Ecotoxicity of Binary Mixtures of ILs and Inorganic Salts of Electrochemical Interest

Juan José Parajó
Universidade de Santiago de Compostela
Pablo Vallet
Universidade de Santiago de Compostela
Luis Miguel Varela
University of Santiago de Compostela: Universidade de Santiago de Compostela
María Villanueva
Universidade de Santiago de Compostela
Josefa SALGADO (j.salgado.carballo@usc.es)
Universidade de Santiago de Compostela https://orcid.org/0000-0002-6806-580X

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Abstract

The applicability of ionic liquids (ILs) has been increased during the last years and even new opportunities are becoming a reality, i.e. mixtures of pure IL and inorganic salt as electrolytes for smart electrochemical devices, but the effects on environment are almost unknown. In this work, the ecotoxicity of two pure protic ILs (Ethylammonium nitrate and Ethylimidazolium nitrate) and two pure aprotic ILs (butylmethylpyrrolidinium bis(trifluoromethylsulfonyl)imide and butyldimethylimidazolium bis(trifluoromethylsulfonyl)imide) and that of their binary mixtures with inorganic salts with common cation was tested towards changes on the bioluminescence of the bacteria *Aliivibrio fischeri*, using the Microtox® standard toxicity test. EC50 of these mixtures was determined over three standard periods of time and compared with the corresponding values to pure ILs. Results indicate that the aprotic ILs are more toxic than protic and that aromatic are more toxic than non-aromatic.

The addition of inorganic mono ($LiNO_3$), di ($Ca(NO_3)_2{\cdot}4H_2O$, $Mg(NO_3)_2{\cdot}6H_2O$) and trivalent ($Al(NO_3)_3{\cdot}9H_2O$) salts in binary mixtures with EAN was firstly analysed, obtaining that the latter induces an important increase on toxicity. Finally, mixtures of IL- inorganic lithium salt ($LiNO_3$, for the protic ILs and LiTFSI for the aprotic ILs) toxicity was also studied, which resulted strongly dependent on the IL of the mixture.

1. Introduction

As it is well known, ionic liquids (ILs) are compounds formed entirely by ions which have low melting points. Their applications seems countless, since they are still not fully studied as pure, in mixtures with other compounds or with active pharmaceutical ingredients (APIs) incorporated in their structures among other possibilities (Rodríguez and Brennecke 2006; Rana et al. 2010; Silva et al. 2016; Toledo-Hijo et al. 2016; Salgado et al. 2019a).

The main characteristic of ILs is the low vapor pressure, which means non-volatility, and therefore they were, firstly, addressed to substitute traditional industrial solvents, most of which are volatile organic compounds (VOCs), one of the most source of environmental pollution in chemical industry (Rogers and Seddon 2003). This fact led to designate them as green solvents, although recent studies concluded that the toxicity of some ILs is similar, or even higher, than traditional solvents (Studzinska and Buszewski 2009; Santos et al. 2014).

Furthermore the non-volatility, other characteristic properties of ILs are, for example, the high thermal and chemical stability, high viscosity, the solubility in water and other solvents, wide electrochemical window and specially the tuneability (Yasuda et al. 2013). All these characteristics make ILs good candidates to be used in high temperature applications, as lubrication [6], as well as desulfurization of fuels (Gutiérrez et al. 2018), batteries (Menne et al. 2013; Balducci 2017; Wang et al. 2020), fuel cells (Nakamoto and Watanabe 2007), fluids in refrigeration systems (Sánchez et al. 2016; Moreno et al. 2018), APIs (Shamshina and Rogers 2020), etc.

ILs can be divided into two different subclasses depending on their structural characteristics: protic (PILs) and aprotic (AILs) ionic liquids. PILs are formed by the proton transfer from acid to base, and hence, they consist of proton-donor and -acceptor sites, which are responsible for building extended three-dimensional hydrogen bond networks as in the case of water, and AILs are mainly based on bulky organic cations (*i.e.* pyrrolidinium, imidazolium…) with long alkyl chain substituents, and on a huge variety of anions (*i.e.* TFSI, FAP, halides). In recent years, significant growth in the structure – property relationships of ILs has been achieved with a better understanding of the intermolecular forces (Salgado et al. 2013; Sánchez et al. 2019).

One of the most cited applications on literature is the electrochemistry, based on mixtures of ILs and inorganic salts that improve some properties that further extend the range of application of these compounds (Salgado et al. 2019b; Yang et al. 2020). Salgado *et al.* (Salgado et al. 2019b) have stated that melting and glass transition temperatures decrease with increasing salt concentration, while thermal stability is not significantly affected. Kim *et al.* (Kim et al. 2011) have studied the mixture n-butyl-n-methylpyrrolidinium TFSI with Li TFSI salt showing slight decrease on ionic conductivity when salt concentration increases. These papers remarks also the applicability to refrigeration besides the most traditional applications on electrochemistry and other uses.

However, together with good physico-chemical properties, current European Union environmental legislation, including REACH (Regulation concerning registration, Evaluation, Authorization and Restriction of Chemicals) (Commission 2006), calls for safety materials, –highlighting the principles of Green Chemistry as prevention, economy, less hazardous chemical synthesis, efficient use of energy, use of renewable raw and biodegradable materials, monitoring of real-time technological processes, and provision of an adequate level of chemical safety.

Therefore, it is urgent to establish evaluation procedures to estimate the toxicity of ILs that can readily provide the needed information and reducing the costs. *Aliivibrio fischeri* (*A. fischeri*) is a well-known marine luminescent bacterium with short reproductive cycle, and whose toxicity inference may be extrapolated to a wide variety of aquatic organisms, and thus can be effectively applied for toxicological risk assessment (Ventura et al. 2013; Parajó et al. 2019).

It is well stablished that the structure of ILs has important influence on the physical and chemical properties and also in toxicity, although some deep studies need to be performed. Thus, the specific choice of cation and anion has an important influence on the ecotoxicity of ionic liquids. It is well known that ILs with aromatic cations are more toxic than non-aromatic ones, mainly due to their water solubility (Ventura et al. 2013). Furthermore imidazolium and pyridinium based ILs with a specific anion show the highest harmful effects among other cations, with EC_{50} of 130 mg/L for the 1-butyl-3-methylpyridinium bromide and 5525 mg/L for 1-butyl-1-methylpyrrolidinium bromide as Ibrahim *et al.* (Ibrahim et al. 2017) underlined in their work. These researchers also highlight that the alkyl chain length has also a strong influence on the ecotoxicity, as well as in other thermophysical properties such as density and viscosity. As a consequence, EC_{50} is reduced by almost four orders of magnitude from 3234 mg/L (relatively harmless) for 1-ethyl-3-methylimidazolium chloride to 0.58 mg/L for 1-hexadecyl-3-methylimidazolium chloride (highly toxic, according Passino and Smith classification (Passino and S. B. Smith 1987)). With regard to the anion, in spite of its undoubted influence, the effects depend on the cation moiety, for example for the cation $[Emim]^+$, the EC_{50} are 9213 mg/L and 1631 mg/L for the $[Cl]^-$ and $[TFSI]^-$ anions, respectively. Nevertheless, for the cation $[C_8mim]^+$ the corresponding values are 2.36 mg/L for chloride and 6.44 mg/L for $[TFSI]^-$ (moderately toxic), which shows that the trend followed for the anions can change with the alkyl chain length.

With the aim to contribute to enlarge de database of toxic effects of ILs and the consequent improvement of the knowledge of relationship between toxicity and structure, the ecotoxicity of two protic ILs (ethylammonium nitrate (EAN) and ethylimidazolium nitrate (EIm NO_3)) and two aprotic ILs (butylmethylpyrrolidinium bis(trifluoromethylsulfonyl)imide (C_4C_1pyrr TFSI) and butyldimethylimidazolium bis(trifluoromethylsulfonyl)imide ($C_4C_1C_1$Im TFSI)) was tested towards changes on the bioluminescence of the bacteria *A. fischeri*, using the Microtox® standard toxicity test. Additionally, changes on the ecotoxicity as consequence of the doping of these ILs with different salts with electrochemical interest were also determined, firstly the doping of the corresponding lithium salt ($LiNO_{3,}$ for the protic ILs and LiTFSI for the aprotic IL) was evaluated, and finally effects of mono ($LiNO_3$), di ($Ca(NO_3)_2{\cdot}4H_2O$, $Mg(NO_3)_2{\cdot}6H_2O$) and trivalent ($Al(NO_3)_3{\cdot}9H_2O$) salts were also estimated for EAN. The effective concentration (EC_{50}) of these mixtures was determined over three standard periods of time, namely 5, 15 and 30 min and compared with the corresponding values to pure ILs.

