Assessing chemical toxicity of ionic liquids on *Vibrio* fi*scheri*: Correlation with structure and composition

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ABSTRACT

One of the most important properties of the ionic liquids (ILs) is their non-volatility. However, they are wide soluble in water. For this reason, they can be released to aquatic ecosystems and to contribute to water pollution. Nevertheless, toxicological data related to ionic liquids is scarce in literature because of the great number of possible ionic liquids synthesized. The present work reports the toxicity of twenty-nine imidazolium-, pyridinium- and ammonium-based ionic liquids towards the luminescent bacteria *Vibrio fischeri*. Some of the effects analyzed on the toxicity have been the type of anion, the length of the alkyl chain of the cation, the cation core and the presence of a functionalized side chain in the cation. These results have showed that the main influence on the toxicity of the ILs is the alkyl chain length. A Quantitative Structure-Activity Relationships (QSAR) method has been used to validate our results obtaining a very good agreement.

Keywords: ionic liquids, toxicity, Microtox® tests, *Vibrio fischeri*, EC_{50}

1. INTRODUCTION

Ionic liquids (ILs) are a class of organic salts made of positively and negatively charged ions with a wide liquid range that have recently gained great attention in a variety of chemical processes, especially, as “green” solvents. Although the conventional organic solvents are widely available and can be obtained without exorbitant prices, issues regarding volatility, toxicity, physical hazards and possibility of environmental pollution can reduce the usage of conventional solvents in the future. When the awareness about the risk of using these solvents increased, the exploration for their alternatives becomes a priority and a new option was found in ILs [1]. In general, the main application areas of ILs are as solvents, electrolytes, lubricants, stationary phases for chromatography, matrices for mass spectrometry, supports for the immobilization of enzymes, in separation technologies, as liquid crystals, templates for

synthesis nano-materials, in preparation of catalytic membranes and in generation of high conductivity materials. As solvents, ILs possess several advantages over conventional solvents, which make them environmentally compatible: low vapor pressures, chemical and thermal stability, non-flammability, high ionic conductivity, large electrochemical window and solvation ability[2].

Non-volatility is an essential common characteristic and for this reason ILs do not evaporate or produce air contamination. For instance, Kabo et al. [3] determined the vapor pressure of $[bmim^+][PF_6^-]$ at 298.15 K as 10^{-11} Pa. If chemical engineers got replace conventional organic solvents for ILs, air pollution and costs for environmental mitigation would be reduced significantly. Nevertheless, while ILs might decrease the costs and environmental damage, introduction of ILs into aquatic environments may involve to water pollution, because of their high solubility in water [4][5][6] [7][8][9]. In fact, Anthony et al [10] showed that the solubility of the ionic liquid $[bmim^+][PF_6^-]$ in water is about 2% (wt). ILs solubility in water depends on the nature of the ILs, specifically, of cation and anion nature. For this reason, water is the most likely medium for ILs to be released into the environment. Moreover, in order to get industrial applications of the ILs it is crucial to know the ILs environmental fate and toxicological behavior. However, lack of ILs toxicological data is a reality in the bibliography although, in the last years, some studies related to these issues have been published. On the context of aquatic toxicity, several publications have studied ILs toxicity through different aquatic organisms such as bacteria (specially, *Vibrio fischeri*) [11][12][5][13][14][15][16][17][18], green algae (*e.g. Pseudokirchneriella subcapitata*) [19][20], aquatic plants (*e.g.* duckweed *Lemna minor*) [15][21][22], invertebrates (mainly the freshwater crustacean *Daphnia magna*) [23][19] or vertebrates like fish (the zebrafish *Danio rerio*) [24][19] or frogs (*Rana nigromaculata*) [25]. However, although the number of studies which evaluate ILs aquatic toxicity is increasing, only limited information is available yet. In addition, the number of possible synthesized ILs is really enormous. Alvarez-Guerra et al[26] established that "more than 10^6 different ILs may be synthesized, with 10^{12} binary combinations and 10^{18} ternary systems possible".

The most used methods to determine the toxicological risk in an aqueous media are inhibition assays [16]. According to Parvez et al [27], *Vibrio fischeri* (formerly known as *Photobacterium Phosphoreum*) luminescence inhibition test is the most common bacterial bioassay. *Vibrio fischeri* is a marine gram negative bacterium. This assay is characterized for being the most rapid, cost effective, sensitive, reproducible and well-established. In addition, this method constitutes a standard (eco) toxicological bioassay in Europe (DIN EN ISO 11348) [28]. As a matter of fact, many different luminescence inhibition tests of *Vibrio fischeri* designed for analysis of aqueous samples, have been developed [16]. In this paper, ILs (eco) toxicity has been determined by Microtox® Toxicity Test. This is one of the most widely used bioassay tests because of its intense and stable light emission, the high sensitivity to different compounds and the flexibility which provides a marine bacterium [29]. In fact, this test was used widely to determine conventional organic compounds toxicity more than two decades ago [30]. In addition, this test provides a rapid means of determining the acute toxicity of aqueous compounds by measuring decreases in light output from the luminescent bacterium *Vibrio fischeri*. Light emission is directly proportional to the metabolic activity of the bacterial population and any inhibition of enzymatic activity causes a corresponding decrease in luminescence[27].

