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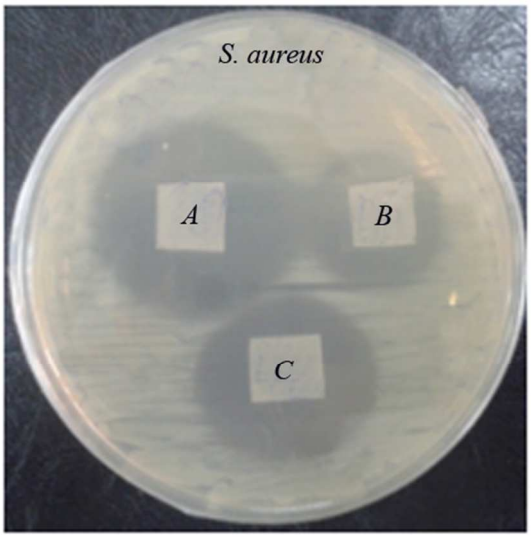

An example of agar disk diffusion result for A) [BMIM][NTf_2] B) [BMIM][I] and C) [BMIM][HSO_4] on *S. aureus*.

Antibacterial and antiadhesive properties of butyl-methylimidazolium ionic liquids toward pathogenic bacteria

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Abstract

Interest in ionic liquids (ILs) for their potential in different area is increasing, as they are claimed to possess a range of bioactive properties, which may be exploited in the design of antibacterial and antiadhesive compounds. However, quantitative information on the antimicrobial and antiadhesive properties of ILs is very scarce. This paper reports, for the first time, on a comprehensive study into the antibacterial and antiadhesive activity of a broad range of 1-Butyl-3-methylimidazolium ILs including [BMIM][$N(CN)_2$], [BMIM][PTS], [BMIM][NO_3], [BMIM][SCN], [BMIM][I], [BMIM][Cl], [BMIM][PF_6], [BMIM][$MeOSO_3$], [BMIM][HSO_4], [BMIM][BF_4] and [BMIM][NTf_2] against a wide range of pathogenic and semi-pathogenic, Gram positive and Gram negative bacteria. These pathogens include *P. aeruginosa*, *S. aureus, E. coli, B. cereus, B. subtilis, S. typhimurium,* and *K. pneumonia.* Antimicrobial activity was comprehensively evaluated qualitatively and quantitatively using agar disk diffusion assay, agar well diffusion assay as well as determining Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). Antiadhesive activity was also evaluated using 96 well microtitre plate assay. The results of the study demonstrated that almost all ILs have antimicrobial activity against mentioned pathogens, however in different degrees. Among them, the IL with NTf_2 anion showed very strong antimicrobial activity against all pathogens. ILs with HSO_4 and SCN anions were also great antimicrobials. Weak antimicrobial activity was observed for IL with PTS anion. In addition, not all ionic liquids showed antiadhesive activity.

Keywords: Ionic liquids, Pathogens, Antibacterial, Antiadhesive

Introduction

Ionic liquids (ILs) are a novel class of low temperature (typically <100 °C) molten salts comprised of an organic cation (e.g. imidazolium, pyridinium), and a polyatomic inorganic anion (e.g. tetrafluoroborate, hexafluorophosphate).[1,2] Interesting properties associated with ILs, i.e. high polarity, ionic conductivity, chemical and thermal stability as well as nonvolatile and nonflammable nature, put them at the center of attention.[3] More important is their "tuneable" nature, whereby physical, chemical, and biological properties could be altered by modification of their anionic or cationic parts. Recently, it has been demonstrated that many ILs have a certain amount of toxicity. However, toxicity itself can be a tunable property which may be useful in a number of other applications, such as antiseptics, biocides, disinfectants, antibacterial, antiadhesive and antifouling reagents.[4,5] Therefore, 'tuneability' of ILs introduces an unparalleled flexibility in particular applications**.**

The antimicrobial activity of various kinds of ionic liquids against both environmental and clinically important microorganisms have been demonstrated.[6,7] In our previous work, the effect of various kinds of ILs on the cell growth of a probiotic strain was examined qualitatively.[8] However, quantitative studies on the antimicrobial properties of ILs are still very scarce.