2. Materials And Methods

2.1. Chemicals

The main characteristics of the four selected ILs (EAN, EIm NO_3, C_4C_1pyrr TFSI and $C_4C_1C_1$Im TFSI) and the inorganic salt ($LiNO_3$, $Ca(NO_3)_2{\cdot}4H_2O$, $Mg(NO_3)_2{\cdot}6H_2O$, $Al(NO_3)_3{\cdot}9H_2O$ and LiTFSI) are indicated in Table 1. Ionic liquids were dried into high vacuum under constant stirring during at least 24 h and the water content, measured by Karl Fischer titration, for all them was below 100 ppm. Calcium, magnesium and aluminium nitrate are hydrated salts with four, six and nine water molecules respectively, and their purity was analysed by EDTA titration. Saturated mixtures of these ILs with lithium salt with common anion were prepared and salt concentration, in molality and molar fraction, and mixture molecular mass are presented in Table 2. Similarly, saturated mixtures of EAN with lithium, calcium, magnesium and aluminium nitrate salts were also prepared and saturated concentrations and the molecular mass of the mixtures were summarized in Table 3. All these mixtures were obtained by mixing both components, ionic liquid and salt as supplied, with the help of an ultrasound bath and a magnetic stirrer during, at least, 48 h. The saturated concentrations have been determined by increasing molality in intervals of 0.5 mol/kg until precipitation is observed at room temperature [5].

Table 1. Chemical structure, identification number, molecular mass, and purity of ILs and salts.

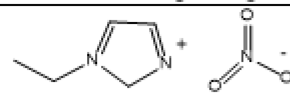
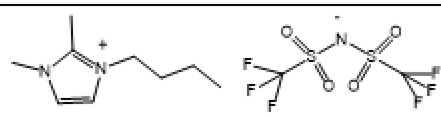
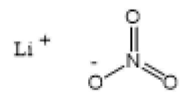
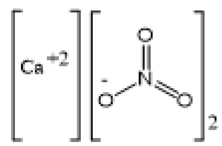
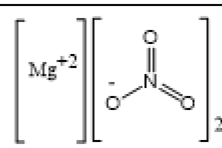
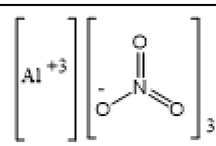
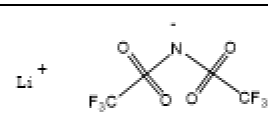
Name Molecular Mass (g/mol)	Abbreviation CAS Number	Chemical Structure	Purity Provenance
Ethylammonium Nitrate 108.10	EAN 22113-86-6		>0.97 Iolitec
Ethylimidazolium nitrate 159.14	EIm NO_3 501693-38-5		>0.98 Iolitec
Butylmethylpyrrolidinium bis(trifluoromethylsulfonyl)imide 422.41	C_4C_1pyrr TFSI 223437-11-4		>0.99 IoLiTec
Butylmethylmethylimidazolium bis(trifluoromethylsulfonyl)imide 433.39	$C_4C_1C_1$Im TFSI 350493-08-2		>0.99 IoLiTec
Lithium Nitrate 68.95	$LiNO_3$ 7790-69-4		>0.999 Merck
Calcium Nitrate Tetrahydrate 236.088	$Ca(NO_3)_2{\cdot}4H_2O$ 13477-34-4		>0.999 Merck
Magnesium Nitrate Hexahydrate 256.3	$Mg(NO_3)_2{\cdot}6H_2O$ 13446-18-9		>0.999 Merck
Aluminium Nitrate Nonahydrate 374.996	$Al(NO_3)_3{\cdot}9H_2O$ 7784-27-2		>0.999 Merck
Lithium bis(trifluoromethylsulfonyl)imide 287.09	LiTFSI 90076-65-6		>0.99 Acros organics

Table 2

Lithium salt saturation concentration (molality and molar fraction) in mixtures IL + salt and molecular mass (M_m) for the mixtures.

Mixture	EAN + $LiNO_3$	C_4C_1pyrr TFSI + LiTFSI	EIm NO_3 + $LiNO_3$	[$C_4C_1C_1$Im][TFSI] + LiTFSI
Molality (mol/l)	2.000	1.500	2.000	1.000
x_{salt}	0.178	0.388	0.241	0.399
M_m (g/mol)	123.01	604.31	181.08	204.83

Table 3
Salt saturation concentration (molality and molar fraction) in mixtures EAN + salt and molecular mass (M_m) for the mixtures

Mixture	EAN + $LiNO_3$	EAN + $Ca(NO_3)_2 \cdot 4H_2O$	EAN + $Mg(NO_3)_2 \cdot 6H_2O$	EAN + $Al(NO_3)_3 \cdot 9H_2O$
Molality (mol/l)	2.000	1.000	2.000	2.000
x_{salt}	0.178	0.270	0.446	0.540
M_m (g/mol)	123.01	133.62	163.51	189.17

2.2. Experimental Section

Acute toxicity was assessed by determining the luminescence inhibition of the rod-shaped Gram-negative marine bacteria *Aliivibrio fischeri* (*A. fischeri*), that bioluminesces through a population-dependent mechanism called quorum sensing sensitive to a wide variety of toxic substances (Ibrahim et al. 2017). Standard Microtox® liquid phase assays (M500 Analyzer - Modern water) was used for this propose. After exposing the bacteria at 15 ºC to each different IL or IL + salt aqueous solutions (from 0 to 81.9%), the light output at 5, 15 and 30 min was measured and compared with a blank control sample. The concentration of the sample (mg/L) which produces a 50%, 20% and 10% luminescence inhibition after exposure at the three selected times (5, 15 and 30 min) is designated as Effective Concentration (EC_{50}, EC_{20} and EC_{10} respectively) and is calculated, together with the corresponding 95% confidence intervals, through a non-linear regression, using the least-squares method to fit the data to the logistic equation (Parajó et al. 2019). The bioluminescence decrease with the increasing concentration of the sample constitutes and integrate measure of the physiological impairment of the bacteria, hence demonstrate the toxic effect of the studied compound (Ventura et al. 2014).

With the aim to clarify the methodology followed, Table 4 indicates the initial concentrations (mg/L) of the mixture stock solutions prepared for the Microtox® measurements.

In this work two classifications were used to distinguish the toxicity of the compounds. The first one is the widely used and proposed by Passino and Smith (Passino and S. B. Smith 1987) based on the values of EC_{50} at 30 minutes: EC_{50} > 1000 mg/L relatively harmless; 100 mg/L < EC_{50} < 1000 mg/L practically harmless; 1 mg/L < EC_{50} < 100 mg/L toxic; 0.1 mg/L < EC_{50} < 1 mg/L highly toxic; 0.01 mg/L < EC_{50} < 0.1 mg/L Extremely toxic.

The other classification is based on the studies of Chang *et al.* (Chang et al. 2013), who used the concept of toxicity units, calculated by:

$$TU = \frac{100}{EC_{50}}$$

being the EC_{50} (in mg/L) measured after 15 minutes of exposition. Thus the toxicity steps are defined as follows: TU < 1 Non-toxic; 1 < TU < 10 Toxic; 10 < TU < 100 Very Toxic; TU > 100 Extremely Toxic.

Table 4
Initial concentrations (mg/L) of pure ILs and binary mixture stock solutions prepared for the Microtox® measurements.

