Environmental properties of phosphonium, imidazolium and ammonium cation-based ionic liquids as potential lubricant additives

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Abstract

This research compares the environmental properties (bacterial toxicity and biodegradability) of 12 ionic liquids -ILs- (7 phosphonium, 2 imidazolium and 3 ammonium cation-based ones), potentially applicable as lubricant additive, with two types of the traditional lubricant additive ZDDP. Aquatic toxicity was determined by means of *Vibrio fischeri* and *Escherichia coli* bacteria, while biodegradability was evaluated through biological oxygen demand (BOD_5) and chemical oxygen demand (COD) measurements. Regarding toxicity results, $[P_{4442}][DEP]$ was the least toxic IL (acute 3 according to GHS) for both bacteria, whereas ZDDP fell into the acute 1 category (very toxic). All samples tested turned out to be poorly biodegradable, showing BOD_5/COD values below 0.1. Two ILs showed better combined tribological and environmental properties than ZDDP.

Keywords: ionic liquids, lubricant additive, bacterial toxicity, biodegradability

1. Introduction

Industry is constantly developing new manufacturing processes to improve speed, efficiency and other parameters. In addition, minimizing environmental risks and contributing to sustainable development in agreement with the ideals of green chemistry are also important objectives during the design and optimization of any industrial process [1]. This is partly because the increasing awareness of the environmental impact of the chemical industry has led to a more restrictive legislation in this field [2].

ILs are molten salts, thermally stable, which melt under 100 °C. In addition, their useful physical and chemical properties (non-flammability, low vapour pressure, remarkable thermal and oxidative stability, ashless character, etc) is the reason why their applications are rapidly growing in number [3-8]. Although these compounds have long been thought of as "green solvents" [9], there is an urgent necessity to explore and identify their effect on living beings in order to avoid negative consequences after unintentional exposure [10]. Research papers dealing with the environmental impact of ILs have become more numerous recently, although our understanding of the biodegradability and toxicity for ecosystems of most ILs is still limited [11-19].

Nowadays, the environmental legislation of the EU is mainly focused on the safety of chemicals, an objective that clearly requires ecotoxicological and biodegradation data [20].

Using ILs in lubrication has been a promising option since 2001 [21-26]. These melting salts have excellent properties, which are desirable for their utilization as either lubricant base stock or lubricant additives. Research using ILs as neat lubricants has been conducted with different tribological contacts: steel-aluminium [27, 28], steel-cast iron [29], steel-steel [30-37], and even with coatings [38-42]. The current high cost of ILs means that although they may well be employed in systems under extreme conditions [43, 44] at which conventional lubricants fail (aerospace and spacecraft applications), they are often too expensive to use under less severe conditions in which other products perform adequately. This is why much research focuses on their use as a lubricant additive in order to enhance their cost effectiveness [26]. Moreover, the solubility problems of ILs in common non-polar oils have tended to direct research towards their utilization as additives at low concentrations in these base stocks [29, 39-42, 45-50] and few studies have tested their use as additive of a non-polar fully-formulated oil [51-53]. Otherwise, various research studies focused on polar oils have used ionic liquids as additive with the aim of overcoming the solubility problem [54-68]. However, the significant amounts of lubricant released into the environment, affecting flora, fauna and, by extension, human life, make it necessary to conduct biodegradability and toxicity studies in order to verify that these recently developed products are environmentally friendly [69-72].

