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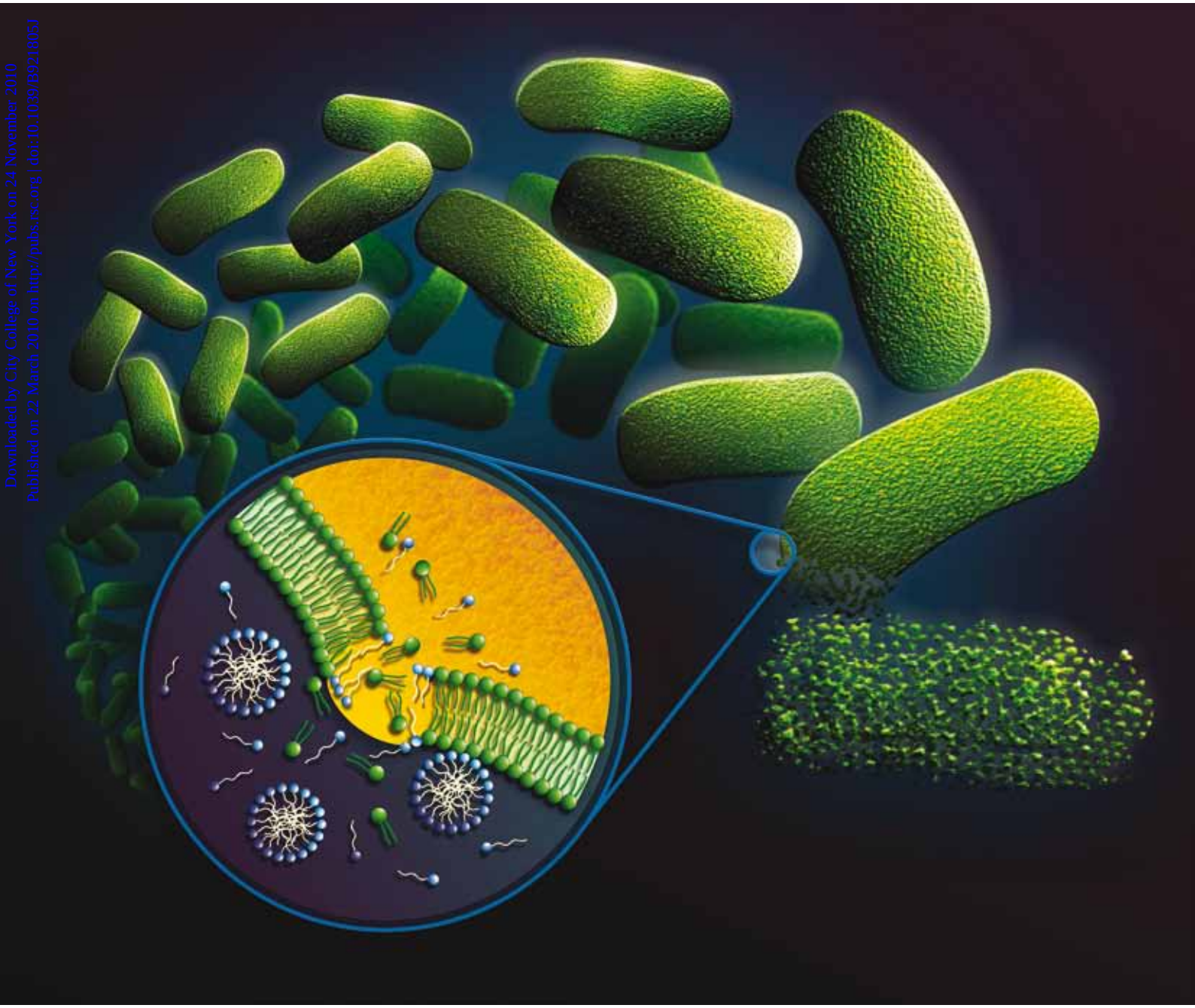

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Antimicrobial and surface activity of 1-alkyl-3-methylimidazolium derivatives

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Knowledge of the structure–activity relationship (SAR) allows for the possibility to design and synthesize new cationic amphiphiles with optimized antimicrobial activities for future development of new disinfectants, sanitizers or preservatives. The need to design and identify new compounds, possessing antimicrobial properties, results from the emergence of more resistant micro-organisms in our globalized society. Hitherto, most studies which analyse the biological activity of ionic liquids (ILs) investigate the effect of the cation, whereas the knowledge of the effect of the anion is limited. The present study confirms the existence of a strong relationship among structure, surface activity and biological action of imidazolium ionic liquids on bacteria and fungi. The dependence of the antimicrobial activity on chemical structure–chain length and anion type of 30 compounds was determined. The anion is an important structural element which partakes in the definition of the phyiscochemical properties of the IL, and in consequence the technological applications and mode of action of the compound. The introduction of a longer substituent on the imidazolium cation results in a lower minimal inhibitory concentration (MIC). Thus, antifungal and antibacterial activities were found to increase with chain length, very often up to a point exhibiting a cut-off effect at chain lengths of 16 or 18 for the imidazolium cation and the [Cl] anion. The efficiency of surface tension reduction circumscribed by the pC_{20} and the relationship between antimicrobial activity and pC_{20} is described herein. The relationship indicates an antimicrobial mode of action dependant on the surface activity of the molecule, inferring that surface activity may contribute to the cut-off effect in the biological activity of ILs.

Introduction

The properties of certain ionic liquids (ILs) such as high extractive selectivity, negligible volatility, inflammability, thermal stability *etc.* allow ILs to play a promising role as alternative media in diverse areas. These can include synthesis and catalytic chemistry,[1] separation[2] and electrochemical[3] processes, and combined reaction-separation processes. One of the main aspects gaining attention in IL 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 fulfil the technological demands of a variety of applications.

