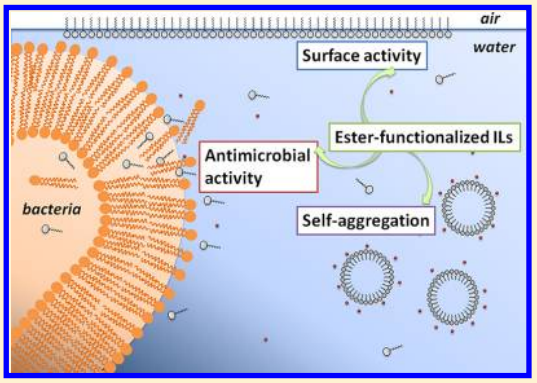
Supporting Information

ABSTRACT: Two series of long chain imidazolium- and pyridinium-based ionic liquids containing an ester functional group in the alkyl side chain, 3-methyl-1-alkyloxycarbonylmethylimidazolium bromides (C_nEMeImBr) and 1-alkyloxycarbonylmethylpyridinium bromides (C_nEPyrBr), were synthesized and their thermal stability, aggregation behavior in aqueous medium, and antimicrobial activity investigated. The introduction of an ester group decreased the thermal stability of the functionalized ILs compared to simple alkyl chain containing ILs (1-alkyl-3-methylimidazolium bromides and 1-alkylpyridinium bromides). Tensiometry, conductimetry, and spectrofluorimetry were applied to study the self-aggregation of the amphiphilic ILs in aqueous solution. The ILs investigated displayed surface activity and the characteristic chain length dependence of the micellization process of surfactants. As compared to simple alkyl chain containing ILs bearing the same hydrocarbon chain, ester-functionalized ILs possess higher adsorption efficiency (pC_{20}) and significantly lower critical micelle concentration (cmc) and surface tension at the cmc (γ_{cmc}), indicating that the incorporation of an ester group promotes adsorption at the air/water interface and micelle formation. The antimicrobial activity was evaluated against Gram-negative and Gram-positive bacteria and fungi. ILs containing more than eight carbon atoms in the alkyl chain showed antimicrobial activity. Their efficiency as antimicrobial agents increased with the hydrophobicity of the amphiphilic cation being the C_{12} homologous the most active compounds. The incorporation of an ester group particularly increased the biological activity against fungi.



1. INTRODUCTION

The study of ionic liquids (ILs) as a new class of nonmolecular, ionic solvents for use in sustainable processes as solvents, catalysts, and electrolytes[1−3] has increased exponentially during the past years. Both the industrial and the academic sectors have realized their potential, resulting in the constant development of novel applications.[4−9] Most widely studied ILs[10,11] are comprised of bulky, asymmetric N-containing organic cations in combination with any wide variety of anions, ranging from simple inorganic anions to more complex organic species. One of the main aspects gaining attention in ILs research is the enormous range of cation−anion combinations, which results in a large potential for adjustability of structure−properties. ILs are often called "designer solvents" or considered "task-specific" because of their possibility to be tailored to fulfill the technological demands of a variety of applications.[1,12,13]

ILs emerged as a possible "green" alternative to common organic solvent due to extremely low vapor pressures. However, other release routes aside evaporation to the environment must be addressed before ILs can be considered as environmental acceptable compounds. Most of the commonly used ILs are not readily biodegradable compounds. Previous studies within our group have focused on synthesizing nontoxic ILs that undergo aerobic biodegradation as a pathway that represents a minimal environmental impact and a means of generating truly green compounds. Our studies on both imidazolium- and pyridinium-based ILs[14,15] highlighted that the introduction of a cleavable ester functional group in the side chain leads to a significant increase of the biodegradability of the ionic liquid molecules in comparison to those ILs bearing simple alkyl chains.[16]

Long-chain imidazolium- and pyridinium-based ionic liquids consist of a charged hydrophilic headgroup and a hydrophobic tail and consequently possess an inherent amphiphilic nature. Several studies[17−22] have reported that these compounds exhibit an interfacial and aggregation behavior analogous to that displayed by conventional cationic surfactants. Their ability to form self-assembled structures may have consequences in a

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variety of areas such as the extractions of products from IL-containing systems, the synthesis and purification of bulk ILs, the solvation properties of the ILs molecules, the formation of dispersed or phase-separated systems, etc. In addition, it can be expected that the introduction of specific functional groups in the long-chain IL structure affects the surface properties of amphiphilic ILs. Recent studies show that COOH-functionalized imidazolium ILs[23] and long-chain β-hydroxy-γ-alkyloxy-*N*-methylimidazolium ILs[24] have superior surface activity compared with the simple alkyl-substituted derivatives.

Most of quaternary ammonium-based surfactants (QACs) are known to possess, besides interfacial activity, prominent biological activity against bacteria and fungi. Kopecky[25] found that antimicrobial activity of QACs is closely related to their surfactant properties. Because of the structural resemblances between QACs and long-chain pyridinium and imidazolium ILs, it was expected that this type of IL might also exhibit antimicrobial activity. This anticipation has been widely confirmed by different authors.[22,26,27] Likewise, recent toxicology data indicate a strong correlation between the length of the alkyl chain appended to the IL cation and the resulting toxicity.[28] Some authors have analyzed the effect of incorporating a specific functional group in the IL structure on its biological activity. For instance, Morrison et al.[29] observed a clear reduction in toxicity for all the imidazolium ILs containing ether or polyether side chains compared with the alkyl-substituted derivatives.

The previous findings above-mentioned showing that imidazolium and pyridinium derivatives with long alkyl chains have surface activity and biological activity and our encouraging data on the effect of the ester functionality on promoting IL biodegradability have led us to synthesize and investigate the self-aggregation and antimicrobial activity in aqueous solution of a series of long-chain ester-containing ILs.