There are various qualitative and quantitative methods for measuring antimicrobial or antiadhesive activity. Such methods provide useful information regarding fundamental sensitivity or tolerance to a given antimicrobial agents and are therefore vital to the successful treatment and management of microbial infections. Agar diffusion techniques including disk or well diffusion assays are simple methods for rapid determination of relative antimicrobial activity of ionic liquids. The Agar disk diffusion method (or Kirby-Bauer method) is usually used for antimicrobial susceptibility testing. Rebrose and co-workers evaluated ILs in this way.[9]

In addition, the terms 'Resistant' or 'Susceptible' can have a realistic interpretation. Thus when in doubt, the way to a precise assessment is dilution tests. Dilution tests are routinely used for the determination of the two most fundamental parameters in antimicrobial susceptibility testing; the minimum inhibitory concentration (MIC) and the minimum bactericidal/fungicidal concentration (MBC/MFC), sometimes referred to as the minimum lethal concentration (MLC). The MBC test determines the lowest concentration at which an antimicrobial agent will kill a particular microorganism. The MBC is determined using a series of steps, undertaken after MIC test has been completed and defined as the lowest concentration of antimicrobial that results in ≥ 99.9% killing of the bacterium under test. MBC testing is useful for comparing the germ-killing activity of several antimicrobial agents at once.

Microorganisms could form biofilms as a survival strategy and it is a predominant mode of growth for microorganisms in natural ecosystems.[10] Infectious diseases could be prevented by preventing biofilm formation using antiadhesive agents. Ionic liquids may someday be used as novel antiadhesive agents for such applications. In addition, no assessment of either microbiological/environmental toxicity or potential utility of ILs as antimicrobial agents would be complete without attention to the antiadhesive efficacy of ionic liquids. Therefore, antiadhesion evaluation of ILs is necessary. There are some methods to evaluate anti-biofilm or antiadhesive activity of ILs. Determination of minimum biofilm eradication concentration (MBEC) and use of 96 well microtitre plate are among them.[10,11]

Although there are a few intensive studies on the antimicrobial activities of ILs, much work is still needed to develop new types of antimicrobial ILs. He et al.[12] evaluated antimicrobial activity of imidazolium based ILs with fumarate anion using agar diffusion assay which is a qualitative test and do not provide the real amount of antimicrobial activity. Docherty et al.[13]

determined MIC values for ILs with Br anionic part. However there is no quantitative information on antimicrobial activity of 1-Butyl-3-methylimidazolium ILs with anions like $N(CN)_2$, PTS, NO_3, SCN, I, PF_6, $MeOSO_3$, HSO_4, BF_4 and NTf_2. On the other hand there is not any single report on the antiadhesive properties of the ILs under investigation.

In this study, a wide series of imidazolium based ILs with various anion types were employed to investigate their antimicrobial and antiadhesive activity against pathogenic bacteria. To our knowledge, this is the first assessment of the antimicrobial and antiadhesive efficacy of the mentioned ionic liquids in the literature. This work provides useful information about ILs, prior to their widespread use.

Results and Discussion

Antimicrobial properties of ILs were evaluated using four kinds of methods including agar disk diffusion, agar well diffusion, MIC and MBC. The results will be presented here.

Antimicrobial properties

All liquid ILs (As shown in Table 1) were tested for their antimicrobial activity using qualitative method of agar disk diffusion. Nine ILs were spread on PP films and placed on agar plates cultured with seven pathogens as described before. The results have been presented in Table 2. As an example, the schematic results of agar disk diffusion for [BMIM][NTf_2], [BMIM][HSO_4] and [BMIM][SCN] on *S. aureus,* have been illustrated in Fig. 1. As can be seen in the Table, all ILs have antimicrobial activity however in different degrees. In an overall view, we can say that [BMIM][NTf_2] resulted in the best antimicrobial activity according to agar disk diffusion results. The clear zone diameter using [BMIM][NTf_2] were almost good and strong.

Among microorganisms, *E. coli* showed the most resistance against ILs as the inhibition zone was not significant in most cases. Similarly, *S. typhimurium*, *K. Pneumonia* and *P.*

aeruginosa were also resistant toward some ILs. Surprisingly, in some cases no inhibition zone was revealed. However, as we are going to explain further, in MIC experiments, all ILs can inhibit the growth of all these bacteria in a certain concentration. Therefore, it indicates that some ILs can not show their capability to inhibit bacterial growth in agar disk diffusion assay. *B. subtilis*, *B. cereus* and *S. aureus* were among sensitive microorganisms as the inhibition zone over them were almost strong.

