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Synthesis and anti-microbial potencies of 1-(2-hydroxyethyl) -3-alkylimidazolium chloride ionic liquids: Microbial viabilities at different ionic liquids concentrations

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ABSTRACT

Three 1-(2-hydroxyethyl)-3-alkylimidazolium chloride room temperature ionic liquids (ILs) [2OHimC_n][Cl]; (n=0, 1, 4) have been synthesized from the appropriate imidazole precursors and characterized by IR and NMR spectroscopies and elemental analysis. Their anti-microbial activities were investigated using the well-diffusion method. The viabilities of *Escherichia coli*, *Aeromonas hydrophila*, *Listeria monocytogenes* and *Salmonella enterica* as a function of IL concentrations were studied. The minimal inhibitory concentrations (MICs) and EC_{50} values for the present ILs were within the concentration range from 60 to 125 mM and 23 to 73 mM. The anti-microbial potencies of the present ILs were compared to a standard antibiotic, gentamicin. The finding affords additional perspective on the level of ILs toxicity to aquatic lifeforms and yet, this characteristic can be readily harnessed to detect microbial growth and activity.

1. Introduction

Room temperature ionic liquids (ILs) are generally salts that bear an organic cation, obtained by the extension of a valence of a nitrogen, phosphorus or sulfur atom, and an organic or inorganic anion with melting points below or not far above ambient temperatures. They are in demand as substitutes to conventional molecular solvents due to their high chemical and thermal stabilities as well as their very low flammability and vapor pressures. ILs are generally excellent solvents for a wide range of inorganic and organic materials (Fuller et al., 1997; Suarez et al., 1998; Brennecke and Maginn, 2001; Gathergood et al., 2004; Couling et al., 2006; Hossain et al., 2011a). Despite their good industrial applicability, the high solubility of some ILs in water raises concerns as they may be potentially toxic to aquatic organisms. Correspondingly, IL researchers have focused their attention on determining IL toxicities on soil/aquatic-based organisms such as earthworms (Luo et al., 2009; Li et al., 2010), *Selenastrum capricornutum* (Cho et al., 2007) and *Vibrio fischeri* (Docherty and Kulpa, 2005) in order to afford a better understanding of their impact to the surrounding environment.

The notoriety of ILs as being toxic to aquatic organisms is, nonetheless, partially negated by the observation that their toxicities can be readily harnessed to detect microbial growth and activity. This dichotomy of the drawbacks and plus points affords IL researchers a rather intriguing perspective as can be seen from different applications. Bacteria are essentially a good foundation to inspect IL toxicity as they have short generation times (Pham et al., 2010). Previous studies concluded that some pyridinium, imidazolium and quaternary ammonium ILs show toxicity towards a range of bacteria (Pernak et al., 2003, 2004; Roslonkiewicz et al., 2005). It has been suggested that some quaternary ammonium compounds can even be considered for disinfection of medical equipment (Demberelnyamba et al., 2004). In this regard, Saadeh et al. (2009) synthesized tetrabutylammonium-based ILs and reported that they were effective against Gram-positive and Gram-negative bacteria. We had previously established that hydroxylammonium-based ILs exhibited toxicity to a wide spectrum of human pathogens and in some cases, showed inhibition effectiveness comparable to a standard antibiotic, gentamicin (Hossain et al., 2011b). On this basis, it is

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postulated that hydroxyl-functionalized imidazolium ILs may also exhibit anti-microbial characteristics.

The aims of the present study are therefore to synthesize and characterize a series of new 1-(2-hydroxyethyl)-3-alkylimidazolium chloride ILs and to investigate their anti-microbial activities, particularly in the context of microbial viabilities as a function of ILs concentrations. Four types of human pathogens, namely, *Escherichia coli*, *Aeromonas hydrophila*, *Listeria monocytogenes* and *Salmonella enterica* were selected to assess the potential toxicities of these ILs and their effectiveness as anti-microbial agents, which indirectly reflected their aquatic toxicities.