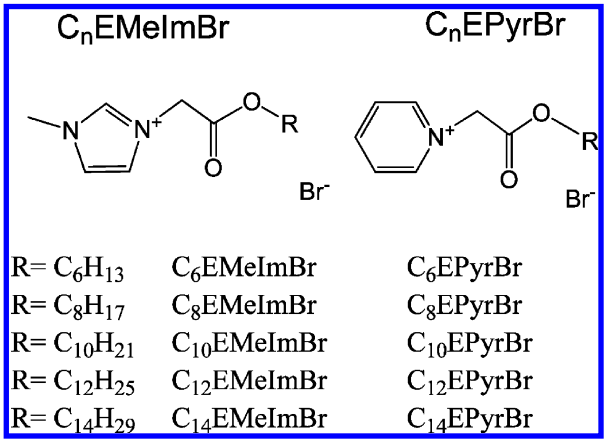
In the present work, two series of ionic liquids based on imidazolium and pyridinium cations containing a hydrolytically cleavable ester group in the hydrophobic side chain, 3-methyl-1-alkyloxycarbonylmethylimidazolium bromides (C_nEMeImBr) and 1-alkyloxycarbonylmethylpyridinium bromides (C_nEPyrBr), have been synthesized in order to investigate the effect of the incorporation of a functional group that enhances IL biodegradation on the aggregation behavior and antimicrobial activity of these amphiphilic ionic liquids in aqueous solution as well as to know whether their biological activity is related to their surfactant properties. The research is expected to contribute to further development of biodegradable-improved ionic liquids as polyfunctional compounds as well as to their fate assessment in the environment.

2. EXPERIMENTAL SECTION

2.1. Synthesis of Ionic Liquids. The synthesis of the 3-methyl-1-alkyloxycarbonylmethylimidazolium bromides [C_nEmimBr] and 1-alkyloxycarbonylmethylpyridinium bromides [C_nEPyrBr] was carried out in two steps using standard methodology.[15,16,29,30] In the first step, the commercially available alcohols were reacted with bromoacetyl bromide to form the desired alkylating agents. Subsequent alkylation of either methylimidazole or pyridine led respectively to the imidazolium- or piridinium-based ionic liquids. Experimental details of the synthetic procedures and analytical data and spectra of the ILs synthesized are described in the Supporting Information. The molecular structures of the ester-functionalized imidazolium- and pyridinium-based ionic liquids are given in Scheme 1.

2.2. Thermal Stability Measurements. Thermal stability of the ester-functionalized ILs was measured with a Mettler Toledo TGA/STGA 851 thermal gravimetric analyzer using a nitrogen atmosphere. All samples were run in aluminum pans by using a nitrogen flow rate of 20 mL/min. Thermograms were recorded using a heating rate of 10 °C/min from 25 to 550 °C.

Scheme 1. Structure of the Ester-Functionalized Imidazolium (C_nEmimBr)- and Pyridinium (C_nEPyrBr)-Based ILs



R= C_6H_{13}	C_6EMeImBr	C_6EPyrBr
R= C_8H_{17}	C_8EMeImBr	C_8EPyrBr
R= $C_{10}H_{21}$	C_{10}EMeImBr	C_{10}EPyrBr
R= $C_{12}H_{25}$	C_{12}EMeImBr	C_{12}EPyrBr
R= $C_{14}H_{29}$	C_{14}EMeImBr	C_{14}EPyrBr

2.3. Surface Tension Measurements. Surface tension measurements were made at 25 °C by the Wilhelmy plate technique using a Krüss K-12 tensiometer. Glass containers and plate were cleaned with chromic acid solution and rinsed thoroughly with distilled water. The plate was flame-dried before each measurement. Surface tension was considered to be at equilibrium when the standard deviation of five consecutive measurements did not exceed 0.10 mN/m.

2.4. Conductivity Measurements. Conductivity was measured at 25 °C using an Orion Conductivity Cell 913005MD with epoxy/graphite electrode in conjunction with a Thermo Orion 5 Star multiparameter instrument with a cell constant of 0.475 cm^{-1}.

2.5. Fluorescence Measurements. Steady-state fluorescence measurements were carried out with a Shidmadzu RF 540 spectrofluorometer equipped with a thermostated cell holder at 25 °C. Both excitation and emission band slits were fixed at 2 nm. All the data were acquired using quartz cells with 1 cm path length.

2.5.1. Steady-State Fluorescence Measurements for C_nEMeImBr. The fluorescence emission spectra of pyrene dissolved in C_nEMeImBr aqueous solutions were recorded from 340 to 450 nm after excitation at 332 nm. Pyrene exhibits fine structure in 370−400 nm region of the steady-state fluorescence emission spectra. The nature and the intensity are extremely dependent on the polarity of the environment. The ratio of the first to the third vibronic peaks, i.e., I_1/I_3, shows the greatest solvent dependency and, hence, can be used to probe the micropolarity of the aggregates and obtain the cmc of the long-chain imidazolium-based ILs in aqueous solution.[31,32]

2.5.2. Steady-State Fluorescence Measurements for C_nEPyrBr. The fluorescence probe pyrene was used in order to ascertain the onset of the aggregation of pyridinium-based ionic liquids in water.[21] The excitation wavelength used for pyrene-containing samples was 332 nm. The fluorescence was collected at 373 nm, the wavelength of the first vibronic emission band.

2.6. Antimicrobial Activity. Antimicrobial tests were carried out using bacteria and fungi which are stored in our laboratory. Microorganisms were *Micrococcus luteus* ATCC9341, *Staphylococcus epidermis* ATCC12228, *Staphylococcus aureus* ATCC5638, *Escherichia coli* ATCC27325, *Klebsiella pneumonia* ATCC13882, *Pseudomonas aeruginosa* ATCC9027, *Candida albicans* ATCC10231, and *Bacillus subtilis* ATCC6633. The antimicrobial activities were determined in vitro on the basis of the minimum inhibitory concentration (MIC) values[33] defined as the lowest concentration of antimicrobial agent that inhibits the development of visible growth after 24 h of incubation at 37 °C. The ionic liquids tested were dissolved in Mueller−Hinton broth (MBH) in the concentration range of 0.1−256 μg/mL, and no

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precipitate was observed at the highest concentration of the ILs. The MHB was prepared according to the manufacturer instructions. Then 10 μL of a nutrient broth starter culture of each bacterial strain was added to achieve final inoculums of ca. 5×10^{-4}–5×10^{-5} colony forming units per mL. The cultures were incubated overnight at 37 °C. Nutrient broth medium without the compound served as control. The growth of the microorganisms was determined visually after incubation for 24 h at 37 °C. The development of turbidity in an inoculated medium is a function of growth. A rise in turbidity reflects increases in both mass and cell number. Changes in turbidity were correlated with changes in cell numbers. All the experiments were performed in triplicate. The lowest concentration of antimicrobial agent at which no visible turbidity was observed was taken as the minimum inhibitory concentration.

