



Toxicity of ionic liquids prepared from biomaterials



W. Gouveia^a, T.F. Jorge^a, S. Martins^a, M. Meireles^a, M. Carolino^b, C. Cruz^b,
T.V. Almeida^c, M.E.M. Araújo^{a,*}

^a CQB and Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Lisbon, Portugal

^b Centro de Biologia Ambiental, Faculdade de Ciências, Universidade de Lisboa, Lisbon, Portugal

^c CBIOS – Universidade Lusófona's Research Center for Health Science and Technologies, Lisbon, Portugal

HIGHLIGHTS

- Eight ionic liquids from biosources, were prepared with moderate to good yields.
- The toxicity of ILs was checked against organisms of various levels of organization.
- The toxicity was observed to depend on both the cation and anion.
- Choline-amino acid ILs showed a remarkable low toxicity to *A. salina* and HeLa cell.
- None of ionic liquids exhibited marked toxicity to bacteria.

ARTICLE INFO

Article history:

Received 8 May 2013

Received in revised form 9 October 2013

Accepted 16 October 2013

Available online 21 November 2013

Keywords:

Ionic liquids
Imidazolium
Biomaterials
Toxicity
Green chemistry

ABSTRACT

In search of environmentally-friendly ionic liquids (ILs), 14 were prepared based on the imidazolium, pyridinium and choline cations, with bromide and several amino acids as anions. Good yields were obtained in the synthesis of pyridinium ILs and those prepared from choline and amino acids. Four of the ILs synthesized from choline and the amino acids arginine, glutamine, glutamic acid and cystine are described here for the first time.

The toxicity of the synthesized ILs was checked against organisms of various levels of organization: the crustacean *Artemia salina*; Human cell HeLa (cervical carcinoma); and bacteria with different types of cell wall, *Bacillus subtilis* and *Escherichia coli*. The toxicity was observed to depend on both the cation and anion. Choline-amino acid ILs showed a remarkable low toxicity to *A. salina* and HeLa cell culture, ten times less than imidazolium and pyridinium ILs. None of ionic liquids exhibited marked toxicity to bacteria, and the effect was 2–3 orders of magnitude smaller than that of the antibiotic chloramphenicol.

© 2013 Elsevier Ltd. All rights reserved.

1. Introduction

Room Temperature Ionic Liquids (RTILs) are chemicals, recently developed, that have attracted a lot of attention from chemists. They are liquids composed not of molecules, but ions, an organic cation and an anion that can be organic or inorganic. Since they are composed of charged units, they have low vapor pressures and are considered non-volatile. RTILs are usually viscous liquids with a great capacity to dissolve inorganic and organic substances. They are miscible, immiscible or partially miscible with water, depending on the cation or anion, and they can be tailored to have the appropriate solvent properties by changing the cation or the anion (Brennecke et al., 2007; Kokorin, 2011).

Ionic liquids (ILs) and RTILs have been object of intense investigation towards many applications, for example, as catalysts

and thermal fluids. This class of materials, considered non-noxious and suitable for green processes in the early days of their application, has been subjected to toxicity studies at several biological levels in order to evaluate the risks to the environment from their use in production processes (Kärkkäinen, 2007; Pham et al., 2010). In fact, it has already been shown that some ILs, such as those based on imidazole, are more toxic than certain volatile organic compounds already used in the chemical industry, such as methanol and dichloromethane (Garcia et al., 2005). ILs containing cations or anions derived from biomaterials such as amino acids (AAs) have been developed (Fukumoto et al., 2005), and are expected to be less toxic and more biodegradable than ILs not derived from biosources (Brennecke et al., 2007). Recognizing that ILs may be sustainable products, it is of major importance to understand, the individual toxic effects of their substructures. Generally, three substructures are considered in the evaluation of their toxicity: (1) a positive portion designated as head-group, (2) the substituents present in that head-group and (3) the anion (Brennecke et al., 2007).

* Corresponding author. Tel.: +351 217500068.