2. Materials and Methods

2.1. Synthesis of 1-(2-hydroxyethyl)-3-alkylimidazolium ILs

All the ILs were synthesized according to previously reported methods (Branco et al., 2002; Yeon et al., 2005; Hossain et al., 2011b). By way of example, ~0.14 mol of 1-methylimidazole (Sigma-Aldrich, USA, AR grade, 99 percent) and 0.2 mol of 2-chloroethanol (Merck KGaA, Darmstadt, Germany, synthesis grade) were added to a round-bottomed flask containing 200 mL of acetonitrile (Sigma-Aldrich, 98 percent). After fitting a reflux condenser, the flask was maintained at 343 K under nitrogen for 24 h with magnetic stirring. The reaction progress was monitored by a thin layer chromatography using aluminum sheets coated with silica gel, with methanol as the mobile phase. This product was kept at ~353 K under vacuum (1 Pa) overnight to remove volatiles and moisture. An analogous procedure was used to synthesize the other two ILs, using 0.11 mol imidazole (Merck, synthesis grade) and 0.6 mol 1-butylimidazole (Merck, synthesis grade). The reactions employed and the structures of the ILs so obtained are presented in Fig. 1. All in all, three ILs were synthesized and characterized: 1-(2-hydroxylethyl)imidazolium chloride, [2OHim][Cl], 1-(2-hydroxylethyl)-3-methylimidazolium chloride, [2OHimC][Cl] and 1-(2-hydroxylethyl)-3-butylimidazolium chloride, [2OHimC_4][Cl]. [2OHimC][Cl] was obtained as a solid-like IL while the other two ILs were in liquid form.

2.2. Characterization

The synthesized ILs were characterized using the Fourier-transform infrared (FTIR) spectroscopy (Shimadzu 8400S), ^{1}H-nuclear magnetic resonance (NMR) spectroscopy (Bruker Avance, 400 MHz) and elemental analysis (Leco 932). For NMR, 5 mg of the sample was dissolved in 0.7 mL of deuterated methanol (CD_3OD). The observed peaks (as seen in Table 1) are abbreviated as s (singlet), d (doublet) t (triplet) and m (multiplet). Elemental analyses were performed according to a standard procedure (ASTM-D5219, 2009). The solid samples (< 2 mg) were enclosed in a silver capsule whereas the liquid samples were analyzed in a silver capsule containing a sorbit pad. Analyses were performed in triplicate and averaged.

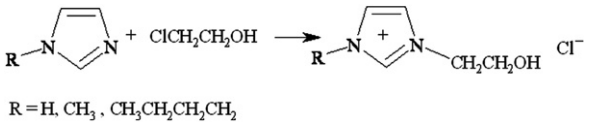

Fig. 1. Synthesis reaction and structures of the present ILs.

2.3. Anti-microbial activity

The ILs were assayed for anti-microbial activity against four registered microbial strains obtained from the Institute of Medical Research (IMR), Kuala Lumpur, Malaysia. These were: gram positive *L. monocytogenes* L 49 as well as gram negative *E. coli*, *A. hydrophila* and *S. enterica* S 1180. This test was carried out using the well-diffusion method (Tagg and McGiven, 1971; Benkerroum et al., 1993). Muller-Hinton agar (20 mL) (Merck, Germany) was dissolved, melted and cooled to 55 °C and subsequently inoculated with 1 mL of the bacterial suspension. The inoculated agar was transferred to a petri-plate and allowed to cool. Upon solidification of the medium, 6 mm diameter holes were created on the agar plate and 20 μL of the IL solution at different concentrations in deionized water were poured into the wells. The plates were then incubated at 37 °C for 24 h or until visible growth was established and the diameter of the inhibition-cleared zone around each well was determined. The screening results were compared with a standard antibiotic, namely, gentamicin (Atlantis Laboratory, Thailand).