Many of the 12 ILs that have been chosen for testing here ($[P_{6,6,6,14}]^+[(iC_8)_2PO_2]^-$, $[P_{6,6,6,14}]^+[BEHP]^-$, $[P_{6,6,6,14}]^+[TFSI]^-$, $[P_{4,4,4,14}]^+[DBS]^-$, $[P_{4442}]^+[DEP]^-$, $[P_{6,6,6,14}]^+[DCA]^-$, $[P_{6,6,6,14}]^+[Cl]^-$, $[C_{12}C_1IM]^+[TFSI]^-$, $[C_{10}C_1C_{10}IM]^+[TFSI]^-$, $[N_{4441}]^+[TFSI]^-$, $[N_{8881}]^+[TFSI]^-$ and $[N_{4441}]^+[DCA]^-$) have been previously studied as lubricant additive. The complete names and CAS numbers of the ILs can be found in Table 1. Qu *et al.* [42, 45], Somers *et al.* [25, 54] and Gonzalez *et al.* [48] stated that $[P_{6,6,6,14}]^+[BEHP]^-$ and $[P_{6,6,6,14}]^+[(iC_8)_2PO_2]^-$ are completely miscible in a wide variety of oils (synthetic and mineral ones), showing similar tribological characteristics to ZDDP in different contacts (steel-steel, steel-aluminium, steel-cast iron), even outperforming this traditional additive at 100 ºC. $[P_{6,6,6,14}]^+[TFSI]^-$ was studied as an additive in different types of oils by González *et al*. [48] and Blanco *et al.* [66], showing the mixtures exhibited better tribological behaviour in a steel-steel contact than the corresponding base oils. Viesca *et al*. [73] and Battez *et al.* [74] proved that the addition of $[C_{12}C_1IM]^+[TFSI]^-$ and $[N_{8881}]^+[TFSI]^-$ to a diester oil showed tribological results similar to those of

the base oil itself, maybe because of competition between base oil and additive in the contact surface, because of their similar polarity. In addition, Blanco *et al.* [75, 76] studied the tribological performance of $[P_{4,4,4,14}]^+[DBS]^-$ $[P_{6,6,6,14}]^+[TFSI]^-$ and $[P_{6,6,6,14}]^+[DCA]^-$ as additives in a polar oil, finding results suggesting the same competition between the base oil and the ionic liquids mentioned above.

Despite their increasing use, there are just a couple of studies about the environmental properties of ILs synthesized for lubrication purposes [77, 78]; and the importance of extending existing knowledge of the toxicity and biodegradability of ILs has been underlined by Qu *et al*. [26]. That is the reason why the aim of this research work is to determine the biodegradability and toxicity of the 12 above-mentioned ILs. In order to verify the obtained results, two different samples of the traditional lubricant additive ZDDP: Phosphorodithioic acid, mixed O,O-bis (1,3-dimethylbutyl and iso-Pr) esters, zinc salts) and phosphorodithioic acid, mixed O,O-bis (diisooctyl) esters, zinc salts), named as ZDDP1 and ZDDP2 respectively, were used as comparison samples [79].

2. Experimental details

2.1. Ionic liquids and ZDDPs

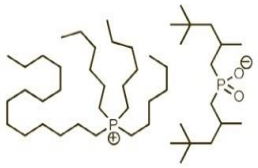
Table 1 shows the chemical descriptions of 14 substances selected due to their use or potential use as additive in lubricants, whilst Fig.1 displays their chemical structure. All ILs are commercially available (Ionic Liquid Technologies GmbH and Sigma-Aldrich S.A.) and Repsol, S.A. kindly supplied both zinc dialkyldithiophosphates (ZDDP).

Table 1. Chemical descriptions of the ILs and ZDDPs.

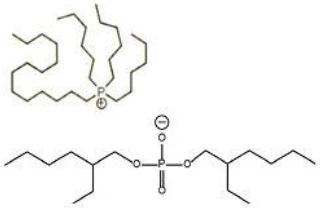
Abbreviation	IUPAC name	CAS Number	Purity (%)	Molecular weight
$[P_{6,6,6,14}]^+[(iC_8)_2PO_2]^-$	Trihexyltetradecylphosphonium bis(2,4,4-trimethylpentyl) phosphinate	465527-59-7	90	773.3
$[P_{6,6,6,14}]^+[BEHP]^-$	Trihexyltetradecylphosphonium bis(2-ethylhexyl)phosphate	1092655-30-5	98	837.3
$[P_{6,6,6,14}]^+[TFSI]^-$	Trihexyltetradecylphosphonium bis(trifluoromethylsulfonyl) imide	460092-03-9	98	763.2
$[P_{4,4,4,14}]^+[DBS]^-$	Tributyltetradecylphosphonium dodecylbenzenesulfonate	959685-58-6	95	725.3
$[P_{4442}]^+[DEP]^-$	Tributylethylphosphonium diethylphosphate	20445-94-7	95	384.5
$[P_{6,6,6,14}]^+[DCA]^-$	Trihexyltetradecylphosphonium dicyanamide	701921-71-3	95	549.9