E-mail address: eduaraujo@fc.ul.pt (M.E.M. Araújo).

In search of environmentally-friendly ionic liquids, 14 compounds (Fig. 1) were prepared and evaluated for their ecotoxicity to the crustacean *Artemia salina*, which is an invertebrate organism inhabiting estuarine ecosystems, widely employed in laboratory bioassays for toxicological applications (Parra et al., 2001). The ILs were also tested against the HeLa cellular line, to evaluate the possible damage to human cells due to direct contact. The effect of ILs on microorganisms was tested against two types of bacteria with distinct cell wall structures, to evaluate the potential impact of leakage to the environment. *Bacillus subtilis* is a ubiquitous species found in soils, associated with plant roots, in aquatic environments and also in animal gastrointestinal tracts (Ashlee et al., 2008), while *Escherichia coli* is a natural inhabitant of the intestinal tract of warm-blooded organisms, consistently associated with humans. The presence of *E. coli* in water is used as an indicator of fecal contamination, but recent reports indicate that soil populations can also be detected in tropical, subtropical, and some temperate environments (Ishii et al., 2006).

2. Material and methods

2.1. Equipment and reagents

FTIR spectra were obtained using a Mattson Satellite spectrophotometer, between 4000 and 450 cm^{-1} , with a resolution of 4 cm^{-1} . 100 Scans were accumulated. Samples were analyzed as discs obtained from a dispersion of material on anhydrous KBr.

^1H NMR and ^{13}C NMR spectra were acquired in a Bruker Avance 400 apparatus at 400 and 100.4 MHz, using D_2O , CDCl_3 or DMSO as solvents. The chemical shifts are reported in parts per million (ppm, δ), using the appropriate signal for residual solvent protons as reference. The RPMI 1640 cell culture medium, fetal bovine serum (FBS) and supplements were purchased from LONZA Co. Reagents and solvents were of analytical purity and provided by Sigma–Aldrich.

2.2. Synthesis of ionic liquids

The halogenated ionic liquids, 1-butylpyridinium bromide [C4py][Br], 1-hexylpyridinium bromide [C6py][Br], 1-butyl-3-methylimidazolium bromide [C4mim][Br] and 1-hexyl-3-methylimidazolium bromide [C6mim][Br] were prepared according to the procedures described in literature (Owens and Abu-Omar, 2002).

The amino acid derived ionic liquids (2-hydroxyethyl)trimethylammonium glycinate [Cho][Gly], (2-hydroxyethyl)trimethylammonium DL-alaninate [Cho][Ala], (2-hydroxyethyl)trimethylammonium DL-phenylalaninate [Cho][Phe], (2-hydroxyethyl)trimethylammonium glutamate [Cho][Glu], (2-hydroxyethyl)trimethylammonium methionate [Cho][Met], (2-hydroxyethyl)trimethylammonium arginine [Cho][Arg], (2-hydroxyethyl)trimethylammonium glutamate[(R, R)-3,3'-ditiobis(2-aminopropanoate)] [Cho][Cyst] were prepared according to the procedures described in

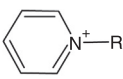
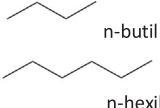
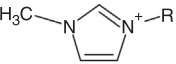
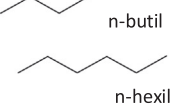
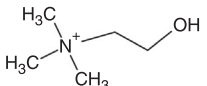
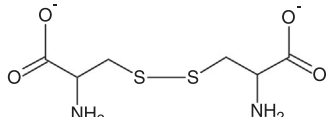
1-alkylpyridinium	
Cation	  R = n-butyl, n-hexyl
Anion	Br-
1-alkyl-3-methylimidazolium	
Cation	  R = n-butyl, n-hexyl
Anion	Br-, alaninate, phenylalaninate
Cholinium RTILs	
Cation	
Anion	Amino-acids: glycinate, D,L alaninate, D,L phenylalaninate, glutamate, methionate, arginine, glutamate Amino-acid dimer: 