3. RESULTS AND DISCUSSION

3.1. Thermal Stability. Decomposition temperatures of all the ester-functionalized ILs have been determined by TGA analysis. The characteristic thermal weight loss (TGA) curves for ester-containing imidazolium and pyridinium ILs in nitrogen atmosphere are given in the Supporting Information. The onset decomposition temperature is the intersection of the baseline weight and the tangent of the weight versus temperature curve as decomposition occurs.[34] Table 1 shows the onset temperatures for the ester-functionalized ILs investigated as well as comparisons to reported onset temperatures for nonfunctionalized ILs (1-alkyl-3-methylimidazolium and 1-alkylpyridinium bromides, C_nMeImBr and C_nPyrBr, respectively).

Table 1. Thermal Decomposition Temperatures (T_{onset}) of Ester-Functionalized ILs (C_nEMeImBr and C_nEPyrBr) and Simple Alkyl-Chain-Containing ILs (C_nMeImBr and C_nPyrBr)

	ionic liquid	T_{onset}/°C
ester-functionalized imidazolium ILs	C_6EMeImBr	210
	C_8EMeImBr	212
	C_{10}EMeImBr	220
	C_{12}EMeImBr	220
	C_{14}EMeImBr	228
ester-functionalized pyridinium ILs	C_6EPyrBr	158
	C_8EPyrBr	158
	C_{10}EPyrBr	162
	C_{12}EPyrBr	160
	C_{14}EPyrBr	162
nonfunctionalized imidazolium ILs	C_4MeImBr	273,[34] 278[35]
	C_8MeImBr	282[35]
nonfunctionalized pyridinium ILs	C_{12}PyrBr	259[36]

The onset of thermal degradation of the ester-contaning imidazolium-based ILs occurs at 210–228 °C, whereas that for the ester-containing pyridinium ILs the thermal degradation occurs at 158–162 °C. Therefore, the ester-functionalized imidazolium ILs exhibit substantially higher thermal stabilities than the ester-functionalized pyridinium compounds. The alkyl chain length does not significantly affect the thermal stability of the functionalized pyridinium ILs, but increasing the cation size produces a slight increase in the onset decomposition temperature of the imidazolium compounds. These results are in good agreement with those reported by Arellano[35] and Huddleston[37] about the effect of increasing the cation size on thermal stability of 1-alkyl-3-methylimidazolium salts.

Compared to simple alkyl-chain-containing ILs, the introduction of an ester group in the alkyl side chain results in a decrease of 50–70 °C in the decomposition onset temperature for the imidazolium-based ILs and about 100 °C for the pyridinium-based ILs (Table 1).

3.2. Self-Aggregation in Aqueous Solution. The aggregation properties of the ester-functionalized ionic liquids in aqueous media were determined by means of different experimental techniques involving conductivity, surface tension, and fluorescence. The results are summarized in Table 2.

3.2.1. Conductivity Measurements. The change of specific conductivity (κ) for aqueous solutions of C_nEMeImBr and C_nEPyrBr series as a function of the ionic liquid concentration was investigated. Representative results are displayed in Figure 1 for two of the homologues investigated.

The specific conductivity values fit into two straight lines of different slopes, and from the location of the abrupt change of slopes the corresponding value was derived for the cmc. In the low concentration range the raise of κ is due to the increase of free C_nEMeIm$^+$ and Br$^-$ ions for C_nEMeImBr series and C_nEPyr$^+$ and Br$^-$ for C_nEPyrBr series. Above the cmc the augmentation of the specific conductivity has a smaller slope because of two reasons: (i) the micelles can contribute to the charge transport to lesser extent than free ions owing to their

Table 2. Aggregation Parameters of 3-Methyl-1-alkyloxycarbonylmethylimidazolium Bromides (C_nEMeImBr) and 1-Alkyloxycarbonylmethylpyridinium Bromides (C_nEPyrBr) in Aqueous Solution at 25 °C Obtained by Different Techniques[a]

	conductivity			surface tension				fluorescence
ILs	cmc (mM)	β	ΔG°_{mic} (kJ/mol)	cmc (mM)	π_{cmc} (mN/m)	pC_{20}	A_{min} (nm^2)	cmc (mM)
C_6EMeImBr	195	0.30	−18.1	90	37	1.6	77	
C_8EMeImBr	77	0.59	−26.0	43	41	2.2	64	74
C_{10}EMeImBr	17	0.67	−33.5	12	44	2.3	61	16
C_{12}EMeImBr	3.8	0.72	−40.8	2.5	43	3.6	56	3.8
C_{14}EMeImBr	0.94	0.67	−45.6	0.90	47	4.3	46	1.0
C_6EPyrBr	200	0.30	−18.1	190	38	1.6	91	
C_8EPyrBr	73	0.57	−25.8	60	39	2.1	70	
C_{10}EPyrBr	17	0.64	−32.9	14	41	2.7	71	14
C_{12}EPyrBr	4.1	0.73	−40.7	3.4	43	3.5	64	3.4
C_{14}EPyrBr	0.91	0.74	−47.6	0.90	48	4.0	45	0.87

[a]Critical micelle concentration (cmc), degree of counterion association (β), Gibbs free energy of micellization (ΔG°_{mic}), adsorption effectiveness (π_{cmc}), adsorption efficiency (pC_{20}), and area per molecule at the interface (A_{min}) of the ester-functionalized imidazolium- and pyridinium-based ILs. The coefficient of variation of cmc values determined by conductivity, surface tension, and fluorescence were found to be ≤6%, ≤14%, and ≤8%, respectively.