$[P_{6,6,6,14}]^+[Cl]^-$	Trihexyltetradecylphosphonium chloride	258864-54-9	95	519.3
$[C_{12}C_1IM]^+[TFSI]^-$	1-Dodecyl-3-methylimidazolium bis(trifluoromethylsulfonyl) imide	404001-48-5	98	531.6
$[C_{10}C_1C_{10}IM]^+[TFSI]^-$	1,3-Didecyl-2-methylimidazolium bis(trifluoromethylsulfonyl) imide	-	95	643.8
$[N_{4441}]^+[TFSI]^-$	Tributylmethylammonium bis(trifluoromethylsulfonyl) imide	405514-94-5	99	480.5
$[N_{8881}]^+[TFSI]^-$	Methyl-trioctylammonium bis(trifluoromethylsulfonyl)imide	375395-33-8	99	648.8
$[N_{4441}]^+[DCA]^-$	Tributylmethylammonium dicyanamide	1262230-03-4	95	252.0
ZDDP1	Phosphorodithioic acid, mixed O,O-bis (1,3-dimethylbutyl and iso-Pr) esters, zinc salts	84605-29-8	-	576.0
ZDDP2	Phosphorodithioic acid, mixed O,O-bis (diisooctyl) esters, zinc salts	28629-66-5	-	772.5