In the studies mentioned previously, authors have established some IL toxicity trends according to different cation cores, alkyl chain length and short second alkyl chain of the cation, side chains' functionalization and types of anion. The effect of the cation core of the IL in the overall *Vibrio fischeri* toxicity has not been studied in depth yet. However, Costa et al [31] observed an apparent higher toxicity of the pyridinium core than of the imidazolium core in their assays. Their results are in concordance with those obtained for Ventura et al [32] who deduced that cations with six member ring (pyridinium) are more toxic than with five member ring (imidazolium). On the one hand, the general tendency is that IL toxicity increases when alkyl chain length does it [5][11][12][17][14][33][34]. For instance, Romero et al[14] showed that "the shorter the chain length of side chain R_2, the lower the toxic effect is". The reason which explains this trend is that an increase in alkyl chain length produces an augment in the cation lipophilicity and, consequently, the entrance of IL through cellular membranes is easier [35][36]. In addition, the structures of the ILs tested (specially, imidazolium-based ILs) are quite similar to cationic surfactants which are known to produce an increase in membrane permeability and cause narcotic effects when alkyl chain length increases [34][16][37][5]. On the other hand, varying the anion contributes to ILs toxicity too. However, a general disagreement exists between authors. In those publications in which anion influence is relevant for IL toxicity, the general trend in increasing anion toxicity would be as follows: bis(trifluoromethylsulfonyl)imide > hexafluorophosphate > tetrafluoroborate > chloride [12][38][39]. According to Pinto et al [12], from [$bmim^+$][Cl^-] to [$bmim^+$][BF_4^-] *Vibrio fischeri* toxicity increased above 12 times. Nevertheless, several authors think that anion influence in ionic liquid toxicity is negligible and the most important influence is due to side alkyl chain of cation [5][14][40]. Finally, literature results suggest that side chains' functionalization (e.g. oxygenated chains) leads to lower toxicity values[39][15]. These and other influences in *Vibrio fischeri* toxicity of ILs such as the influence of a second short alkyl chain in the ionic liquid cation and the presence of double bonds in it are studied in this paper.

During the last years, some authors have developed mathematical models based on the structural features of the ILs to predict their toxicity. These models are called Quantitative Structure-Activity Relationships (QSAR) and consist of group contribution methods [17][35][41][42][40][43][26][44][45][46]. The influence of three main components, namely the cation ring, the alkyl chain and the anion on the toxicity of IL is studied towards these methods. However, this type of models has some limitations yet due to the lack of experimental data for some specific families of ILs. Recently, a critical review has underlined the necessity of extensive and systematic studies which contribute to the enlargement of the toxicological databases, in order to better understand the toxicity of ILs [47]. According with this lack of toxicological information data for ILs, some previous studies available in literature have tried to establish relations or mathematical correlations between toxicity and lipophilicity or hydrophobicity of ILs by 1-octanol-water partition coefficient [5][15][48].

The main objective of this work has been to measure aquatic toxicity by *Vibrio fischeri* inhibition test for a set of imidazolium, pyridinium and ammonium-based ILs, specifically, twenty-nine ILs: 1-ethyl-3-methylimidazolium hexafluorophosphate ([$emim^+$][PF_6^-]), 1-butyl-3-methylimidazolium hexafluorophosphate ([$bmim^+$][PF_6^-]), 1-hexyl-3-methylimidazolium hexafluorophosphate ([$hmim^+$][PF_6^-]), 1-methyl-3-octylimidazolium hexafluorophosphate ([$omim^+$][PF_6^-]), 1-butyl-3-methylimidazolium tetrafluoroborate ([$bmim^+$][BF_4^-]), 1-methyl-3-octylimidazolium tetrafluoroborate ([$omim^+$][BF_4^-]), 1-ethyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide ([$emim^+$][NTf_2^-]), 1-butyl-3-methylimidazolium

