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Ecotoxicity assessment of dicationic *versus* monocationic ionic liquids as a more environmentally friendly alternative

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ABSTRACT

One of the reasons why ionic liquids have received growing interest from researchers is their environmentally interesting characteristics, such as their negligible vapour pressure and their good chemical and thermal properties. In particular, dicationic ionic liquids whose thermal and electrochemical stability is higher than that of monocationic ionic liquids have begun to gain attention during recent years. In this work, monocationic and dicationic ionic liquids were synthesized, characterized and tested for their toxicity, which was assessed using the luminescent bacterium *Vibrio fischeri*. The results revealed that the toxicity of the ionic liquids mainly depends on the head groups and linkage chain length of their cationic structure. Introduction of a new cationic head decreased the EC_{50} (concentration which leads to 50% reduction in bioluminescence of the bacteria) of the ionic liquids. The results present a promising picture of dicationic ionic liquids as alternatives with lower environmental impact than their monocationic counterparts and underline the significance of designing particular structures for ionic liquids.

1. Introduction

Ionic liquids (ILs) are organic salts which are liquids at or near ambient conditions ($T < 100\,^{\circ}C$). ILs have gained much attention as alternative solvents during recent years due to their rather unique combination of physical and chemical properties, which include negligible vapour pressure, non-flammability, wide liquid state temperature range, high thermal and chemical stability and high ionic conductivity (Lee and Lin, 2014). Moreover, the properties of ILs can be tuned by adjusting the structures of their anion and cation, or both, leading ILs to become known as “designer solvents” (Viswanathan et al., 2006). In this way, it is possible to control several physical properties, such as hydrophobicity, viscosity, density, solubility and their biodegradability and toxicological behaviour (Ventura et al., 2012). Because of these properties, monocationic ILs can be used in a wide variety of applications in different inter-disciplinary research areas, such as in the field of chemical engineering, organic synthesis, separation process, material science, catalysis, biocatalysis, green chemistry, sensoristics, medicine, electrochemistry, electronic devices (Galinski et al., 2006; Hough et al., 2007; Kogelnig et al., 2010; Kore and Srivastava, 2013; Wasserscheid and Welton, 2008) and supported liquid membranes (Víllora, 2013).

Geminal (symmetrical) dicationic ILs are generally composed of two distal cationic head groups linked by some form of linking fragment, which may be composed of simple alkyl chain, or more functional in nature. These compounds are especially interesting because they generally possess higher thermal and electrochemical stability, making them more suitable than monocationic ILs for use in high-temperature applications (Steudte et al., 2014). In this context, the suitability of dicationic ILs as reaction media has been studied when high temperatures are necessary (Han and Armstrong, 2005). They have great potential to be used as solvents for high temperature uses, surfactants, lubricants, nanoparticle coating, gas chromatography stationary phases, separation media and catalyst for esterification and transesterification reactions (Wei-Li et al., 2014; Anderson et al., 2005).

Asymmetrical dicationic ILs are another type of dicationic ILs which consist of different head groups of cation which are also attached via a linking fragment such as an alkyl chain. These asymmetrical ILs can be said to have dual functionality as they have two different head groups (Masri et al., 2016). Asymmetrical dicationic ILs based on both imidazolium and aliphatic ammonium have been synthesized as potential electrolyte additives applied to lithium secondary batteries (Zhang et al., 2008).

On the other hand, heteroanionic dicationic ILs can be symmetrical

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or asymmetrical (head groups), but they have one dication with two different anions. Currently, dicationic ILs are extensively investigated as separation material (supported liquid membrane technology) and catalyst candidates (hydrolysis, biodiesel production, esterification of carboxylic acid…) (Masri et al., 2016).

Although ILs should not contribute to atmospheric pollution due to their non-volatility (Pham et al., 2010), they can be easily soluble in water and therefore could be toxic to aquatic organisms (Freire et al., 2009) in cases of accidental release or at the end of their life cycle. For this reason, the environmental behaviour and toxicological effects of ILs need to be evaluated. However, more studies related to the ecotoxicological risk profiles of ILs are required because of the huge number of ILs that can be synthesized (Ventura et al., 2011; Lee and Lee, 2009). During the past few years, some publications have studied IL toxicity using different aquatic organisms such as bacteria (especially, *Vibrio fischeri*) (Montalbán et al., 2016; Samorì et al., 2007; Pinto et al., 2012; García et al., 2005; Docherty and Kulpa, 2005; Romero et al., 2008; Stolte et al., 2007; Ventura et al., 2011, 2012, 2014; Luis et al., 2007), green algae (*e.g. Pseudokirchneriella subcapitata*) (Pretti et al., 2011; Pham et al., 2008), aquatic plants (*e.g.* duckweed *Lemna minor*) (Stolte et al., 2007; Larson et al., 2008; Zhang et al., 2013), invertebrates (mainly the freshwater crustacean *Daphnia magna* and *Artemia salina*) (Pretti et al., 2011; Wells and Coombe, 2006; Steudte et al., 2014; Gouveia et al., 2014; Vraneš et al., 2016) or vertebrates like fish (the zebrafish *Danio rerio*) (Pretti et al., 2006, 2011) or frogs (*Rana nigromaculata*) (Li et al., 2009). In these studies, ILs show a varying hazard potential, depending on their individual chemical structure and morphology. Regardless of the studied test system, the strongest effect on IL toxicity seems to be their lipophilicity (Steudte et al., 2014). However, despite the increasing number of studies which evaluate IL aquatic toxicity, the information available is still limited, especially information related to dicationic ILs.