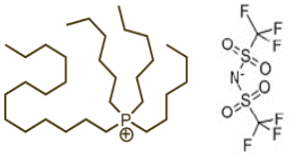
$[P_{6,6,6,14}]^+[(iC_8)_2PO_2]^-$



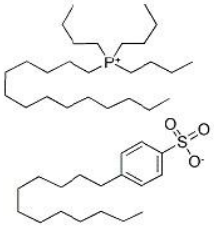
$[P_{6,6,6,14}]^+[BEHP]^-$



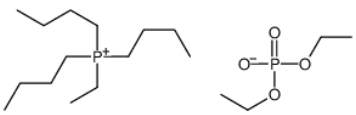
$[P_{6,6,6,14}]^+[TFSI]^-$



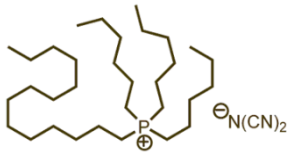
$[P_{4,4,4,14}]^+[DBS]^-$



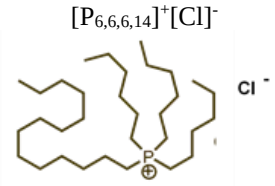
$[P_{4442}]^+[DEP]^-$



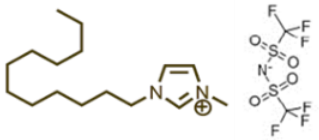
$[P_{6,6,6,14}]^+[DCA]^-$



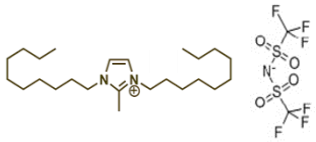
$[P_{6,6,6,14}]^+[Cl]^-$



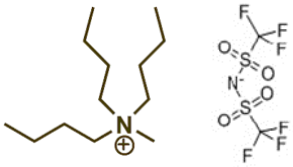
$[C_{12}C_1IM]^+[TFSI]^-$



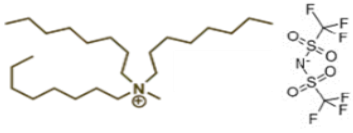
$[C_{10}C_1C_{10}IM]^+[TFSI]^-$



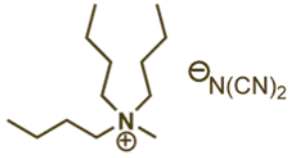
$[N_{4441}]^+[TFSI]^-$



$[N_{8881}]^+[TFSI]^-$



$[N_{4441}]^+[DCA]^-$



ZDDP1 ZDDP2

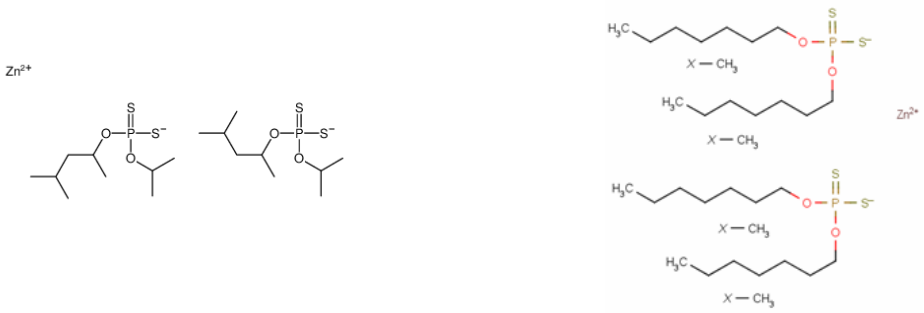


Fig. 1. Chemical structures and abbreviation of the ILs and ZDDPs.

2.2. Preparation of the water solutions of ILs

The aqueous solutions were obtained by mixing 1 g of each substance to 2 g of distilled water and the resulting samples were then gently stirred for 1 minute in a vortex mixer and centrifuged (5000 rpm, 3 minutes). Phase separation was clearly observed in all cases, except for $[P_{4442}]^+[DEP]^-$, where separation was minimal. The minority phase (additive) was discarded and the main phase (aqueous) was used to prepare the stock solutions that were to be employed in the determination of bacterial toxicity and biodegradability. After a visual inspection, it could be seen that $[P_{4442}]^+[DEP]^-$, $[N_{4441}]^+[DCA]^-$and $[P_{4,4,4,14}]^+[DBS]^-$, were soluble and the remaining ILs were partially soluble. In order to determine the additive concentrations (Table 2), total organic carbon (TOC) of the main phase was calculated with a Shimadzu TOC-V_{CSH} analyser. These concentrations were determined basing on the TOC data, the number of carbon atoms and the molecular weight of each of the ILs (see Table 1) according to eq. (1):

$$IL_{conc}\ (g/L) = TOC_{conc}\left({}^{g\ C}/_{L}\right) \times \frac{1\ mol-g\ C}{12\ g\ C} \times \frac{1\ mol-g\ IL}{n\ mol-g\ C} \times \frac{IL_{MW}}{1\ mol-g\ IL} \quad (1)$$

where: IL_{conc} is the IL concentration, TOC_{conc} is the TOC concentration, n is the number of carbon atoms of the IL and IL_{MW} is the molecular weight of the IL.

Once the concentration of the IL was known, the stock solution used in the bacterial toxicity and biodegradability tests (0.1 g/L and 1 g/L) was prepared by dilution of the former in distilled water.

Table 2. Solubility data, TOC and IL concentrations of the different ILs and ZDDPs studied.