Compound	Concentration / $mg{\cdot}L^{-1}$
EAN	44.461×10^3
EAN + $LiNO_3$ 2m	50.850×10^3
EAN + $Ca(NO_3)_2$ 1m	43.346×10^3
EAN + $Mg(NO_3)_2$ 2m	36.893×10^3
EAN + $Al(NO_3)_3$ 2m	3.340×10^3
C_4C_1pyrr TFSI	5.394×10^3
C_4C_1pyrr TFSI + LiTFSI 1.5m	5.929×10^3
EIm NO_3	61.172×10^3
EIm NO_3 + $LiNO_3$ 2m	15.915×10^3
$C_4C_1C_1$Im TFSI	14.590×10^3
$C_4C_1C_1$Im TFSI + LiTFSI 1m	2.689×10^3

3. Results And Discussion

3.1. Toxicity of pure ILs

A set of ecotoxicity parameters obtained (EC_{10}, EC_{20} and EC_{50}) for four ILs, two protic and two aprotic, towards the toxicity endpoint of bioluminescence of the bacteria *A. fischeri* is reported in this work. Although the most used parameter is EC_{50}, that is the concentration for a 50% of reduction in the luminescence of the bacteria, EC_{10} and EC_{20} (concentrations to reduce 10% and 20% regarding the initial luminescence, respectively) also provide intermediate toxicity references, and, hence, a more complete ecotoxicological characterization of these compounds. Furthermore, EC_{10} and EC_{20} are starting points for the estimation of the lowest observed effect concentration, and especially EC_{10} can be used as a reliable parameter of the effects independent of concentration or for the lowest environmental risk compounds (Ventura et al. 2014). Tables 5–7 present the values of EC_{10}, EC_{20} and EC_{50}, respectively, of pure ILs for 5 min, 15 min and 30 min of exposition.

Figure 1 shows the behavior of inhibition of the bioluminiscente of *Aliivibrio fischeri* bacteria versus concentration of the four pure ionic liquids. As expected, the inhibition increases with the concentration following a logistic equation. It is well known that the bacterial bioluminescence reactions are indicative of cellular metabolism of the bacteria, and a reduction in bioluminescence implies a decrease in cellular respiration (Perales et al. 2016). Thus, the trend followed by these four ILs is $C_4C_1C_1$Im TFSI > C_4C_1pyrr TFSI ≈ EIm NO_3 > EAN. A Similar tendency was obtained for EC_{20} 30 min, but for the values EC_{50} at 30 min the trend obtained is slightly different $C_4C_1C_1$Im TFSI > EIm NO_3 > C_4C_1pyrr TFSI > EAN. These results are in agreement with the previous idea that the protic and non-aromatic ILs are less toxic that the aprotic and aromatic ones.

Taking into account the two criteria exposed in the previous section, EAN, with EC_{50} at 30 min of 12582 mg/L value, higher than 1000 mg/L and TU ≈ 9.10^{-3}, is placed in the lowest group of toxicity, being harmless, whereas the harmfulness of $TFSI^-$ based aprotic ILs depends on the cation, being for the C_4C_1pyrr TFSI practically harmless (1464 mg/L and TU = 0.1) and for the $C_4C_1C_1$Im TFSI almost toxic according to the second criterion (TU ≈ 1). A comparison of toxicity of 32 ILs between the most used as well as toluene (one of the most used VOCs) is shown in Fig. 2. The main part of the these ILs are essentially harmless, and imidazolium cations with long alkyl chain length and $TFSI^-$ anion seems to be the most toxic, even more than toluene.

Microtox toxicity screening has been widely applied to different ionic liquids families; nevertheless results of the selected ILs of this work are scarce, being specially striking the case of EAN, which is the first synthetized IL, more than century ago. Ventura *et al.* (Ventura et al. 2013) obtained values of EC_{50} at 5 min and 15 min of 130.85 mg/L and 87.23 mg/L respectively for $C_4C_1C_1$Im TFSI, that are in very good concordance with our results for this IL and with that of Delgado-Mellado *et al.* (Delgado-Mellado et al. 2019) who found values of 141 mg/L and 100 mg/l for the EC_{50} at 5 and 15 min respectively. Montalbán *et al.* (Montalbán et al. 2016) also have found lower values for EC_{50} at 30 min for pure EAN, being relatively harmless and non-toxic according to both classifications indicated in this work. In the case of C_4C_1pyrr TFSI Viboud *et al.* (Viboud et al. 2012) have found EC_{50} at 15 min of 219 mg/L which is lower values than our value but corresponding to the same toxicity group. Additionally, Ventura *et al.* (Ventura et al. 2013) analysed the effect of some TFSI based ILs with the same alkyl length, being the pyrrolidinium one the most harmless of these aprotic ILs, also in agreement with our observations.

As a general conclusion, the results reported here confirm the idea that the protic ILs are generally less toxic than aprotic ones and the non-aromatic are less toxic that aromatic ones. Also the role of water solubility is important, being lower toxicity related with higher hydrophilicity in every group (Peric et al. 2013; Ventura et al. 2013).

Table 5
EC_{10} effective concentration values in mg/L and the respective 95% confidence intervals, obtained after 5, 15 and 30 min of exposure of the marine bacteria *A. fischeri*. Some values are repeated to facilitate the comparison.

IL/Mixture	EC_{10} 5 min/ mg/L	EC_{10} 15 min/ mg/L	EC_{10} 30 min/ mg/L
	(Lower; Upper) limits	(Lower; Upper) limits	(Lower; Upper) limits
PURE IONIC LIQUIDS			
EAN	2304.89	1609.79	1517.65
	(248.43; 4361.05)	(560.06; 3163.56)	(332.07; 2703.22)
EIm NO_3	100.10	103.80	127.39
	(21.33; 179.99)	(22.16; 184.19)	(37.59; 214.25)
C_4C_1pyrr TFSI	438.08	254.32	170.23
	(225.18; 650.98)	(146.51; 362.18)	(93.44; 247.12))
$C_4C_1C_1$Im TFSI	23.25	20.96	20.34
	(0.00; 50.07)	(7.96; 33.95)	(12.63; 28.05)
IL + INORGANIC SALT SATURATED MIXTURES			
EAN + $LiNO_3$ 2m	6842.44	5920.72	4701.17
	5316.88; 8368.00)	(3841.85; 7999.89)	(1744.45; 7658.88)
EAN + $Ca(NO_3)_2{\cdot}4H_2O$ 1m	2732.16	1938.08	1059.24
	(1304.74; 4159.58)	(632.90; 3046.27)	(263.33; 1849.14)
EAN + $Mg(NO_3)_2{\cdot}6H_2O$ 2m	5469.35	7049.21	8409.28
	(3551.43; 7387.26)	(5925.65; 8174.77)	(6357.53; 10461.04)
EAN + $Al(NO_3)_3{\cdot}9H_2O$ 2m	8.04	17.96	14.30
	(3.01; 13.06)	(12.40; 21.53)	(10.29; 18.31)
EIm NO_3 + $LiNO_3$ 2m	232.85	251.51	263.96
	(9.66; 455.18)	(6.98; 496.73)	(10.87; 515.83)
C_4C_1pyrr TFSI + LiTFSI 1.5m	35.88	23.22	21.12
	(17.01; 51.49)	(6.87; 39.12)	(8.52 ;35.82)
$C_4C_1C_1$Im TFSI + LiTFSI 1m	6.35	5.26	5.45
	(0.15; 12.56)	(2.46; 8.07)	(3.64; 7.26)

Table 6
EC_{20} effective concentration values in mg/L and the respective 95% confidence intervals, obtained after 5, 15 and 30 min of exposure of the marine bacteria *A. fischeri*. Some values are repeated to facilitate the comparison.