bis(trifluoromethylsulfonyl)imide ([bmim$^+$][NTf$_2^-$]), 1-hexyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide ([hmim$^+$][NTf$_2^-$]), 1-methyl-3-octylimidazolium bis(trifluoromethylsulfonyl)imide ([omim$^+$][NTf$_2^-$]), 1-butyl-2,3-dimethylimidazolium bis(trifluoromethylsulfonyl)imide ([bdmim$^+$][NTf$_2^-$]), 1-ethyl-3-methylimidazolium triflate ([emim$^+$][TfO$^-$]), 1-ethyl-3-methylimidazolium ethylsulphate ([emim$^+$][EtSO$_4^-$]), 1-ethyl-3-methylimidazolium acetate ([emim$^+$][CH$_3$COO$^-$]), 1-butyl-3-methylimidazolium methylsulphate ([bmim$^+$][MeSO$_4^-$]), 1-butyl-3-methylimidazolium 2-(2-methoxiethoxi) ethylsulphate ([bmim$^+$][MDEGSO$_4^-$]), 1-methylimidazolium chloride ([mim$^+$][Cl$^-$]), 1-ethyl-3-methylimidazolium chloride ([emim$^+$][Cl$^-$]), 1-methyl-3-propylimidazolium chloride ([pmim$^+$][Cl$^-$]), 1-allyl-3-methylimidazolium chloride ([amim$^+$][Cl$^-$]), 1-(2-hydroxi-ethyl)-3-methylimidazolium chloride ([hemim$^+$][Cl$^-$]), 1,2-dimethylimidazolium chloride ([dmim$^+$][Cl$^-$]), 1-ethylimidazolium chloride ([eim$^+$][Cl$^-$]), 1-butyl-3-methylimidazolium chloride ([bmim$^+$][Cl$^-$]), 1-hexyl-3-methylimidazolium chloride ([hmim$^+$][Cl$^-$]), 1-methyl-3-octylimidazolium chloride ([omim$^+$][Cl$^-$]), 1-ethyl-3-methylpyridinium ethylsulphate ([empy$^+$][EtSO$_4^-$]), 1-butyl-3-methylpyridinium tetrafluoroborate ([bmpy$^+$][BF$_4^-$]) and ethylammonium nitrate (ETAN). In the present paper ILs toxicity is determined as effective nominal concentration EC_{50} which is defined such as the IL concentration that produces a mortality of 50 per cent of de bacteria population. With these measurements, we have presented an accurate study of the different influences on ILs toxicities and established a correlation between EC_{50} values and 1-octanol-water partition coefficients (K_{ow}) obtained in a previous paper.

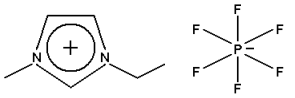
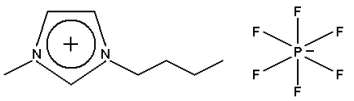
2. MATERIALS AND METHODS

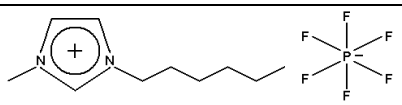
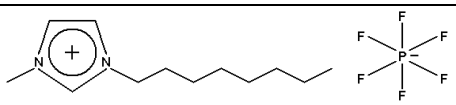
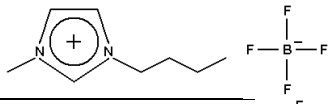
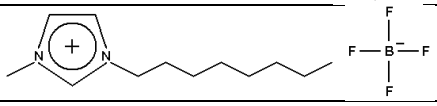
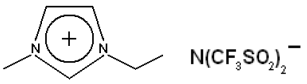
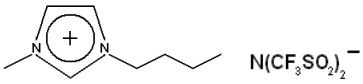
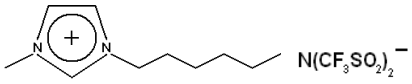
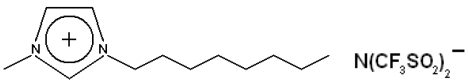
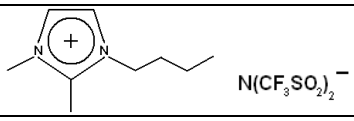
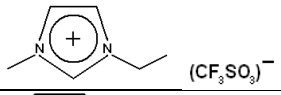
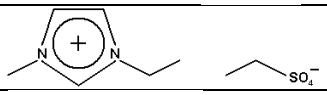
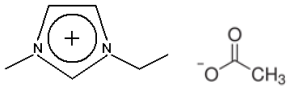
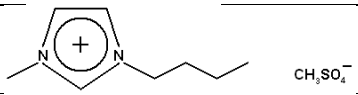
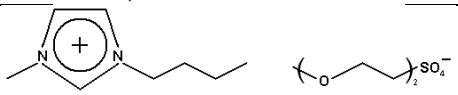
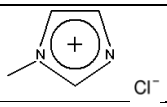
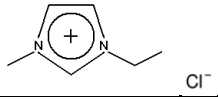
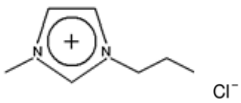
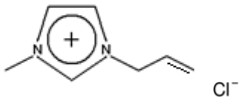
2.1. Test Chemicals