IL sample	Solubility at 20ºC (g of IL/mL of H_2O)	TOC concentration (g C/L)	IL concentration (g/L)
$[P_{6,6,6,14}]^+[(iC_8)_2PO_2]^-$	$2.1 \cdot 10^{-3} \pm 1 \cdot 10^{-4}$	1.03 ± 0.03	1.39 ± 0.04
$[P_{6,6,6,14}]^+[BEHP]^-$	$5.8 \cdot 10^{-3} \pm 3 \cdot 10^{-4}$	2.7 ± 0.1	3.9 ± 0.1
$[P_{6,6,6,14}]^+[TFSI]^-$	$5.8 \cdot 10^{-4} \pm 2 \cdot 10^{-5}$	0.206 ± 0.002	0.385 ± 0.003
$[P_{6,6,6,14}]^+[DCA]^-$	$7.0 \cdot 10^{-4} \pm 1 \cdot 10^{-5}$	0.355 ± 0.001	0.479 ± 0.001
$[P_{6,6,6,14}]^+[Cl]^-$	$2.3 \cdot 10^{-3} \pm 3 \cdot 10^{-4}$	1.14 ± 0.03	1.54 ± 0.04
$[P_{4,4,4,14}]^+[DBS]^-$	$4.3 \cdot 10^{-2} \pm 5 \cdot 10^{-3}$	21.0 ± 0.3	28.9 ± 0.4
$[P_{4442}]^+[DEP]^-$	$4.8 \cdot 10^{-1} \pm 4 \cdot 10^{-2}$	179.8 ± 0.7	320 ± 1
$[C_{12}C_1IM]^+[TFSI]^-$	$7.0 \cdot 10^{-4} \pm 1 \cdot 10^{-5}$	0.189 ± 0.008	0.46 ± 0.02
$[C_{10}C_1C_{10}IM]^+[TFSI]^-$	$3.0 \cdot 10^{-4} \pm 1 \cdot 10^{-5}$	0.10 ± 0.01	0.22 ± 0.02
$[N_{8881}]^+[TFSI]^-$	$1.5 \cdot 10^{-4} \pm 2 \cdot 10^{-5}$	0.0515 ± 0.0001	0.1032 ± 0.0001
$[N_{4441}]^+[TFSI]^-$	$1.7 \cdot 10^{-3} \pm 2 \cdot 10^{-4}$	0.421 ± 0.002	1.123 ± 0.005
$[N_{4441}]^+[DCA]^-$	$1.2 \cdot 10^{-1} \pm 3 \cdot 10^{-2}$	58.5 ± 0.7	81.9 ± 0.9
ZDDP1	$3.0 \cdot 10^{-3} \pm 4 \cdot 10^{-4}$	0.76 ± 0.01	2.03 ± 0.03
ZDDP2	$4.9 \cdot 10^{-3} \pm 2 \cdot 10^{-4}$	1.66 ± 0.03	3.35 ± 0.07

2.3. Biodegradability: chemical and biochemical oxygen demand

The dichromate methodology at 600 nm was used to determine the chemical oxygen demand (COD), employing a Hach DR/2500 spectrophotometer [80]. In addition, a respirometric method was employed for measuring the biological oxygen demand (BOD_5) by means of Lovibond BOD system. Solutions containing 0.1 g/L of each additive were prepared and the pH was adjusted until it was within the range of 6.5-7 using a Jenway 3510 pH-meter. Sodium acetate solution (0.1 g/L) was used as the control sample (free of toxicants). Finally, the effluent from an aerobic bioreactor (1 mL) was employed to inoculate 157 mL of each sample solution and then, after a mixing step, poured into the BOD bottles and incubated at 20ºC for 5 days (dark conditions). All analytical measurements were repeated at least three times.

2.4. Toxicity assays

2.4.1. Vibrio fischeri tests

Bacterial toxicity assessment was conducted with *Vibrio fischeri*. This bioluminescence assay is a European standard ecotoxicological test (DIN EN ISO 11348) [81] and one of the methods most widely applied to appraise the potential environmental risk of a compound in aquatic environments, since it is cost-effective, rapid and well-established [82]. The Biofix®Lumi-10 commercial test was used with a specially selected lyophilized strain (NRRL number B-11177) and the bacterial toxicity was determined in serial two-fold aqueous dilutions

