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(Eco)toxicity and biodegradability of protic ionic liquids

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HIGHLIGHTS

- The toxicity and biodegradability of four PILs was tested.
- The elongation of the alkyl chain tends to increase the toxic effect of PILs.
- The PILs m-2-HEAA and m-2-HEAP are the less and the most toxic.
- The four PILs have low biodegradability.

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ABSTRACT

Ionic liquids (ILs) are often claimed to be "environmentally friendly" compounds however, the knowledge of their potential toxicity towards different organisms and trophic levels is still limited, in particular when protic ionic liquids (PILs) are addressed. This study aims to evaluate the toxicity against various microorganisms and the biodegradability of four PILs namely, N-methyl-2-hydroxyethylammonium acetate, m-2-HEAA; N-methyl-2-hydroxyethylammonium propionate, m-2-HEAPr; N-methyl-2-hydroxyethylammonium butyrate, m-2-HEAB; and N-methyl-2-hydroxyethylammonium pentanoate, m-2-HEAP. The antimicrobial activity was determined against the two bacteria, *Sthaplylococcus aureus* ATCC-6533 and *Escherichia coli* CCT-0355; the yeast *Candida albicans* ATCC-76645; and the fungi *Fusarium* sp. LM03. The toxicity of all PILs was tested against the aquatic luminescent marine bacterium *Vibrio fischeri* using the Microtox® test. The impact of the PILs was also studied regarding their effect on lettuce seeds (*Lactuta sativa*). The biodegradability of these PILs was evaluated using the ratio between the biochemical oxygen demand (BOD) and the chemical oxygen demand (COD). The results show that, in general, the elongation of the alkyl chain tends to increase the negative impact of the PILs towards the organisms and biological systems under study. According to these results, m-2-HEAA and m-2-HEAP are the less and most toxic PILs studied in this work, respectively. Additionally, all the PILs have demonstrated low biodegradability.

1. Introduction

Ionic liquids (ILs) are molten salts at low temperature which properties can be tuned for a specific application, by the adequate cation/anion/alkyl chain combination (Hussey, 1988). Because of their unique properties, including non-volatility and non-flammability, variable solubility, high chemical and thermal stability (Domanska, 2006), high ionic conductivity and wide electrochemical potential window, ILs have been widely studied, used and recognized as promising alternatives for various applications, some of them with high industrial potential (Wasserscheid and Keim, 2000). In this scenario, ILs have been applied as promising alternatives in organic synthesis (Wang et al., 2003), catalysis (Souza et al., 2003), electrochemical (Jiang et al., 2004), biocatalysis

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(Ventura et al., 2012b; Sintra et al., 2014), enzyme immobilization (Souza et al., 2013b; Oliveira et al., 2014) and in various extraction processes (Freire et al., 2012; Ventura et al., 2012a, 2012c).

Recently, the protic ILs (PILs) have been the principal focus of several studies (Chen et al., 2014; Huang et al., 2014; Kusano et al., 2014; Peric et al., 2014; Santos et al., 2014). They are synthesized by proton transfer from a Brønsted acid to a Brønsted base (Anouti et al., 2008; Mirjafari et al., 2013). A key property of PILs is their capacity to promote hydrogen bonds, in which proton acceptance and proton donation are included (Austen et al., 2012). The interest in this class of ILs stems from their simple synthesis, low cost of preparation and purification, and also their claimed biodegradable nature (Hussey, 1988). They have been applied in organic synthesis (Hangarge et al., 2002), chromatography techniques (Poole, 2004), as proton conducting electrolytes (Menne et al., 2013), catalysts (Jiang et al., 2004), and solvents (Achinivu et al., 2014). PILs are normally considered as of good technical performance (Greaves et al., 2006), however the current European Union environmental legislation concerning the registration, evaluation, authorization and restriction of chemicals (REACH, 2006) imposes the safety assessment of new chemicals in which (eco)toxicological and biodegradation demands are included. Despite the widespread idea that PILs should be less toxic than the aprotic ILs (Peric et al., 2014) considering the aquatic compartment, they are poorly studied and new structures require new and adequate tests. Peric and collaborators (Peric et al., 2011, 2013, 2014, 2015) are one of the most active groups in the study of the toxicological profile of PILs. The authors have performed tests to assess the toxicity of different chemical structures considering aquatic and terrestrial organisms and also on their biodegradability profile (Peric et al., 2013). They found that one of the most sensitive species studied considering the evaluation of the toxicity of different PILs was the macrophyte *Lemna minor* (Peric et al., 2013). These PILs were also found to be more toxic for *Lemna minor* than the aliphatic ILs studied in the same work (Peric et al., 2013), which the authors attributed to the higher hydrophilicity of PILs. As a result of their investigation, the authors classified 2-hydroxytriethanolamine pentanoate (2-HDEAP) as harmful regarding the three plants analyzed, namely Allium cepa (onion), Lolium perenne (grass) and the dicotyledonae Raphanus sativus (radish) (Peric et al., 2014). Moreover, the authors have also performed tests considering the acetylcholinesterase inhibition (Peric et al., 2013) which are normally attributed to the study of the potential biochemical toxic mechanisms imposed by toxicants. These results have highlighted the incapacity of the PILs tested to inhibit acetylcholinesterase, being the contrary conclusion obtained for the aprotic ILs under study (Peric et al., 2013).

