chemistry proceedings

Proceeding Paper

Ecotoxicity of Mixtures of IL and Lithium Salt †

Juan José Parajó [1,2,*], Pablo Vallet [1], Lois Fernádez-Míguez [1], María Villanueva [1] and Josefa Salgado [1]

[1] NAFOMAT Group, Departamentos de Física Aplicada y Física de Partículas, Universidade de Santiago de Compostela, 15782 Santiago de Compostela, Spain; Pablo.vallet.moreno@usc.es (P.V.); Lois.fernandez.miguez@usc.es (L.F.-M.); Maria.villanueva@usc.es (M.V.); J.salgado.carballo@usc.es (J.S.)

[2] Departamento de Química e Bioquímica, CIQUP-Centro de Investigaçao em Química da Universidade do Porto, Universidade do Porto, P-4169-007 Porto, Portugal

* Correspondence: juanjose.parajo@usc.es

† Presented at the 24th International Electronic Conference on Synthetic Organic Chemistry, 15 November–15 December 2020; Available online: https://ecsoc-24.sciforum.net/.

Abstract: The applicability of ionic liquids (IL) 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. In this work, the ecotoxicity of two protic ILs (Ethylammonium nitrate and Ethylimidazolium nitrate) and one aprotic IL (butylmethylpyrrolidinium bis(trifluoro omethylsulfonyl)imide) doped with the corresponding Lithium salt was tested towards changes on the bioluminescence of the bacteria *Aliivibrio fischeri*, using the Microtox® standard toxicity test. Half maximal effective concentration (EC_{50}) of these mixtures was determined over three standard periods of time and compared with the corresponding values to pure ILs.

Keywords: ionic liquids; ecotoxicity; electrolyte; Microtox; doped

Citation: Parajó, J.J.; Vallet, P.; Fernádez-Míguez, L.; Villanueva, M.; Salgado, J. Ecotoxicity of Mixtures of IL and Lithium Salt. *Chem. Proc.* **2021**, *3*, 84. https://doi.org/10.3390/ecsoc-24-08361

Academic Editors: Julio A. Seijas and M. Pilar Vázquez-Tato

Published: 14 November 2020

1. Introduction

The applicability of Ionic liquids (IL) seems never-ending, since they are still not fully studied and mixtures of pure IL and inorganic salt burst in as electrolytes for smart electrochemical devices [1,2]. ILs can be divided into two different subclasses depending in their structural characteristics: protic (PIL) and aprotic (AIL) ionic liquids. PILs are formed by the transfer of proton 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 huge variety of anions (i.e., bis trifluoromethylsulfonyl imide (TFSI), tris(pentafluoroethyl)trifluorophosphate (FAP), halides). Despite having many useful properties (arising from the protic nature) and potential applicability, the literature on PILs is still scarce compared to their AIL [3,4].

In recent years, significant growth in the structure−property relationships of ILs has been achieved with a better understanding of the intermolecular forces [5,6]. As mentioned above, when IL properties are not still fully known, mixtures of ILs and inorganic salts increases gives new possibilities improving, in some particular cases, some of their properties [1,7]. Salgado et al. [1] have stated how melting and glass transition temperatures present lower values when salt concentration increases and thermal stability remains equal on mixture than pure IL. Kim et al. [8] have studied the mixture n-butyl-n-methylpyrrolidinium TFSI with Li TFSI salt showing slight decrease on ionic conductivity when salt concentration increases.

However, apart from good physico-chemical properties, current European Union environmental legislation including REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) [9] makes firm demands for safety materials, that sometimes, unfortunately ecotoxicity test are skipped since they are usually expensive and time-consuming [10]. Therefore, it is urgent to establish evaluation procedures to estimate the toxicity of ILs that can provide the needed information without taking too long and reducing the costs. *Aliivibrio fischeri* (*A. fischeri*) is a well-known marine luminescent bacterium with short reproductive cycle, and the toxicity inference for *A. fischeri* may be extrapolated for a wide variety of aquatic organisms and thus can be effectively applied for toxicological risk assessment [11,12].