Fig. 1. Structures of the ionic liquids prepared.

the literature (Moriel et al., 2010) with a slight modification. Briefly, 15.6 mL of a solution of cholinium hydroxide in methanol [Cho][OH]/MeOH 45%, 57.76 mmol) was evaporated under vacuum at 40 °C to remove methanol. Then an almost equivalent quantity of the corresponding amino acid (57.79 mmol) in 50 mL of water was added and the mixture was cooled in an ice bath and stirred for 12 h. Water was then removed under vacuum at 50 °C. A mixture of acetonitrile and methanol (9:1) was then added to precipitate the unreacted amino acid. The mixture was stirred vigorously and then filtered. The solvents were evaporated under reduced pressure. The purified ionic liquid was dried in vacuum overnight at 60 °C and stored under moisture-free conditions until use.

The methylimidazolium-amino acid ionic liquids, (1-butyl-3-methylimidazolium alalaninate [C4mim][Ala] and 1-butyl-3-methylimidazolium phenylalalaninate [C4mim][Phe]), were prepared according to the procedures described in the literature (Fukumoto et al., 2005; Ranu and Banerjee, 2005) with a slight modification. Briefly, the intermediate [C4mim][OH] was prepared reacting [C4mim][Br] (20.70 mmol) with KOH (21.85 mmol) in methanol (30 mL). The reaction occurred in a water bath at 60 °C during 12 h. Water (120 mL) was added to the mixture and then methanol was evaporated. The amino acid (20.68 mmol) aqueous solution (20 mL) was added to the [C4mim][OH] solution, and the resulting mixture was stirred vigorously for 12 h at room temperature. The product was then dried in a vacuum for one day.

The yields obtained are presented in Table 1. All ionic liquids were characterized by FTIR, ¹H NMR and ¹³C NMR spectroscopy, Tables SM-1, SM-2 and SM-3, Supplementary material (SM). The spectroscopic data of the previously described ILs ([C4py][Br], [C6py][Br], [C4mim][Br], [C6mim][Br], [C4mim][Ala], [C4mim][Phe], [Cho][Gly], [Cho][Ala] and [Cho][Phe]) agreed with the literature.

2.3. Toxicity assays

2.3.1. Aquatic microcrustacean *A. salina*

Mean lethal concentrations (LC₅₀) were determined by a modification of the *in vitro* test described by Parra et al. (2001). Artificial salty water (larvae medium) was obtained by dissolving 1.9 g marine salt from the Ria Formosa Natural Park in Algarve, Portugal, in 250 mL of distilled water. The pH of the solution was adjusted to 9 using Na₂CO₃ to avoid the pH rising during incubation, since this would be lethal to *A. salina* larvae. Larvae were incubated for 36–48 h at 25 °C under aerobic conditions, then 15 were transferred with micropipettes to a test tube which was filled to 5 mL with the ionic liquid (IL), so each tube contained 15 larvae, artificial salty water (larvae medium) and one IL. In the control tubes, the

Table 1
Physical state and yields of synthesized ionic liquids.

Ionic liquid	Physical state at rt ^a	Colour	η (%)
[C ₄ mim][Br]	Solid	White	62
[C ₆ mim][Br]	Liquid	Light yellow	67
[C ₄ mim][Ala]	Liquid	Light yellow	75
[C ₄ mim][Phe]	Solid	Light yellow	73
[C ₄ py][Br]	Solid	Dark yellow	94
[C ₆ py][Br]	Liquid	Colourless	95
[Cho][Ala]	Liquid	Dark yellow	78
[Cho][Arg]	Liquid	Orange	35
[Cho][Cys]	Liquid	Dark yellow	35
[Cho][Gln]	Liquid	Light yellow	65
[Cho][Glu]	Liquid	Light yellow	65
[Cho][Gly]	Liquid	Light yellow	90
[Cho][Met]	Liquid	Light yellow	90
[Cho][Phe]	Liquid	Orange	52

^a rt – room temperature (≤25 °C).

