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Toxicity assessment of various ionic liquid families towards *Vibrio fischeri* marine bacteria

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ABSTRACT

The increasing interest on the application of ionic liquids (ILs) to a wide range of processes and products has been hampered by a lack of toxicological data, mainly in what concerns novel cations, such as guanidinium, phosphonium, and functionalized and non-functionalized imidazolium-based ILs. The present study reports the toxicity of five guanidinium-, six phosphonium, and six imidazolium-based ILs, towards the luminescent marine bacteria *Vibrio fischeri*. These new results clearly show that guanidinium-, unlike the imidazolium- and phosphonium-based ILs, do not follow the trend of increasing toxicity with the increase in the alkyl chain length. Moreover, the introduction of oxygenated groups on the alkyl chains, such as ether and ester, leads to a decrease of the toxicity of guanidinium and also imidazolium compounds. In what respects the effect of the different cations, it is possible to recognize that the phosphonium-based ILs seem to be more toxic when compared to the analog imidazolium-based ILs (with the same anion and alkyl chains).

1. Introduction

Ionic liquids (ILs) are attracting increasing attention due to their unique properties, such as negligible vapor pressure, chemical and thermal stability, non-flammability, high ionic conductivity, wide electrochemical potential window and solvation ability. A huge number of different ILs can be synthesized by the combination of different anion moieties and cation cores, or by the manipulation of their characteristics such as varying their alkyl chain length or by the introduction of oxygenated groups. This makes possible to control physical properties such as, hydrophobicity, viscosity, density, their solubility behavior, and also, to influence their biodegradation ability or toxicological features (Earl and Seddon, 2000; Ranke et al., 2007b; Wasserscheid and Welton, 2007; Welton, 1999).

In the light of their recent widespread commercial availability, the synthesis of ILs has been object of a huge number of developments. If in the past, the synthesis of ILs was focused on obtaining unique physico-chemical properties (1st IL generation); to achieve a specific behavior considering the potential final industrial application (2nd ILs generation); nowadays the main goal is to produce ILs with the desired biological features (3rd ILs generation) (Hough et al., 2007) for the final application and also to facilitate the REACH registration processes. Although ILs can lessen the risk of air pollution due to their virtually zero vapor pressure, they do present some water solubility (Anthony et al., 2001; Freire et al., 2007, 2008, 2009, 2010), which was already correlated with the different ILs' parameters, such as the anion (Ranke et al., 2007a) and cation hydrophobicity (Ranke et al., 2009). This is, consequently, the most likely medium for ILs to be released into the environment. In view of the crescent number of industrial applications, their accidental discharge and environmental contamination are realities to take into consideration

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(Frade and Afonso, 2010; Pham et al., 2009; Ranke et al., 2007b). The properties that make them of industrial interest (high chemical and thermal stabilities and non-volatility) suggest potential problems with degradation or persistence in the environment. Moreover, due to the use of non-renewable resources as starting materials, the economic/environmental impact and the cumulative energy demand, the environmental fate and the (eco)toxicological behavior are extremely important parameters to take into account for a complete analysis of ILs sustainability, above all in order to improve the chances of large-scale applications (Kralisch et al., 2005; Zhang et al., 2008). As a consequence, the number of studies involving the environmental fate analysis (chemical degradation, bioaccumulation, biodegradability and the distribution of ILs in different environmental compartments, such as soil, water and sediments) and the toxicological effects (cytotoxicity, (eco)toxicity, phytotoxicity, and antimicrobial and antibacterial activities) of ILs are increasing (Frade and Afonso, 2010; Pham et al., 2009; Ranke et al., 2007b). The toxicological impact of ILs has been analyzed in a series of interdisciplinary studies (Jastorff et al., 2003; Matzke et al., 2007; Ranke et al., 2007b; Stolte et al., 2007a), which have underlined the importance of a preventive evaluation in the ILs design, because of the influence of different structures on pertinent technological and (eco)toxicological properties. Moreover, as previously demonstrated by our group (Gonçalves et al., 2011; Ventura et al., 2010) the need of a full understanding of the biological effects at different biological levels has a fundamental importance, and such knowledge should became a key factor in the modulation and selection of ILs features.