In previous research, it has been shown that the toxicity is not only comparable to common molecular solvents, but also to highly toxic biocides. The structure–activity relationships (SARs) of ILs have indicated in almost all investigated test-systems (*in vitro* assays,[4,5] organism studies comprising *e.g.* bacteria,[6] earthworms,[7] water fleas,[8,9] zebrafish,[10] and algae[11]) that the length of the side chain is the most significant indicator of biological activity.

A number of studies were published which analyse also the microbial inhibition of ILs. Demberelnyamba *et al.* described antimicrobial activity of nine ILs from the imidazolium and pyrrolidinonium families against seven strains of bacteria and fungi.[12] The antimicrobial effects of butyl-, hexyl- and octylimidazolium and pyridinium bromide ILs on the growth of a group of microorganisms (*Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas fluorescens* and *Saccharomyces cerevisiae*) was investigated by Docherty *et al.*[13] Both groups presented biological activity dependence on the chain length in the investigated cations of ILs. Cieniecka-Roslonkiewicz *et al.* tested a broad range of phosphonium ILs with diverse anions, revealing that the biological activity of phosphonium salts depend on both cation structure and anion type. Replacement of halide anions with other, more complicated, moieties was found to decrease the antimicrobial properties.[14] Pernak *et al.* established the correlation between MIC (minimal inhibitory concentration) and hydrophobicity (octanol–water partition coefficient) of *N*-alkoxymethylpyridinium and alkylthiomethylimidazolium derivatives,[6,15] where it was found that the higher

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the hydrophobicity of a surface-active compound, the lower the MIC. Carson *et al.* investigated the antimicrobial and antibiofilm activity of 1-alkyl-3-methylimidazolium chlorides, against 11 microbial pathogens.[16]

In general, it was shown that compounds with short functionalised side chains have weaker biological efficacy than those with long apolar alkyl chains in all organisms and strains tested. Moreover, an increase in the number of alkyl groups substituted on the cation ring resulted in an elevated biological effect. It should be mentioned that limited studies were performed that discuss the systematic effect of the anion.

Moreover, in a number of previous studies the relationship between hydrophobicity of the cation[15,17-20] and biological effect has been described. The hydrophobicity is applied as a measure to estimate interaction potentials with membranes. However, for amphiphilic compounds the additional descriptor of the surface activity provides a more thorough picture of the interaction potential.[21-23]

The latest findings show that imidazolium derivatives with long alkyl chains behave as amphiphilic compounds, displaying surface activity similar to quaternary ammonium compounds (QACs).[24-26] The cationic headgroup of ILs may contain a charged ammonium and one or more hydrophobic 'tails', and thus may find comparable applications to QACs. Surface-active compounds (surfactants) decrease surface activity of solutions and self-assemble, forming aggregates with a number of shapes and sizes depending on the molecular structure of the surfactant, its concentration, solution temperature *etc.*[27] A variety of cationic surfactants with long alkyl chains are used to control microbes as a component of disinfectant, sanitizer and preservative products, among others.[28]

The aim of the present paper is to investigate the microbial inhibition of imidazolium derivatives with a variety of alkyl side chain lengths and anion types. Due to their unique tunability, ILs allow us to create a SAR which takes into account the structural elements of the cations and anions, and correlates these with the antimicrobial activity. To this end, a number of microbial inhibition tests for a variety of imidazolium ILs with diverse chain lengths and anions were carried out. We investigated 30 compounds on 13 microbial strains, including bacterial and fungal species. In addition, for the first time, the efficiency of surface tension reduction (pC_{20}) for these ILs was correlated with the MIC to quantify the surface activity of these compounds.

Results and discussion

Antimicrobial activity

Biological activities of a variety of imidazolium ionic liquids (shown in Table 1) were estimated using Gram-positive and Gram-negative bacteria as well as fungi. The strains used in this study are regarded as omnipresent and commonly occurring in industrial processes or health services. Table 2 and 3 present calculated average MICs of bacteria and fungi respectively. All ionic liquids applied in the experiment exhibited biological activity against all microorganisms used. Relevant MIC values were related to the number of carbon atoms of ILs, as presented in Fig. 1 and Fig. 2.

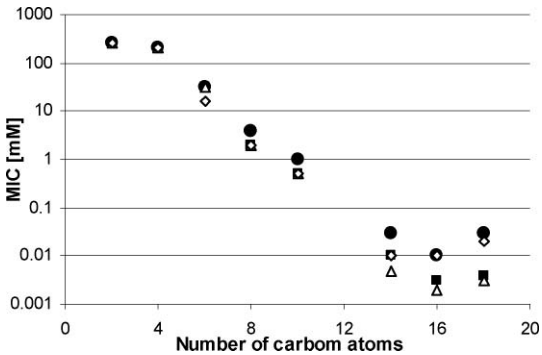


Fig. 1 Relationship between antibacterial activities of 1-alkyl-3-methylimidazolium chlorides and the alkyl chain length of the imidazolium cation: (●) *E. coli*, (◇) *S. aureus*, (■) *E. faecium*, (△) *E. hirae*.

The results demonstrate that imidazolium salts are active against bacteria and fungi. Short-chained ILs exhibit weak biological activity compared to the strong, wide-ranging antimicrobial activity of long-chained compounds. Our results were in agreement with those published by Carson *et al.*,[16] Pernak *et al.* [29] (for alkoxy methylimidazolium compounds), and Demberelnyamba *et al.*[12] However, the range of micro-organisms is much greater than those tested by other researchers.