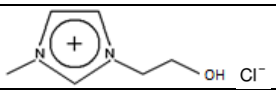
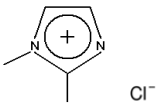
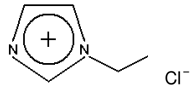
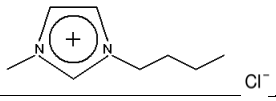
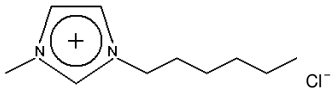
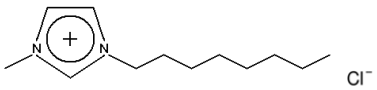
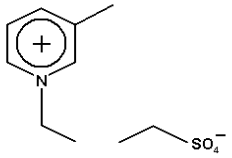
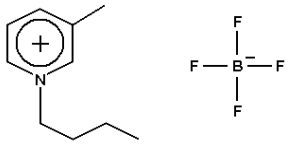
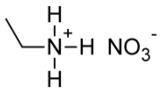
The structures of the ILs used in this study are listed in Table 1. The ILs [emim$^+$][PF$_6^-$] (purity>99%), [bmim$^+$][PF$_6^-$] (purity>99%), [hmim$^+$][PF$_6^-$] (purity>99%), [omim$^+$][PF$_6^-$] (purity>99%), [bmim$^+$][BF$_4^-$] (purity>99%), [emim$^+$][NTf$_2^-$] (purity>99%), [bmim$^+$][NTf$_2^-$] (purity>99%), [hmim$^+$][NTf$_2^-$] (purity>99%), [omim$^+$][NTf$_2^-$] (purity>99%), [bdmim$^+$][NTf$_2^-$] (purity>99%), [emim$^+$][TfO$^-$] (purity>99%), [emim$^+$][EtSO$_4^-$] (purity>99%), [emim$^+$][CH$_3$COO$^-$] (purity>95%), [mim$^+$][Cl$^-$] (purity>98%), [emim$^+$][Cl$^-$] (purity>98%), [pmim$^+$][Cl$^-$] (purity>98%), [amim$^+$][Cl$^-$] (purity>98%), [hemim$^+$][Cl$^-$] (purity>99%), [dmim$^+$][Cl$^-$] (purity>98%), [eim$^+$][Cl$^-$] (purity>98%), [bmim$^+$][Cl$^-$] (purity>99%), [hmim$^+$][Cl$^-$] (purity>98%), [omim$^+$][Cl$^-$] (purity>98%) and ETAN (purity>97%) were purchased from Iolitec (Germany). The ILs [omim$^+$][BF$_4^-$] (purity>99%), [bmim$^+$][MeSO$_4^-$] (purity>99%), [bmim$^+$][MDEGSO$_4^-$] (purity>98%), [empy$^+$][EtSO$_4^-$] (purity>99%) and [bmpy$^+$][BF$_4^-$] (purity>99%) were purchased from Solvent Innovation GmbH (Cologne, Germany).

[Insert Table 1 about here]

Table 1. Abbreviations and structures of the studied ILs.

Abbreviation	Molecular weight (g mol^{-1})	Structure
[emim$^+$][PF$_6^-$]	256.13	
[bmim$^+$][PF$_6^-$]	284.18	

[hmim$^+$][PF$_6^-$]	312.08	
[omim$^+$][PF$_6^-$]	340.29	
[bmim$^+$][BF$_4^-$]	226.02	
[omim$^+$][BF$_4^-$]	282.13	
[emim$^+$][NTf$_2^-$]	391.31	
[bmim$^+$][NTf$_2^-$]	419.37	
[hmim$^+$][NTf$_2^-$]	447.42	
[omim$^+$][NTf$_2^-$]	475.47	
[bdmim$^+$][NTf$_2^-$]	433.39	
[emim$^+$][TfO$^-$]	260.24	
[emim$^+$][EtSO$_4^-$]	236.29	
[emim$^+$][CH$_3$COO$^-$]	170.21	
[bmim$^+$][MeSO$_4^-$]	250.32	
[bmim$^+$][MDEGSO$_4^-$]	338.43	
[mim$^+$][Cl$^-$]	118.57	
[emim$^+$][Cl$^-$]	146.02	
[pmim$^+$][Cl$^-$]	160.64	
[amim$^+$][Cl$^-$]	158.63	

[hemim$^+$][Cl$^-$]	162.62	
[dmim$^+$][Cl$^-$]	131.58	
[eim$^+$][Cl$^-$]	131.58	
[bmim$^+$][Cl$^-$]	174.67	
[hmim$^+$][Cl$^-$]	202.72	
[omim$^+$][Cl$^-$]	230.78	
[empy$^+$][EtSO$_4^-$]	247.32	
[bmpy$^+$][BF$_4^-$]	237.05	
ETAN	108.1	

2.2. Microtox® tests

The Microtox® Toxicity Test evaluates the luminescence inhibition in the marine bacteria *Vibrio Fischeri*. This bacterium was purchased from. A Microtox® M500 Analyzer (Azur Environmental) was used. In this test a range of diluted aqueous solutions (from 5.625 to 45 percent) of each IL was used. Concentration of 100 percent corresponds to a known concentration of an IL stock solution. After 15 minutes of bacteria exposition to the IL solution (depending on the IL), the light output of the luminescent was measured and compared with the light output of a blank control sample. The toxicity was evaluated and a 50 percent reduction in luminescence was computed using. In this work toxicity values reported in the text and in tables are expressed as Log EC_{50} (μM) that is the toxicity value measured 15 min after *Vibrio fischeri* comes in contact with an IL. For each IL the measurement was taken at least three times.

