Synthesis and anti-microbial activity of hydroxylammonium ionic liquids

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Abstract

Eight hydroxylammonium-based room temperature ionic liquids (ILs) have been synthesized by acid-base neutralization of ethanolamines with organic acids. The ILs were characterized by infrared and nuclear magnetic resonance spectroscopies and elemental analysis. Their anti-microbial activities were determined using the well-diffusion method. All eight ILs were toxic to *Staphylococcus aureus*, while 2-*hydroxyethylammonium lactate* and *2-hydroxy-N-(2-hydroxyethyl)-N-methylethanaminium acetate* showed high anti-microbial activity against a wide range of human pathogens.

Keywords: Hydroxyl ammonium ionic liquids; Anti-microbial screening; Inhibition potential; Human pathogens

1. Introduction

Room temperature ionic liquids (ILs) are low melting point organic salts with many interesting properties including potential as 'green' substitutes for volatile organic compounds in process chemistry and the food industry (Welton, 1999). Specific application of ILs include catalysis (Kalkhambkar et al., 2011), CO_2 absorption (Yokozeki et al., 2008), synthesis of nanoparticles (Antonietti et al., 2004) and usage as industrial solvents (Welton, 1999; Luo et al., 2009). Typically, ILs involve a combination of heterocyclic organic cations with inorganic or organic anions, which provides their unique properties. However, the high solubility of some ILs in water, coupled with several studies (Bernot et al., 2005, Pretti et al., 2009) highlighting their toxicity to aquatic organisms, raises questions about their long-term utility.

Bacteria are a good starting point to examine IL toxicity as they have short generation times (Pham et al., 2010) compared with other living organisms. This has indirectly led to the realization that some ILs exhibit anti-microbial characteristics. For example, Pernak et al. (2003, 2004) and Roslonkiewicz et al. (2005) have reported a trend of increasing toxicity towards a range of bacteria (including rods, cocci and fungi) with increasing chain length of alkyl substituents in pyridinium, imidazolium and quaternary ammonium salts. Quaternary ammonium compounds (QACs) are generally considered to be bioactive and have been used for environmental and medical equipment disinfection (Demberelnyamba et al., 2004). On this basis, it is reasonable to assume that hydroxylammonium ILs should also exhibit anti-microbial characteristics.

The objectives of this study are therefore to synthesize and characterize a series of hydroxylammonium ILs and to investigate their anti-microbial activities. Five types of human pathogens were selected to assess the potential toxicities of these ILs and their effectiveness as anti-microbial agents.

2. Materials and Methods

2.1. Synthesis and characterization of hydroxylammonium ILs

Eight hydroxylammonium ILs (hereafter referred to as **ILs 1** to **8**) were synthesized by acid-base neutralization of ethanolamines (**ILs 1**, **2**, **3** from 2-ethanolamine; **ILs 4**, **5**, **6** from 2,2'-iminodiethanol; **ILs 7** & **8** from bis(2-hydroxyethyl)methylamine) with three carboxylic acids (formic, acetic or lactic acid). Ethanolamines (ACS reagent grade) and acids (AR grade) were obtained from Merck (USA). In a typical preparation, a stoicheometric amount of the acid was added drop-wise to the ethanolamine contained in a round-bottomed flask