An important note is that the more resistant bacteria in agar disk diffusion test are all Gram negative (*E. coli*, *K. pneumonia*, *S. typhimurium* and *P. aeruginosa)*. This could be very interesting while Gram positive bacteria were very sensitive to most ILs. This is because of the interaction of IL with the cell membrane. As discussed in our previous study comprehensively[8], the outer membrane of Gram negative bacteria is composed of lipopolysaccharides (LPS), phospholipids and lipoproteins, covalently linked to the peptidoglycan layer while Gram positive bacteria have no lipopolysaccharides in their membrane and the outer layer of their cell membrane is composed of a thick peptidoglycan layer. The outer membrane of Gram negative bacteria may serve as a barrier to the entry of antimicrobial molecules. Hence, the difference between cell membrane is the main reason for difference in their susceptibility toward antimicrobial agents.[10,14,15]

Agar well diffusion assays also confirmed the antimicrobial activity of ILs. Fig. 2 shows an example of the result of agar well diffusion assay for [BMIM][NO_3] on *K. pneumonia.* Agar well diffusion results were in agreement with agar disk diffusion assay (data not shown).

Information on minimum inhibitory concentration of ILs is necessary as these compounds may someday be in widespread use in related industries. To our knowledge there are many qualitative reports that prove antimicrobial activity of ILs, however, such quantitative

information is so scarce. On the other hand, the majority of researches on antimicrobial activity of ILs, evaluated the effect of alkyl chain length of IL on its antimicrobial activity. Busetti et al.[10] measured antimicrobial and antibiofilm properties of a series of 1-alkylquinolinium bromides against a range of clinically relevant microorganisms. They showed that the longer the alkyl chain length, the stronger the antimicrobial activity. Here, the antimicrobial properties of 1-Butyl-3-methylimidazolium with broad range of common anions is reported. In this condition, we will be able to compare ionic liquids according to their anions.

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values have been determined for butyl imidazolium ionic liquids against a range of pathogenic organisms and the results are shown in Table 3. Interesting results were obtained after determining MIC values. As can be seen in Table 3, the IL with NTf_2 anion showed very strong antimicrobial activity against all bacteria. It was in general agreement with previous experiments of agar disk and well diffusion assays. [BMIM][NTf_2] showed the MIC value lower than 0.048 g/L on *E. coli*. This amount was the lowest MIC between all MIC values obtained. *S. typhimurium* was also among the susceptible microorganisms when contacted with [BMIM][NTf_2] as the obtained MIC value was 0.39 g/L. MIC value of 3.12 g/L was obtained over all other bacteria using the IL with NTf_2 anion.

[BMIM][HSO_4] was another great antimicrobial after [BMIM][NTf_2]. The MIC values of 3.12 or 6.25 g/L were obtained against all microorganisms using this IL. On the other hand, [BMIM][PTS] showed the lowest antimicrobial activity. This observation is in agreement with our previous work[8] using *P. freudenreichii* as a probiotic microorganism. [BMIM][$N(CN)_2$] was another IL with low antimicrobial activity compared to other ILs. Surprisingly, the MIC values for this IL was 25 g/L and was the same against all bacteria tested. [BMIM][$MeOSO_3$] also

showed similar behavior. The MIC values for this IL (12.5 g/L), was the same against all pathogens.

MIC is used by diagnostic laboratories mainly to confirm resistance, but most often as a research tool to determine the *in vitro* activity of new antimicrobials. Another important parameter is MBC. Table 3 also shows the MBC values for ILs against investigated bacteria. The results showed that the value of MBC in many cases are the same as MIC. In a few cases, however, the MBC value was one order higher than MIC value.

Anti adhesive activity

In order to control the infection, inhibition of microbial adherence to the surfaces is one of the effective means.[16] Bacteria can adhere to many natural and synthetic surfaces. There are some reasons why bacteria adhere to the surface, however the best answer is ‘‘Adhesion to a surface is a survival mechanism for bacteria’’.[17] By means of specific adhesion molecules, the microorganism is able to recognize specific receptors located at the surface and then attaches itself.