3. RESULTS AND DISCUSSION

This study is focused on the relevance of some ILs structural features such as the cation core (with tetrafluoroborate and ethylsulphate anions), the alkyl chain length (with hexafluorophosphate, tetrafluoroborate, bis(trifluoromethylsulfonyl)imide and chloride anions),

the impact of different anions (with imidazolium family), the presence of a functionalized lateral chain (with chloride anion), the influence of a second short alkyl chain (with bis(trifluoromethylsulfonyl)imide and chloride anions) and the effect of a double bond in the alkyl chain (with chloride anion) in their toxicity towards the marine luminescent bacteria *Vibrio fischeri*. Table 2 shows the EC_{50} values for the ILs measured in this work experimentally and EC_{50} values for some of them from literature.

[Insert Table 2 about here]

Table 2. Log EC_{50} values for ILs tested in each trial after 15 min of exposure to the luminescent bacteria *Vibrio fischeri*, with respective 95 percent confidence limits (in brackets) and Log EC_{50} mean values. Log EC_{50} obtained with a group contribution method QSAR [35], Log EC_{50} found in literature and octanol-water partition coefficient (Log K_{ow}) are showed too. Some Volatile Organic Compounds (VOCs) appear in italics at the end of the table with their toxicities [30].Toxicity is expressed as Log EC_{50} (μM) in all cases.

Ionic liquid	**Log EC_{50}, this work** (lower limit, upper limit)	**Log EC_{50}, this work** (average)	**Log EC_{50}** [35]	**Log EC_{50}** from literature	**Log K_{ow}**
$[emim^+][PF_6^-]$	4.11 (3.11, 5.11) 4.29 (4.13, 4.44) 4.27 (4.12, 4.43)	4.22	4.38	-	
$[bmim^+][PF_6^-]$	3.43 (3.25, 3.61) 3.34 (3.29, 3.38) 3.09 (2.69, 3.50)	3.29	3.32	3.07[13]	
$[hmim^+][PF_6^-]$	2.29 (2.29, 2.36) 2.51 (2.48, 2.55) 2.23 (2.21, 2.26) 2.43 (2.39, 2.46)	2.36	2.24	2.17[13]; 2.11[14]	
$[omim^+][PF_6^-]$	1.33 (1.18, 1.62) 1.44 (0.27, 2.61) 0.96 (0.74, 1.18)	1.25	1.18	0.95[13]; 0.70[14]	
$[bmim^+][BF_4^-]$	3.55 (3.41, 3.69) 3.54 (3.34, 3.73) 3.21 (2.38, 4.04) 3.51 (2.03, 5.00) 3.49 (3.39, 3.60)	3.46	3.32	3.56[34]; 3.10[13]; 3.54[38], 3.20[39]	
$[omim^+][BF_4^-]$	1.07 (0.80, 1.34) 0.92 (0.77, 1.07) 0.73 (0.48, 0.98) 0.94 (0.85, 1.04)	0.91	1.18	1.41[34]	
$[emim^+][NTf_2^-]$	3.62 (3.32, 3.91) 3.61 (3.27, 3.95) 3.62 (3.61, 3.63)	3.62	3.97	2.57[18]; 3.41[31]	
$[bmim^+][NTf_2^-]$	2.56 (2.30, 2.81) 2.49 (2.42, 2.56) 2.50 (2.43, 2.56)	2.51	2.90	2.48[38]; 2.53[18]	
$[hmim^+][NTf_2^-]$	1.76 (1.61, 1.90) 1.91 (1.85, 1.97) 1.78 (1.77, 1.79)	1.82	1.82	-	
$[omim^+][NTf_2^-]$	0.91 (0.72, 1.10) 1.02 (0.82, 1.22) 1.06 (0.73, 1.38)	0.99	0.76	-	
$[bdmim^+][NTf_2^-]$	3.03 (2.95, 3.12) 2.81 (2.49, 3.14) 2.76 (2.67, 2.84)	2.87	2.69	-	
$[emim^+][TfO^-]$	3.70 (3.15, 4.24) 3.76 (3.20, 4.32) 3.77 (3.32, 4.22)	3.74	2.77	-	
$[emim^+][EtSO_4^-]$	4.02 (3.66, 4.38) 4.12 (3.64, 4.60) 4.17 (3.98, 4.36)	4.10	4.38	4.02[14]	
$[emim^+][CH_3COO^-]$	3.81 (3.49, 4.14) 3.92 (3.89, 3.95)	3.89	2.77	4.17[31]	