ranging from 2^0 to 2^{10}. Two starting solutions (stock solutions) were employed, the concentrations being 0.1 g/L and 1 g/L. These values were selected based on the IL concentration determined (see Table 2) and with the aim of using the same initial concentration in order for the results to be easily comparable. Therefore, the concentrations of the stock solutions were the following: 0.1 g/L for $[P_{6,6,6,14}]^+[TFSI]^-$, $[P_{6,6,6,14}]^+[DCA]^-$, $[C_{12}C_1IM]^+[TFSI]^-$, $[C_{10}C_1C_{10}IM\ ]^+[TFSI]^-$, $[N_{8881}]^+[TFSI]^-$, ZDDP1 and ZDDP2 and 1 g/L for $[P_{6,6,6,14}]^+[(iC_8)_2PO_2]^-$, $[P_{6,6,6,14}]^+[Cl]^-$, $[P_{6,6,6,14}]^+[BEHP]^-$, $[N_{4441}]^+[TFSI]^-$, $[P_{4,4,4,14}]^+[DBS]^-$, $[N_{4441}]^+[DCA]^-$ and $[P_{4442}]^+[DEP]^-$ ILs. The temperature was hold at 15 ºC using a water bath and salinity was fixed at 2% after a pH adjustment from 6.5 to 7.5 was carried out. The equipment employed was a Luminometer BioFix® Lumi-10. Results were expressed in accordance with the ISO 11348-3 [81]. The inhibition percentage (IL) was determined comparing the fall in light emission with a toxic-free 2% NaCl solution (control sample) after 15 min of contact with the bacteria. Since bacterial luminescence (light emission) is proportional to cellular respiration in a direct way, a decrease in light emission indicates reduced respiration due to the presence of substances that are toxic to the bacteria. The concentration of additive, which reduces the luminescence of bacteria by 50% after 15 min of exposure (EC_{50}), was determined [83]. In addition, the toxic units (TU) were calculated after a contact time of 15 min. This unit is the reciprocal of the IL concentration that provoke the death of 50% of the bacteria after the exposure period. It is determined dividing the concentration of the IL, which is 100%, by lethal end-point (EC_{50}), according to eq. (2) [84]. These units make easier the understanding of toxicity measurements, since an increase in the TU value implies an increase in the toxicity.

$$TU = \frac{1}{EC_{50}} \times 100 \quad (2)$$

TU is a unitless parameter, which indicates relative toxicity and classifies the substances into four categories: i) non-toxic: TU values lower than 1, ii) toxic: TU values from 1 to 10, iii) very toxic: TU values between 10 and 100, and iv) extremely toxic: TU values higher than 100 [83, 85]. All analytical measurements were carried out at least three times.

2.4.2. Staining procedure and fluorescence measurements

This procedure was carried out to assess the viability of *Vibrio fischeri* after performing the toxicity tests. The solution (IL and bacteria) was centrifuged at 15 000 g for 5 min in order to separate the bacteria from the IL. Before staining, it was necessary to wash the bacteria twice with sterile-filtered PBS (phosphate-buffered saline; pH 7.4), and sonicate them for 2 seconds to avoid cellular aggregation. Afterwards, the supernatant was

removed and the bacteria were resuspended in PBS. Next, 250 µL of this suspension were added to two staining solutions prepared beforehand. Two fluorescents dyes, propidium iodide (PI, Invitrogen) and carboxyfluorescein diacetate (cFDA, Invitrogen), were employed in a dual-staining procedure. The working solutions of PI and cFDA were prepared according to Alonso et al. [86]. Firstly, the sample was stained with PI and incubated for 30 min in darkness at 20ºC. Then, the PI-stained sample was incubated with cFDA for 15 min in darkness at 20ºC. Thus, the metabolic activity and membrane integrity of Vibrio fischeri, i.e. its physiological status, was evaluated using cFDA and PI, respectively.

Fluorescence measurements were carried out using a Leica DMR-XA fluorescence microscope equipped with HBO100 Mercury Arc Lamp. Green fluorescence from the suspensions, which corresponds to cFDA-stained bacteria, was registered on the L5 blue filter, whereas the PI-stained ones were collected with N2.1 green filter. Each analysis was conducted in duplicate. Images acquisition was performed using the software QFluoro from Leica Microsystems.