IL was replaced by distilled water. 8.5×10^{-5} mol L⁻¹ potassium dichromate was used as a positive control (Svensson et al., 2005). After 24 h exposure, live larvae were counted and the LC₅₀ value (Table 2) was calculated using the probit method (Rath et al., 2011). Mortality was corrected using the Abbott formula:

$$\text{Corrected mortality (\%)} = [1 - (n_t/n_c)] * 100$$

where n_t is the treated larvae population, n_c is the non treated control larvae population.

Tests were performed in triplicate and results are present as IC₅₀ ± SD ($p < 0.05$).

2.4. Cell cultures

The HeLa (cervical carcinoma) cell line was maintained in RPMI 1640 supplemented with 10% FBS, 200 U mL⁻¹ penicillin, 100 μg mL⁻¹ streptomycin and 0.3 g mL⁻¹ L-glutamine in a humidified atmosphere of 95% CO₂ at 37 °C.

2.4.1. Cell cultures and cytotoxicity assay by MTT

The MTT assay was used to determine cell viability to indicate the sensitivity of the cells to the ionic liquids tested. Exponentially growing cells were seeded at a density of approximately 4×10^5 cells mL⁻¹, in a 96-well flat bottomed microplate, and 48 h later they were incubated with the ILs. These compounds were dissolved in RPMI 1640 medium and tested in concentrations ranging from 1 to 4 mM. Each experiment included ten replicates for each concentration of each compound, and results represent three independent experiments. The cytotoxicity of test compounds was evaluated by the MTT method (Mossman, 1983). The optical density was measured at 570 nm using a 96-well multiscanner autoreader.

2.4.2. Antibacterial activity

The toxicity of ILs to culture bacteria was measured at a range of concentrations (0.1, 0.5 and 1 M) and their effect compared with that of a solution of the antibiotic chloramphenicol.

The bacteria used were Gram-positive *B. subtilis* subsp. *subtilis* strain 168 and Gram-negative *E. coli* K-12 MG1655. Toxicity was assayed by agar diffusion (Faleiro et al., 2005). Sterile filter paper disks of 6 mm containing 20 μL of each tested IL were distributed on Mueller Hinton agar plates, then inhibition zones were determined after an incubation period of 48 h at 28 °C. Twenty μL chloramphenicol (0.001 M disc⁻¹) was used as a positive reference. The assays were performed in duplicate in two independent experiments.

Table 2
Median lethal concentrations of the synthesized ionic liquids to *Artemia salina*.

Cation-based	Ionic liquid	LC ₅₀ (mM) ^a
1-Alkyl-3-methylimidazolium	[C4mim][Br]	0.092 ± 0.005
	[C4mim][Ala]	0.114 ± 0.009
	[C4mim][Phe]	0.094 ± 0.006
	[C6mim][Br]	0.079 ± 0.003
1-Alkylpyridinium	[C4py][Br]	0.117 ± 0.005
	[C6py][Br]	0.086 ± 0.001
	[Cho][Ala]	9.001 ± 0.319
	[Cho][Arg]	2.896 ± 0.223
	[Cho][Cys]	5.437 ± 0.070
Cholinium	[Cho][Gln]	6.468 ± 0.161
	[Cho][Glu]	6.278 ± 0.166
	[Cho][Gly]	9.517 ± 0.261
	[Cho][Met]	6.816 ± 0.262
	[Cho][Phe]	6.764 ± 0.300
AAs ^b (10×10^5 μM)		≤7%
K ₂ Cr ₂ O ₇ (85 μM)		100%

^a Data presented as LC₅₀ ± STD ($n = 3$).

^b AAs: alanine, arginine, cystine, glutamine, glutamic acid, glycine, methionine and phenylalanine.