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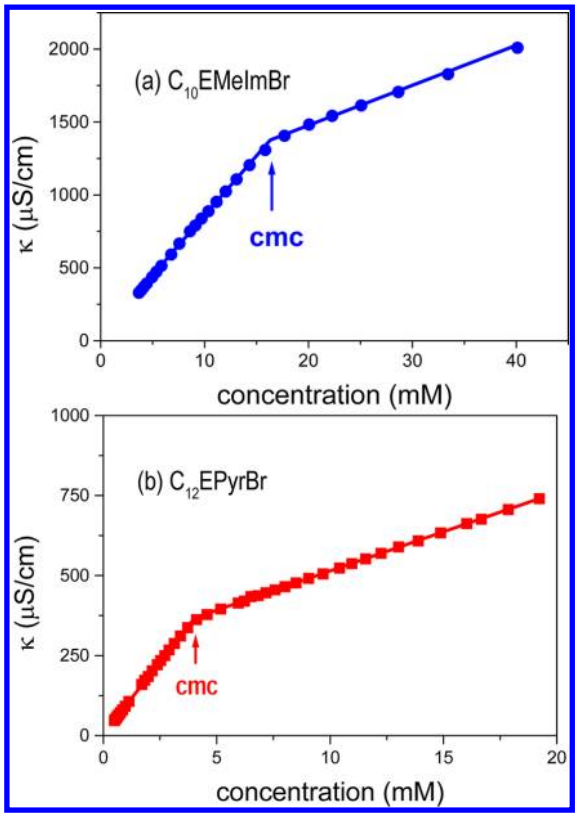

Figure 1. Specific conductivity versus ionic liquid concentration in water at 25 °C for C_{10}EMeImBr (a) and C_{12}EPyrBr (b).

lower mobility, and (ii) the binding of a fraction of the counterions to the micellar surface results in an effective loss of ionic charges.[22,38] The counterion binding parameter (β) gives the average number of counterions per surfactant ion in the micelle and can be estimated from the ratio of the slopes.[39] The values of the cmc and β obtained by a least-squares analysis are presented in Table 2.

A progressive cmc diminution when the number of carbon atoms of the alkyl chain increased was observed for both series of ester-functionalized ionic liquids (Table 2). This behavior is analogous to that described for different ionic surfactant families[40,41] as well as for nonfunctionalized imidazolium[20,22,42−44] and pyridinium-based ILs.[22,45] For these ester-containing imidazolium- and pyridinium-based ILs, there is a linear relationship between log cmc and the number of carbon atoms in the alkyl chain (Figure 2).

Ester-functionalized ILs show the characteristic slope values (0.28−0.30) reported for simple long-chain alkyl imidazolium[22,43,44] and pyrdinium[22] ILs and conventional cationic surfactants.[41]

One more CH_2 group in the alkyl tail lowers the cmc by about 0.3. It also means that one more CH_2 group approximately halves the cmc values of the corresponding ionic liquid. The increment of one CH_2 group to −log cmc is proportional to the free energy of the transfer of this group from water to the micellar core. The free energy change involved in transferring a methylene unit of the hydrocarbon chain from the aqueous environment to a micelle or aggregate was calculated from the slope of the linear correlation between log cmc and n,[41] obtaining a value of −1.71 kJ/mol for both series of ester-functionalized ILs. This value is very similar to those reported for n-alkyl ionic surfactants with a single ionic

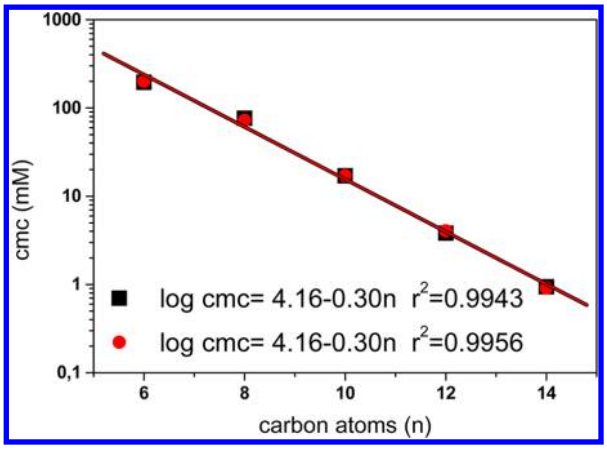

Figure 2. Effect of the alkyl chain length on the cmc of 3-methyl-1-alkyloxycarbonylmethylimidazolium (■) and 1-alkyloxycarbonylmethylpyridinium bromides (●) in aqueous solution at 25 °C.

head[41] and also to the reported for simple alkyl chain containing imidazolium- and pyridinium-based ILs 1.83 and −1.77 kJ/mol, respectively.[22]

Comparing the two series of ester-functionalized ILs, C_nEMeImBr and C_nEPyrBr, it can be observed (Table 2) that very similar cmc values are obtained for the homologues with the same alkyl chain length. It suggested that both polar head groups possess a very similar hydrophobic character that does not affect the tendency to form micelles as already reported for simple alkyl-chain-containing imidazolium and pyridinium ILs.[22]

Imidazolium- and pyridinium-based ionic liquids with an ester moiety possess lower cmc values (Table 2) than the corresponding nonfunctionalized homologues.[22] Thus, ester-containing imidazolium and pyridinium ILs exhibit a 2−3-fold decrease in cmc values compared to the simple alkyl-chain-containing ILs. The introduction of an ester functional group in the hydrophobic chain close to the polar headgroup leads to a reduction in cmc values that could be attributed to the increased H-bonding in the headgroup region.[46,47]