Ionic liquids as antimicrobial agents may inhibit bacterial adhesion to surfaces. However, there is no information on the antiadhesive activity of the ILs under investigation. To examine that, antiadhesive activity of a wide range of 1-Butyl-3-methylimidazolium ionic liquids were evaluated using 96-well microtitre plates under in vitro conditions. The results have been presented in Table 4. As can be seen, the antiadhesive activity strongly depends on the type of microorganism and the concentration of IL. In addition, very different behavior can be observed among ILs. Some ILs showed strong antiadhesive activity. On the other hand, some other not only did not inhibit cell adhesion, but improve cell adhesion to the surface. [BMIM][SCN], [BMIM][HSO_4], [BMIM][NO_3] and [BMIM][$N(CN)_2$] were among ILs with good antiadhesive

activity. The best antiadhesive activity was observed against *B. subtilis*. As can be seen, 100% inhibition of cell adhesion was obtained using [BMIM][NO_3] and [BMIM][$N(CN)_2$] at concentration of 50 g/L . In many cases, with increasing the concentration of IL, the antiadhesive activity was improved. However, over some microorganisms this trend can not be observed. For example, [BMIM][$N(CN)_2$] showed almost similar antiadhesive activity over *E. coli* in all concentrations.

[BMIM][NTf_2], [BMIM][I], [BMIM][Cl] and [BMIM][PTS] showed limited antiadhesive activity over a few tested microorganisms. Surprisingly, in some cases, the IL improved the cell adhesion. For example, as can be seen for [BMIM][NTf_2], the cell adhesion of *B. cereus* improved for 80% compared to the control (PBS only). The results say that not all ILs have antiadhesive activity. Therefore, more research on antiadhesive activity of ILs is needed.

Conclusion

Ionic liquids could prove attractive reagents for the control and prevention of infection and may be employed in a diverse range of antimicrobial applications. Antimicrobial and antiadhesive properties of various kinds of butyl methylimidazolium ionic liquids were obtained. All ILs showed antimicrobial activity against indicator microorganisms. Among ILs, [BMIM][NTf_2] demonstrated the best antimicrobial capability. On the other hand [BMIM][PTS] resulted in weak activity. The result of the study showed that not all ILs have antiadhesive activity. However, more work is still needed to complete the knowledge of scientific community towards ionic liquids characteristics in terms of antimicrobial and antiadhesive properties.

Experimental

Chemicals and Microorganisms

All chemicals were obtained from Merck (Germany) unless otherwise stated. ILs which have been used in this work are presented in Table 1. All ILs were synthesized in our laboratory according to our previous works (Mokhtarani et al. 2009a, 2009b; Hajfarajollah et al. 2014). ILs are imidazolium based with different anions.

For antimicrobial and antiadhesive assays, the following Gram positive and Gram negative strains were kindly provided by the Faculty of Genetic engineering, National Institute of Genetic Engineering and Biotechnology (Iran): *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus, Escherichia coli, Bacillus cereus*, *Salmonella typhimurium* and *Klebsiella pneumonia.* All microbial strains were stored at -70 ˚C in glycerol stocks, and subcultured in Muller Hinton Broth (MHB) before testing.

Antimicrobial activity

Qualitative assays

Agar disk diffusion.

The test was initiated by pouring the Muller Hinton agar onto sterilized Petri dishes and was allowed to solidify. 100 µL of incubated testing bacterial solution (10^8 CFU/mL) was spread uniformly over the plate. 20 µL of each IL was spread on polypropylene (PP) films (1.5 cm × 1.5 cm) and was placed on the Muller Hinton agar surface. The Petri dishes were incubated for 16-18 h at 37 °C. The clear zone formed around the samples was recorded as an indication of inhibition of the microbial species. Control experiments were performed with uncoated polyethylene films. The diameter of the inhibition zone surrounding the PP film was then measured. The diameter of the zone was scored as follows: since the side of PP film is 15 mm,

15 mm equals no inhibition (–), diameter lower than 17 mm (weak, +), diameter between 17 and 20 mm (good, ++) and diameter larger than 20 mm (strong, +++). All experiments were performed in three independent experiments. It should be noted that this test was performed only for liquid ILs. ILs with Cl and PTS anions are solid in room temperature.

Agar well diffusion.