Several published works have attempted to predict the aquatic toxicity of ILs by Quantitative Structure-Activity Relationships (QSAR) methods (Luis et al., 2007, 2010; Lacrămă et al., 2007; Ismail Hossain et al., 2011; Couling et al., 2006; Bruzzone et al., 2011) which are mathematical models mainly based on the anion, the cation core and the length of the alkyl chain of the cation of the IL. Nevertheless, this kind of models is not sufficiently well developed to be applied to more complex or functional structures including relatively simple dicationic ILs. For this reason, more experimental data concerning the toxicity of dicationic ILs are required for incorporation in the respective databases, which will help predict their toxicity.

According to Ventura et al. (2011), one of the most widely used toxicological tests is the Microtox® Acute Toxicity Test, which uses the gram negative marine bacterium *Vibrio fischeri* (formerly known as *Photobacterium phosphoreum*). In 2007, *Vibrio fischeri* was renamed to *Aliivibrio fischeri* (Urbanczyk et al., 2007). This test is one of the most commonly used bioassay tests due to the intense and stable light emission of these bacteria and because it is highly sensitive to different compounds (Fuentes et al., 2006), and has been used for more than two decades in a large number of studies to determine the toxicity of conventional organic compounds toxicity for more than two decades (Kaiser and Palabrica, 1991). In fact, this method constitutes a standard (eco) toxicological inhibition assay in Europe (DIN EN ISO 11348) (ISO 11348-3, 2007), which determines the toxicity of a substance toward *Vibrio fischeri* by measuring the diminution in their light output. This decrease in *Vibrio fischeri* light emission is due to a reduction in enzymatic activity, so that any luminescence is directly proportional to the metabolic activity of the bacterial population (Parvez et al., 2006).

The main goal of this work was to measure the aquatic toxicity of a set of twenty-six imidazolium, pyrrolidinium and pyridinium-based ILs (9 monocationic and 17 dicationic) (see Table 1 in Montalbán et al. (2017)) using the *Vibrio fischeri* inhibition test as the effective nominal EC_{50} concentration (concentration necessary to decrease 50% of luminescence produced by the bacteria population). To date, the synthesis of some of these compounds has not been reported and hence no report in the literature has studied the toxicity of the most of these ILs (22 out of 26) towards *Vibrio fischeri*. The results represent an accurate study of the influence of the composition and structure on ILs toxicities.

2. Materials and methods

2.1. Test chemicals

1-methylimidazole (> 99%), 1-methylpyrrolidine (> 98%), pyridine (> 99%), 1-bromooctane (> 99%), 1,2-dibromoethane (> 98%), 1,3-dibromopropane (> 99%), 1,4-dibromobutane (> 99%), 1,6-dibromohexane (> 96%), 1,8-dibromooctane (> 98%), 1,12-dibromododecane (98 > %), lithium bis(trifluoromethane)sulfonylimide (99.95 > %) sodium hexafluoroantimonate (V) (technical grade), acetonitrile, ethyl acetate, dichloromethane and methanol were purchased from Sigma Aldrich (Steinheim, Germany). All chemical products were used without additional purification.

2.2. Synthesis and characterization

All ILs investigated herein were prepared in the laboratories of the University of Nottingham. A full description of each chemical synthesis is provided in Montalbán et al. (2017). Full characterization data of all the compounds, including ^{1}H NMR, ^{13}C NMR and ^{19}F NMR spectroscopy and mass spectrometry (MS), are also provided in Montalbán et al. (2017). Melting point temperature or thermal decomposition temperature of the solid ILs at room temperature was measured and included in Montalbán et al. (2017) (see Table 2).

2.3. Toxicity tests

The Microtox® Toxicity Test evaluates any inhibition in luminescence of the marine Gram-negative bacterium *Vibrio fischeri*. This bacterium was purchased in lyophilized form from Modern Water and activated by rehydration with a restorative solution of MilliQ water. A control sample of the bacterial suspension without the test substance was included along with the sample. Both, standard and samples were used in 2% NaCl to adjust the osmotic pressure. A Microtox® M500 Analyzer (Azur Environmental) was used to measure the light emission of the bacterium in contact with the samples. In this test, a range of diluted aqueous solutions (from 5.625% to 45.000%) of each IL was used. A concentration of 100% corresponds to a known concentration of an IL stock solution. After 15 min of exposure to the IL solution, whose concentration depends on the IL, the light output of the luminescent bacterium was measured and compared with the light output of a blank control sample. The toxicity was evaluated and a 50% reduction in luminescence was computed. The toxicity values reported in the text and tables are expressed as Log EC_{50} (μM), representing the toxicity value measured 15 min after *Vibrio fischeri* comes in contact with an IL. The measurement was taken at least three times for most ILs.

3. Results and discussion

The above mentioned mono- and dicationic ILs were synthesized and their ecotoxicity towards the luminescent marine bacterium *Vibrio fischeri* was evaluated. The yield of the synthesis reactions varied between 35.4% and 99.9%.

Tables 1 and 2 show the experimental results of the EC_{50} for monocationic and dicationic ILs, respectively. Table 1 also includes the EC_{50} values found in the literature and estimated by a QSAR method. There are no data available for dicationic ILs (Table 2). Table S1 shows the EC_{50} values of common volatile organic compounds (VOCs) collected from the literature. Fig. 1 depicts the EC_{50} values collated in Tables 1 and 2 and S1 for comparison.

To obtain less toxic ILs, several structural changes affecting the