The values of the counterion binding parameter β increased with increasing the alkyl chain length from C_6 to C_{14} similarly to that observed for nonfunctionalized ILs.[19,20,22,32] Thus, the counterion is stronger bonded to the aggregate as its alkyl chain becomes longer. The similarity between the values of the β parameter for C_nEMeImBr, C_nEPyrBr (Table 2), C_nMeImBr,[22,48] and C_nPyrBr[22] suggests that the counterion binding degree to the aggregate does not depend significantly on the nature of the cationic group but on the length of the alkyl chain bonded to the cationic group. The micelle formation of the ionic liquids in aqueous solution is a spontaneous process, and it means that the free energy change of this phenomenon is negative. Applying the phase separation model to the monomer−micelle equilibrium for cationic amphiphile, the standard Gibbs energy of micellization (ΔG°_{mic}) can be calculated from the equation[49]

$$\Delta G^\circ_{mic} = (2 - \beta)RT \ln x_{cmc}$$

where β is the ionization degree and x_{cmc} is the critical micellar concentration expressed as mole fraction. ΔG°_{mic} indicates the free energy difference per mole between molecules in water and in micelles and also the free energy of transfer 1 mol of IL from the aqueous phase to micellar pseudophase. The values of ΔG°_{mic} calculated for the ILs are given in Table 2. These values

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are negative and give evidence that the micelle formation in aqueous solution of the ester-functionalized ionic liquids investigated is a spontaneous process. As observed, the longer the alkyl chain length, the more negative the free energy of Gibbs, indicating that the aggregation process takes place easier with the increase of the alkyl chain length.

3.2.2. Surface Tension Measurements. The plot of surface tension (γ) versus the logarithm of the ionic liquid concentration for the C_nEMeImBr and C_nEPyrBr series is shown in Figure 3. Critical micelle concentration (cmc),

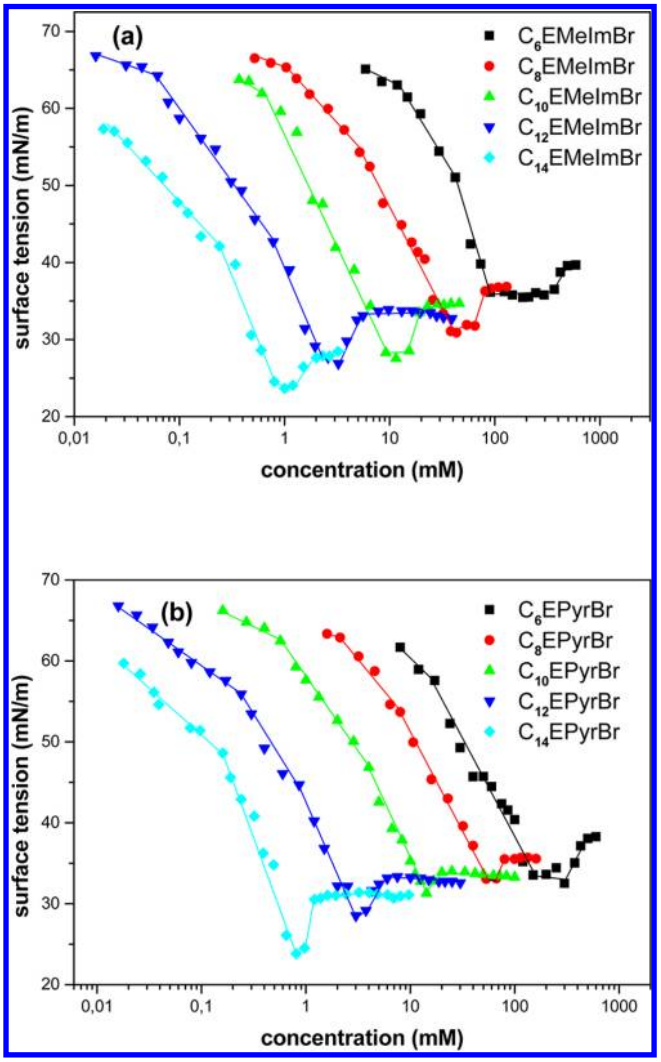

Figure 3. Surface tension as a function of concentration for ester-functionalized imidazolium (a) and ester-functionalized pyridinium (b) ILs.

adsorption efficiency adsorption effectiveness (π_{cmc}), and area per molecule residing at the surface (A_{min}) (Table 2) were obtained from the surface tension isotherms displayed in Figure 3.

The surface tension decreases with increasing the IL concentration as a consequence of the adsorption on the air/liquid interface of the C_nEMeImBr and C_nEPyrBr compounds. For most of the C_nEMeImBr and C_nEPyrBr salts a minimum of surface tension appears before attaining a plateau. This is a common behavior of classical ionic or nonionic surfactants containing a certain amount of impurities that adsorb strongly in the liquid/air interface,[41] and for these compounds the minimum can be easily reduced or eliminated by purification. However, in the case of the ionic liquids investigated, the additional steps of purification did not lead to a significant reduction of the surface tension minimum. The presence of a minimum on the surface tension curve was already reported by other authors for simple alkyl-chain-containing ILs (1-alkyl-3-methylimidazolium salts with alkyl chains of 8 and 10 carbon atoms[17,22,50−52] and for 1-alkylpyridinium[22] with the same alkyl chain lengths). Goodchild et al.[50] tentatively attributed the presence of a minimum on the surface pressure curve to the formation of surface micelles prior to bulk aggregation and the re-establishment of a surface monolayer at concentrations greater than the cmc. This phenomena coinciding with aggregation may lower the accuracy of the aggregation onset determination. In this work the concentration at which the minimum appeared was assigned to the cmc value.

The cmc values of ester-containing ILs decrease with increasing the alkyl chain length for both C_nEMeImBr and C_nEPyrBr series. The cmc decreases by increasing the hydrophobicity which makes the ionic liquids with longer alkyl chain more surface active.