IL/Mixture	EC_{50} 5 min/ mg/L	EC_{50} 15 min/ mg/L	EC_{50} 30 min/ mg/L
	(Lower; Upper) limits	(Lower; Upper) limits	(Lower; Upper) limits
PURE IONIC LIQUIDS			
EAN	4314.31	3236.68	3012.33
	(1548.95; 7081.66)	(951.77; 5522.60)	(1264.99; 4761.67)
EIm NO_3	195.44	194.19	223.45
	(79.12; 312.90)	(79.98; 310.53)	(105.10; 342.82)
C_4C_1pyrr TFSI	684.04	416.73	289.18
	(441.90; 926.09)	(286.18; 545.93)	(192.91; 386.85)
$C_4C_1C_1$Im TFSI	46.34	39.09	36.45
	(5.74; 86.95)	(20.78; 57.40)	(26.05; 46.85)
IL + INORGANIC SALT SATURATED MIXTURES			
EAN + $LiNO_3$ 2m	8892.60	7495.94	6145.81
	(7412.30; 10373.90)	(5603.85; 9386.04)	(3301.99; 8988.58)
EAN + $Ca(NO_3)_2{\cdot}4H_2O$ 1m	3939.68	2858.82	1804.56
	(2427.26; 5450.11)	(1475.52; 4240.12)	(800.29; 2808.82)
EAN + $Mg(NO_3)_2{\cdot}6H_2O$ 2m	7471.07	8933.00	10222.13
	(5520.95; 9421.19)	(7887.15; 9979.85)	(8450.60; 11994.66)
EAN + $Al(NO_3)_3{\cdot}9H_2O$ 2m	15.21	23.30	19.25
	(8.47; 22.96)	(18.29; 27.31)	(15.84; 23.65)
EIm NO_3 + $LiNO_3$ 2m	423.67	435.29	442.20
	(119.10; 727.77)	(118.19; 753.03)	(125.48; 759.18)
C_4C_1pyrr TFSI + LiTFSI 1.5m	89.11	51.51	44.11
	(56.09; 123.44)	(23.58; 79.99)	(23.08; 64.29)
$C_4C_1C_1$Im TFSI + LiTFSI 1m	13.01	10.09	9.88
	(3.31; 22.72)	(6.00; 14.18)	(7.39; 12.37)

Table 7
EC_{50} effective concentration values in mg/L and the respective 95% confidence intervals, obtained after 5, 15 and 30 min of exposure of the marine bacteria *A. fischeri*. Some values are repeated to facilitate the comparison.

IL/Mixture	EC_{50} 5 min/ mg/L	EC_{50} 15 min/ mg/L	EC_{50} 30 min/ mg/L
	(Lower; Upper) limits	(Lower; Upper) limits	(Lower; Upper) limits
PURE IONIC LIQUIDS			
EAN	12582.07 (8186.64; 16977.50)	10665.47 (6650.14; 14680.80)	9711.63 (6561.46; 12860.79)
EIm NO_3	612.55 (395.90; 828.01)	573.77 (372.29; 774.55)	597.89 (408.00; 785.08)
C_4C_1pyrr TFSI	1463.91 (1162.13; 1765.69)	964.58 (791.32; 1137.88)	714.43 (577.92; 851.21)
$C_4C_1C_1$Im TFSI	150.44 (72.43; 228.49)	113.32 (82.29; 144.35)	98.70 (82.39; 115.01)
IL + INORGANIC SALT SATURATED MIXTURES			
EAN + $LiNO_3$ 2m	13911.23 (12469.75; 15232.70)	11210.37 (9613.17; 12808.57)	9706.72 (7233.87; 12179.58)
EAN + $Ca(NO_3)_2{\cdot}4H_2O$ 1m	7354.41 (5672.26; 9036.56)	6064.77 (4343.18; 7784.36)	4502.32 (3067.63; 5937.02)
EAN + $Mg(NO_3)_2{\cdot}6H_2O$ 2m	12724.84 (10834.31; 14615.38)	13384.92 (12522.40; 14247.45)	14266.92 (13128.37; 15403.46)
EAN + $Al(NO_3)_3{\cdot}9H_2O$ 2m	45.22 (32.45; 58.98)	37.34 (33.10; 41.59)	32.25 (29.84; 36.65)
EIm NO_3 + $LiNO_3$ 2m	1178.11 (691.19; 1665.78)	1114.08 (644.19; 1583.21)	1073.03 (626.44; 1520.48)
C_4C_1pyrr TFSI + LiTFSI 1.5m	453.19 (360.94; 547.22)	208.10 (142.51; 274.71)	149.60 (108.86; 189.91)
$C_4C_1C_1$Im TFSI + LiTFSI 1m	44.26 (24.69; 63.82)	30.63 (23.26; 40.00)	27.24 (23.17; 31.32)

3.2. Toxicity of salts –EAN mixtures

The second part of this work is the knowledge of the effect on toxicity towards *A. Fischeri* bioluminiscence inhibition of different salt addition to EAN IL. Figure 3 presents the percentage of bioluminescence inhibition of the bacteria with regards to the concentration of EAN - nitrate salt saturated solutions following, all of them, a logistic equation. Additionally, the values of EC_{10}, EC_{20} and EC_{50} of these samples for 5 min, 15 min and 30 min of exposition were also exposed in Tables 5–7. Results showed that mixtures of EAN with the mono and divalent salts did not affect significantly to the toxicity of the mixture with regards to the pure ionic liquid, but the addition of the aluminium salt had an important effect on bioluminescence of *A. Fischeri*, being this mixture toxic in both classifications used in this paper, as it can be seen in the Table 8. This behavior can be related to the well-known antimicrobial and antibacterial effects of aluminium salts which is also related with the acidification of the sample as consequence of this salt addition (being the pH lower than 4 for this salt, whereas for the other salt is always higher than 5) (Guida et al. 1991). As it is well known, aluminium in solid state plays a key role in environment, but due to high reactivity it is difficult to find in free State in nature. At neutral or weakly acidic pH, it is present in the form of insoluble oxides and aluminosilicates. However, for the high acidic media, aluminium is solubilized into a phytotoxic form (Matsumoto 2000), since, under these acidic conditions, it is a polyvalent cation that binds strongly to negative charges, usually the carboxyl groups in cell wall molecules, affecting to cell division, cell extension or nutrient transport and provoking the cell growth disruption and serious perturbation of the normal metabolism of living organism (Haug 1984; Jones and Ryan 2016). Contrarily, Li^{+}, Ca^{+2} and Mg^{+2} cations do not exert any appreciable influence in the toxicity of the IL. Indeed, these cations play different beneficial functions in cells, since they are oligoelements, i.e. metal or metalloids ions that have an important roles as, for example, constituents of cell tissues or as catalyzers of chemical reactions of the cell metabolism (Carvalho et al. 2015). Specifically, lithium has an especial role on brain diseases, as for example through the inhibition of the inositol, sugar involved in the ability of neurons to exchange signals; lowering inositol levels (using Li^{+}) can calm overactive neurons (Pilcher 2003). Mg^{+2} and Ca^{+2} have intermediate binding strengths to organic ligands and in the particular case of Ca^{+2}, works for a charge carrier and for signal transmission inside the cells (Crichton 2017).

Table 8
Toxicity identification of the EAN- nitrate salt mixtures using the criteria of Passino and Smith (Passino and S. B. Smith 1987) and Chang *et al.* (Chang et al. 2013)

IL/mixture	**Passino and Smith (Passino and S. B. Smith 1987)**	**Chang *et al.* (Chang et al. 2013)**
EAN	Relatively Harmless	Non toxic
EAN + $LiNO_3$ 2m	Relatively Harmless	Non toxic
EAN + $Ca(NO_3)_2$ 1m	Relatively Harmless	Non toxic
EAN + $Mg(NO_3)_2$ 2m	Relatively Harmless	Non toxic
EAN + $Al(NO_3)_3$ 2m	Toxic	Toxic

3.3. Toxicity of IL-lithium salt mixtures

The last part of this paper is the analysis of the effect of mixture of lithium salt and different ionic liquids. Figure 4 shows the comparison of the inhibition of bioluminescence of *A. fischeri* against logarithm of concentration for 30 min of exposure of the four pure ionic liquids EAN, EIm NO_3, C_4C_1pyrr TFSI and $C_4C_1C_1$Im TFSI with the corresponding saturated lithium salt binary mixture, Li NO_3 for the protic ILs and Li TFSI for the aprotic ones. Additionally, the values of EC_{10}, EC_{20} and EC_{50} of these samples, pure ILs and mixtures, for 5 min, 15 min and 30 min of exposition were also exposed in Tables 5–7.