The alkyl chain length dependency of the biological effect was observed in both bacteria and fungi. An increase of the chain length results in a decrease of the MICs, meaning that elongation of the alkyl substituent strongly increases antimicrobial activity of the ILs. The set of long-chain 1-alkyl-3-methylimidazolium salts were the most efficient biocides among these tested. Highly

Table 1 Abbreviations and names of all investigated ions

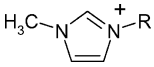
Cation		Anion	
			
		X^-	
[EMIM]	1-Ethyl-3-methylimidazolium	[Cl]	Chloride
[BMIM]	1-Butyl-3-methylimidazolium	[BF_4]	Tetrafluoroborate
[HMIM]	1-Hexyl-3-methylimidazolium	[$MeOSO_3$]	Methyl sulfate
[OMIM]	1-Methyl-3-octylimidazolium	[$OctOSO_3$]	Octyl sulfate
[DMIM]	1-Decyl-3-methylimidazolium	[Tf_2N]	Bis(trifluoromethylsulfonyl)imide
[TDMIM]	1-Methyl-3-tetradecylimidazolium	[TFMS]	Trifluoromethanesulfonate
[HDMIM]	1-Hexadecyl-3-methylimidazolium	[*p*Ts]	*p*-Toluenesulfonate
[ODMIM]	1-Methyl-3-octadecylimidazolium		

Table 2 Antibacterial activities of imidazolium ionic liquids measured as MICs [mM]

	Gram-negative bacteria	Gram-positive bacteria		
Ionic liquid	*Escherichia coli* ATCC 10231	*Staphylococcus aureus* ATCC 9763	*Enterococcus faecium* ATCC 6057	*Enterococcus hirae* ATCC 10541
[BIM]	14.7	21.9	58.4	58.4
[EMIM][Cl]	261.8	261.8	261.8	261.8
[BMIM][Cl]	207.5	207.5	207.5	207.5
[HMIM][Cl]	31.5	15.6	31.2	31.2
[OMIM][Cl]	4	2	2	2
[DMIM][Cl]	1	0.5	0.5	0.5
[TDMIM][Cl]	0.03	0.01	0.01	0.001
[HDMIM][Cl]	0.01	0.01	0.003	0.003
[ODMIM][Cl]	0.03	0.02	0.004	0.002
[Na][BF_4]	31	15.3	15.3	7.7
[EMIM][BF_4]	31.8	30.6	61.2	15.3
[BMIM][BF_4]	15.7	15.7	15.6	15.6
[HMIM][BF_4]	7.8	7.8	7.8	3.9
[OMIM][BF_4]	4.4	2.1	3.9	3.9
[DMIM][BF_4]	2.1	0.8	0.5	0.8
[Na][$MeSO_4$]	15.6	11.6	15.5	15.5
[EMIM][$MeOSO_3$]	267.5	267.5	267.5	267.5
[BMIM][$MeOSO_3$]	123.6	62.5	62.5	62.5
[BMIM][Tf_2N]	2	>2	0.5	0.5
[Na][TFMS]	217.1	>217.1	212.8	212.8
[BMIM][TFMS]	62.63	62.5	31.2	62.5
[OMIM][TFMS]	3.8	1.9	1.5	1.5
[Na][$OctOSO_3$]	14.6	7.2	7.2	7.2
[EMIM][$OctOSO_3$]	30.1	22.3	29.8	14.9
[BMIM][$OctOSO_3$]	31.3	11.5	15.7	15.7
[OMIM][$OctOSO_3$]	4	2	1	2
[BMIM][*p*Ts]	250	125	125	125

Table 3 Antifungal activities of imidazolium ionic liquids measured as MIC [mM]

	Eumycota							
	Ascomycetes							Basidiomycetes
Ionic liquid	*Candida albicans* ATCC 10231	*Candida glabrata* DSM 11226	*Candida tropicalis* KKP 334	*Saccharomyces cerevisiae* ATCC 9763	*Saccharomyces cerevisiae* JG	*Saccharomyces cerevisiae* JG CDR1	*Geotrichum candidum*	*Rhodotorula mucilaginosa*
[MIM]	66	66	66	33	66	66	66	66
[BIM]	11	14.7	14.7	14.7	11	11	14.6	14.6
[EMIM][Cl]	>261.8	261.8	261.8	261.8	261.8	261.8	261.8	261.8
[BMIM][Cl]	207.5	207.5	207.5	207.5	207.5	207.5	207.5	207.5
[HMIM][Cl]	31.2	7.8	15.6	7.8	15.6	15.6	15.6	4
[OMIM][Cl]	2	1	2	0.5	0.5	1	0.5	2
[DMIM][Cl]	0.5	0.5	0.5	0.2	0.1	0.5	0.1	0.5
[TDMIM][Cl]	0.06	0.06	0.2	0.03	0.03	0.06	0.003	0.015
[HDMIM][Cl]	0.02	0.02	0.12	0.02	0.02	0.02	0.0015	0.0015
[ODMIM][Cl]	0.01	0.004	0.004	0.01	0.004	0.004	0.004	0.01
[Na][BF_4]	24.6	123.85	61.95	31	15.5	31	23	7.7
[EMIM][BF_4]	49.3	247.5	124	61.95	31	31	45.9	7.7
[BMIM][BF_4]	62.5	125	62.5	31.25	31.25	31.25	15.63	15.63
[HMIM][BF_4]	31.4	15.7	31.4	15.7	15.7	15.7	7.8	7.8
[OMIM][BF_4]	4.3	4.3	4.3	4.3	4.3	4.3	4.3	1.6
[DMIM][BF_4]	2.1	2.1	2.1	2.1	2.1	2.1	3.2	1.6
[Na][$MeOSO_3$]	61.8	61.8	61.8	61.8	15.6	7.8	77.3	5.8
[EMIM][$MeOSO_3$]	>267.5	>267.5	>267.5	>267.5	>267.5	>267.5	>267.5	267.5
[BMIM][$MeOSO_3$]	>92	>92	>92	46	46	46	62.5	62.5
[BMIM][Tf_2N]	>2	>2	>2	1.3	>2	>2	>2	>2
[Na][TFMS]	>217.1	217.1	>217.1	>217.1	217.1	217.1	212.8	80.9
[BMIM][TFMS]	250	62.5	125	62.5	62.5	62.5	125	62.5
[OMIM][TFMS]	11.4	15.2	11.4	15.2	7.6	7.6	15.1	15.1
[Na][$OctOSO_3$]	58.3	16.3	28.9	58.3	7.2	7.2	28.9	3.6
[EMIM][$OctOSO_3$]	60.2	30	45.25	30	22.45	30	29.7	22.3
[BMIM][$OctOSO_3$]	62.5	62.5	31.25	31.25	15.7	15.7	15.7	15.7
[OMIM][$OctOSO_3$]	1	1	1	1	1	1	0.5	1
[BMIM][*p*Ts]	62.5	125	125	62.5	62.5	62.5	62.5	62.5