From the surface tension plots, two additional parameters, the effectiveness of surfactant to decrease surface tension of solvent (π_{cmc}) and the adsorption efficiency (pC_{20}), and can also be estimated.[41] π_{cmc} can be determined as follows:

$$\pi_{cmc} = \gamma_0 - \gamma_{cmc}$$

where γ_0 is the surface tension of pure solvent and γ_{cmc} is the surface tension at cmc. The pC_{20} parameter is determined by using the following equation:

$$pC_{20} = -\log C_{20}$$

where C_{20} is the concentration of surfactant to reduce the surface tension of pure solvent by 20 mN/m. The negative logarithm of the concentration of surfactant in the bulk phase required to produce a 20 mN/m reduction in the surface tension of the solvent is a convenient measure of the efficiency of adsorption of the surfactant; i.e., it is close to the minimum concentration needed to produce saturation adsorption at the interface. The larger the value of pC_{20}, the more efficiently the surfactant is adsorbed at the interface and the more efficiently it reduces surface tension.[18,50]

The π_{cmc} and pC_{20} values of the ester-functionalized ILs are displayed in Table 2. The data show that, as a function of increasing alkyl chain length, both the effectiveness (π_{cmc}) and the adsorption efficiency (pC_{20}) increase and that, at the same alky chain length, π_{cmc} and pC_{20} parameters of the ester−imidazolium and ester−pyridinium homologues are very similar.

Comparing the efficiency of adsorption and the effectiveness of the ester-functionalized ILs to the simple alkyl-chain-containing ILs,[22,32] it is clear that the introduction of an ester group improves the surface activity of the IL. As compared with COOH-functionalized imidazolium-based ILs,[23] the ester derivatives also possess a superior ability to reduce the surface tension of pure water.

The efficiency of adsorption (pC_{20}) of the two series of ester-functionalized ILs increases linearly with the increase in the number of carbon atoms in the alkyl side chain (see graph of pC_{20} versus alkyl chain length in the Supporting Information), reflecting the negative free energy of adsorption of a methylene group at the interfaces. From these linear relationships standard free energy values associated with the transfer of a methylene

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group of −1.93 and −1.77 kJ/mol can be calculated for the $C_nEMeImBr$ and $C_nEPyrBr$ series.

The Gibbs adsorption isotherm equation was applied to the surface tension versus concentration data to estimate the average area per molecule residing at the surface (A_{min}).[41] As not correction for nonideality has been made despite the high ionic strengths of the solution near the cmc, the A_{min} values must be considered as rough estimates. The resulting A_{min} are given in Table 2. As expected, the A_{min} decreases as a function of increasing the length of the alkyl chain due to concomitant closer packing of monomer at the interface.

At the same chain length, the A_{min} values found for the ester-containing ILs are lower than those reported for simple alkyl-chain containing ILs $C_nMeImBr$[18,23,32] and for COOH-functionalized ILs,[23] implying a more compact arrangement for the molecules at the air/water interface.

The relative values of the A_{min} can be used to estimate aggregates shapes near the cmc. An approach of the shape of the aggregate may be obtained from geometric arguments using the Israelachvilís ratio

$$f = v/la$$

where v is the tail group volume, a is the headgroup area, and l is the tail group chain length of the amphiphile.[53] If $f < 1/3$, spherical micelles are expected. Using the A_{min} values determined from the surface tension data (Table 2) and the values of l and v established using the Tanford formula,[54] we found values of f lower than 1/3 for all the homologues studied. Therefore, at concentrations close to the cmc, spherical aggregates may be expected for all the ionic liquids investigated.

As discussed above, the presence of an ester linkage between the cationic headgroup and the hydrophobic chain of the imidazolium- and pyridinium-based ILs affects their micellization as well as their adsorption at the aqueous solution−air interface. In order to evaluate the relative effect of the ester functional group on these two processes, the cmc/C_{20} ratio[41] was calculated. The introduction of an ester group lead to a significant increase in the cmc/C_{20} ratio as compared to this ratio for single alkyl chain substituent homologues,[18] which indicates that for ester-functionalized ILs adsorption is facilitated more than micellization probably due to the introduction of a larger hydrophilic group.

3.2.3. Fluorescence Measurements. Steady-state fluorescence measurements using pyrene as the solvatochromic probe were applied to study the micelle aggregation behavior of these ester-functionalized long-chain imidazolium- and pyridinium-based ILs in aqueous solution although different protocols were applied to imidazolium and pyridinium salts.

Imidazolium-Based Ionic Liquids: The intensity ratio of the first to the third vibronic peaks of pyrene, i.e. I_1/I_3, was measured as a function of the IL concentrations. Results are showed in Figure 4. The abrupt sigmoidal decrease in I_1/I_3 intensity indicates the formation of IL aggregates and the preferential residence of pyrene molecules in the more hydrophobic environment of the micelles relative to water. The cmc values were taken as the concentration that corresponds to the intersection between the linear extrapolation of the relative stabilized portion corresponding to low IL concentrations and the abruptly varied portion of the curve. The cmc values obtained are shown in Table 2.

Clearly these values derived from fluorescence measurements are in good agreement with those obtained from the rest of techniques (Table 2), especially with those obtained by conductivity.

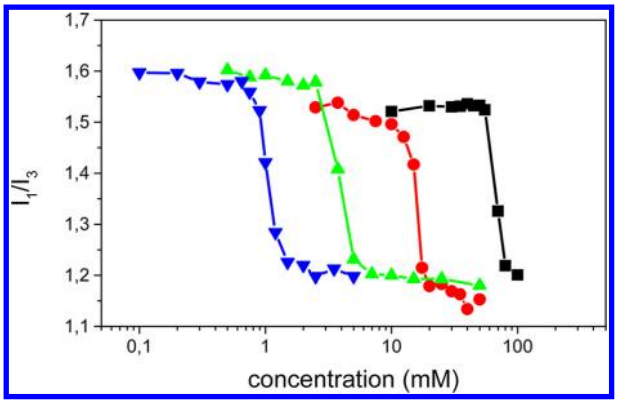

Figure 4. Variation of the I_1/I_3 ratio with the IL concentration for $C_8EMeImBr$ (■), $C_{10}EMeImBr$ (●), $C_{12}EMeImBr$ (▲), and $C_{14}EMeImBr$ (▼) in aqueous solution at 25 °C.

