proceedings

MDPI

Proceedings

ECOTOXICITY OF EAN AND DOPED EAN IONIC LIQUIDS

J.J. Parajó*, M. Villanueva, L.M. Varela, J. Salgado

NaFoMat Group. Applied Physic and Particle Physics Departments.University of Santiago de Compostela.

Campus Vida. 15782 Santiago. Spain

*e-mail: juanjose.parajo@usc.es

Abstract

The ecotoxicity of a nitrate-based Ionic Liquid (IL) and saturated mixtures with four nitrate salts was tested in this work, towards the bioluminescent bacteria *Vibrio fischeri*, using the Microtox® standard toxicity test [1]. The selected IL was Ethylammonium Nitrate (EAN) and the nitrate salts were Lithium Nitrate ($LiNO_3$), Calcium Nitrate ($Ca(NO_3)_2$), Magnesium Nitrate ($Mg(NO_3)_2$) and Aluminum Nitrate ($Al(NO_3)_3$). The effective concentration (EC_{50}) of these mixtures was determined over three standard periods of time, namely 5, 15 and 30 min.

Results of EC_{50} for pure EAN at 15 minutes are in relatively good concordance with the literature. To the best of our knowledge, no ecotoxicity studies have been performed for doped EAN.

Similar results have been found for pure EAN and for EAN doped with $LiNO_3$ and $Mg(NO_3)_2$, whose values indicated low toxicity. Nevertheless the addition of $Ca(NO_3)_2$ and $Al(NO_3)_3$ cause an increase on the ecotoxicity of EAN, specially for the IL doped with $Al(NO_3)_3$ which present values associated to highly toxic compounds, even comparable with benzene or atrazine .

Keywords: Ecotoxicity; Ionic Liquids; *Vibrio fischeri*; Microtox; Doped.

1. Introduction

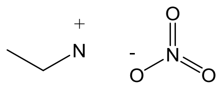
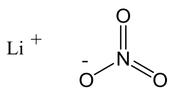
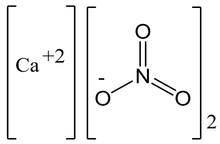
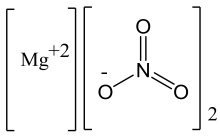
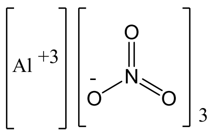
Ionic Liquids (ILs) are generically regarded as environmentally “harmless” and thus, accepted as “non-toxic” [1]. However, owing to the limitless possibilities in their design, their ecotoxicity is still poorly known, and due to their ionic character, almost all ILs are soluble in water [2], which can represent an environmental problem. Different studies showed that ILs present some toxicity [3,4], being their environmental impact strongly dependent on the cation and anion used to synthesized them [5,6].Under the scoop of the REACH regulation [7] an accurate knowledge on the toxicity of a substance is required before their application and/or commercialization. Focusing on the use of ILs as: electrolyte for chemical devices, base lubricants or lubricant additives, or even as potential working fluids for absorption heat pumps, a spill of IL can occur and it is necessary to know their possible environmental effects. Although, so far, toxicity of ILs has been evaluated through different models, it is extremely important to study the effects of their ecotoxicity across various trophic levels. Also, the knowledge on the relation between the chemical structure and the toxic effect is essential for the future design of greener solvents. The ecotoxicity of pure and doped ethylammonium nitrate (EAN) with four different ionic salts has been analyzed using the standard organism *Vibrio fischeri*.

2. Materials and methods

2.1. Chemicals

Main characteristics of selected Ionic Liquid (IL), EAN, are indicated in Table I. To obtain doped EAN, lithium, calcium, magnesium and aluminum nitrate salts were used.

Table I. Chemical structure, identification number, molecular mass and purity of EAN and nitrate salts.