2.4.3. E. coli tests

A colorimetric assay: *Toxi-ChromoTest* (epbi - version 3.6) was employed in order to obtain an additional value of bacterial toxicity of the samples. According to Chow *et al*. [87], this assay is based on the capacity of different substances to inhibit the β-galactosidase enzyme in a greatly sensitive *Escherichia coli* strain. These assays were carried out with serial two-fold aqueous dilutions ranging from 2^0 to 2^{10} (triplicate samples). The concentrations of the starting solutions (stock solutions) were the same as those used in the *Vibrio fischeri* bioassays (0.1 g/L or 1 g/L). The exposure of the bacteria to the additive solutions was 90 min. After this incubation period, a chromogenic substrate (blue chromogen) was added. If the sample is found to be toxic, no colour will be visible. Nevertheless, a distinctive blue colour quickly appears with non-toxic samples. To quantify the colour observed, the optical density was tested with a Cary Uv/Vis spectrophotometer at 615 nm (for blue colour). The toxicity (degree of inhibition) was calculated by comparison with the optical density of the control sample free of toxicants. Mercury chloride (4 mg/L) was used as a standard toxic substance in order to check the viability of the *E. coli* strain. Both the values of EC_{50}, that is to say the concentration that inhibits enzymatic activity by 50%, and TU units were calculated after 90 min of incubation.

3. Results and discussion

3.1. Ecotoxicity analysis

3.1.1. Vibrio fischeri tests: calculation

Table 3 shows the values of bacterial toxicity for the marine bacteria after 15 min of exposure in terms of EC_{50} (mg/L). Toxicity values followed this sequence: $[P_{6,6,6,14}]^+[TFSI]^-$ > $[P_{6,6,6,14}]^+[DCA]^-$ > $[C_{10}C_1C_{10}IM]^+[TFSI]^-$ > $[C_{12}C_1IM]^+[TFSI]^-$ > $[P_{6,6,6,14}]^+[Cl]^-$ > $[N_{8881}]^+[TFSI]^-$ > $[P_{4,4,4,14}]^+[DBS]^-$ > $[N_{4441}]^+[TFSI]^-$ > $[N_{4441}]^+[DCA]^-$ > $[P_{6,6,6,14}]^+[(iC_8)_2PO_2]^-$ > $[P_{6,6,6,14}]^+[BEHP]^-$ > $[P_{4442}]^+[DEP]^-$.

It is worth noting that EC_{50} values of the samples studied are mainly controlled by the cationic part, especially if it contains long carbon chains [88-92]. Although the influence of the anionic part on toxicity is less predictable [2, 93], from these data it can be concluded that the toxicity of these ILs also depends considerably on the anion. In this sense, the EC_{50} of $[P_{6,6,6,14}]^+[(iC_8)_2PO_2]^-$ was approximately twenty two times higher than that of $[P_{6,6,6,14}]^+[TFSI]^-$ IL (1.3 mg/L). On the other hand, the "side chain effect" found by various authors in imidazolium ILs was also observed in the ILs here analysed. This effect refers to the observation that longer alkyl chain length causes a rise in toxicity [11, 94]. This is the reason why the EC_{50} value for $[C_{10}C_1C_{10}IM]^+[TFSI]^-$ was slightly higher than that for $[C_{12}C_1IM]^+[TFSI]^-$. Therefore, the nature of both ions had a significant influence on bacterial toxicity of ionic liquids. It should be reported that ZDDP1 and ZDDP2 were found to be the most toxic samples. This can be mainly explained by the presence of zinc in their composition, which is highly toxic to *Vibrio fischeri* [95]. Table 3 shows the toxic units (TU), which can also be used as a measurement of the toxic action of the ILs and ZDDPs.

According to GHS (Globally Harmonized System of Classification and Labelling of Chemicals) [96, 97], the $[P_{6,6,6,14}]^+[TFSI]^-$, $[P_{6,6,6,14}]^+[DCA]^-$, $[P_{6,6,6,14}]^+[Cl]^-$, $[C_{12}C_1IM]^+[TFSI]^-$, $[C_{10}C_1C_{10}IM]^+[TFSI]^-$ and ZDDP1 can be placed in the category of "toxic to the aquatic environment": namely, category Acute 2 (EC_{50} between 1 and 10 mg/L). In addition, ZDDP2 can be included in the category Acute 1 (EC_{50} < 1 mg/L). The other analyzed ILs can be classified as harmful (category Acute 3), with EC_{50} values ranging from 10-100 mg/L.