Pyridinium-Based Ionic Liquids: The comparison of intensities of the first and the third vibronic bands of the pyrene emission spectrum (the polarity index I_1/I_3 of pyrene) cannot be applied to the determination of the cmc of $C_nEPyrBr$ ionic liquids because the fluorescence of the pyrene is quenched by the ionic liquid itself.[21] The aggregation behavior of the 1-alkyloxycarbonylmethylpyridinium bromides was studied by the method proposed by Blesic et al.[21] to determine the cmc of 1-alkyl-3-methylpyridinium chlorides. The onset of micellization of pyridinium-based ionic liquids studied can be determined as a pronounced break-point in the plot of I_0/I (fluorescence intensity in the absence, I_0, and presence, I, of ionic liquid) versus the concentration of ionic liquid (Figure 5). The cmc of C_6 and C_8 cannot be determined by this method because the cmc of these ionic liquids is higher than the concentration at which complete quenching of pyrene occurs.

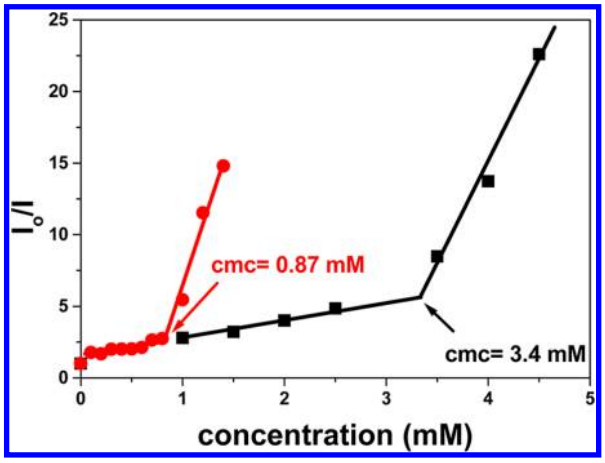

Figure 5. Monitoring the self-aggregation of ester-functionalized pyridinium-based ILs in aqueous solution: $C_{12}EPyr$ Br (■) and $C_{14}EPyrBr$ (●).

With the limit of cmc quenching this method provides results that are in good agreement with those obtained from other well-established conductivity and surface tension techniques (Table 2).

3.3. Antimicrobial Activity. Seven strains of bacteria, four Gram-positive and three Gram-negative, and one strain of fungi

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Table 3. MICs Values for 3-Methyl-1-alkyloxycarbonylmethylimidazolium Bromides (C_nEMeImBr), 1-Alkyloxycarbonylmethylpyridinium Bromides (C_nEPyrBr), 1-Alkyl-3-methylimidazolium Bromides (C_nMeImBr), 1-Alkylpyridinium Bromides (C_nPyrBr), and Hexadecyltrimethylammonium Bromide (HTAB)[a]

	MIC* (μM)							
	Gram-positive cocci			Gram-negative rods			fungi	bacillus
compound	*M. luteus*	*S. epidermidis*	*S. aureus*	*E. coli*	*K.pneumoniae*	*P. aeruginosa*	*C. albicans*	*B. subtilis*
C_6EMeImBr	R	R	R	R	R	R	R	R
C_8EMeImBr	R	R	R	R	R	R	R	R
C_{10}EMeImBr	354	89	177	709	354	709	354	177
C_{12}EMeImBr	82	10	82	164	82	R	329	41
C_{14}EMeImBr	615	154	154	615	308	R	308	615
C_6EPyrBr	R	R	R	R	R	R	R	R
C_8EPyrBr	R	R	R	R	R	R	R	R
C_{10}EPyrBr	357	89	179	714	357	R	357	179
C_{12}EPyrBr	166	28	83	166	166	R	83	83
C_{14}EPyrBr	77	39	39	618	309	R	154	77
C_{10}MeImBr[22]	R	844	106	R	R	R	R	422
C_{12}MeImBr[22]	R	193	97	386	773	R	R	48
C_{14}MeImBr[22]	178	6	45	356	356	356	178	6
C_{10}PyrBr[22]	R	428	428	428	R	R	R	428
C_{12}PyrBr[22]	R	49	195	97	780	780	R	24
C_{14}PyrBr[22]	90	6	22	45	359	359	359	6
HTAB[55]	44	10		44	44			44

[a]MIC*: the lowest concentration of compound at which the microorganism tested does not show visible growth. R: resistant microorganism at the highest concentration tested (256 mg/L).

were used to assess the antimicrobial activity of the ILs investigated. The concentrations tested ranged from 0 to 256 mg/L. Minimum inhibitory concentration (MIC) values determined for imidazolium and pyridinium ILs containing an ester functional group are summarized in Table 3. For the sake of comparison, the MICs corresponding to the simple alkyl-chain-substituted imidazolium and pyridinium ILs, as well as the MICs values corresponding to the hexadecyltrimethylammonium bromide (HTAB), a classical antimicrobial surfactant agent, have been included.

From the MIC values obtained (Table 3) it is obvious that the antimicrobial activity of the imidazolium- and pyridinium-based ionic liquids with an ester moiety depends on the alkyl chain length as also reported for cationic surfactants[25,56−58] and other ionic liquids.[22,26,27] The shorter homologues of each series tested (C_6−C_8EmimBr and C_6−C_8EPyrBr) showed low activity (MIC ≥ 256 mg/L) against all the microorganisms tested. Ester-containing imidazolium and pyridinium salts with more than eight carbon atoms in the alkyl chain showed biological activity against a wide range of Gram-positive microorganisms. These C_{10}, C_{12}, and C_{14} homologous also exhibited activity against most of Gram-negative bacteria, although their efficiency is higher against Gram-positive bacteria. In addition, C_{10}−C_{14} homologues with an ester linkage in the hydrocarbon side chain showed significant antifungal activity.