	3.95 (3.88, 4.01)				
[bmim$^+$][MeSO$_4^-$]	3.44 (3.37, 3.51) 3.80 (3.15, 4.45) 3.49 (3.37, 3.60)	3.57	3.32	3.56[16]	
[bmim$^+$][MDEGSO$_4^-$]	3.36 (3.32, 3.41) 3.59 (3.52, 3.66) 3.49 (3.47, 3.50)	3.48	-	-	
[mim$^+$][Cl$^-$]	4.04 (3.47, 4.60) 3.75 (3.64, 3.87) 4.07 (3.94, 4.21) 3.90 (3.59, 4.21)	3.94	-	-	
[emim$^+$][Cl$^-$]	4.76 (4.39, 5.13) 4.77 (4.67, 4.88) 4.87 (4.78, 4.95)	4.80	4.38	4.55[17];4.33[15]; 4.60[49]	
[pmim$^+$][Cl$^-$]	3.67 (3.58, 3.77) 3.84 (3.72, 3.93) 3.83 (3.38, 4.30)	3.78	3.86	-	
[amim$^+$][Cl$^-$]	4.28 (3.15, 5.41) 4.33 (4.26, 4.41) 4.45 (4.39, 4.51)	4.36	3.86	-	
[hemim$^+$][Cl$^-$]	5.30 (4.93, 5.67) 5.35 (5.18, 5.52) 5.36 (5.28, 5.45) 5.34 (5.23, 5.45)	5.34	-	-	
[dmim$^+$][Cl$^-$]	4.33 (4.13, 4.54) 4.34 (4.21, 4.47) 4.24 (4.12, 4.37)	4.31	4.92	-	
[eim$^+$][Cl$^-$]	4.33 (4.10, 4.56) 4.02 (3.93, 4.12) 4.12 (3.92, 4.32)	4.16	4.67	-	
[bmim$^+$][Cl$^-$]	3.48 (3.35, 3.61) 3.50 (3.06, 3.93) 3.41 (3.24, 3.59)	3.46	3.32	3.71[5]; 3.39[14]; 3.40[38]; 3.47[15]; 3.34[13]; 2.95[4]; 3.21[50]	
[hmim$^+$][Cl$^-$]	2.37 (1.84, 2.90) 2.53 (2.15, 2.90) 2.33 (2.16, 2.49) 2.45 (2.13, 2.37)	2.37	2.25	1.94[17]; 2.18[14]; 2.32[13]	
[omim$^+$][Cl$^-$]	0.79 (0.63, 0.94) 0.73 (0.48, 0.98) 0.64 (0.49, 0.79) 0.65 (0.29, 1.01) 0.63 (0.57, 0.69)	0.69	1.18	1.19[13]; 0.94[14]; 1.01[15]; 0.30[50]	
[empy$^+$][EtSO$_4^-$]	3.79 (3.68, 3.90) 3.71 (3.41, 4.01) 3.94 (3.47, 4.40) 3.88 (3.44, 4.32)	3.83	3.87	-	
[bmpy$^+$][BF$_4^-$]	2.44 (2.32, 2.55) 2.54 (2.46, 2.63) 2.34 (2.20, 2.47)	2.44	2.80	2.38[31]	
ETAN	4.27 (2.79, 5.74) 4.29 (3.96, 4.62) 4.41 (3.12, 5.69) 4.33 (3.86, 4.79)	4.32	-	-	
Methanol	-	-	-	6.51[30]	-
Ethanol	-	-	-	5.89[30]	-
Isopropanol	-	-	-	5.72[30]	-
Acetonitrile	-	-	-	5.57[30]	-
Acetone	-	-	-	5.17[30]	-
Dichlorometane	-	-	-	4.57[30]	-
Chloroform	-	-	-	4.16[30]	-

Data in Table 2 showed that the measured mean Log EC_{50} values vary from 0.69 ([omim$^+$][Cl$^-$]) to 5.34 ([hemim$^+$][Cl$^-$]) μM depending on the ionic liquid chemical structure. It is very important to underline that an excellent agreement exists between toxicity values obtained in this paper and values from literature. For instance, García et al [13] obtained Log EC_{50} values for [hmim$^+$][Cl$^-$] and [bmim$^+$][Cl$^-$] of 2.32 and 3.34 and in this work we have measured values

of 2.37 and 3.46, respectively. Other example of this is given by Matzke et al [38] who measured Log EC_{50} values for [$bmim^+$][BF_4^-] and [$bmim^+$][NTf_2^-] of 3.54 and 2.48 and we have obtained values of 3.46 and 2.51, respectively. In addition, toxicity values estimated by a QSAR method [35] appears in Table 2 and it should be emphasized that Log EC_{50} values obtained in this work experimentally show a very good agreement with these estimated values as it could be observed in Figure 1. For example, according to Luis et al [35], estimated Log EC_{50} for [$bmim^+$][PF_6^-] and [$pmim^+$][Cl^-] would be 3.32 and 3.86 and we have measured values of 3.29 and 3.78, respectively. In addition, *Vibrio fischeri* toxicity of fourteen out twenty-nine ILs tested was not previously analyzed in literature. However, these values are consistent with the rest of the Log EC_{50} values obtained in this work.