Changes on the bioluminescence inhibition as a consequence of salt addition are strongly dependent of the ionic liquid – salt mixture. Pure EAN and EAN + $LiNO_3$ mixture present results of EC_{50} at 30 minutes that indicates that the toxicity of the mixture is similar than the pure IL, although the inhibition of the lowest concentrations (EC_{20} and EC_{10}) is lower for the mixture than for the pure IL. It is especially interesting the case of the mixture of EIm NO_3 + Li salt which presents lower toxicity than pure IL following the Passino and Smith classification, since the EC_{50} at 30 min increase from 598 to 1073 mg/L for pure IL and mixture respectively. Aprotic IL- lithium salt mixtures present the same behavior: the toxicity increases significantly with addition of lithium salt, although for the case of $C_4C_1C_1$Im TFSI, which present the lowest values of EC_{50} and so the highest toxicity of the four pure ILs here studied, the addition of Li TFSI reduced in four times the EC_{50} for all the exposure times, but not enough to change the toxicity group following the two classifications chosen in this work, as can be seen in Table 9. Taking into account that Viboud *et al.* (Viboud et al. 2012) classify the Li TFSI salt in the lowest toxicity group ($EC_{50} > 1000$ mg/L), this last statement can be unexpected, but this can be explained from the increment of the concentration of $TFSI^-$ ion after the addition of the salt to the IL with the common anion, which increases the hydrophobicity of the sample, closely related with the toxicity towards *A. Fischeri* bioluminescence, as it was pointed out previously in the 3.1 section.

Table 9. Toxicity identification of the IL-lithium salt mixtures using the criteria of Passino and Smith (Passino and S. B. Smith 1987) and Chang *et al.* (Chang et al. 2013)

IL/mixture	Passino and Smith (Passino and S. B. Smith 1987)	Chang *et al.* (Chang et al. 2013)
EAN	Relatively Harmless	Non toxic
EAN + $LiNO_3$	Relatively Harmless	Non toxic
EIm NO_3	Practically Harmless	Non toxic
EIm NO_3 + $LiNO_3$	Relatively Harmless	Non toxic
C_4C_1pyrr TFSI	Practically Harmless	Non toxic
C_4C_1pyrr TFSI + LiTFSI	Slightly toxic	Non toxic
$C_4C_1C_1$Im TFSI	Slightly toxic	Toxic
$C_4C_1C_1$Im TFSI + LiTFSI	Slightly toxic	Toxic

As a general observation, the addition of the lithium salt does not change the effect of these compound on the bioluminescence of *A. Fischeri* bacteria, although the extrapolation of these result to other toxicity endpoint cannot be done easily, because the response is highly dependent on the trophic levels (Perales et al. 2018). Although there are not references about the ecotoxicity changes on ILs after the salt addition, it is especially interesting the results of Sixto *et al.* (Sixto et al. 2021), who analysed the effect of EAN and EAN-lithium nitrate mixture (equally harmless to *A. Fischeri*) on the respiration of two soils with different organic matter content, concluding that both, pure IL and mixture, strongly affect the soil respiration and that the organic matter content can mitigate the negative effect of lithium salt. All these remarks highlight the need to increase and precise the knowledge of impact of these ionic compounds and their mixtures in order to set up appropriate recovery and recycling procedures.

4. Conclusions

In this work, the ecotoxicity of two protic ILs (Ethylammonium nitrate and Ethylimidazolium nitrate) and two aprotic ILs (Butylmethylpyrrolidinium bis (trifluoromethylsulfonyl)imide and Butyldimethylimidazolium bis (trifluoromethylsulfonyl)imide) pure and binary mixtures with different inorganic salt of electrochemical interest were tested towards changes on the bioluminescence of the bacteria *Aliivibrio fischeri*, using the Microtox® standard toxicity test.

The main conclusions of this work are

- Protic ILs are less toxic than aprotic ones and that non-aromatic are generally less toxic than aromatic ones. Moreover, the role of water solubility is important, being lower toxicity related with higher hydrophilicity in every group.

- Mixtures of EAN with the mono- and divalent salts does not affect significantly the toxicity of the mixture with regards to the pure ionic liquid, but the addition of an aluminium salt has an important effect on bioluminescence of *Fischeri*, being this mixture the most toxic of the studied ones.

- Lithium salt addition toxic effects strongly depend on the IL of the mixture: protic ILs do not significantly modify the EC_{50} with regard to that of the pure IL, or even lead to slight increases of it indicating a reduction of toxicity, nevertheless for the aprotic ILs, especially for pyrrolidinium one, the effect of salt addition clearly increases the toxicity of the mixture.

- The two criteria used in this paper to classify the toxicity concerning marine bacteria *A. Fischeri* bioluminescence inhibition are comparable.

5. Declarations

Ethics approval and consent to participate:

Not applicable.

Consent for publication:

Not applicable.

Competing interests:

The authors declare no competing interests

Availability of data and material:

Not applicable

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Authors Contributions

Conceptualization: J.J.P., J.S., and L.M.V.; Methodology and data measurements, P.V., J.J.P.and M.V.; Data analysis, tables and figures preparation: J.J.P.,P.V.,M.V. and J.S.; Writing—original draft preparation: J.J.P.,P.V., L.M.V., M.V., and J.S.; Funding acquisition: M.V, J.S. and L.M.V.; All authors have read and agreed to the published version of the manuscript

6. References

1. Balducci A (2017) Ionic Liquids in Lithium-Ion Batteries. Top Curr Chem 375:1–27. https://doi.org/10.1007/s41061-017-0109-8
2. Carvalho DC, Coelho LM, Acevedo MSMSF, Coelho NMM (2015) The oligoelements. In: Guardia M, de la, Garrigues S (eds) Handbook of Mineral Elements in Food, First Edit. John Wiley & Sons, Ltd, pp 109–122
3. Chang SC, Wang YF, You SJ et al (2013) Toxicity evaluation of fly ash by Microtox®. Aerosol Air Qual Res 13:1002–1008. https://doi.org/10.4209/aaqr.2012.10.0267
4. Commission E (2006) Regulation No. 1907/2006 of the European Parliament and of the Council Concerning the Registration, Evaluation, Authorization and Restriction of Chemicals
5. Crichton RR (2017) Metal Toxicity - An Introduction. In: Crichton RR, Ward RJ, Hider RC (eds) Metal Chelation in Medicine, First edit. The Royal Society of Chemistry, pp 1–23
6. Delgado-Mellado N, Ayuso M, Villar-Chavero MM et al (2019) Ecotoxicity evaluation towards Vibrio fischeri of imidazolium- and pyridinium-based ionic liquids for their use in separation processes. SN Appl Sci 1:896. https://doi.org/10.1007/s42452-019-0916-3
7. Guida L, Saidi Z, Hughes MN, Poole RK (1991) Aluminium toxicity and binding to Escherichia coli. Arch Microbiol 156:507–512
8. Gutiérrez A, Atilhan M, Aparicio S (2018) Theoretical Study of Oil Desulfuration by Ammonium-Based Deep Eutectic Solvents. Energy Fuels 32:7497–7507. https://doi.org/10.1021/acs.energyfuels.8b01403
9. Haug A (1984) Molecular aspects of aluminum toxicity. CRC Crit Rev Plant Sci 1:345–373. https://doi.org/10.1080/07352688409382184
10. Hernández-Fernández FJ, Bayo J, Ríos APDL et al (2015) Ecotoxicology and Environmental Safety Discovering less toxic ionic liquids by using the Microtox s toxicity test. Ecotoxicol Environ Saf 116:29–33. https://doi.org/10.1016/j.ecoenv.2015.02.034
11. Ibrahim M, Mutalib A, Mutalib A (2017) Ecotoxicity of and Ionic Liquids Towards Vibrio fischeri: Experimental and QSAR Studies. In: Handy S (ed) Progress and Developments in Ionic Liquids. INTECH, pp 429–449
12. Jones DL, Ryan PR (2016) Aluminum Toxicity. In: Encyclopedia of Applied Plant Sciences, Second Edi. Elsevier, pp 211–218
13. Kim J-K, Lim D-H, Scheers J et al (2011) Properties of N-butyl-N-methyl-pyrrolidinium Bis(trifluoromethanesulfonyl) Imide Based Electrolytes as a Function of Lithium