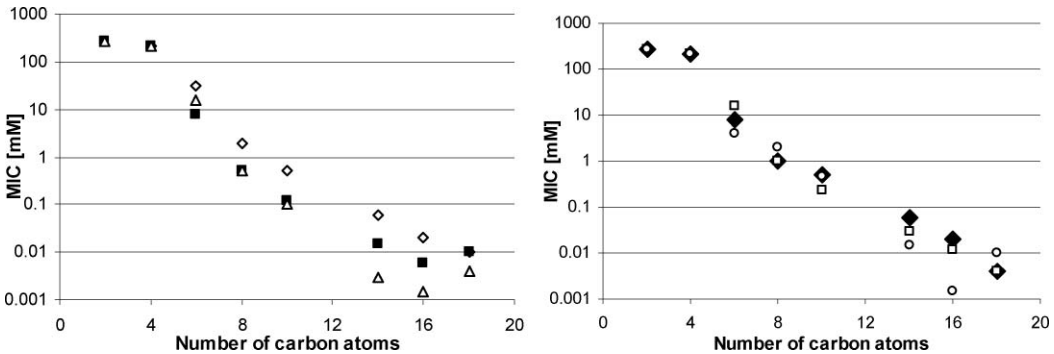


Fig. 2 Relationship between antifungal activities of 1-alkyl-3-methylimidazolium chlorides and the alkyl chain length of the imidazolium cation: (◇) *C. albicans*, (△) *G. candidum*, (■) *S. cerevisiae* ATCC9763, (□) *S. cerevisiae* JG, (♦) *C. glabrata*, (○) *R. mucilaginosa*.

bacterio- and fungistatic effects were demonstrated by halides with octyl to octadecyl substituents on the imidazolium cation.

The MICs of ILs with constant anion, [Cl], and variable cations displayed an observable minimum with increasing alkyl chain lengths for diverse microorganism strains, as shown in Fig. 1 and 2. Thus, the dependency between increasing alkyl side chain length and increasing biological activity no longer holds true for very long alkyl side chains; at a certain chain length the biological efficacy can no longer be increased. This phenomenon has been labelled in the literature as a 'cut-off effect'.

The highest antifungal and antibacterial activity was observed for alkyl chain lengths with 16 or 18 carbon atoms. The similar locations of the minima for all microorganism strains facilitates selection of the compound for specific applications, as only one compound exhibits the highest activity with the lowest concentration.

The cut-off phenomenon is consistent with literature findings concerning the biological activity of long-chained amphiphilic molecules including ILs.[22,29] It was observed for ionic liquids in several test systems *e.g.* for *Vibrio fischeri* the toxicity decreased from 1-dodecyl-3-methylimidazolium chloride to 1-methyl-3-tetradecylimidazolium chloride.[20] The minima were also observed in the biological activity tests of ILs possessing appended alkoxy groups and [Cl], [BF_4] and [PF_6] anions investigated with bacteria by Pernak *et al.*[29]

For this cut-off phenomenon, several explanations were postulated based either on insufficient solubility, a decrease in perturbation, or kinetic aspects.[30] It was proposed that with the elongation of the hydrocarbon substituent, solubility in the oil phase increases faster than the change in oil–water partition coefficient. As a result, for long-chained compounds, partitioning is limited and the concentration becomes insufficient to have a significant effect on the cell membrane.[20,22,31] Another theory describes the decrease in the perturbation of the membrane caused by long-chained molecules, which imitate components of the lipid bilayer, limiting disruption in the membrane.[22] Kinetic aspects may involve steric effects for compounds with a high molecular volume or aggregate formation.[30] In the case of surface-active agents, the micellization process was suggested as the explanation of the cut-off effect. It was postulated that the tendency to self-assemble limits the rate of diffusion to the surface of the cell, resulting in a decrease of the compound concentration at the site of action as well as its permeation ability.[22]

Short-chained imidazolium salts [EMIM] and [BMIM] with a chloride anion demonstrate weak antimicrobial activity. Exchange of the halide by other anions resulted in an increase of antibacterial and antifungal activities of ILs for these two cations. Examples of biological activities of ILs possessing the stable cation [BMIM] and different anions are presented in Fig. 3. For example, *Candida albicans* antifungal activity follows the trend below:

$$Tf_2N > OctOSO_3 \approx BF_4 \approx p\text{Ts} > MeOSO_3 > Cl > TFMS$$

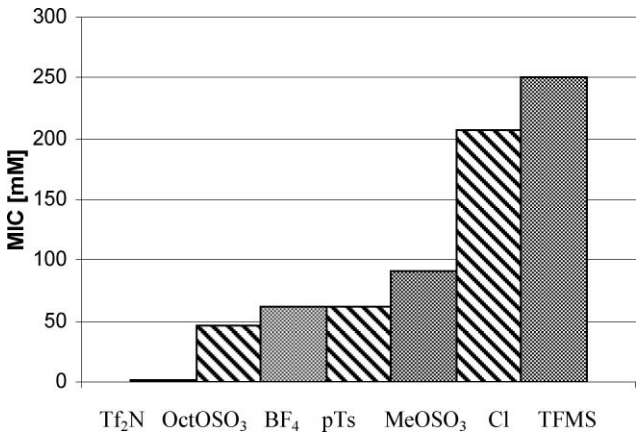


Fig. 3 Antifungal activities of compounds with cation [BMIM] and various types of anions, determined for *C. albicans*.