77 equipped with a reflux condenser, a magnetic stirrer and an inlet and outlet for N_2 gas. The
78 purpose of this procedure was to reduce the production of heat since the reaction is strongly
79 exothermic. After the acid had been added the mixture was maintained at room temperature
80 for 2 hours with stirring then heated to 60 ^{o}C to ensure complete reaction. Reaction progress
81 was monitored by thin layer chromatography, using aluminium sheets coated with silica gel
82 with methanol as the mobile phase. The resultant colourless, strongly hygroscopic, viscous
83 liquids were kept at 80 ^{o}C under vacuum overnight to remove volatiles (the present ILs are
84 thermally stable to ~300 ^{o}C). The synthesized ILs were characterized via infrared (Shimadzu
85 8400S) and ^{1}H-nuclear magnetic resonance (Bruker, 400 MHz) spectrometry and elemental
86 analyses (Leco 932). The water content of the ILs was ~1000 ppm, determined with a
87 coulometric Karl Fischer Titrator DL 39 (Mettler Toledo).
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89 *2.2. Anti-microbial activity*
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91 The ILs were assayed for anti-microbial activity against five registered microbial isolates
92 obtained from the Institute of Medical Research (IMR), Kuala Lumpur, Malaysia. These
93 were: gram-positive *Staphylococcus aureus* S 1426 and *Listeria monocytogenes* L 49 as well
94 as gram-negative *Salmonella typhi* S 1180, *Vibrio cholera*e V 116 and *Klebsiella pneumonia*
95 K 41. This test was conducted at the Department of Cell and Molecular Biology, University
96 Putra Malaysia, using the well diffusion method (Magaldi et al., 1998, 1999). Test plates
97 were prepared as follows. Muller Hinton agar (20 mL) (Merck, Germany) was melted and
98 cooled to 55 °C and then inoculated with 1 mL of the bacterial suspension. The inoculated
99 agar was transferred onto a petri-plate and allowed to cool. Upon solidification of the
100 medium, 6 mm diameter holes were created in the central part of the agar plate and 20 µL of
101 IL solution, at concentrations 1, 10 and 20% (v/v) 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: gentamicin (Roche Diagnostics, Germany).

3. Results and Discussion

3.1. Chemical structures of ILs

The reactions employed in this study are encapsulated in the scheme shown in Fig. 1. Eight hydroxylammonium ILs were prepared: *2-hydroxyethylammonium acetate* (**1**), *formate* (**2**) and *lactate* (**3**); *bis-(2-hydroxyethyl)ammonium acetate* (**4**), *formate* (**5**) and *lactate* (**6**); *2-hydroxy-N-(2-hydroxyethyl)-N-methylethanaminium acetate* (**7**) and *formate* (**8**) were synthesized and characterized. The product yields ranged from 70 to 90 % as shown in Table 1, which also includes the IR, ^{1}H-NMR and elemental analysis data.

A representative IR spectrum (of IL **2**, 2-hydroxylethylammonium formate) is shown in Fig. 2. The IR spectra of these compounds confirm the presence of the carboxylate ($–COO^{–}$) group from the symmetric and anti-symmetric stretching peaks at ~1595 cm^{-1} and ~1390 cm^{-1} respectively, with the former being overlapped by N–H vibrations. The broad absorption around 3350 cm^{-1} can be assigned to the presence of OH moieties. The peaks at approximately 2970 and 2930 cm^{-1} correspond to methyl and methylene C–H bonds. The broad band at 2400-3500 cm^{-1} is characteristic of the ammonium structure of the present ILs. The amine, methyl and hydroxyl structures detected via ^{1}H-NMR are consistent with the IR data. The calculated carbon, hydrogen and nitrogen percentages are in good agreement with

126 the corresponding observed values (Table 1). The structures of the synthesized hydroxyl
127 ammonium ILs are shown in Fig. 3.
128
129 *3.2. Anti-microbial activities of ILs*
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131 The anti-microbial activities of the synthesized ILs were established by evaluating the
132 inhibition zones on the agar plates. This zone is defined as the area on the agar plate where
133 growth of a microbe is prevented by the test compound. The results obtained are summarized
134 in Table 2. An IL concentration of 1 % v/v does not appear to show anti-microbial activity,
135 which may be contrasted with the findings of Ganske and Bornscheuer (2006) who found that
136 *1-butyl-3-methylimidazolium hexafluorophosphate* is toxic at 1 % v/v on *Escherichia coli*,
137 *Pichia pastoris* and *Bacillus cereus.*
138 At higher concentrations all the present ILs exhibited anti-microbial activity, with **IL 6**
139 being the least active. Both **IL 2** and **IL 7** showed anti-microbial activities to a wide range of
140 microbes, exhibiting noteworthy inhibition compared with gentamicin. *Staphylococcus*
141 *aureus* seemed to be susceptible to all the present ILs. Overall, the level of toxicity of the
142 studied ILs was: **ILs 2**, **7** (highest) **> ILs 4**, **8 > ILs 1, 3**, **5 > IL 6**. It has been established
143 (Pernak et al., 2003, 2004; Roslonkiewicz et al., 2005; Docherty and Kulpa, 2005) that an
144 increase in the chain length of alkyl substituents correlates with increased IL toxicity or
145 growth inhibition for both imidazolium- and ammonium-based ILs but no such correlation is
146 apparent in the present study.
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4. Conclusions