Name **Molecular mass** **($g\cdot mol^{-1}$)**	**Abbreviation** **CAS number**	**Chemical structure**	**Purity** **Provenance**	**Water Content**
Ethylammonium Nitrate 108.0965	EAN 22113-86-6		>0.999 Iolitec	<100 ppm
Lithium Nitrate 68.946	$LiNO_3$ 7790-69-4		>0.999 Merck	<100 ppm
Calcium Nitrate Tetrahydrate 236.088	$Ca(NO_3)_2{\cdot}4H_2O$ 13477-34-4		>0.999 Merck	$30_{wt}\%$
Magnesium Nitrate Hexahydrate 256.3	$Mg(NO_3)_2\ 6H_2O$ 13446-18-9		>0.999 Merck	$42_{wt}\%$
Aluminium Nitrate Nonahydrate 374.996	$Al(NO_3)_3\ 9H_2O$ 7784-27-2		>0.999 Merck	$43_{wt}\%$

The different solutions of salts have been prepared by mixing both components with the help of an ultrasound bath and a magnetic stirrer during 24 to 48 hours. Saturated solutions have been reached using the hydrated salts by increasing molality in 0.5 $mol\cdot kg^{-1}$ intervals till saturation point, at room temperature.

2.2. Experimental section

Microtox® Toxicity Test [8] was used to assess the toxicity of the tested chemical compounds, through the inhibition of the luminescence of the marine bacteria *Vibrio fischeri.* Exposure to a toxic substance causes a disruption of the respiratory process of the bacteria, and the light production is directly proportional to the metabolic activity of the bacterial population. This test was performed using a range of diluted aqueous solutions (from 0 to 81.9%) of each tested compound (100% corresponds to a known concentration of a stock solution). After 5, 15 and 30 min of exposure to the IL, the light output of the luminescent bacteria was measured and compared with the light output of a blank control sample (without the presence of the IL). These data were used to estimate the concentrations that promote 50%, 20% and 10% of luminescence inhibition (corresponding to the effective concentration, EC_{50}, EC_{20} and EC_{10}, respectively) and the corresponding 95% confidence intervals through a non-linear regression, using the least-squares method to fit the data to the logistic equation.

3. Results and discussion

Figure 1 show the typical behavior of *Vibrio fischeri* bacteria when it is exposed to a toxic, in this case % of luminescence relative to control vs concentration of EAN + Al 2m is represented. Furthermore, the effective concentration values (EC_{10}, EC_{20}, and EC_{50}) of the different compounds have been published on Table 2.

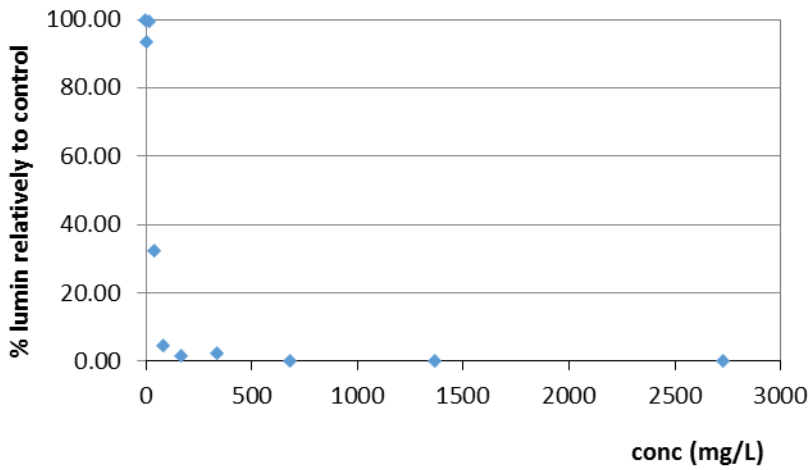


Figure 1.EC_{50} at 30 mins for EAN + Al 2m.

Table 2. Mean effective concentration values (EC_{10}, EC_{20}, EC_{50}) in mg L^{-1} and the respective 95% confidence intervals (CI), obtained after 5, 10 and 30 minutes of exposure of the marine bacteria *Vibrio 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	12582 (8186; 16977)	4314 (1548; 7081)	2304 (248; 4361)
	15	10665 (6650; 14680)	3236 (951; 5522)	1609 (56; 3163)
	30	9711 (6561; 12860)	3012 (1264; 4761)	1517 (332; 2703)
EAN + Li 2m	5	13911 (12469; 15232)	8892 (7412; 10373)	6842 (5316; 8368)
	15	11210 (9613; 12808)	7495 (5603; 9386)	5920 (3841; 8000)
	30	9706 (7233; 12179)	6145 (3301; 8988)	4701 (1744; 7658)
EAN + Ca 1m	5	7354 (5672; 9036)	3939 (2427; 5450)	2732 (1304; 4159)
	15	6064 (4343; 7784)	2858 (1475; 4240)	1938 (632; 3046)
	30	4502 (3067; 5937)	1804 (800; 2808)	1059 (263; 1849)
EAN + Mg 2m	5	12724 (10834; 14615)	7471 (5520; 9421)	5469 (3551; 7387)
	15	13384 (12522; 14247)	8933 (7887; 9979)	7049 (5925; 8174)
	30	14266 (13128; 15403)	10222 (8450; 11994)	8409 (6357; 10461)
EAN + Al 2m	5	45 (32; 58)	15 (8; 22)	8 (3; 13)
	15	37 (33; 41)	23 (18;27)	17 (12; 21)
	30	32 (29; 36)	19 (15;23)	14 (10; 18)