Muller Hinton agar (MHA) medium plates were seeded with 18-24-hour-old cultures of microbial inocula (a standardized inoculum of 10^8 CFU.ml^{-1} 0.5 McFarland Standard). 8 mm in diameter wells were cut into the agar media with a sterilized pipette Pasteur and then ILs in concentration ranging from 100 to 0.78 g/L poured into the wells. The plates were kept in 4 °C for 3-4 h to better penetration of ILs in agar. Inoculated plates were then incubated at 37 ºC for 18 h and zones of inhibition were measured. Three replicates were prepared for each microorganism.

Quantitative assay

MIC/MBC determination.

The microtitre plate for determination of MIC and MBC was set up as described elsewhere.[10] An original working solution of each ionic liquid was initially prepared and 0.22 µm sterile filtered. From this stock solution, serial two-fold dilutions in MHB were carried out in 96-well microtitre plates over the concentration range 50-0.048 g/L. Bacterial suspensions were prepared and adjusted to the logarithmic-phase growth to match the turbidity of a 0.5 McFarland standard, yielding approximately 10^8 CFU/ml. 2.5 µl of the bacterial solution was inoculated to each well. Positive and negative controls included in each plate. All controls and test concentrations were prepared as three replicates. The microtitre plates were then incubated for 48 h at 37 °C in a stationary incubator. Following determination of the MIC for each compound, the

minimum bactericidal concentrations (MBC) were derived by transferring 20 µL of the suspension from the wells, which displayed no signs of growth to MHA plates. The MHA plates were then incubated in a stationary incubator at 37 °C for 24 h and examined for 99.9% killing.

Antiadhesion assay

The antiadhesive activity of the ILs against seven bacterial strains; *S. aureus, P. aeruginosa, B. cereus*, *B. subtilis*, *E. coli*, *S. typhimurium* and *K. pneumonia* was evaluated according to a previously reported adhesion assay.[18] Briefly, the wells of a sterile 96-well flat-bottomed plastic tissue culture plate with a lid were filled with 200 µl of the IL solution. Five concentrations were tested ranging from 3.12 to 50 g/l. The plate was incubated for 18 h at 4 °C and subsequently washed twice with PBS. Control wells contained buffer (PBS) only. A 200 µl aliquot of a washed bacterial suspension (0.5 McFarland standard) was added and incubated in the wells for 4 h at 4 °C. Unattached organisms were removed by washing the wells three times with PBS. The adherent microorganisms were fixed with 200 µl of 99% methanol per well, and after 15 min the plates were emptied and left to dry. Then the plates were stained for 5 min with 200 µl of 2% crystal violet used for Gram staining per well. Excess stain was rinsed by placing the plate under running tap water. Subsequently the plates were air dried, the dye bound to the adherent microorganisms was resolubilized with 200 µl of 33% (v/v) glacial acetic acid per well and the optical density readings of each well were taken at 595 nm. The microbial inhibition percentages at different biosurfactant concentrations for each microorganism were calculated as Eq. (1):

$$\%\text{Microbial inhibition}=\left[1-\frac{A_c}{A_0}\right]\times 100 \quad (1)$$

Where A_c represents the absorbance of the well with IL concentration c and A_0 the absorbance of the control well.

Statistical Analysis

All data were analyzed using the SPSS 11.5 statistical analysis system. A one-way analysis of variance was used to determine whether a significant difference existed between the treated groups and controls. Data were expressed as mean values of three replicates and differences were considered statistically significant if $P<0.05$.

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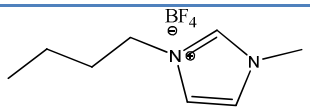
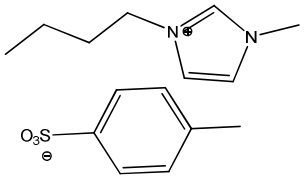
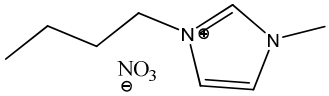
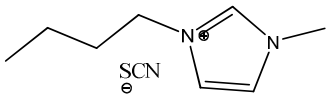
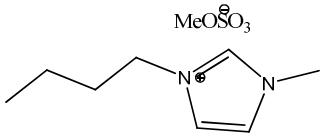
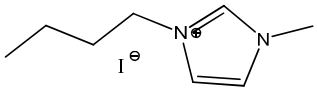
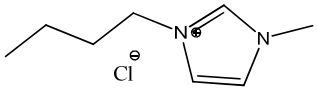
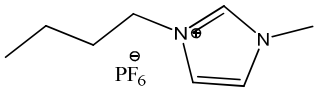
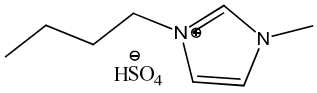
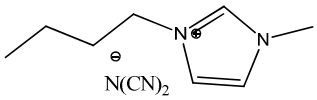
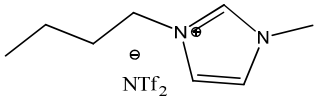
Figure captions