Compared to simple alkyl-chain-containing ILs, the introduction of an ester group in the alkyl chain close to the cationic core leads to a significant increase of the antimicrobial activity for C_{10}−C_{12} homologues, whereas C_{14} homologues show similar or slightly lower biological activity (Table 3). It is noteworthy that the antifungal activity increased significantly by introducing an ester group in the hydrophobic side chain of the imidazolium and pyridinium ionic liquids as compared to nonfunctionalized ILs. The effect of the ester moiety on increasing the antifungal efficacy of both imidazolium- and pyridinium-based ILs is consistent with data reported by Kanjilal et al.[46] on antimicrobial activity of some ester-containing imidazolium salts. When compared with ILs containing other functional groups, these ester-functionalized ILs resulted to be slightly more active as antimicrobial agents than 1-alkyloxymethyl-3-methylimidazolium salts[26] and clearly much more active than imidazolium derivatives with polyether functional groups in the side chain.[29] Finally, compared with the classical quaternary ammonium surfactant HTAB (Table 3), these ester-functionalized ILs show a high to moderate activity level against Gram-positive and Gram-negative bacteria, respectively.

As discussed above, the elongation of the alkyl substituent increases the biological activity of the ester-functionalized ILs. However, for most of the microorganism strains the MIC values displayed a minimum with increasing alkyl chain length. Thus, ester-containing ionic liquids with an alkyl chain length of 12 carbon atoms showed the highest efficiency as antimicrobial agents. The biological activity decreased or remained practically constant for ester-functionalized C_{14} homologues. The optimum biological effect at a specific chain length can be attributed to the combination of several physicochemical parameters: hydrophobicity, adsorption, cmc, aqueous solubility, and transport in the test medium, the solubility being the limiting step for the transport.[13,59] For the ILs here investigated, the cooperative interaction of these variables determined that the homologous of 12 carbon atoms have the largest tendency to be adsorbed at the bacterial/water interface and therefore exert their antimicrobial action at the lowest concentrations. The optimum efficacy at a certain chain length is consistent with data reported in the literature concerning the biological activity of long-chain amphiphilic ionic liquids.[26] Thus, maximum efficiency as antimicrobial agents was reported for alkylimidazolium and alkyloxyimidazolium lactates containing 11 or 12 carbon atoms in the alkyl group,[26] for alkylimidazolium salts with 12 or 14 carbon atoms

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in the alkyl chain,[22,27] and for alkylpyridinium salts with 14 carbon atoms in the alkyl chain.[22] On the other hand, it is noteworthy that the nature of the hydrophilic group, imidazolium- or pyridinium-based cations, has not a significant effect on their biological activity (Table 3). This corroborates that the hydrophilic characters of both polar head groups are very similar as previously suggested by the study of the surface properties for both functionalized IL series (Table 2).

Kopecky[25] reported that the antimicrobial activity of QACs was closely related to their surfactant properties. Because of their amphiphilic nature, the ester-functionalized ILs here investigated exhibit many characteristic features of cationic surfactants. As reported for QACs[25] and for single-alkyl-chain-containing ILs,[22,26] the ester−imidazolium and ester−pyridinium derivatives with and alkyl chain below a certain length, and thus weak surfactant properties, were found to be ineffective as antimicrobial agents. The increase of biological activity observed for the C_{10}−C_{12} ester-functionalized homologues as compared to simple alkyl-chain-containing ILs (Table 3) could be attributed to the increase in surface activity, resulting in introducing an ester functional group in the hydrophobic side chain (Table 2). However, for C_{14} ester derivatives the elongation of the chain length did not lead to an increase in their biological efficacy. As suggested by Luczak et al.,[13] the micellization process could be a reasonable explanation for the cutoff effect on biological activity observed as a function of the alkyl chain length for ester-containing ILs. Thus, although C_{14} ester derivatives exhibit better surface properties than the shorter ester-derivative homologues (Table 2) and the corresponding nonfunctionalized C_{14}-homologues,[22] their low cmc values (which can even be lower in salty systems[18,24,44] as the aqueous medium of the MIC tests) led to the formation of aggregates at very low concentrations, resulting in a decrease of the compound concentration at the site of action as well as in its permeation ability.[13]

4. CONCLUSIONS

The introduction of an ester group in the alkyl tail attached to the polar headgroup leads to ionic liquids with higher surface activity as compared to simple alkyl-chain-containing ILs and to conventional cationic surfactants like alkyltrimethylammonium compounds. In the homologous series of ionic liquids investigated the tendency to micellize increases and cmc decreases regularly with the length of the hydrophobic alkyl chain. Regarding thermal stability, ester-functionalized ILs present lower decomposition temperatures than ILs without an ester moiety in the alkyl side chain. The ester-functionalized ILs display antimicrobial activity, and their efficacy as antimicrobial agents depends on the alkyl chain length. The compounds with short alkyl chains are not active against bacteria and fungi, whereas the ILs containing from 10 to 14 carbon atoms in the alkyl chain show significant antimicrobial activity. The C_{12} homologous are the most active compounds. Imidazolium and pyridinium derivatives with an ester linkage show similar or slightly higher antimicrobial activity against Gram-positive and Gram-negative microorganisms and display a significant increase of antifungal activity as compared to simple alkyl-chain-containing ILs. The antimicrobial activity of ester-functionalized ILs against Gram-positive microorganisms was similar to that shown by the cationic surfactant cetyltrimethylammonium chloride, a classical antimicrobial agent.

This study shows that the introduction of a hydrolytically cleavable ester functionality in the hydrophobic alkyl side chain improves not only IL biodegradation but also surface and biological activities of ILs. The better understanding of the structural parameters affecting self-aggregation and biological activity of the long chain ionic liquids described in this work is expected to aid in the design and selection of ionic liquids with improved physicochemical and biological properties for new pharmaceutical, engineering, or nanotechnology applications as well as for the environmental fate assessment of these compounds since the interfacial phenomena play a crucial role in the biodegradation processes.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Synthesis procedures and characterization of alkyl bromoacetates (^{1}H NMR, ^{13}C NMR) and ionic liquids (^{1}H NMR, ^{13}C NMR, mass spectroscopic analysis, ESI-MS data, elemental analysis); characteristic thermal weight loss (TGA) curves for C_nEMeImBr and C_nEPyrBr and graphs of adsorption efficiency versus alkyl chain length for C_nEMeImBr and C_nEPyrBr. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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