Bis(trifluoromethanesulfonyl) Imide Doping. J Korean Electrochem Soc 14:92–97. https://doi.org/10.5229/JKES.2011.14.2.092

14. Matsumoto H (2000) Cell Biology of Aluminum Toxicity and Tolerance in higher plants. Int Rev Cytol 200:1–46
15. Menne S, Pires J, Anouti M, Balducci A (2013) Protic ionic liquids as electrolytes for lithium-ion batteries. Electrochem Comm 31:39–41. https://doi.org/10.1016/j.elecom.2013.02.026
16. Montalbán MG, Hidalgo JM, Collado-González M et al (2016) Assessing chemical toxicity of ionic liquids on Vibrio fischeri: Correlation with structure and composition. Chemosphere 155:405–414. https://doi.org/10.1016/j.chemosphere.2016.04.042
17. Moreno D, Ferro VR, de Riva J et al (2018) Absorption refrigeration cycles based on ionic liquids: Refrigerant/absorbent selection by thermodynamic and process analysis. Appl Energy 213:179–194. https://doi.org/10.1016/j.apenergy.2018.01.034
18. Nakamoto H, Watanabe M (2007) Brønsted acid–base ionic liquids for fuel cell electrolytes. Chem Commun 2539–2541. https://doi.org/10.1039/B618953A
19. Parajó JJ, Macário IPE, De Gaetano Y et al (2019) Glycine-betaine-derived ionic liquids: Synthesis, characterization and ecotoxicological evaluation. Ecotoxicol Environ Saf 184:109580. https://doi.org/https://doi.org/10.1016/j.ecoenv.2019.109580
20. Passino DRM, Smith SB (1987) Acute bioassays and hazard evaluation of representative contaminants detected in great lakes fish. Environ Toxicol Chem 6:901–907. https://doi.org/10.1002/etc.5620061111
21. Perales E, García CB, Lomba L et al (2016) Comparative ecotoxicology study of two neoteric solvents: Imidazolium ionic liquid vs. glycerol derivative. Ecotoxicol Environ Saf 132:429–434. https://doi.org/10.1016/j.ecoenv.2016.05.021
22. Perales E, Lomba L, García-escudero M et al (2018) Toxicological study of some ionic liquids. Green Process Synth 7:287–295
23. Peric B, Sierra J, Martí E et al (2013) (Eco)toxicity and biodegradability of selected protic and aprotic ionic liquids. J Hazard Mater 261:99–105
24. Pilcher HR (2003) The ups and downs of lithium. Nature 425:118–120
25. Rana UA, Bayley PM, Vijayaraghavan R et al (2010) Proton transport in choline dihydrogen phosphate/H3PO4 mixtures. Phys Chem Chem Phys 12:11291. https://doi.org/10.1039/c0cp00156b
26. Rodríguez H, Brennecke J (2006) Temperature and composition dependence of the density and viscosity of binary mixtures of water + ionic liquid. J Chem Eng Data 51:2145–2155. https://doi.org/10.1021/je0602824
27. Rogers RD, Seddon KR (2003) Ionic Liquids - Solvents of the Future? Science (80-) 302:792–793
28. Salgado J, Parajó JJ, Villanueva M et al (2019a) Liquid range of ionic liquid – Metal salt mixtures for electrochemical applications. J Chem Thermodyn 134:164–174. https://doi.org/10.1016/j.jct.2019.03.012

29. Salgado J, Parajó JJ, Villanueva M et al (2019b) Liquid range of ionic liquid – Metal salt mixtures for electrochemical applications. J Chem Thermodyn 134:164–174. https://doi.org/https://doi.org/10.1016/j.jct.2019.03.012
30. Salgado J, Villanueva M, Parajó JJ, Fernández J (2013) Long-term thermal stability of five imidazolium ionic liquids. J Chem Thermodyn 65:. https://doi.org/10.1016/j.jct.2013.05.049
31. Sánchez PB, García J, Salgado J, González-Romero E (2016) Studies of Volumetric and Transport Properties of Ionic Liquid-Water Mixtures and Its Viability to Be Used in Absorption Systems. ACS Sustain Chem Eng 4:. https://doi.org/10.1021/acssuschemeng.6b01541
32. Sánchez PB, González B, Salgado J et al (2019) Physical properties of seven deep eutectic solvents based on L-proline or betaine. J Chem Thermodyn 131:. https://doi.org/10.1016/j.jct.2018.12.017
33. Santos AG, Ribeiro BD, Alviano DS, Coelho MAZ (2014) Toxicity of ionic liquids toward microorganisms interesting to the food industry. RSC Adv 4:37157–37163. https://doi.org/10.1007/s13595-014-0427-8
34. Shamshina JL, Rogers RD (2020) Are myths and preconceptions preventing us from applying ionic liquid forms of antiviral medicines to the current health crisis? Int J Mol Sci 21:1–16. https://doi.org/10.3390/ijms21176002
35. Silva FAe, Caban M, Stepnowski P et al (2016) Recovery of ibuprofen from pharmaceutical wastes using ionic liquids. Green Chem 18:3749–3757. https://doi.org/10.1039/c6gc00261g
36. Sixto T, Priano E, Reyes O et al (2021) Does soil organic matter affect the impact of the ionic liquid ethylammonium nitrate in the pure state and as mixture with lithium salt on soil basal respiration? Chem Proc 3:1–9. https://doi.org/10.3390/ecsoc-24-08376
37. Studzinska S, Buszewski B (2009) Study of toxicity of imidazolium ionic liquids to watercress (Lepidium sativum L.). Anal Bioanal Chem 393:983–990. https://doi.org/10.1007/s00216-008-2523-9
38. Toledo-Hijo AAC, Maximo GJ, Costa MC et al (2016) Applications of ionic liquids in the food and bioproducts industries. ACS Sustain Chem Eng 4:5347–5369. https://doi.org/10.1021/acssuschemeng.6b00560
39. Ventura SPM, Gonçalves AMM, Sintra T et al (2013) Designing ionic liquids: The chemical structure role in the toxicity. Ecotoxicology 22:1–12. https://doi.org/10.1007/s10646-012-0997-x
40. Ventura SPM, Silva FA e, Gonçalves AMM et al (2014) Ecotoxicity analysis of cholinium-based ionic liquids to Vibrio fischeri marine bacteria. Ecotoxicol Environ Saf 102:48–54. https://doi.org/10.1016/j.ecoenv.2014.01.003
41. Viboud S, Papaiconomou N, Cortesi A et al (2012) Correlating the structure and composition of ionic liquids with their toxicity on Vibrio fischeri: A systematic study. J Hazard Mater 215–216:40–48. https://doi.org/10.1016/j.jhazmat.2012.02.019
42. Wang Y, Wang Z, Sun J et al (2020) Flexible, stable, fast-ion-conducting composite electrolyte composed of nanostructured Na-super-ion-conductor framework and continuous Poly (ethylene

oxide) for all-solid-state Na battery. J Power Sources 454:227949/1–227949/10. https://doi.org/10.1016/j.jpowsour.2020.227949

43. Yang H, Luo X-F, Matsumoto K et al (2020) Physicochemical and electrochemical properties of the (fluorosulfonyl)(trifluoromethylsulfonyl)amide ionic liquid for Na secondary batteries. J Power Sources 470:228406. https://doi.org/https://doi.org/10.1016/j.jpowsour.2020.228406
44. Yasuda T, Kinoshita H, Miran MS et al (2013) Comparative Study on Physicochemical Properties of Protic Ionic Liquids Based on Allylammonium and Propylammonium Cations. J Chem Eng Data 58:2724–2732. https://doi.org/10.1021/je301284x