Fig. 1. Agar disk diffusion result for A) [BMIM][NTf_2] B) [BMIM][SCN] and C) [BMIM][HSO_4] on *S. aureus.*

Fig. 2. Agar well diffusion result for [BMIM][NO_3] on *K. pneumonia.*

1 Table 1. Ionic liquids that used in this work; their structure and their physical characterization

Ionic Liquid	Chemical Structure	Acronym	Viscosity (pa.s)	Density (g/cm^3)	Molecular Weight
1-butyl-3-methylimidazolium tetrafluoroborate		[BMIM][BF_4]	233(25 °C)	1.12 (25 °C)	226.02
1-butyl-3-methylimidazolium tosylate		[BMIM][PTS]	b	a	310.41
1-butyl-3-methylimidazolium nitrate		[BMIM][NO_3]	266 (20 °C)	1.15(20 °C)	201.22
1-butyl-3-methylimidazolium thiocyanate		[BMIM][SCN]	a	1.02(20 °C)	197.30
1-butyl-3-methylimidazolium methyl sulfate		[BMIM][$MeOSO_3$]	a	1.21 (20 °C)	250.32
1-butyl -3-methylimidazolium iodide		[BMIM][I]	a	a	266.12
1-butyl -3-methylimidazolium chloride		[BMIM][Cl]	b	a	174.67
1-butyl -3-methylimidazolium hexafluorophosphate		[BMIM][PF_6]	a	1.38(20 °C)	284.18
1-butyl -3-methylimidazolium hydrogen sulfate		[BMIM][HSO_4]	a	1.27(25 °C)	236.29
1-butyl -3-methylimidazolium dicyanamide		[BMIM][$N(CN)_2$]	a	a	205.26
1-butyl -3-methylimidazolium bis(trifluoromethanesulfonyl)imide		[BMIM][NTf_2]	a	1.44(20 °C)	419.36

a: no data available; b: solid form

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Table 2. Antimicrobial activity of ILs against some indicator microorganisms in agar disk diffusion assay.

Ionic liquid	*S. aureus* (PTCC 1112)	*B. subtilis* (PTCC 1715)	*B. cereus* (PTCC 1015)	*E. coli* (PTCC 1338)	*S. typhimurium* (wild type)	*K. Pneumonia* (PTCC 1290)	*P. aeruginosa* (PTCC 1310)
[BMIM][$N(CN)_2$]	+++	+	+	-	-	+	-
[BMIM][NO_3]	-	+++	++	+	-	+	+
[BMIM][SCN]	+	+	+++	-	+	+	+
[BMIM][I]	+++	+	++	-	-	+	+
[BMIM][PF_6]	+++	+	+	+	++	+	++
[BMIM][$MeOSO_3$]	++	+++	+++	-	+	-	+
[BMIM][HSO_4]	+++	+++	+++	+	+	++	++
[BMIM][BF_4]	++	++	++	-	-	+	+
[BMIM][NTf_2]	+++	+++	+++	++	+	+	++

Antimicrobial activity detected as zones of inhibition with diameters of (-), no inhibition; (+), < 17 mm; (++), 17-20 mm; (+++) > 20 mm.

Table 3. MIC and MBC values (g/L) of the ILs against pathogenic bacteria.