3. Results and discussion

Fourteen ionic liquids were obtained with good purity levels, their physical appearance and yield is presented in Table 1. Good yields were obtained in the synthesis of pyridinium ILs and those prepared from choline and the amino acids glycine [Cho][Gly] and methionine [Cho][Met]. With exception of [C4py][Br] [C4mim][Br] and [C4mim][Phe], all the compounds were viscous liquids at room temperature. Synthesis of [Cho][Arg], [Cho][Cys], [Cho][Gln], [Cho][Glu] and [Cho][Met] is reported for the first time.

3.1. Toxicity to aquatic microcrustaceans

The crustacean *Artemia salina* L. (Artemiidae), is an invertebrate organism, component of the fauna of saline aquatic and marine ecosystems. It has been used in laboratory bioassays to determine toxicity through the estimation of the medium lethal concentration (LC₅₀). This organism has also been used to predict the toxicity of plant extracts through comparison with LD₅₀ value-results obtained from oral acute toxicity tests in mice (Parra et al., 2001).

Data presented in Table 2 show that the pyridinium-based ILs were slightly less toxic to *A. salina* than the imidazolium-based ILs. For the same bromide anion, toxicity increases slightly with the increasing length of alkyl chain (butyl to hexyl) present in both head-groups, imidazolium and pyridinium. These results indicate that the effect of the studied ILs on *A. salina* is similar to that on other marine organisms (Frade and Afonso, 2010). For ILs containing the same cation [C4mim]⁺, a change of the bromide anion to an amino acid carboxylate has practically no effect on toxicity. However, a marked difference appears when both the cation and the

anion were derived only from biomaterials (choline and amino acids, [Cho][AA]). In those compounds the toxicity can be two orders of magnitude lower (LC₅₀ is one hundred times bigger) than that of the imidazolium and pyridinium based ILs: the LC₅₀ of [Cho][Ala] is only 9.001 ± 0.319 mM, while that of [C₄mim][Ala] is 0.114 ± 0.009 mM. However, it is important to note that the ILs whose cation and anion derived from biomaterials [Cho][Arg] were found to be three times more toxic than [Cho][Ala] and [Cho][Gly]. Although they were derived from amino acids, the arginate anion is longer than alaninate and glycinate, and one can speculate if that also influenced toxicity, in a similar manner to the size of alkyl chain of the head groups.

3.2. Toxicity to the Human HeLa cell line

The ILs were assayed for cytotoxic activity to HeLa cells, which provide a model to evaluate the damage they might cause to human cells by direct exposure. The cells were exposed to each compound for 48 h. Using the colorimetric mitochondrial function-based MTT assay, it was possible to observe a decrease in cell viability with increase concentration of ionic liquids (Figs. 2 and 3). Nevertheless it was not possible to determine the IC₅₀ value (concentration required to inhibit cell proliferation by 50%, compared to the control viability) of any compound as none of the tested ILs was very effective as cytotoxic agents.

However, the results indicated some interesting structure-activity relationships. An increase of alkyl chain (butyl to hexyl) for ionic liquids containing the same bromide anion and imidazolium/pyridinium head groups (Fig. 2) results in a higher toxicity, as it does to *A. salina*. 50% viability is reached near 4 × 10³ μM for

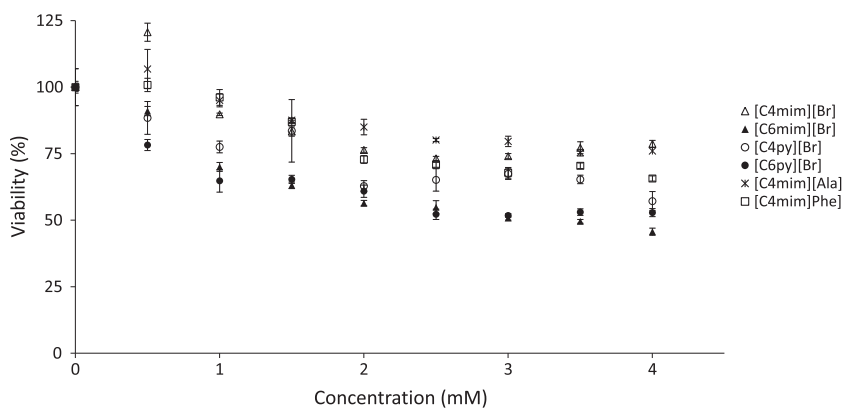


Fig. 2. Viability of HeLa cells exposed to imidazolium and pyridinium-based ionic liquids.