All eight hydroxylammonium ILs exhibited anti-microbial activity (especially against *Staphylococcus aureus*) with *2-hydroxyethylammonium lactate* and *2-hydroxy-N-(2-hydroxyethyl)-N-methylethanaminium acetate* being toxic to a wide spectrum of human pathogens and exhibiting inhibition effectiveness comparable to gentamicin. The IL *bis-(2-hydroxyethyl)ammonium lactate* was the least toxic amongst the eight liquids studied.

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Figure captions

Fig.1. Reaction scheme for the synthesis of the ammonium-containing ILs

Fig.2. IR spectrum of IL**2** (2-hydroxylethylammonium formate)

Fig.3. Chemical structures of the synthesized hydroxylammonium ILs.

Table 1. Characterization data for the synthesized ILs

Ionic Liquid	Formula	Molecular weight	Yield (%)	IR (cm^{-1})	^{1}H-NMR	Elemental analysis	
						Experimental (%)	Calculated (%)
1	$C_4H_{11}O_3N$	121	86	3350, 2970, 2930, 1594, 1390	δ_H 1.88 (s, 3H, –CH_3 acetate), 3.00 (t, 2H, –N–CH_2–), 3.30 (s, 3H, -NH_3), 3.74 (t, 2H, –O–CH_2–), 5.37 (s, 1H, OH)	C 40.07 H 9.27 N 12.12	C 39.67 H 9.09 N 11.57
2	$C_3H_9O_3N$	107	82	3290, 3058, 2939, 2879, 1650, 1531, 1382, 1174, 1062	δ_H 3.10 (t, 2H, –N–CH_2–), 3.50 (s, 3H, -NH_3), 3.74 (t, 2H, –O–CH_2–), 5.60 (s, 1H, OH)	C 32.98 H 8.34 N 12.56	C 33.67 H 8.41 N 13.08
3	$C_5H_{13}O_4N$	151	78	2970, 2931, 2877, 1566, 1411, 1355, 1309, 1117, 1070, 1022	δ_H 1.29 (s, 3H, -CH_3), 2.97 (t, 2H, -N-CH_2), 3.27 (q, 1H, -CH), 3.71 (t, 2H, -O-CH_2), 5.02 (m, 3H, -OH)	C 40.21 H 8.45 N 9.38	C 39.73 H 8.61 N 9.27
4	$C_6H_{15}O_4N$	165	90	3400, 2050, 1670, 1590, 1415,1080, 955	δ_H 1.91 (s, 3H, –CH_3 acetate), 3.14 (t, 4H, –N–CH_2–), 3.60 (s, 2H, -NH_2), 3.80 (t, 4H, –O–CH_2–), 5.37 (s, 2H, -OH)	C 42.85 H 9.25 N 7.96	C 43.64 H 9.09 N 8.48
5	$C_5H_{13}O_4N$	151	80	3188, 2923, 2852, 1554, 1395, 1334, 1066, 1043, 1016, 956	δ_H 1.30 (s, 3H, -CH_3), 3.08 (t, 4H, -N-CH_2), 3.80 (t, 4H, -O-CH_2), 3.98 (q, 1H, -CH),5.02 (m, 4H, -OH)	C 40.51 H 9.24 N 9.15	C 39.73 H 8.61 N 9.27
6	$C_7H_{17}O_5N$	195	81	3208, 2893, 2852, 1564, 1395, 1344, 1026, 1011	δ_H 1.91 (s, 3H, -CH_3), 2.88 (s, 3H, -CH_3), 3.26 (t, 4H, -N-CH_2), 3.87 (t, 4H, -O-CH_2), 5.27 (m, 4H, -OH)	C 42.69 H 8.59 N 7.65	C 43.08 H 8.71 N 7.18
7	$C_7H_{17}O_4N$	179	70	3217, 2788, 2696, 1587, 1463, 1375, 1340, 1137, 1074, 1008, 759	δ_H 1.87 (s, 3H, -CH_3), 2.80 (t, 4H, -N-CH_2), 3.27 (s, 3H, -NMe), 3.70 (bs, 1H, NH), 3.90 (t, 4H, -O-CH_2), 5.21 (m, 4H, -OH)	C 45.62 H 9.58 N 7.56	C 46.93 H 9.50 N 7.82
8	$C_6H_{15}O_4N$	165	84	3225, 2680, 2190, 1470, 1120, 956	δ_H 2.56 (t, 4H, -N-CH_2), 3.31 (s, 3H, -NMe), 3.60 (bs, 1H, NH), 3.79 (t, 4H, -O-CH_2), 5.38 (m, 4H, -OH)	C 44.21 H 9.26 N 8.47	C 43.64 H 9.09 N 8.48