Despite the lack of current data in the biodegradability of PILs, there are some results already being provided in the open literature, as recently reviewed by Gathergood and collaborators (Jordan and Gathergood, 2015). In this work, the authors mentioned that the majority of the PILs being classified as biodegradable, are those synthesized from analogues of cholinium salts (Jordan and Gathergood, 2015). Thus, the low toxicity and good biodegradability of shorter PILs (Peric et al., 2013) claimed still for a very limited number of chemical structures, allowed the general conclusion that together with their low production costs, simple preparation and still numerous applications, they are environmental benign alternatives considering the ILs class.

The main objective of this work is the toxicity and biodegradability evaluation of four PILs, in which we have applied experimental tests to determine *i)* their antimicrobial activity, towards a Gram-negative and a Gram-positive bacteria, one fungus and one yeast, *ii)* their toxicity *via* Microtox®, *iii)* their phytotoxicity tested in lettuce seeds and *iv)* their inherent biodegradation in water. Their toxic effect was studied regarding six biological systems, five microorganisms, the bacteria *Vibrio fischeri*, *Escherichia coli* CCT-0355 and *Staphylococcus aureus* ATCC-6533, the yeast *Candida albicans* ATCC-76645 and the mold *Fusarium* sp. LM03 and one plant (*Lactuta sativa*) was assessed. Here, model organisms that represent entire classes were selected being assumed that their reaction to the toxic compound can be extrapolated to other organisms of the same class. The IL concentration which prevented the germination of lettuce seeds in 50% (LD_{50}), analyzed by the final germination percentage (FG) and LD_{50} parameters were also determined considering the phytotoxic tests done.

The biodegradability was tested by the determination of the Chemical Oxygen Demand (COD) which is a parameter that measures the amount of organic matter capable to be oxidized by a chemical *via* when in a liquid sample (expressed in mg O_2 L^{-1}). The Biological Oxygen Demand (BOD) represents the amount of oxygen consumed in the biodegradation of the organic matter in the aquatic environment by biological processes. Behind the COD and BOD parameters, the percentage of oxygen consumed (%O_2) was also determined. This method is recurrently used in wastewater treatment studies (Anastasi et al., 2010). These parameters are being used to evaluate the biodegradation profile of different ILs (Jordan and Gathergood, 2015).

2. Experimental section

2.1. Materials

In this work, four PILs were used, namely the N-methyl-2-hydroxyethylammonium acetate (m-2-HEAA), the N-methyl-2-hydroxyethylammonium propionate (m-2-HEAPr), the N-methyl-2-hydroxyethylammonium butyrate (m-2-HEAB) and the N-methyl-2-hydroxyethylammonium pentanoate (m-2-HEAP), whose chemical structures are depicted in Fig. 1. Those were synthesized at our laboratory, by reacting equimolar amounts of the amine and the respective organic acids, as detailed elsewhere (Matzke et al., 2010). All ILs were used in this study in their pure form (99%). Tetracycline (purity of 95–100%) and miconazole (purity of 99.77%), compounds used as positive controls in the antimicrobial experiments for bacteria and fungus, were purchased at DEG Farmacêutica and Genix Farmacêutica, respectively. NaCl (purity of 99%) from Quimex was used as the negative control in the antimicrobial experiments. The lettuce seeds were purchased in the Central Market from Aracaju, Sergipe, Brazil.

2.2. (Eco)toxicity evaluation

2.2.1. Antimicrobial activity tests

To test the antimicrobial activity of the PILs, a standard protocol validated by us for aprotic ILs (Ventura et al., 2012d) and adopted from literature (Rebros et al., 2009; Biczak et al., 2014) was followed. Briefly, aqueous solutions of miconazole (50 μg L^{-1}) and tetracycline (50 μg L^{-1}) were used as reference compounds and positive control for fungi and bacteria, respectively. The negative control used in this work was an aqueous solution of NaCl at 0.9% (w/v). The microorganisms, *E. coli* CCT-0355 (Gram-negative bacteria), *Staphylococcus aureus* ATCC-6533 (Gram-positive bacteria), *Fusarium* sp. LM03 (mold) and *C. albicans* ATCC-76645 (yeast). These microorganisms were grown in a Bushell-Hass medium (total composition, g L^{-1}: $MgSO_4$, 0.2; $CaCl_2$, 0.02; KH_2PO_4, 1.0; $(NH_4)_2HPO_4$, 1.0; KNO_3, 1.0; and $FeCl_3$, 0.05) until an optical density of 1.0, taking into account the MacFallen scale (Ventura et al., 2012d). Suspensions of 1 mL of these microorganisms were uniformly spread on the plates [samples prepared with Nutrient Agar (total composition (g L^{-1}): meat extract 3.0; peptone 5.0; agar 15;