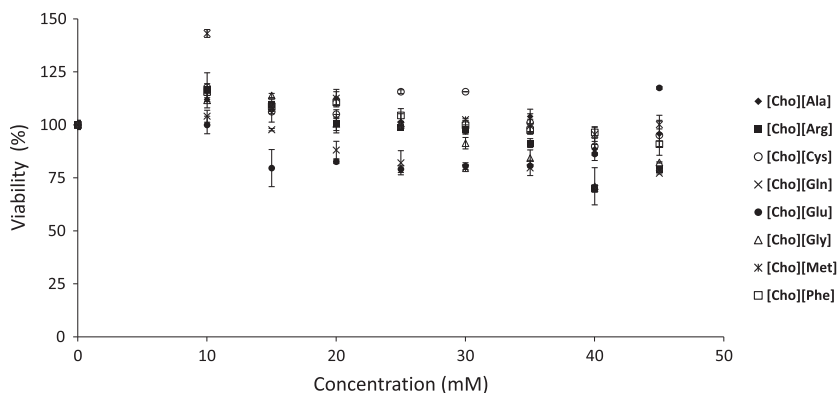


Fig. 3. Viability of HeLa cells exposed to cholinium-based ionic liquids.

Table 3

Bacterial inhibition halo (cm) due to exposure to ionic liquids or chloramphenicol (control). Inhibition values represent the mean \pm STD ($n = 4$).

Ionic liquids	Concentration (M)	Inhibition halo (cm)	
		<i>B. subtilis</i> (<i>B. subtilis</i> 168)	<i>E. coli</i> (<i>E. coli</i> K-12 MG1655)
[C ₄ mim][Ala]	0.1	0.90 \pm 0.14	0.85 \pm 0.07
[C ₆ mim][Br]	0.5	1.25 \pm 0.21	1.35 \pm 0.07
[C ₄ mim][Phe]	0.5	0.95 \pm 0.07	0.75 \pm 0.07
[Cho][Gly]	0.5	1.05 \pm 0.21	– ^a
[C ₆ py]Br	0.5	1.17 \pm 0.12	1.53 \pm 0.05
[Cho][Phe]	0.5	0.95 \pm 0.05	– ^a
[C ₄ mim][Br]	1.0	1.05 \pm 0.07	0.70 \pm 0.00
[Cho][Gln]	1.0	1.00 \pm 0.00	– ^a
[Cho][Met]	1.0	0.90 \pm 0.07	– ^a
[C ₄ py]Br	1.0	1.05 \pm 0.06	0.95 \pm 0.07
Chloramphenicol	0.001	1.93 \pm 0.12	1.80 \pm 0.10

^a No growth inhibition.

[C₆mim][Br] and [C₆py][Br]. For the same cation [C₄mim]⁺, changing the bromide anion to an amino-acid carboxylate [AA] reduces slightly the toxicity of the [C₄mim][AA] ionic liquids could be slightly less toxic such that they can be considered moderately toxic (Fatemi and Izadiyan, 2011). The 50% viability of [Cho][AA] was above $40 \times 10^3 \mu\text{M}$ (Fig. 3), indicating that these bio-ionic liquids are at least an order of magnitude less toxic than the imidazolium- and pyridinium-based ionic liquids, and can be considered to have low toxicity (Fatemi and Izadiyan, 2011).

3.3. Toxicity for soil bacteria

In general (Table 3), ILs were more toxic to *B. subtilis* (Gram + bacteria) than to *E. coli* (Gram–bacteria). Our results agree with those of Docherty and Kulpa (2005), who found *B. subtilis* to be more sensitive to 1-alkyl-3-methyl imidazolium and 1-alkyl pyridinium ILs than *E. coli*. Other authors (Saadeh et al., 2009; Khungar et al., 2012) also found that Gram-positive bacteria was more susceptible to various ionic liquids solutions as compared to the Gram-negative *E. coli*, which may be due to different interactions between these compounds and the peptidoglycan and lipid components of the cell wall of the Gram positive cells.