The wide range of anions used in the investigation revealed that both cation structure and the type of anion have effects on the biological activity. However, it should be noticed that the anion influence was comparatively small. Similar results were obtained by Pernak *et al.* for imidazolium ionic liquids with an appended alkoxy functional group and anions ([Cl], [BF_4] and [PF_6]).[29] Some differential antibacterial activities, observed for short-chained cations, were shown in Fig. 4.

An analysis of the MICs for compounds with different anions and variable chain length also support these findings. For example, the introduction of seven carbon atoms ([$MeOSO_3$] → [$OctOSO_3$]) in the anion resulted in a 4–6-fold increase in the MIC. In comparison, a six carbon atom increase in the cation ([EMIM] → [OMIM]) resulted in a 15- and 130-fold increase in the MIC for compounds with [$OctOSO_3$] and [Cl] anions, respectively, using *C. albicans* as an example. These

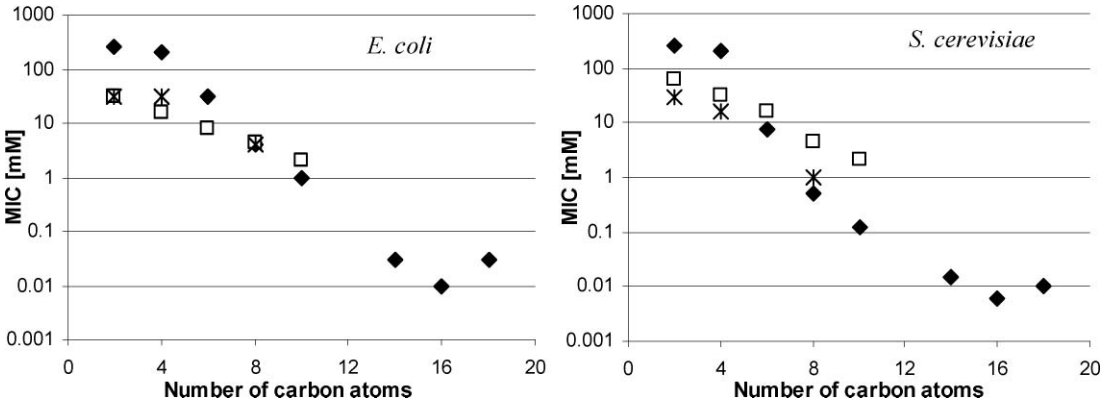


Fig. 4 Relationship of the anion type and the alkyl chain length on antibacterial activities against *E. coli and S. cerevisiae*: (◆) [Cl], (□) [BF_4], (*) [$OctOSO_3$].

results also confirm that the antimicrobial effectiveness of ILs is a consequence of the presence of the positive charge on the nitrogen atom in the imidazolium ring and its affinity to the microbial surface. In addition, the smaller biostatic activity exhibited by compounds with bulky anions may be due to steric hindrance, which limits the rate of transport to the site of action.

For compounds with chain lengths of six and more in the imidazolium cation, practically no difference in antimicrobial activity for the diverse anions was observed. This may corroborate that the surface activity of the cation is the primary factor for determining their antiseptic activity.

Some differences between the antimicrobial activity between Gram-positive, Gram-negative bacteria and fungi was also observed. These differences would indicate that microbial inhibition by the IL is not only dependant on its structure, but also upon the structure of the cell membrane. Gram-negative bacteria were found to be equal or slightly more resistant towards ILs than Gram-positive ones. This might be attributed to the membrane and cell wall differences between bacterial types and strains. These results are consistent with the findings by Carson *et al.*[16] The cell wall of Gram-negative bacteria is surrounded by an additional lipopolysaccharide membrane. This outer membrane barrier is one of the factors that explain the increased tolerance of Gram-negative organisms to ionic liquids.[21]

According to the literature, QACs target mainly the cytoplasmic (inner) membrane in bacteria and the plasma membrane in yeasts.[32] The mode of action of surfactants includes their interaction with phospholipid components in the membrane followed by loss of permeability of the membrane. An analogous mechanism might be proposed for ILs.

Surface activity

To correlate the MIC to the surface activity of the ILs, we observed the influence of these salts on surface tension of an aqueous system and determined their pC_{20}. In our previous study we presented the role of the alkyl chain length of the 1-alkyl-3-methylimidazolium chlorides on their surface activity and aggregation behaviour in aqueous solutions.[25] As a result of their amphiphilic characteristics and positive charge on the nitrogen atom, these compounds have properties such as reduction of surface tension and an attraction to negatively charged surfaces. These characteristics promote adsorption onto many surfaces within the medium, as most natural surfaces are negatively charged (*e.g.* microbial cells and pollutant particles).[33,34]

In this work we aimed to continue the investigation of the surface activity of diverse imidazolium cations with a variety of anions. The results of the tensiometry experiments revealed that all tested ILs reduce the surface tension of the aqueous solutions and therefore present some surface activity (numerically presented as pC_{20} in Table 4). In the investigated set of compounds, only salts possessing [Cl] and [$OctOSO_3$] anions were found to self-assemble into micelles. The micelles formation was seen as a typical 'breakpoint' in the surface tension isotherm (as shown in Fig. 5). This discontinuity is termed the critical micelle concentration (CMC). The obtained CMC values are listed in Table 4.

Micelle formation was detected for imidazolium chlorides possessing eight and more carbon atoms in the hydrocarbon chain. However, replacing the [Cl] anion with large hydrophobic anions such as [OMIM][BF_4], [DMIM][BF_4] and [OMIM][Tf_2N] the cationic micelle formation was inhibited, as shown in Fig. 6, 7 and 8.