2.4. Determination of minimal inhibitory concentration (MIC)

Determination of minimal inhibitory concentration (MIC) was conducted according to the standard procedure (CLSI-M07-A9, 2008) developed by the Clinical and Laboratory Standards Institute (CLSI), Pennsylvania, USA. The microbial strains were cultured on a Muller-Hinton broth (MHB) for 24 h. The ILs were added to the first two wells of two horizontal rows in the 96-well plate and twofold dilutions were made from the second set of wells while the last wells were kept untreated. Three replicates and seven different concentrations were studied for each IL. For turbid suspensions (optical density *ca* 0.1 to 0.3 at 530 nm), a 1:1000 dilution was used. The final bacterial inocula ranged from 10^5 to 10^7 colony-forming units (CFU) per milliliter. Gentamicin was used as a positive control while anhydrous dimethylsulfoxide (DMSO) (99.9 percent, Sigma-Aldrich) was used as a negative control. Microbial growth was visually determined after incubation for 24 h at 37 °C. The lowest concentration at which there was no visible growth (turbidity) was taken as the MIC. The 96 well-plate was subsequently kept in an ELISA plate reader to establish the EC_{50} (effective concentration of IL required for 50 percent toxicity within a specified exposure time) at wavelength 530 nm.

3. Results and discussion

3.1. Characterization

The IR, ^{1}H-NMR and elemental analysis data are listed in Table 1. The IL product yields ranged from 88 to 95 percent. For the ^{1}H-NMR spectrum of [2OHim][Cl], the peaks at δ 3.91 and 4.35 ppm indicate two two-proton triplets for –CH_2OH and –NCH_2– of the side-alkyl chain of the imidazolium ring, respectively. The –CH protons at C-4 and C-5 provide two doublet-doublets at δ 7.53 (J=0.16 Hz, 4-CH) and 7.67 (J=0.28 Hz, 5-CH). The other two singlet peaks correspond to the –NH and –CH proton on C-2 at δ 8.80 and 9.08, respectively. the hydroxyl peak of the studied ILs disappeared, which could be explained by a dynamic proton exchange reaction between the labile proton of the –OH group for the ILs and the labile deuterium of the –OD group for the solvent CD_3OD. This can be confirmed by the appearance of a peak with weak intensity at δ 3.31 ppm, which

Table 1
FTIR and NMR spectroscopic and elemental data for the synthesized ILs.

Ionic liquid	MW (g/mol)	Yield (%)	FTIR (cm^{-1})	^{1}H-NMR (δ ppm)	Elemental analysis (%)	
					Experimental	Calculated
[2OHim][Cl]	148.5	86	626.8, 759.9, 829.3, 1066.6, 1163.0, 1446.5, 1581.5, 1629.7, 2846.7, 2958.6, 3143.8, 3357.8	3.91 (t, 2H, CH_2OH), 4.35 (t, 2H, N$^+$-CH_2), 7.53 (dd, J=0.16 Hz, 4-CH), 7.67 (dd, J=0.28 Hz, 5-CH), 8.80 (s, 1H, 3-NH), 9.08 (s, 1H, 2-CH)	C 39.81 H 5.96 N 20.12	C 40.40 H 6.06 N 18.85
[2OHimC][Cl]	162.5	95	754.1, 867.9, 1072.3, 1164.9, 1448.4, 1568.0, 3072.4, 3145.7, 3232.5	3.90–3.93 (t, 2H, CH_2–OH), 4.00 (s, 3H, CH_3–N), 4.36–4.39 (t, 2H, CH_2–N), 7.62 (s, 1H, 4-CH), 7.68 (s, 1H, -5-CH), 9.01 (s, 1H, 2-CH)	C 44.53 H 7.03 N 17.11	C 44.30 H 6.77 N 17.23
[2OHimC_4][Cl]	204.5	88	752.2, 869.8, 1076.2, 1163.0, 1456.2, 1562.2, 2871.8, 3068.5, 3137.9, 3232.5	1.01 (t, 3H, CH_3), 1.39–1.42 (m, 2H, CH_2), 1.88–1.95 (m, 2H, CH_2), 3.90–3.92 (t, 2H, CH_2–OH), 4.29 (t, 2H, CH_2–N), 4.36 (t, 2H, CH_2–N), 7.70 (s, 1H, 4-CH), 7.72 (s, 1H, 5-CH), 9.10 (s, 1H, 2-CH)	C 52.16 H 8.97 N 12.98	C 52.81 H 8.31 N 13.69