E. coli was not affected by even the highest concentration (1 M) of the choline-amino-acid-derived ILs [Cho][Gln], [Cho][Gly], [Cho][Met] and [Cho][Phe], while *B. subtilis* was inhibited by [Cho][Gln] and [Cho][Met] at the same concentration.

Regarding the effect of the cation, 0.5 M of [C₄mim][Phe] and [Cho][Phe] produced the same halo growth inhibition on *B. subtilis*. However, *E. coli* was only affected by [Cho][Phe], not [C₄mim][Phe], again demonstrating that the toxicities of an IL to Gram-positive and Gram-negative bacteria can be quite different, such that these new chemicals should always be tested on different structural types of bacteria.

The influence of the length of the alkyl chain linked to the methylimidazolium cation also influences toxicity. The IL with a smaller chain, [C₄mim][Br], was less toxic than [C₆mim][Br].

[C₄mim][Phe] presented a similar toxicity to [C₄mim][Ala] for both bacteria at a higher concentration (0.5 M), indicating that toxicity to these microorganisms, is due not only to the cation but also the anion.

We also observed an increase of toxicity of pyridinium bromide with the length of the alkyl chain; [C₆py]Br was more toxic than [C₄py]Br to both tested cultures. Nevertheless, pyridinium ILs were less toxic than imidazolium ILs.

Of all the ILs tested, [C₄mim][Ala] was the most toxic to soil bacteria (*B. subtilis* and *E. coli*), inhibiting growth even at the lowest concentration tested (0.1 M). However, the growth inhibition

observed was smaller than that induced by chloramphenicol at concentrations two orders of magnitude lower (0.001 M).

4. Conclusion

Preparation of ILs from biosources was easily achieved, generally with good yields. Almost all synthesized compounds were liquid at room temperature (RTILs) and all were soluble in water. The toxicity of these compounds was evaluated against three models of different levels of biological organization: a crustacean, the brine shrimp *A. salina*; Human cell line (HeLa); and bacteria (*B. subtilis*, Gram+; *E. coli*, Gram–). The toxicity of the ILs to *A. salina* was found to depend on both cation and anion. However, an exchange of the cation from methylimidazolium to cholinium strongly lowered the toxicity to *A. salina*. The same effect was observed in tests on HeLa cell culture. Nonetheless, the same relation between chemical structure and toxicity was not found in tests on bacteria. None of the ionic liquids studied exhibited marked toxicity to Gram positive and Gram negative bacteria.

Generally, ionic liquids produced from the biocompounds choline and amino acids were found to have low toxicity to humans and to the environment, so they are a promising class of materials for a wide range of uses.

Acknowledgment

Funding for this work, PEst-OE/UI0612/2011, was supported by FCT (Fundação para a Ciência e Tecnologia, Portugal).

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.chemosphere.2013.10.055>.