Table 4 Surface activity of the selected ionic liquids, expressed as the CMC and pC_{20}

	Critical micelle concentration [mM]		
IL	Surface tension	Literature data	Efficiency pC_{20}
[BMIM][Cl]	—	—	–0.61
[HMIM][Cl]	—	900[40]	0.01
[OMIM][Cl]	234 ± 10	220[40]	0.99
[DMIM][Cl]	54 ± 5.0	55[40]; 39.9[41]; 40.47[41]	1.65
[TDMIM][Cl]	3.2 ± 0.5	4[40]; 2.98[41]; 3.68[41]	2.61
[HDMIM][Cl]	1.1 ± 0.1	0.88[42]; 0.87[41]; 0.86[41]	3.18
[ODMIM][Cl]	0.45 ± 0.05	—	3.56
[EMIM][$OctOSO_3$]	70 ± 5.0	—	1.81
[BMIM][$OctOSO_3$]	35 ± 3.0	31[43]	2.13
[OMIM][$OctOSO_3$]	—	—	3.34
[BMIM][BF_4]	—	800[44]; 820[44]	0.37
[OMIM][BF_4]	—	—	1.30
[DMIM][BF_4]	—	—	2.38
[BMIM][$MeOSO_3$]	—	—	–0.03
[BMIM][*p*Ts]	—	—	0.56
[BMIM][TFMS]	—	—	0.94
[BMIM][PF_6]	—	—	1.05
[OMIM][Tf_2N]	—	—	2.43

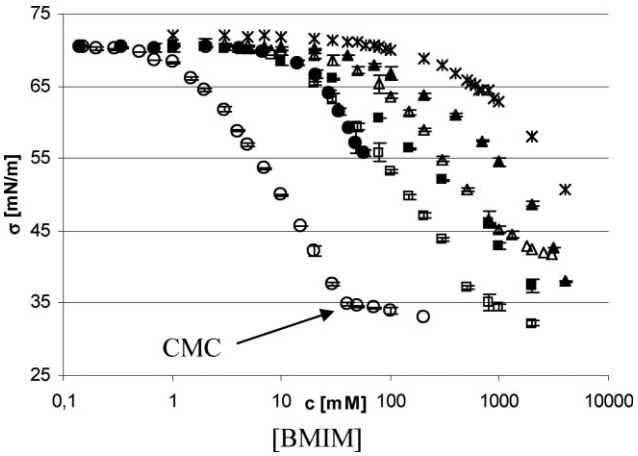


Fig. 5 Surface tension data *vs.* IL concentration isotherms measured at 298 K for aqueous solutions of [BMIM][$OctOSO_3$] (○), [BMIM][PF_6] (●), [BMIM][TFMS] (□), [BMIM][*p*Ts] (■), [BMIM][BF_4] (△), [BMIM][$MeOSO_3$] (▲) and [BMIM][Cl] (*). The position of the CMC is indicated.

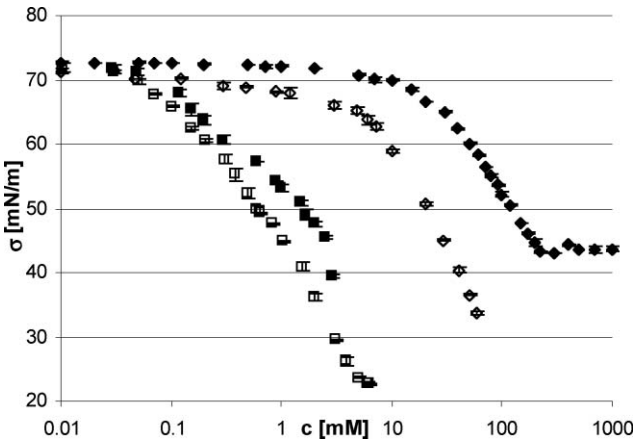


Fig. 6 Surface tension (σ [mN m^{-1}]) data *vs.* IL concentration (*c* [mM]) isotherms measured at 298 K for aqueous solutions of [OMIM][$OctOSO_3$] (□), [OMIM][Tf_2N]] (■), [OMIM][BF_4] (◇), [OMIM][Cl] (◆).

A number of factors may prevent the formation of self-assembled structures. Firstly, the limit of solubility may have been reached before micelle formation could occur (for highly hydrophobic anions such as [Tf_2N]). Secondly, the destabilization of the micelle might be the result of the large volume of the anionic moiety. Hence, it will not fit into the Stern layer, as it is hampered by steric hindrance (for example [BF_4]). Two research groups have found results which support our findings.[35,36]

For ILs possessing the [$OctOSO_3$] anion, micelle formation was observed for the compounds with two and four carbon atoms in the alkyl substituent of the imidazolium cation at 298 K, as shown in Fig. 9. For this homologous series the micelles are thought to be anionic, as [EMIM] and [BMIM] do not form micelles by themselves (analogous to short-chained imidazolium chlorides). Therefore, [EMIM] and [BMIM] cations act as cationic counterions. Surprisingly, [OMIM][$OctOSO_3$] did not form micelles at 298 K. Micelle formation was not observed as the solubility limit was reached and phase separation had taken place. The lack of a breakpoint on the surface tension isotherm

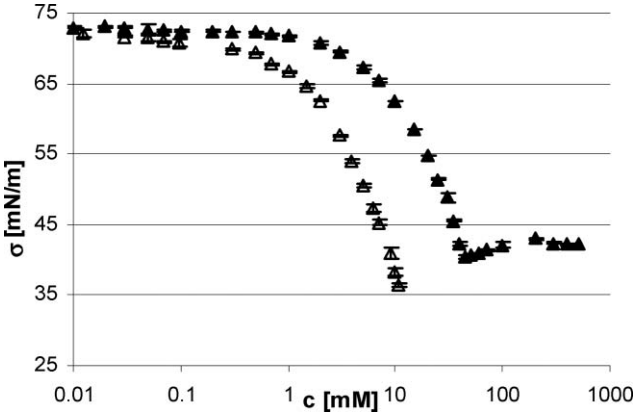


Fig. 7 Surface tension (σ [mN m^{-1}]) data *vs.* IL concentration (*c* [mM]) measured at 298 K for aqueous solutions of [DMIM][BF_4] (△) and [DMIM][Cl]] (▲).