Figures

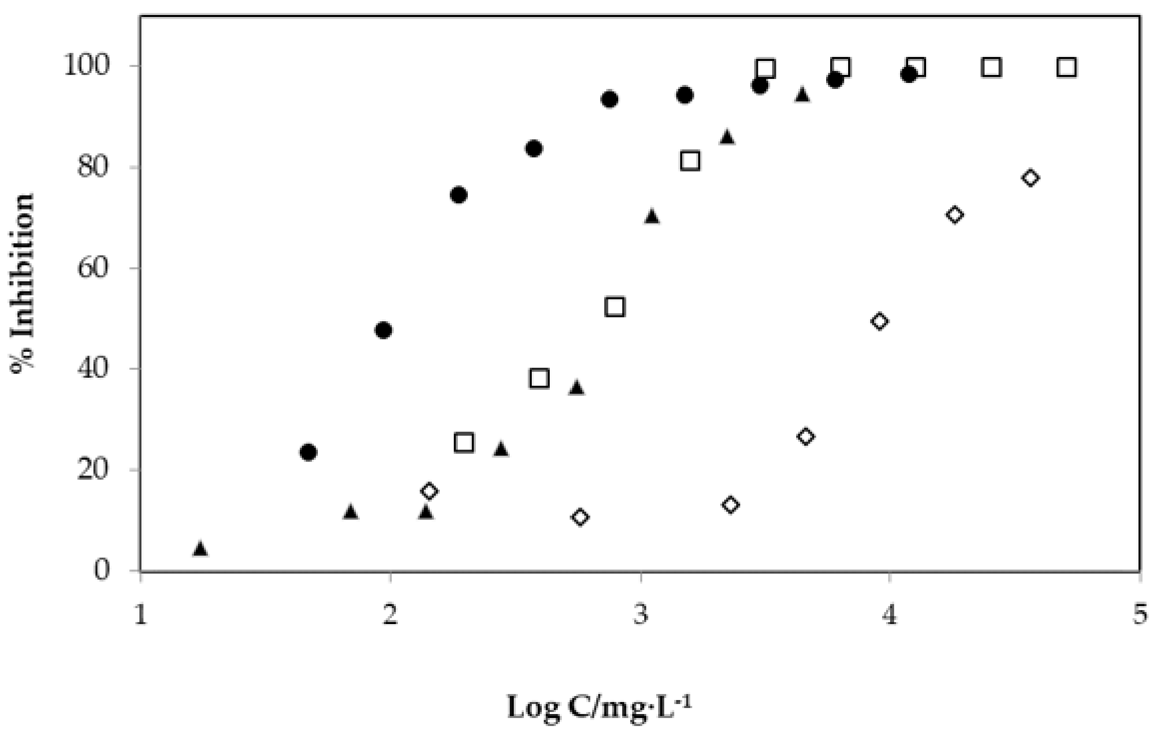

Figure 1

Inhibition of bioluminescence for 30 min of exposure against logarithm of concentration of the four pure ionic liquids: (●) C4C1C1Im TFSI, (▲) C4C1pyrr TFSI, () EIm NO3, () EAN.

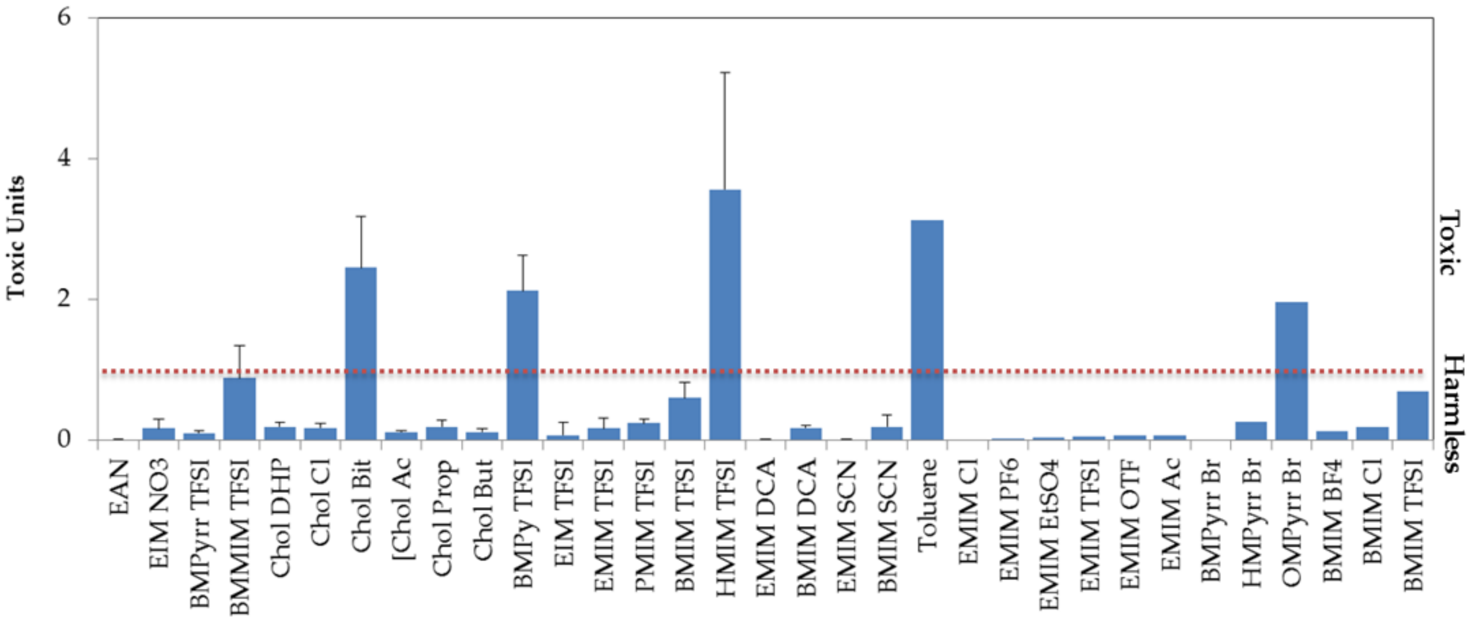

Figure 2

Comparison of the toxicity level (Chang et al. 2013) of the ILs here studied and some of the literature (error bars were included if the uncertainty intervals were published). Cholinium dihidrogen phosphate (Chol DHP), Choline chloride (Chol Cl), cholinium bitartatre (Chol Bit), Choline Acetate (Chol Ac), Choline propanoate (Chol Prop) and Choline Butanoate (Chol But) (Ventura et al. 2014); 1-butyl-4-methylpyridiniumbis (trifluoromethylsulfonyl)imide (BMPy TFSI), 1-ethylimidazolium bis(trifluoromethylsulfonyl) imide (EMIM TFSI), 1-Ethyl-3-methylimidazolium bis(trifluoromethylsulfonyl) imide (EMIM TFSI), 1-Propyl-3-methylimidazolium bis(trifluoromethylsulfonyl) imide (PMIM TFSI), 1-Butyl-3-methylimidazolium bis(trifluoromethylsulfonyl) imide (BMIM TFSI), 1-Ethyl-3-methylimidazolium bis(trifluoromethylsulfonyl) imide (EMIM TFSI), 1-Hexyl-3-methylimidazolium bis(trifluoromethylsulfonyl) imide (HMIM TFSI), 1-Ethyl-3-methylimidazolium dicyanamide (EMIM DCA), 1-Butyl-3-methylimidazolium dicyanamide (BMIM DCA), 1-Ethyl-3-methylimidazolium thiocyanate(EMIM SCN), 1-Butyl-3-methylimidazolium Thiocyanate (BMIM SCN), (Delgado-Mellado et al. 2019) ; Toluene (Hernández-Fernández et al. 2015); 1-Ethyl-3-methylimidazolium chloride (EMIM Cl), 1-Ethyl-3-methylimidazolium hexafluorophosphate (EMIM PF6), 1-Ethyl-3-methylimidazolium ethylsulphate (EMIM EtSO4), 1-Ethyl-3-methylimidazolium bis((trifluoromethyl) sulfonyl)amide (EMIM TFSI), 1-Ethyl-3-methylimidazolium triflate (EMIM OTF), 1-Ethyl-3-methylimidazolium acetate (EMIM Ac), 1-Butyl-1-methylpyrrolidinium bromide (BMPyrr Br), 1-Hexyl-1-methylpyrrolidinium bromide (HMPyrr Br), 1-Octyl-1-methylpyrrolidinium bromide (OMPyrr Br), 1-Butyl-3-methylimidazolium tetrafluoroborate (BMIM BF4), 1-Butyl-3-methylimidazolium Chloride (BMIM Cl), 1-Butyl-3-methylimidazolium bis((trifluoromethyl) sulfonyl)amide (BMIM TFSI) (Ibrahim et al. 2017).