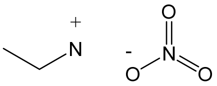
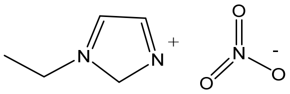
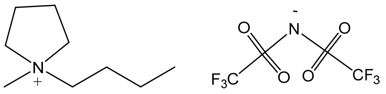
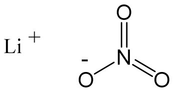
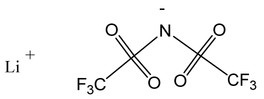
In this work, the ecotoxicity of two protic ILs (Ethylammonium nitrate (EAN) and Ethylimidazolium nitrate (EIm NO_3)) and one aprotic IL (butylmethylpyrrolidinium bis(trifluoromethylsulfonyl)imide (C_4C_1pyrr TFSI)) doped with the corresponding Lithium salt (Li NO_3, for the protic ILs and Li TFSI for the aprotic IL) was tested towards changes on the bioluminescence of the bacteria *Aliivibrio fischeri*, using the Microtox® standard toxicity test. 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 selected ILs (EAN, EIm NO_3, and C_4C_1pyrr TFSI) with the corresponding Lithium salt (Li NO_3 and Li TFSI) are indicated in Table 1. Nitrate ionic liquids were dried into high vacuum. Water content for all the samples was below 100 ppm.

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

Name Molecular Mass ($g\cdot mol^{-1}$)	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 Merck
Lithium Nitrate 68.95	Li NO_3 7790-69-4		>0.999 Merck
Lithium bis(trifluoromethylsulfonyl)imide 287.09	LiTFSI 90076-65-6		>0.99 Acros organics

The different solutions of IL+salt mixtures were prepared by mixing both components with the help of an ultrasound bath and a magnetic stirrer during, at least, 48 h. Saturated solutions have been reached using the hydrated salts by increasing molality in intervals of 0.5 $mol\cdot kg^{-1}$ until saturation point at room temperature [1]. The molar fraction (χ) of metal salts and pure IL and the final molecular mass (M_m) for every saturated mixture are indicated in Table 2.

Table 2. Molar fraction of metal salts and pure IL with final molecular mass (M_m) for the mixtures.

Mixture	EAN + Li NO_3	C_4C_1pyrr TFSI + Li TFSI	EIm NO_3 + $LiNO_3$
$\text{Molality}_{\text{saturation}}$	2.000	1.500	2.000
χ_{molar} sal	0.178	0.388	0.241
M_m/g mol^{-1}	123.01	604.31	181.08

2.2. Experimental Section

Acute toxicity was assessed by determining the luminescence inhibition of the marine bacteria *Aliivibrio fischeri* (*A. fischeri*). A Microtox® M500 Analyzer (Modern water) was used. After exposing the bacteria to each different IL or IL+salt solutions (from 0 to 81.9%) at 5, 15, and 30 min, the light output was measured and compared with a blank control sample. The concentration of the sample (mg L^{-1}) which produces a 50, 20, and 10% luminescence inhibition after exposure at the three selected times (5, 15, and 30 min) is designated as the 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 [11].

3. Results and Discussion

Figure 1 shows the behavior of % of luminescence relative to control *Aliivibrio fischeri* bacteria versus concentration, of C_4C_1pyrr TFSI and C_4C_1pyrr TFSI + TFSI 1.5 m mixture. The luminescence strongly decreases with the concentration of the toxic, following a logistic equation. Furthermore, it can be seen how the salt affects, negatively in this case, to the toxicity of the final solution mixture.

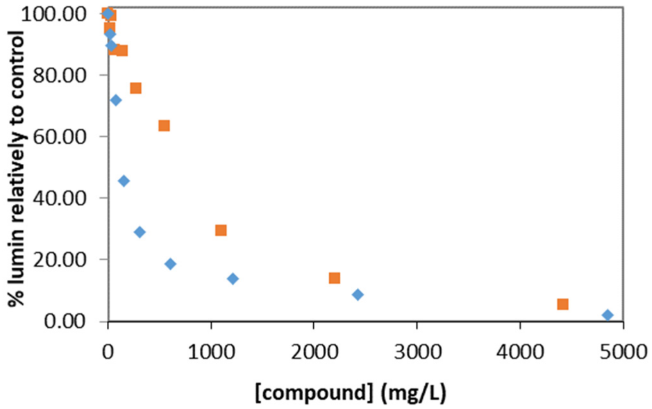


Figure 1. Comparative of the relationship between concentration of: pure C_4C_1pyrr TFSI (■) and C_4C_1pyrr TFSI + Li TFSI 1.5m (♦) mixture and the bioluminescence after 30 min of exposure.

From this curves the effective concentration values (EC_{10}, EC_{20}, and EC_{50}) of the different compounds at different times have been calculated and exposed in Table 3.

Table 3. Mean effective concentration values (EC_{50}, EC_{20}, EC_{10}) in mg L^{-1} and the respective 95% confidence intervals, obtained after 5, 10, and 30 min of exposure of the marine bacteria *A. fischeri*.