References

- Ashlee, M.E., Losick, R., Kolter, R., 2008. Ecology and genomics of *Bacillus subtilis*. *Trends Microbiol.* 16, 269–275.
- Brennecke, J.F.D., Rogers, R.D., Seddon, K.R. (Eds.), 2007. Ionic liquids IV: not just solvents anymore, ACS Symposium Series, American Chemical Society, Washington, 408p.
- Docherty, K.M., Kulpa, C.F., 2005. Toxicity and antimicrobial activity of imidazolium and pyridinium ionic liquids. *Green Chem.* 7, 185–189.
- Faleiro, L., Miguel, G., Gomes, S., Costa, L., Venâncio, F., Teixeira, A., Figueiredo, A.C., Barroso, J.G., Pedro, L.G., 2005. Antibacterial and antioxidant activities of essential oils isolated from *Thymbra capitata* L. (Cav.) and *Origanum vulgare* L. *J. Agr. Food. Chem.* 53, 8162–8168.
- Fatemi, M.H., Izadiyan, P., 2011. Cytotoxicity estimation of ionic liquids based on their effective structural features. *Chemosphere* 84, 553–563.
- Frade, R.F.M., Afonso, C.A.M., 2010. Impact of ionic liquids in environment and humans. *Hum. Exp. Toxicol.* 29, 1038–1054.
- Fukumoto, K., Yoshizawa, M., Ohno, H., 2005. Room temperature ionic liquids from 20 natural amino acids. *J. Am. Chem. Soc.* 127, 2398–2399.
- Garcia, M.T., Gathergood, N., Scammells, P., 2005. Biodegradable ionic liquids, Part II. Effect of the anion and toxicology. *Green Chem.* 7, 9–14.
- Ishii, S., Kroll, W.B., Hicks, R.E., Sadowsky, M.J., 2006. Presence and growth of naturalized *Escherichia coli* in temperate soils from Lake Superior watersheds. *Appl. Environ. Microbiol.* 72, 612–621.
- Kärkkäinen, J., Preparation and characterization of some ionic liquids and their use in the dimerization reaction of 2-methylpropene. *Acta Univ. Oul. A* 480, Oulu, Finland, 2007, 141p.
- Khungar, B., Rao, M.S., Pericherla, K., Nehra, P., Jain, N., Panwar, J., Kumar, A., 2012. Synthesis, characterization and microbiocidal studies of novel ionic liquid tagged Schiff bases. *C. R. Chim.* 15, 669–674.
- Kokorin, A. (Ed.), 2011. Ionic liquids: Applications and perspectives, InTech, 674p. <<http://www.intechopen.com/books/ionic-liquids-applications-and-perspectives>> (accessed April 2013).
- Moriel, P., García-Suárez, E.J., Martínez, M., García, A.B., Montes-Morán, M.A., Calvino-Casilda, V., Bañares, M.A., 2010. Synthesis, characterization and catalytic activity of ionic liquids based on biosources. *Tetrahedron Lett.* 51, 4877–4881.

- Mossman, T., 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Meth.* 65, 55–63.
- Owens, G.S., Abu-Omar, M.M., 2002. Comparative kinetic investigations in ionic liquids using the MTO/peroxide system. *J. Mol. Catal. A: Chem.* 187, 215–225.
- Parra, A.L., Yhebra, R.S., Sardiñas, I.G., Buela, L.I., 2001. Comparative study of the assay of *Artemia salina* L. and the estimate of the medium lethal dose (LD50 value) in mice, to determine oral acute toxicity of plant extracts. *Phytomedicine* 8, 395–400.
- Pham, T.P.T., Cho, C.W., Yun, Y.S., 2010. Environmental fate and toxicity of ionic liquids: a review. *Water Res.* 44, 352–372.
- Ranu, B.C., Banerjee, S., 2005. Ionic liquids as catalyst and reaction medium. The dramatic influence of ionic liquid, [bmimOH], in Michael addition of active methylene carboxylic esters and nitriles. *Org. Lett.* 7, 3049–3052.
- Rath, S., Sahu, M., Dubey, D., Debata, N., Padhy, R., 2011. Which value should be used as the lethal concentration 50 (LC50) with Bacteria? *Interdiscip. Sci. Comput. Life Sci.* 3, 138–143.
- Saadeh, S.M., Yasseen, Z., Sharif, F.A., Shawish, H.M.A., 2009. New Room Temperature Ionic Liquids with interesting ecotoxicological and antimicrobial properties. *Ecotoxicol. Environ. Saf.* 72, 1805–1809.
- Svensson, B.M., Mathiasson, L., Rtensson, M., Bergström, S., 2005. *Artemia salina* as test organism for assessment of acute toxicity of leachatewater from landfills. *Environ. Monit. Assess.* 102, 309–321.