Microorganisms		Ionic Liquids					
		[BMIM][N(CN)$_2$]	[BMIM][PTS]	[BMIM][NO$_3$]	[BMIM][SCN]	[BMIM][I]	[BMIM][Cl]
P. aeruginosa	*MIC*	25	25	12.5	6.25	12.5	12.5
	MBC	25	50	50	6.25	12.5	12.5
S. aureus	*MIC*	25	50	12.5	3.12	12.5	50
	MBC	25	50	12.5	3.12	25	50
E. coli	*MIC*	25	25	12.5	6.25	25	12.5
	MBC	25	25	12.5	6.25	25	12.5
B. cereus	*MIC*	25	50	50	25	50	50
	MBC	25	50	50	25	50	50
S. typhimurium	*MIC*	25	25	12.5	6.25	12.5	12.5
	MBC	25	25	12.5	12.5	25	50
K. pneumonia	*MIC*	25	25	25	6.25	25	25
	MBC	25	25	25	6.25	25	N.D
B. subtilis	*MIC*	25	25	50	25	25	50
	MBC	25	25	50	25	25	N.D

Microorganisms		Ionic Liquids				
		[BMIM][PF$_6$]	[BMIM][MeOSO$_3$]	[BMIM][HSO$_4$]	[BMIM][BF$_4$]	[BMIM][NTf$_2$]
P. aeruginosa	*MIC*	25	12.5	6.25	12.5	3.12
	MBC	50	12.5	12.5	50	3.12
S. aureus	*MIC*	25	12.5	3.12	12.5	3.12
	MBC	25	12.5	3.12	12.5	3.12
E. coli	*MIC*	12.5	12.5	3.12	12.5	<0.04
	MBC	12.5	12.5	3.12	12.5	0.048
B. cereus	*MIC*	50	12.5	3.12	50	3.12
	MBC	50	12.5	3.12	50	3.12
S. typhimurium	*MIC*	50	12.5	6.25	12.5	0.39
	MBC	50	12.5	6.25	12.5	0.39
K. pneumonia	*MIC*	25	12.5	3.12	25	3.12
	MBC	25	50	6.25	N.D	3.12
B. subtilis	*MIC*	50	12.5	3.12	50	3.12
	MBC	50	12.5	3.12	N.D	3.12

N.D: Not Determined, All data groups are significant with P-value<0.05.

Table 4. Microbial inhibition percentages obtained from the microtiter-plate antiadhesion assay with several ILs at different concentrations (g/L).

Microorganism	[BMIM][SCN] (g/L)						[BMIM][HSO_4] (g/L)					
	Control	3.12	6.25	12.5	25	50	control	3.12	6.25	12.5	25	50
P. aeruginosa	0	36	37	41	40	44	0	38	39	42	41	41
S. aureus	0	52	41	43	55	49	0	11	29	33	44	54
E. coli	0	42	44	38	44	41	0	9	26	37	40	52
B. cereus	0	81	38	55	71	78	0	44	49	55	59	71
S. typhimurium	0	33	38	41	46	42	0	1	22	37	36	43
K. pneumonia	0	32	37	43	41	42	0	-1	3	19	31	37
B. subtilis	0	39	100	79	66	89	0	41	50	59	87	88
Microorganism	[BMIM][$N(CN)_2$] (g/L)						[BMIM][NO_3] (g/L)					
	Control	3.12	6.25	12.5	25	50	control	3.12	6.25	12.5	25	50
P. aeruginosa	0	61	68	77	80	79	0	31	39	53	77	79
S. aureus	0	53	55	67	67	68	0	57	59	62	62	60
E. coli	0	55	55	55	56	56	0	48	55	63	62	63
B. cereus	0	44	64	69	72	79	0	54	59	63	72	81
S. typhimurium	0	45	55	59	60	66	0	44	61	64	65	65
K. pneumonia	0	66	66	71	78	80	0	22	21	33	41	71
B. subtilis	0	93	93	93	97	100	0	2	12	47	65	100
Microorganism	[BMIM][I] (g/L)						[BMIM][NTf_2] (g/L)					
	Control	3.12	6.25	12.5	25	50	control	3.12	6.25	12.5	25	50
P. aeruginosa	0	21	39	43	48	51	0	-22	-5	-1	12	18
S. aureus	0	-1	4	12	19	21	0	86	90	79	88	87
E. coli	0	-3	-4	0	1	-1	0	3	0	-1	1	0
B. cereus	0	-44	-35	-12	-3	1	0	0	-3	-15	-63	-80
S. typhimurium	0	1	3	4	3	1	0	-4	5	3	4	4
K. pneumonia	0	12	21	33	41	51	0	7	7	12	23	25
B. subtilis	0	3	13	19	29	38	0	-1	0	1	-11	-25
Microorganism	[BMIM][Cl] (g/L)						[BMIM][PTS] (g/L)					
	control	3.12	6.25	12.5	25	50	control	3.12	6.25	12.5	25	50
P. aeruginosa	0	-1	3	9	8	9	0	17	21	20	30	51
S. aureus	0	2	1	9	19	19	0	6	15	37	42	40
E. coli	0	1	5	-2	0	1	0	-2	1	-5	-9	-1
B. cereus	0	-1	-5	-11	-22	-13	0	6	5	25	21	33
S. typhimurium	0	1	8	12	11	33	0	1	-1	1	1	12
K. pneumonia	0	0	3	1	3	12	0	0	-3	3	3	5
B. subtilis	0	-1	-4	-33	-40	-35	0	1	11	32	56	85