IL	Time/min	EC_{50} (Lower Limit; Upper Limit)/mg·L^{-1}	EC_{20} (Lower Limit; Upper Limit)/mg·L^{-1}	EC_{10} (Lower Limit; Upper Limit)/mg·L^{-1}
EAN	5	12,582 (8186; 16,977)	4314 (1548; 7081)	2304 (248; 4361)
	15	10,665 (6650; 14,680)	3236 (951; 5522)	1609 (56; 3163)
	30	9711 (6561; 12,860)	3012 (1264; 4761)	1517 (332; 2703)
EAN + Li NO_3 2 m	5	13,911 (12,469; 15,232)	8892 (7412; 10,373)	6842 (5316; 8368)
	15	11,210 (9613; 12,808)	7495 (5603; 9386)	5920 (3841; 8000)
	30	9706 (7233; 12,179)	6145 (3301; 8988)	4701 (1744; 7658)
EIm NO_3	5	612 (395; 828)	195 (79; 312)	100 (21; 179)
	15	573 (372; 774)	194 (79; 310)	103 (22; 184)
	30	597 (408; 785)	223 (105; 342)	127 (37; 214)
EIm NO_3 + Li NO_3 2 m	5	1178 (691; 1665)	423 (119; 727)	232 (9; 455)
	15	1114 (644; 1583)	435 (118; 753)	251 (6; 496)
	30	1073 (626; 1520)	442 (125; 759)	263 (10; 515)
C_4C_1pyrr TFSI	5	1463 (1162; 1765)	684 (441; 926)	438 (225; 650)
	15	964 (791; 1137)	416 (286; 545)	254 (146; 362)
	30	714 (577; 851)	289 (192; 386)	170 (93; 247)
C_4C_1pyrr TFSI + Li TFSI 1.5 m	5	453 (360; 547)	89 (56; 123)	35 (17; 51)
	15	208 (142; 274)	51 (23; 79)	23 (6; 39)
	30	149 (108; 189)	44 (23; 64)	21 (8 ;35)

Results found in Table 3 exhibit the well-known effect of time in the toxicity results, which typically describes the lowest EC values for the highest time of exposure. Some authors [13–15] have reported that aromatic cations (imidazolium in this case) are more toxic than non-aromatic based ILs (pyrrolidinium and ammonium); this statement perfectly fits with our results that present the following trend: EAN < < C_4C_1pyrr TFSI < EIm NO_3.

According to Passino & Smith classification [16], pure EAN can be considered as harmless (>1000 mg L^{-1}), and pure C_4C_1pyrr TFSI and EIm NO_3 practically harmless (10 mg L^{-1} < EC_{50} < 100 mg L^{-1}). Scarce values of the toxicity of these compounds can be found in literature, to the best of our knowledge, except for pure EAN [17] and C_4C_1pyrr TFSI [15], who found similar values than the present study.

It can be seen how lithium salt induces different effects on these pure ILs: whereas $LiNO_3$ seems that does not induce changes on EC values for pure EAN, EIm NO_3 + Li NO_3 mixture presents higher EC values than the corresponding to pure one, indicating slightly lower toxicity in mixture than in pure IL [16]. Nevertheless Li TFSI clearly decrease EC values for the pure Aprotic ILs, being the mixture more toxic than pure IL, considered as moderately toxic on EC_{20} and EC_{10} indicators [16]. No previous bibliographic data have been found for the mixtures of IL with salt. The differences between these results can be explained in terms of the saturation concentration of the different salts on the ILs as it is exposed in Table 2. Since EAN + $LiNO_3$ is the mixture with the lowest salt molar fraction and the lowest toxicity effect, and C_4C_1pyrr TFSI + Li TFSI is the mixture with the highest salt molar fraction and, consequently, highest toxicity change.

4. Conclusions

The main conclusion of this work is that there are some differences between PIL and APIL toxicity, and even among the two selected PILs. Salt addition clearly affects to the toxicity and is highly depending on the molar fraction, as expected. Ethylammonium nitrate and its mixture can be considered as non-toxic, EIm NO_3 pure is slightly toxic meanwhile its mixture is non-toxic, and finally, C_4C_1pyrr TFSI goes from practically harmless to moderately toxic when lithium salt is added.