Table 2. Inhibition zones (in mm) for the synthesized ILs[a]

Antibiotic / ionic liquid	Concentration (% v/v)	*Klebsiella pneumoniae*	*Staphylococcus aureus*	*Salmonella typhi*	*Vibrio cholera*e	*Listeria monocytogenes*
Gentamicin	20	10	21	20	10	20
IL 1	1	-	-	-	-	-
	10	-	7	-	-	-
	20	-	11	-	-	-
IL 2	1	-	-	-	-	-
	10	9	12	11	9	-
	20	10	14	14	10	-
IL 3	1	-	-	-	-	-
	10	-	7	-	-	-
	20	-	9	-	-	-
IL 4	1	-	-	-	-	-
	10	-	12	10	-	-
	20	-	14	14	-	-
IL 5	1	-	-	-	-	-
	10	-	7	-	-	-
	20	-	11	-	-	-
IL 6	1	-	-	-	-	-
	10	-	-	-	-	-
	20	-	7	-	-	-
IL 7	1	-	-	-	-	-
	10	-	13	13	8	16
	20	-	17	16	9	18
IL 8	1	-	-	-	-	-
	10	7	9	-	-	-
	20	9	10	-	-	-

[a] Absence of a number indicates that no inhibition zone was detected.

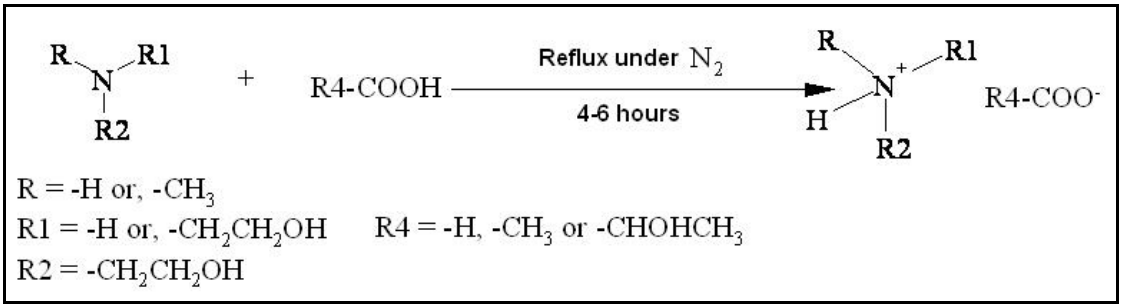

Fig.1. Reaction scheme for the synthesis of the ammonium-containing ILs

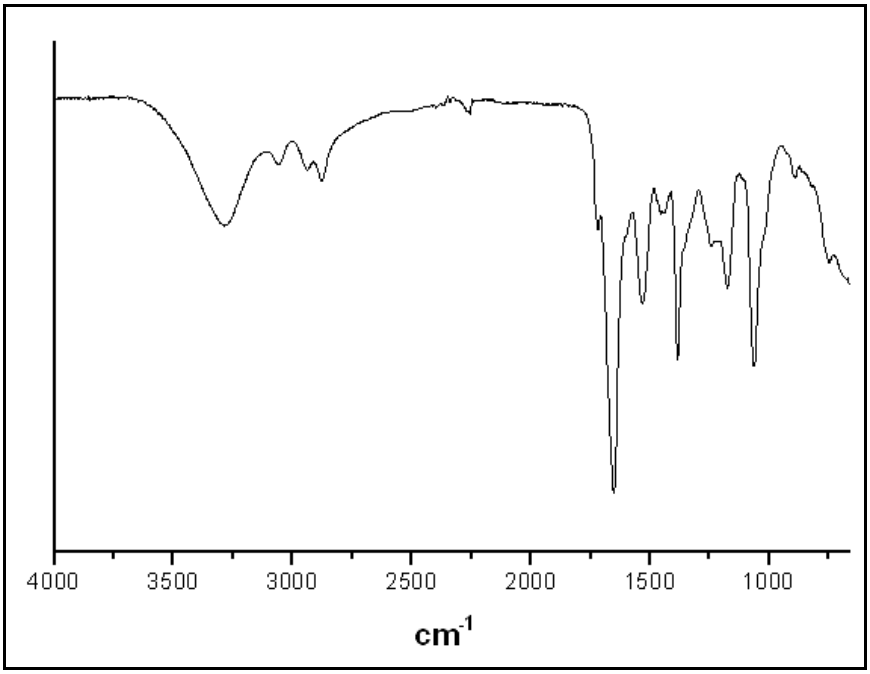

Fig.2. IR spectrum of IL**2** (2-hydroxylethylammonium formate)

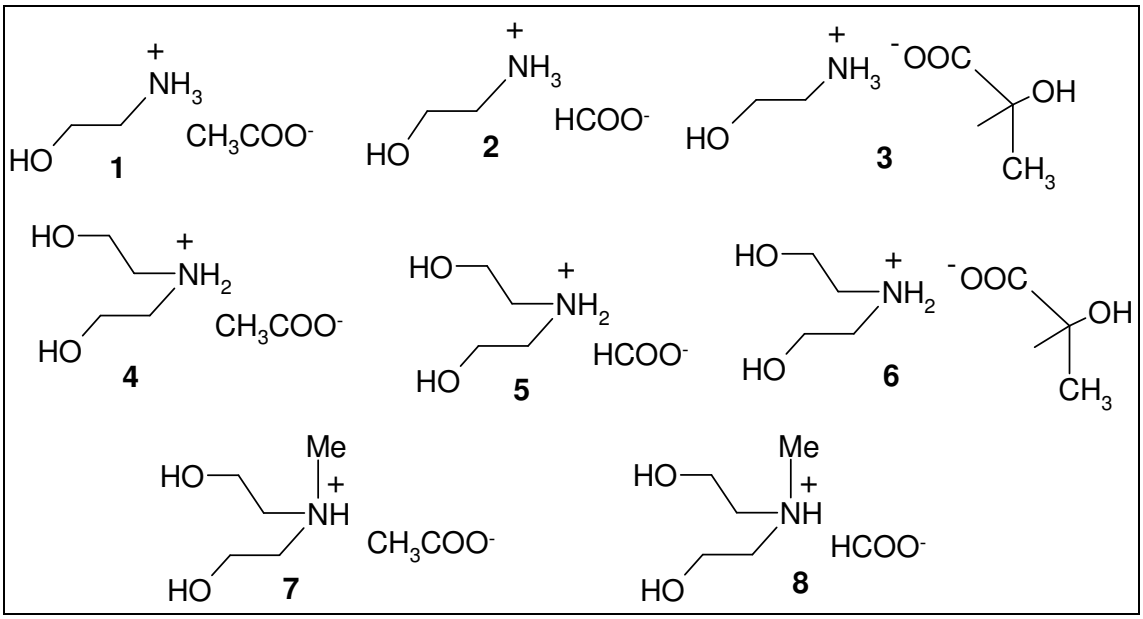

Fig.3. Chemical structures of the synthesized hydroxylammonium ILs.