All data groups are significant with P-value<0.05.

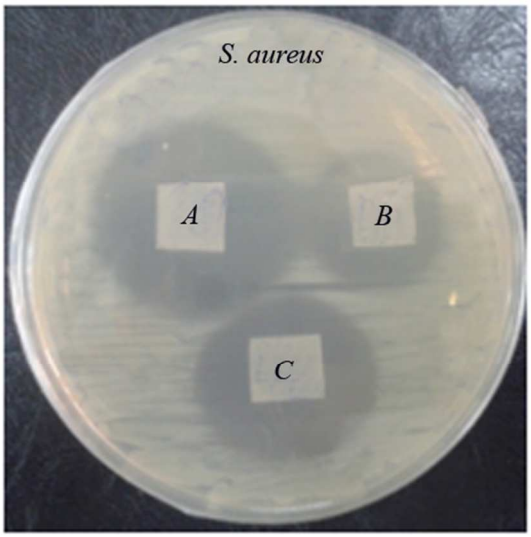

Fig. 1. Agar disk diffusion for A) [BMIM][NTf_2] B) [BMIM][I] and C) [BMIM][HSO_4] on *S. aureus*.

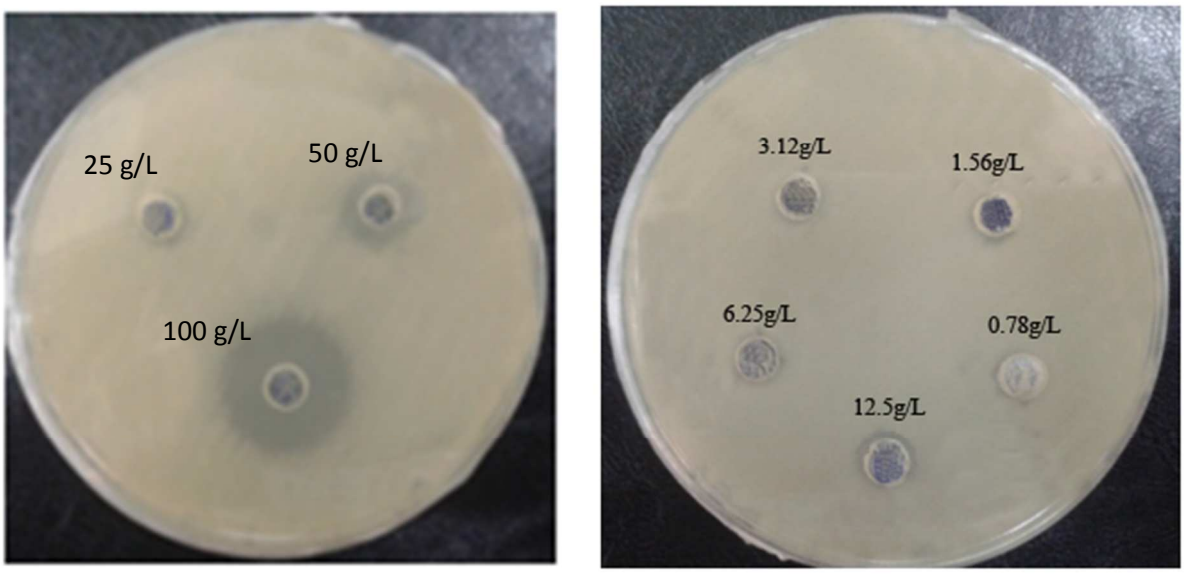

Fig. 2. Agar well diffusion for [BMIM][NO_3] on *K. pneumonia.*