The most used methods to evaluate the toxicological risk of a substance in an aqueous media are those measuring their toxicity by an inhibition assay. Different aquatic species have been used in these inhibition measurements (daphnids, algae and fish) and also the test, which uses the *Vibrio fischeri* (formerly known as *Photobacterium phosphoreum*) bioluminescence inhibition assay. This is a rapid, cost-effective, and well-established method for toxicity determination (Steinberg et al., 1995) focusing on environmental issues, and also a standard (eco)toxicological bioassay in Europe (DIN EN ISO 11348). Several different luminescence inhibition tests of *Vibrio fischeri* have been developed so far, most being designed for analysis of aqueous samples, such as Microtox® Toxicity Test. This test can be used into a wide range of applications, such as the analysis of industrial effluents and discharges, waters, soils and sediments, and new products. This test is normally used as a possible approach for determination of the toxicity for both organic solvents (Lapertot and Pulgarin, 2006; Nirmalakhandan et al., 1994; Ruiz et al., 1997) and ILs (Couling et al., 2006; Docherty and Kulpa, 2005; Frade and Afonso, 2010; Garcia et al., 2005; Gonçalves et al., 2011; Luis et al., 2007; Matzke et al., 2007; Papaiconomou et al., 2010; Pham et al., 2009; Ranke et al., 2004, 2007b; Romero et al., 2008; Stolte et al., 2007a, 2007b; Ventura et al., 2010). There are different approaches in what concerns the use of Microtox® and ILs. The published data on ILs toxicity towards *Vibrio fischeri* were comprehensively interpreted in literature (Peraccini et al., 2007). Several authors have discussed the effect of different alkyl chain lengths, anions and different cations. Usually, the increase in the alkyl chain length leads to a pronounced augment in the toxicity (Couling et al., 2006; Docherty and Kulpa, 2005; Garcia et al., 2005; Luis et al., 2010; Ranke et al., 2004; Romero et al., 2008; Stolte et al., 2007a). This was explained by the increase in the lipophilicity of the cation that, according to the baseline toxicity, is responsible for a non-specific disturbance of the structure functioning of biological membranes (Ranke et al., 2007a). Thus, the long alkyl chains are able to be incorporated into the phospholipidic bilayer of the membranes, the same toxicity mechanism described in literature for some surfactants (Roberts and Costello, 2003). Considering the cation core, some reports show that the aromatic cations (imidazolium and pyridinium) are, in general, more toxic than the non-aromatic ILs, such as pyrrolidinium, piperidinium, phosphonium and ammonium (Couling et al., 2006; Gonçalves et al., 2011; Luis et al., 2007, 2010; Papaiconomou et al., 2010; Pretti et al., 2009; Stolte et al., 2007a, 2007b).

The anion seems to contribute to the toxicity in spite of the large disagreement among the literature data (Bernot et al., 2005; Garcia et al., 2005; Gonçalves et al., 2011; Stolte et al., 2006); tetrafluoroborate and hexafluorophosphate were considered less toxic and bis(trifluoromethylsulfonyl)imide and octylsulfate the most toxic anions (Azimova et al., 2009; Couling et al., 2006; Docherty and Kulpa, 2005; Garcia et al., 2005; Gonçalves et al., 2011; Matzke et al., 2007; Ranke et al., 2004; Romero et al., 2008; Stolte et al., 2007a). Various authors have attempted to find a correlation between EC_{50} and different ILs' properties, such as lipophilicity (Ranke et al., 2007a; Stolte et al., 2006; Stolte et al., 2007a, 2007b), or hydrophobicity (Ranke et al., 2009), and recently, solubility of ILs in water (Gonçalves et al., 2011).

More recently, some authors have applied Quantitative Structure–Activity Relationships (QSAR) models to data sets of ionic liquids to detect general assumptions on the toxicity and behavior of these compounds (Alvarez-Guerra and Irabien, 2011; Couling et al., 2006; Lacrămă et al., 2007; Luis et al., 2007, 2010; Putz et al., 2007). The aim is the development of predictive tools to help in the sustainable design of ILs safer for humans and for the environment (Matzke et al., 2010). The picture that emerges is that a number of characteristics play an active role on toxicity, most of them being still poorly understood (Gonçalves et al., 2011).

In this context, it is easy to understand the necessity of contributing to the enlargement of the toxicological databases considering the different families and structural features of ILs. Imidazolium based ILs appear as the family most studied in the toxicological field and phosphonium-based ILs being one of the less studied, despite their high industrial interest (for example in biotransformation processes such as xenobiotics-degradation (Cieniecka-Roslonkiewicz et al., 2005). Moreover, this family shows some interesting properties such as the possibility to decrease its antimicrobial activity for the longest alkyl chains (phosphonium based in alkyl chains of 8 and 14 carbons and conjugated with the chloride anion) (Cieniecka-Roslonkiewicz et al., 2005). Moreover, it was also focused that the exchange of the halide by other anions has resulted in a loss of antimicrobial activity (Cieniecka-Roslonkiewicz et al., 2005) thus rendering higher interest in this family. The apparently high toxicity of other phosphonium halides against *Vibrio fischeri* (Couling et al., 2006), *Daphnia magna* (Wells and Coombe, 2006), and *Pseudokirchneriella subcapitata* (Cho et al., 2008; Wells and Coombe, 2006) was also demonstrated. Still, the information for this family about its hazards to the environment is still scarce and not conclusive.

Recent toxicological studies have been focused on a new class of ILs with increased biodegradability through the incorporation of oxygenated alkyl chains (Coleman and Gathergood, 2010; Gathergood and Scammells, 2002; Neumann et al., 2010). The oxygenation is usually carried at two different parts of the IL structure, the cation core, represented for example by the morpholinium family, (Frade and Afonso, 2010) or at the cation alkyl chains, which can be achieved by the introduction of hydroxyl (–OH) (Docherty and Kulpa, 2005; Garcia et al., 2005; Ranke et al., 2004), ester (O–C=O) (Garcia et al., 2005) or ether (–O–) (Frade et al., 2007, 2009; Modelli et al., 2008; Ranke et al., 2007b; Samori et al., 2007, 2010; Samori` et al., 2011; Stolte et al., 2007a) groups. The oxygenated imidazolium-based ILs are a promising class of