Author Contributions: Conceptualization, J.J.P. and J.S.; methodology and data, J.J.P., P.V., L.F.-M.; software, J.J.P., P.V., L.F.-M.; writing—original draft preparation, J.J.P., J.S., M.V.; funding acquisition, J.S. and M.V. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by Spanish Ministry of Economy and Competitiveness and European Regional Development Fund. (FEDER) Program through the project, MAT2017-89239-C2-1-P, as well as by Xunta de Galicia through *Grupo de Referencia Competitiva* (GRC) ED431C 2020/10 project and the Galician Network of Ionic Liquids (ReGaLIs) ED431D 2017/06. P.V. and J.J.P. thank funding support of *Formación de Personal Investigador* (FPI) Program from Spanish Ministry of Science, Education and Universities and Postdoctoral Program of Xunta de Galicia, respectively.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The study did not report any supplementary material.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Salgado, J.; Parajó, J.J.; Villanueva, M.; Rodríguez, J.R.; Cabeza, O.; Varela, L.M. Liquid range of ionic liquid–Metal salt mixtures for electrochemical applications. *J. Chem. Thermodyn.* **2019**, *134*, 164–174.
2. Lui, M.Y.; Crowhurst, L.; Hallett, J.P.; Hunt, P.A.; Niedermeyer, H.; Welton, T. Salts dissolved in salts: Ionic liquid mixtures. *Chem. Sci.* **2011**, *2*, 1491–1496.
3. Peric, B.; Sierra, J.; Martí, E.; Cruañas, R.; Garau, M.A.; Arning, J.; Bottin-Weber, U.; Stolte, S. (Eco)toxicity and biodegradability of selected protic and aprotic ionic liquids. *J. Hazard. Mater.* **2013**, *261*, 99–105.
4. Lindberg, S.; Jeschke, S.; Jankowski, P.; Abdelhamid, M.; Brousse, T.; Le Bideau, J.; Johansson, P.; Matic, A. Charge storage mechanism of α-MnO_2 in protic and aprotic ionic liquid electrolytes. *J. Power Sources* **2020**, *460*, 228111.
5. Salgado, J.; Villanueva, M.; Parajó, J.J.; Fernández, J. Long-term thermal stability of five imidazolium ionic liquids. *J. Chem. Thermodyn.* **2013**, *65*, 184–190.
6. Sánchez, P.B.; González, B.; Salgado, J.; José Parajó, J.; Domínguez, Á. Physical properties of seven deep eutectic solvents based on L-proline or betaine. *J. Chem. Thermodyn.* **2019**, *131*, 517–523.
7. Yang, H.; Luo, X.-F.; Matsumoto, K.; Chang, J.-K.; Hagiwara, R. Physicochemical and electrochemical properties of the (fluorosulfonyl)(trifluoromethylsulfonyl)amide ionic liquid for Na secondary batteries. *J. Power Sources* **2020**, *470*, 228406.
8. Kim, J.-K.; Lim, D.-H.; Scheers, J.; Wilken, S.; Johansson, P.; Ahn, J.-H.; Matic, A.; Jacobsson, P. 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.* **2011**, *14*, 92–97.
9. Union OJ of the E (2007) Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).
10. Heckenbach, M.E.; Romero, F.N.; Green, M.D.; Halden, R.U. Meta-analysis of ionic liquid literature and toxicology. *Chemosphere* **2016**, *150*, 266–274.
11. Parajó, J.J.; Macário, I.P.E.; De Gaetano, Y.; Dupont, L.; Salgado, J.; Pereira, J.L.; Gonçalves, F.J.M.; Mohamadou, A.; Ventura, S.P.M. Glycine-betaine-derived ionic liquids: Synthesis, characterization and ecotoxicological evaluation. *Ecotoxicol. Environ. Saf.* **2019**, *184*, 109580.
12. Ventura, S.P.M.; Gonçalves, A.M.M.; Sintra, T.; Pereira, J.L.; Gonçalves, F.; Coutinho, J.A.P. Designing ionic liquids: The chemical structure role in the toxicity. *Ecotoxicology* **2013**, *22*, 1–12.
13. Zhao, D.; Liao, Y.; Zhang, Z. Toxicity of Ionic Liquids. *Clean Soil Air Water* **2007**, *35*, 42–48.
14. Ventura, S.P.M.; Marques, C.S.; Rosatella, A.A.; Afonso, C.A.M.; Gonçalves, F.; Coutinho, J.A.P. Toxicity assessment of various ionic liquid families towards Vibrio fischeri marine bacteria. *Ecotoxicol. Environ. Saf.* **2012**, *76*, 162–168.
15. Stolte, S.; Matzke, M.; Jürgen, A.; Böschen, A.; Pitner, W.-R.; Welz-Biermann, U.; Jastorff, B.; Ranke, J. Effects of different head groups and functionalised side chains on the aquatic toxicity of ionic liquids. *Green Chem.* **2007**, *9*, 1170–1179.
16. Passino, D.R.M.; Smith, S.B. Acute bioassays and hazard evaluation of representative contaminants detected in great lakes fish. *Environ. Toxicol. Chem.* **1987**, *6*, 901–907.
17. Montalbán, M.G.; Hidalgo, J.M.; Collado-González, M.; Díaz Baños, F.G.; Víllora, G. Assessing chemical toxicity of ionic liquids on Vibrio fischeri: Correlation with structure and composition. *Chemosphere* **2016**, *155*, 405–414.