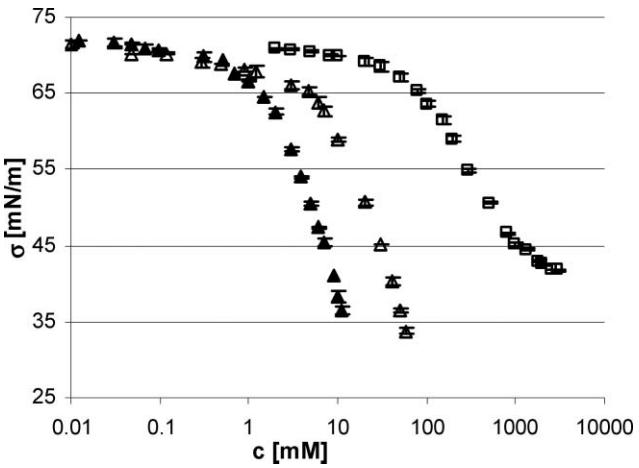


Fig. 8 Surface tension (σ [mN m^{-1}]) data *vs.* IL concentration (*c* [mM]) measured at 298 K for aqueous solutions of [DMIM][BF_4] (▲), [OMIM][BF_4] (△) and [BMIM][BF_4] (□).

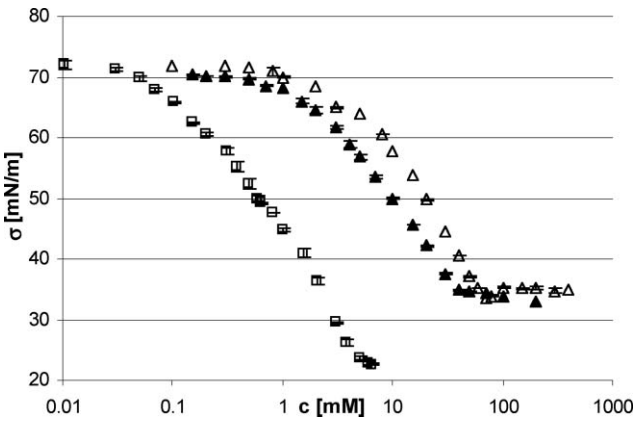


Fig. 9 Surface tension (σ [mN m^{-1}]) data *vs.* IL concentration (*c* [mM]) measured at 298 K for aqueous solutions of [OMIM][$OctOSO_3$] (□), [BMIM][$OctOSO_3$] (▲) and [EMIM][$OctOSO_3$] (△).

of [OMIM][$OctOSO_3$] allows us to assume that the van der Waals interaction of the tails of the anion and cation, and coulombic interaction of the headgroups will result in a neutral dimer. A combination of cationic and anionic amphiphiles results in a non-polar, lipophilic ion pair.

In Fig. 5, it can be seen that the pC_{20} is proportional to the hydrophobicity of the anions and inversely proportional to the solubility of the ionic liquids in water, as was previously described by Rosen.[37] For the [BMIM] cation, the pC_{20} concentration of various anions varied as follows:

$$OctOSO_3 > PF_6 > TFMS > pTs > BF_4 > MeOSO_3 > Cl$$

For the same concentration, the most hydrophobic ionic liquid caused the most significant reduction in surface tension of the aqueous phase[36] and thereby higher antimicrobial activity. Similar results were obtained for longer-chained compounds. For the [OMIM] cation:

$$OctOSO_3 > Tf_2N > BF_4 > Cl$$

and also [DMIM]:

$$BF_4 > Cl$$

Moreover, the efficiency factor pC_{20} linearly depends also on the number of carbon atoms in the hydrophobic chain, as seen in Table 4. Generally, the larger pC_{20} the more efficiently the ionic liquid is adsorbed at the interface and the more efficiently it reduces surface tension. This may be a result of a decrease of electrostatic repulsion between the head groups due to the relatively strong binding of the hydrophobic counterion to the IL cations and the hydrophobicity of the anions themselves. For ionic liquids, a reduction in the effective charge, observed as a higher pC_{20}, might be obtained by use of a more strongly bound (less hydrated) counterion and/or increase in ionic strength of the aqueous phase.

In Fig. 10 the relationship between antimicrobial activity and the surface-active properties of aqueous 1-alkyl-3-methylimidazolium salts is presented. The higher the pC_{20} exhibited by the ionic liquids the lower the MIC observed. The results show a strong correlation for both bacteria and fungi, with a coefficient of determination of 0.80, and 0.84 respectively. These results indicate a strong antimicrobial activity dependence on surface activity of these compounds. A quantitative relationship between the MIC and CMC was earlier demonstrated for 1-alkyl-3-alkylthiomethylimidazolium chlorides by Pernak *et al.*[38]

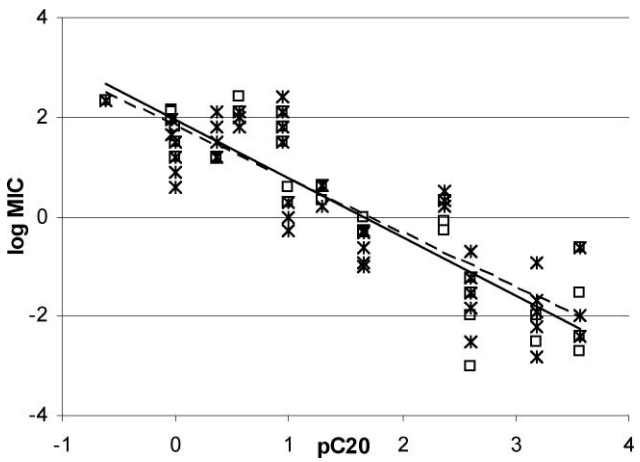


Fig. 10 Comparative graph of log MIC and pC_{20} of both bacteria and fungi.