Results found in Table 2 demonstrate the well-known effect of time in the toxicity results, which normally describes the lowest EC values for the highest time of exposure. Montalbán *et al.* [9] have found lower values than ours for pure EAN in the effective concentration for EC_{50} at 15 minutes of 2256 mg · L^{-1}, this value is quite different than ours, although both of them can be considered as non-toxic according to GHS [10]. Similar results have been found for the solutions of lithium and magnesium (non-toxic). As well as for calcium solution, although in this case is a bit more toxic. On the other hand aluminum solution can be categorized as Acute III, being this value comparable with atrazine and benzene [11].

4. Conclusions

Main conclusion of this work is that ethylammonium nitrate and three of the studied salts solutions are non-toxic, although when the studied solution is the one saturated with aluminum is highly toxic.

Funding

This work was supported by the projects GRC ED431C 2016/001 (Xunta de Galicia, Spain) and MAT2017-89239-C2-1-P (Ministerio de Economía, Industria y Competitividad. Spain)

Acknowledgments

Authors thank to J.M. Sánchez for the technical support.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Rogers, R.D.; Seddon, K.R.; Volkov: Springer Netherlands. Green Industrial Applications of Ionic Liquids. Springer **2002**, ISBN: 9789401001274.
2. Freire, M.G.; Santos, L.M.N.B.F.; Fernandes, A.M.; Coutinho, J.A.P.; Marrucho, I. An overview of the mutual solubilities of water-imidazolium-based ionic liquids systems. *Fluid Phase Equilib* **2007**, 261, 449–454. doi:10.1016/j.fluid.2007.07.033
3. Pawłowska, B.; Biczak, R. Evaluation of the effect of tetraethylammonium bromide and chloride on the growth and development of terrestrial plants. *Chemosphere* **2016**, 149, 24–33. DOI: 10.1016/j.chemosphere.2016.01.072
4. Salgado, J.; Parajó, J.J.; Teijeira, T.; Cruz, O. Proupin, J.; Villanueva, M.; Rodríguez-Añon, J.A.; Verdes, P.V., Reyes, O. New insight into the environmental impact of two imidazolium ionic liquids. Effects on seed germination and soil microbial activity. *Chemosphere* **2017**, 185, 665–672. http://dx.doi.org/10.1016/j.chemosphere.2017.07.065
5. Petkovic, M.; Seddon, K.R.; Rebelo, L.P.N.; Pereira, C.S. Ionic liquids: a pathway to environmental acceptability. *Chem Soc Rev* **2011**, 40, 1383–1403. DOI: 10.1039/c004968a
6. Santos, J.I..; Gonçalves, A.M.M.; Pereira, J.L.; Figueiredo, B.F.H.T.; eSilva, F.A.; Coutinho J.A.P.; Ventura, S.P.M.; Gonçalves, F. Environmental safety of cholinium-based ionic liquids: assessing structure-ecotoxicity relationships. *Green Chem* **2015**, 17:4657–4668. doi:10.1039/C5GC01129A
7. 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)
8. Microbics Corporation; Microtox® Manual—A Toxicity Testing Handbook. Carlsbad, CA, USA (1992) 1–5
9. 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. DOI:10.1016/j.chemosphere.2016.04.042
10. Nations U **2011** Globally harmonized system of classification and labeling of chemicals (GHS), fourth rev. United Nations.

11. Ventura, S.P.M.; eSilva, F.A.; Gonçalves, A.M.M.; Pereira,J.L.;Gonçalves, F.; Coutinho J.A.P. Ecotoxicity analysis of cholinium-based ionic liquids to Vibrio fischeri marine bacteria. *Ecotox. Environ. Saf.* **2014**, 102, 48-54. http://dx.doi.org/10.1016/j.ecoenv.2014.01.003