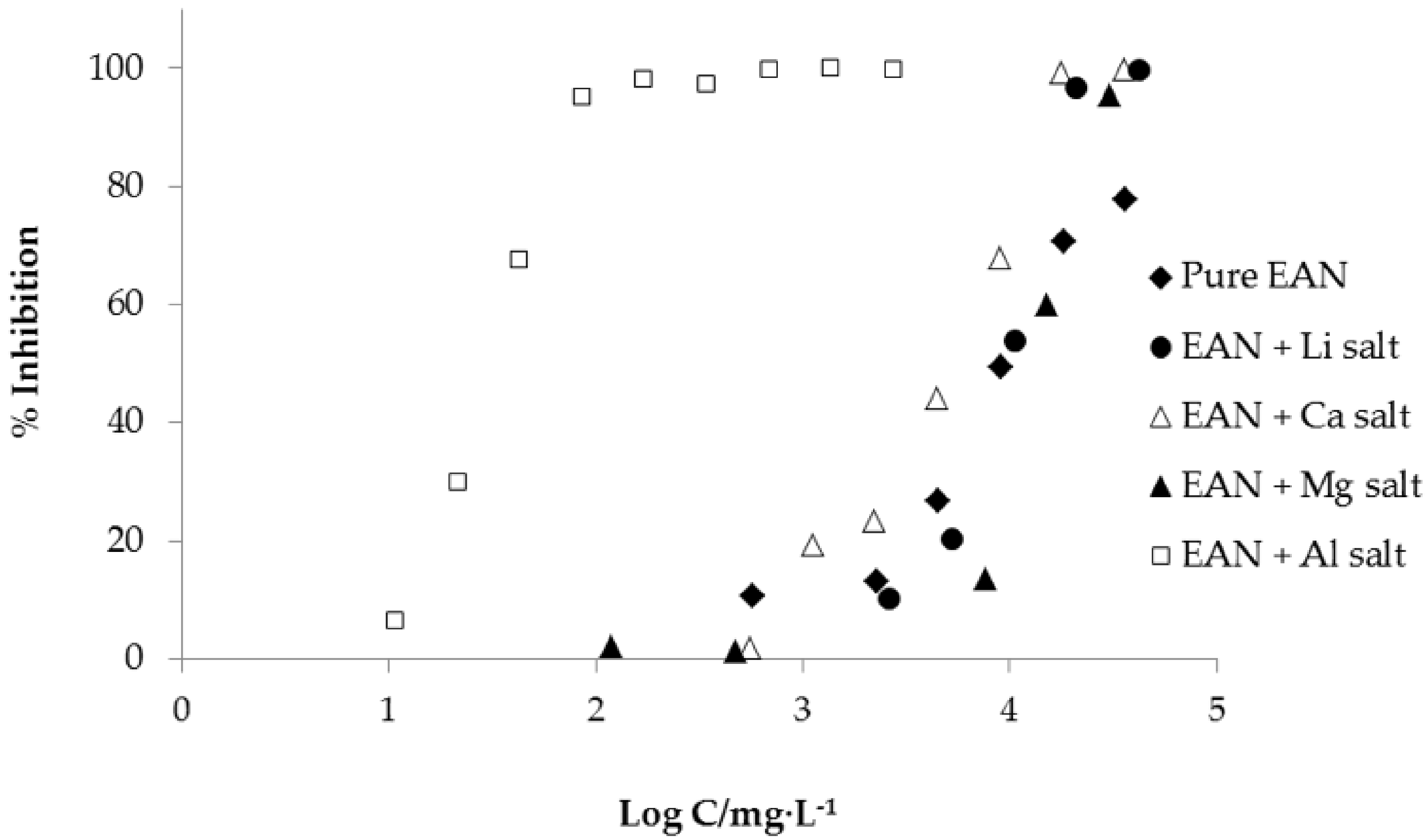

Figure 3

Inhibition of bioluminescence for 30 min of exposure against logarithm of concentration of the EAN and mixtures with the four nitrate salts.

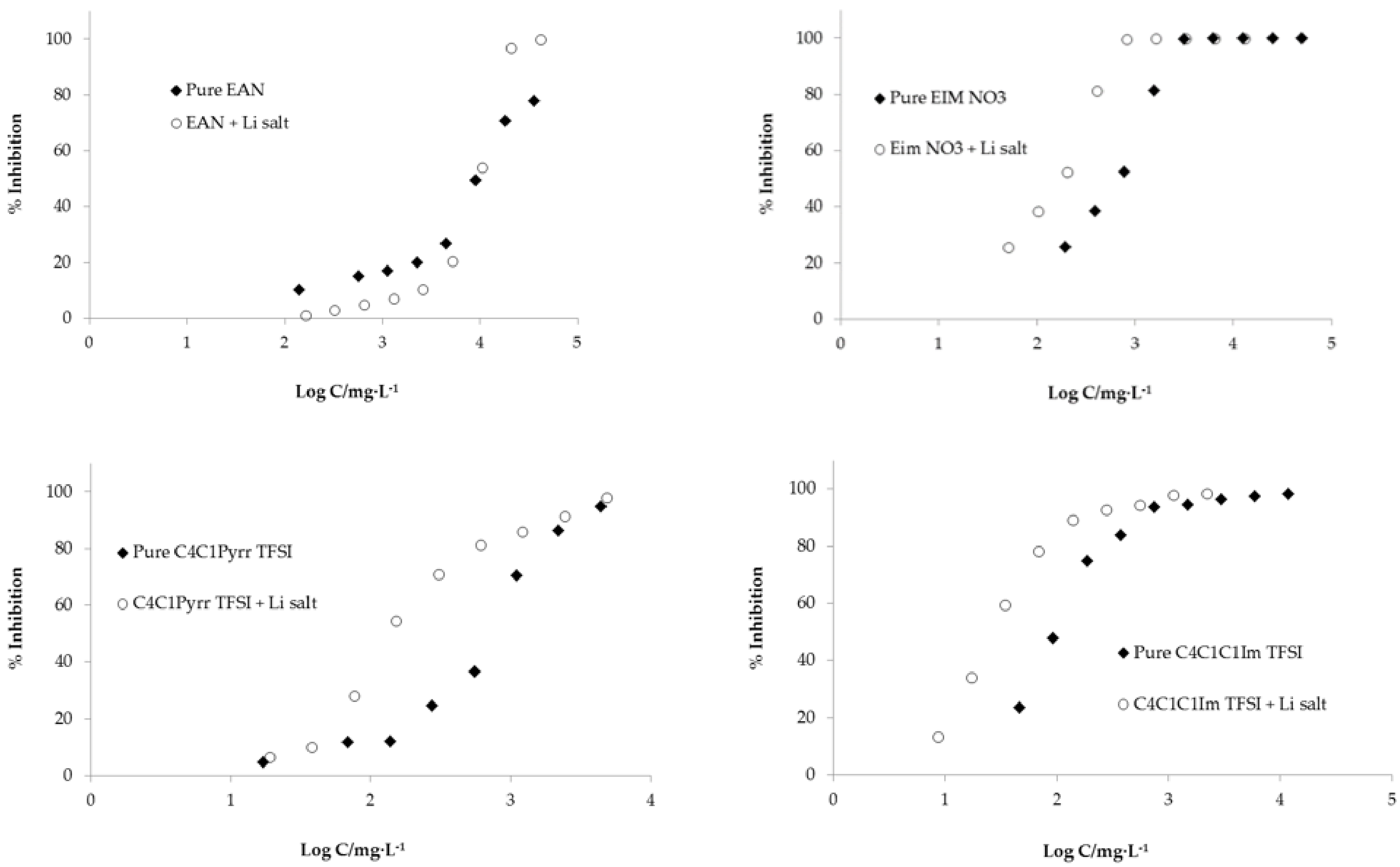

Figure 4

Comparison of the inhibition of bioluminescence of A. fischeri against logarithm of concentration of the four pure ionic liquids C4C1C1Im TFSI, C4C1pyrr TFSI, EIm NO3 and EAN, after 30 min of exposure, with the corresponding saturated lithium salt.