Experimental

Materials

The investigated cations and anions are shown in Table 1. The ionic liquids were obtained from Merck KGaA, Darmstadt, Germany, with a purity of ≥98%. The ionic liquids were degassed and dried prior to use. In addition, two precursors of IL salts were used: 1-methylimidazole [MIM] and 1-butylimidazole [BIM].

The following microorganisms were used in the experiment: *Candida albicans* ATCC 10231, *Candida glabrata* DSM 11226, *Candida tropicalis* KKP 334, *Saccharomyces cerevisiae* ATCC 9763, *Saccharomyces cerevisiae* JG, *Saccharomyces cerevisiae* JG CDR1, *Rhodotorula mucilaginosa*, *Geotrichum candidum*, *Escherichia coli* ATCC 10231, *Staphylococcus aureus* ATCC 9763, *Enterococcus faecium* ATCC 6057, and *Enterococcus hirae* ATCC 10541.

Serial two-fold microdilution method according to CLSI M27-A2

Minimal inhibitory concentrations of the examined compounds were determined by the serial two-fold dilution microtiter plate method in the relevant medium. Wells containing serially diluted examined compounds and a control were inoculated with 10^4 cells/ml of an overnight culture of bacterial and fungal cells, and microtiter plates were incubated for 24 h at 303 K. Bacteria strains were incubated in trypticase soy broth and fungi in Sabouraud medium. Microbial growth was measured using the microplate reader (Victor, Perkin Elmer) at a wavelength of 531 nm. The MIC was defined as the inhibitor concentration preventing at least 80% of microbial growth, as compared to the inhibitor-free control. Every test was carried out in triplicate and the calculated average MIC values are presented. According to the NCCLS document, as a reference substance amphotericin B was used, giving following results: *Candida albicans* ATCC 10231, 0.07 mM; *Candida glabrata* DSM 11226, 1.08 mM; *Candida tropicalis* KKP 334, 0.13 mM; *Saccharomyces cerevisiae* ATCC 9763, 0.07 mM; *Saccharomyces cerevisiae* JG, 0.07 mM, *Saccharomyces cerevisiae* JG CDR1, 0.07 mM.

pC_{20} and CMC determination

The surface tension of the methylimidazolium solutions were measured using the drop volume tensiometer TVT-1 (Lauda, Lauda-Königsberg, Germany) with a precision of ±0.01 mN m^{-1}. The temperature of the samples in the TVT-1 was regulated to 298 ± 0.1 K. The drops were measured in five sets of triplicates for each concentration. A syringe with a volume of 5.0 ml and a needle inner diameter of 1.050 mm was used. The error calculation was performed by the Lauda software.

The ability of a amphiphiles to reduce the surface tension can be discussed in terms of the concentration required to produce a given surface tension reduction (*e.g.* pC_{20}, pC_{25}). pC_{20} was calculated as the negative logarithm of the ionic liquid concentration required to obtain a 20 mN m^{-1} reduction of the surface tension. According to Rosen,[37] at this value the amphiphile concentration is close to the minimum concentration that provides maximum adsorption at the interface (where the surface is 84.0–99.9% saturated).

The CMC was determined from the breakpoint on the surface tension isotherm using Phillips' definition. Phillips defined the CMC as the concentration corresponding to the maximum change in a gradient in the property *versus* concentration curve[39] as shown in eqn (1):

$$\left(\frac{d^3\phi}{dC_T^3}\right)_{C_T=CMC} = 0 \quad (1)$$

where ϕ is a parameter that quantifies the properties that can be used to determine the CMC, and C_T is the total concentration of the amphiphile. The CMC was determined by computing the third derivative of the local polynomial fit, and the minima were recorded.

Conclusions

The imidazolium salts examined in this study exhibit antifungal and antibacterial activities against bacteria and fungi. The structure–activity comparison showed a strong relationship between antimicrobial efficacy and structure of the imidazolium cation. Shorter substituents on the cation result in low biostatic activities, whereas elongation of the alkyl substituents affects antimicrobial activity, observed as a decrease of minimal inhibitory concentrations. The highest activity is shown by compounds with 16 carbon atoms in the alkyl chain and a Cl^- anion. Antimicrobial activities of imidazolium derivatives are also subtly affected by the type of anion.

Altering of the anion type has a smaller effect on antimicrobial activity of imidazolium salts, indicating that the IL biocidal activity is mainly driven by the alkyl chain length in the cation.

Aqueous solutions of the 1-alkyl-3-methylimidazolium chloride family with carbon numbers ranging from 8 to 18 behave as ionic surfactants and self-aggregate into micellar aggregates. For short-chained imidazolium derivatives only compounds possessing the [$OctOSO_3$] anion exhibit a CMC.

A correlation between the pC_{20} and the MIC of ILs was found. These results show that the mechanism of action is a direct result of the surface activity of the compound. Therefore, these results indicate the necessity of further experiments to investigate the interaction of ionic liquids with cellular surfaces, and their mode of inhibition, and thereby allow us to tailor ILs for a specific technological application. Thus, interfacial phenomena play a crucial role in the biodegradation processes, due to changes in affinity and availability of the ILs in the intercellular space.

This study presents polyfunctionality of imidazolium ionic liquids. In commercial applications ILs may fulfil many functions, concurrently exhibiting surface, antimicrobial and antielectrostatic activity. This creates possibilities of developing new disinfectants, antiseptics and preservatives. In addition, the low antimicrobial activities of short-chained salts indicate that ILs seem to be favourable compounds for bioprocesses, *e.g.* biotransformations, enzymatic reactions and separation processes as an alternative to organic solvents.

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