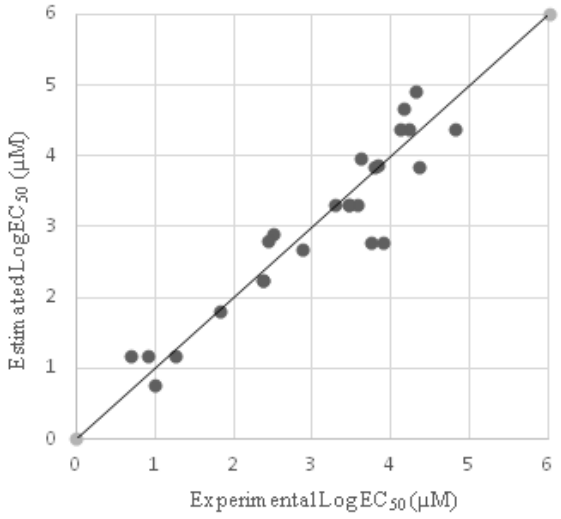


Figure 1. Comparation of estimated Log EC_{50} of *Vibrio fischeri* with the experimental data for some of the ILs tested.

According to the results showed in Table 2, the higher toxicity of pyridinium-based ILs comparing with similar imidazolium-based ILs can be confirmed. It can be seen in two pairs of ILs with the same anion and alkyl chain: [$empy^+$][$EtSO_4^-$]-[$emim^+$][$EtSO_4^-$] and [$bmpy^+$][BF_4^-]-[$bmim^+$][BF_4^-]. These results are in good agreement with literature data [31][32].

Regarding to alkyl chain length effect, it can be observed that all types of anions showed an increase on ionic liquid toxicity when alkyl chain length suffers an augment: [$omim^+$][PF_6^-] > [$hmim^+$][PF_6^-] > [$bmim^+$][PF_6^-] > [$emim^+$][PF_6^-]; [$omim^+$][BF_4^-] > [$bmim^+$][BF_4^-]; [$omim^+$][NTf_2^-] > [$hmim^+$][NTf_2^-] > [$bmim^+$][NTf_2^-] > [$emim^+$][NTf_2^-]; [$omim^+$][Cl^-] > [$hmim^+$][Cl^-] > [$bmim^+$][Cl^-] > [$pmim^+$][Cl^-] > [$emim^+$][Cl^-]. This tendency had been demonstrated for other authors previously as we have established in Introduction Section[5][11][12][14][17][33][34]. In Figure 2 the relationship between alkyl chain length and ionic liquid toxicity is represented.

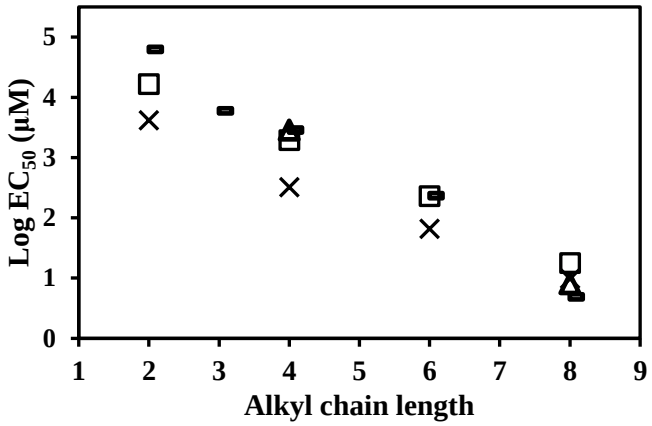


Figure 2. Effect of the number of carbons of the alkyl chain (nC_{R1}) in the aquatic toxicity (Log EC_{50}) for some of the ILs tested; × bis(trifluoromethysulfonyl)imide, □ hexafluorophosphate, △ tetrafluoroborate and ▬ chloride anions.

In this work, the linear regression analysis of Log EC_{50} vs. alkyl chain length has been carried out assuming that the most important factor in the Log EC_{50} estimation is the alkyl chain length and the following equation was obtained:

$$Log\ EC_{50} = 5.22 - 0.52nC_{R1} \qquad (1)$$

This equation has a squared correlation coefficient r^2 of 0.918 and agrees very well with the equation obtained by Romero et al. [14]. Our regression confirms that the effect of the alkyl chain length is more determining than the anion effect in the *Vibrio fischeri* toxicity of ILs.

Our results have confirmed that the anion contributes on the ionic liquid toxicity with lower impact that the alkyl chain length as it can be seen in Figure 2. Ten different anions are present in the studied ILs. For an exhaustive toxicity comparison, the same cation is necessary. However, it is not possible because we have not got identical alkyl chains in the cations for all the anions. For this reason, toxicity comparison is made in a general way, using [$bmim^+$] and [$emim^+$] cations with all types of anions. According to this, the next trend in the ionic liquid toxicity could be established: [NTf_2^-]>[PF_6^-]>[BF_4^-]≈[$MeSO_4^-$]≈[$MDEGSO_4^-$]>[TfO^-]>[CH_3COO^-]>[$EtSO_4^-$]>[Cl^-]. The most toxic anions have been those which have fluorine atoms in their structure due to their hydrolysis to hydrofluoric acid[31]. Specially, bistriflimide [NTf_2^-] anion with six fluorine atoms was the most toxic to *Vibrio fischeri*. The same order was established by Frade et al. [51]. This trend was also observed previously by Pinto et al. [12], Samorì et al. [39] and Costa et al. [31] who presumed a more toxic effect in [BF_4^-] than [Cl^-] anion. The higher toxicity of bistriflimide anion comparing with acetate anion was also observed by Costa et al. [31]. Other authors, such as Stolte et al. [15] also established that most of the ILs with [NTf_2^-] anion tested in their work resulted to be more toxic than ILs with halides anions such as [Cl^-].

Other trend in the *Vibrio fischeri* toxicity of ILs that could be analyzed is the effect of having a functionalized alkyl chain in the cation. For this particular, [$hemim^+$][Cl^-] (log EC_{50}=5.34) and [$emim^+$][Cl^-] (log EC_{50}=4.80) can be compared. Our results presume that the introduction of a hydroxyl group in the alkyl chain could decrease *Vibrio fischeri* toxicity of the ionic liquid. It could be possible because the introduction of one oxygen increases the polarity of the alkyl

chain and reduces the toxicity. This trend has not been studied in literature in depth yet, however, some authors have obtained similar results [39][15].

Furthermore, other influence studied in this work is the presence of a second short (one or two carbon atoms) alkyl chain. This trend could be analyzed comparing [$bdmim^+$][NTf_2^-] (log EC_{50}=2.87) which has two short alkyl (and a long one) chains in the imidazolium ring and [$bmim^+$][NTf_2^-] (log EC_{50}=2.51) with only a short alkyl chain (and a long one). Other examples would be [$dmim^+$][Cl^-] (log EC_{50}=4.31) and [$emim^+$][Cl^-] (log EC_{50}=4.80), both with two short alkyl chains, comparing with [mim^+][Cl^-] (log EC_{50}=3.94) and [eim^+][Cl^-] (log EC_{50}=4.16), respectively, both with only one alkyl chain. According to experimental data present in Table 2, ILs with two short alkyl chains resulted less toxic than those with only one alkyl chain in the cation. As a consequence of this, we can presume that a higher number of short alkyl chains in the imidazolium ring involves a decrease on ionic liquid toxicity towards *Vibrio fischeri*.

Finally, the effect of the presence of a double bond in the alkyl chain of the cation could be analyzed if [$pmim^+$][Cl^-] (log EC_{50}=3.78) and [$amim^+$][Cl^-] (log EC_{50}=4.36) toxicities are compared. [$amim^+$][Cl^-] posses a double bond in its chemical structure as can be seen in Table 1. Concerning to the toxicity measurements, it might be that, for the same anion (in this case, [Cl^-]) and a cation with the same number of carbon atoms (in this case, three), when a double bond is present in the alkyl chain ([$amim^+$][Cl^-]), ionic liquid *Vibrio fischeri* toxicity is lower than with a single bond ([$pmim^+$][Cl^-]).

With respect to some Volatile Organic Compounds (VOCs) whose Log EC_{50} values are presented in Table 1, a mention can be done. Only seven of twenty-nine ILs resulted being inside of the toxicity range of VOCs. The rest are more environmentally harmful.

As we have mentioned in Introduction Section, lipophilicity and hydrophobicity of ILs are terms that are linked to the aquatic toxicity in several studies. For this reason, a relationship between hydrophobicity of ILs (represented as logarithm of octanol-water partition coefficient, Log K_{ow}) and EC_{50} is established in this work. Log K_{ow} for some of these ILs was published in a previous work (referencia artículo Kow) and it can be found in Table 1. In Figure 2 Log EC_{50} is plotted versus Log K_{ow} for some ILs.

[Insert Figure 2 about here]

4. CONCLUSIONS

ACKNOWLEDGEMENTS

This work has been partially supported from the European Commission (FEDER/ERDF) and the Spanish MINECO (Ref. CTQ2011-25613 and Ref. CTQ2014-57467-R). Mercedes G. Montalbán acknowledges support from Spanish MINECO (FPI grant, BES-2012-053267).

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