Table 3. EC_{50} values for the *Vibrio fischeri* of ILs and ZDDPs (exposure: 15 min).

IL sample	EC_{50} (mg/L)[a]	TU[b]
$[P_{6,6,6,14}]^+[(iC_8)_2PO_2]^-$	28.3 ± 0.7	3.53
$[P_{6,6,6,14}]^+[BEHP]^-$	30.2 ± 0.9	3.31
$[P_{6,6,6,14}]^+[TFSI]^-$	1.3 ± 0.6	76.92
$[P_{6,6,6,14}]^+[DCA]^-$	4.4 ± 0.5	22.73
$[P_{6,6,6,14}]^+[Cl]^-$	7.8 ± 0.3	12.82
$[P_{4,4,4,14}]^+[DBS]^-$	14.4 ± 0.5	6.94
$[P_{4442}]^+[DEP]^-$	55.2 ± 0.7	1.81
$[C_{12}C_1IM]^+[TFSI]^-$	7.3 ± 0.6	13.70
$[C_{10}C_1C_{10}IM]^+[TFSI]^-$	6.8 ± 0.9	14.71
$[N_{8881}]^+[TFSI]^-$	12.9 ± 0.3	7.75
$[N_{4441}]^+[TFSI]^-$	17.6 ± 0.4	5.68
$[N_{4441}]^+[DCA]^-$	21.1 ± 0.6	4.74
$[P_{6,6,6,14}]^+[(iC_8)_2PO_2]^-$	1.0 ± 0.2	100.00
$[P_{6,6,6,14}]^+[BEHP]^-$	0.9 ± 0.1	111.11

[a] **EC_{50} classification for aquatic life**: non-toxic: EC_{50} > 100 mg/L; harmful (acute 3): EC_{50}: 10 - 100 mg/L; toxic (acute 2): EC_{50} : 1 - 10 mg/L; and very toxic (acute 1): EC_{50} < 1 mg/L.

[b] **TU classification:** non-toxic: TU < 1; toxic: TU: 1 - 10; very toxic: TU: 10 - 100 and extremely toxic: TU > 100

Figure 2 shows the green and red fluorescence merged images of *Vibrio fischeri* after being in contact with the control sample (2% NaCl solution), $[P_{4442}]^+[DEP]^-$ and $[P_{6,6,6,14}]^+[TFSI]^-$ (at a concentration of 0.1 g/L) for 15 min. The use of a dual staining, cFDA-PI, enabled to identify three different subpopulations of *Vibrio fischeri*: active, damaged and dead ones. In this sense, PI is a red-fluorescent nucleic acid dye, which stains damaged and dead cells, and cFDA is a fluorogenic ester that can only diffuse into living cells. It is hydrolised by an intracellular enzyme (estearase) to yield fluorescein, a green fluorescent product [86, 98]. Therefore, the green colour indicates active/living cells, the red one represents dead cells and the yellow one depicts damaged cells. As expected, in the control sample, only active/healthy cells can be distinguished due to the absence of toxicity (Fig. 2a). In this case, the bacteria were not stained by PI since the membrane remained intact. Nevertheless, all of the bacteria displayed in Fig 2b and c are dead. This can be attributed to the fact that $[P_{6,6,6,14}]^+[TFSI]^-$ and ZDDP2 caused the loss of metabolic activity and adversely affected the integrity of the cell membrane, thus allowing PI to diffuse into the membrane. Therefore, these images allowed us to validate the toxicity results, in which $[P_{6,6,6,14}]^+[TFSI]^-$ and ZDDP2 were revealed as the most toxic substances. In the case of $[P_{4442}]^+[DEP]^-$, all the subpopulations (active, damaged and dead cells) were identified (Figure 2d). This can be explained considering that this IL has a lower impact on both the metabolic activity and the integrity of the cell membrane.

The information disclosed by this image is in agreement with the toxicity tests, which showed that $[P_{4442}][DEP]$ was the least toxic among the studied ILs.

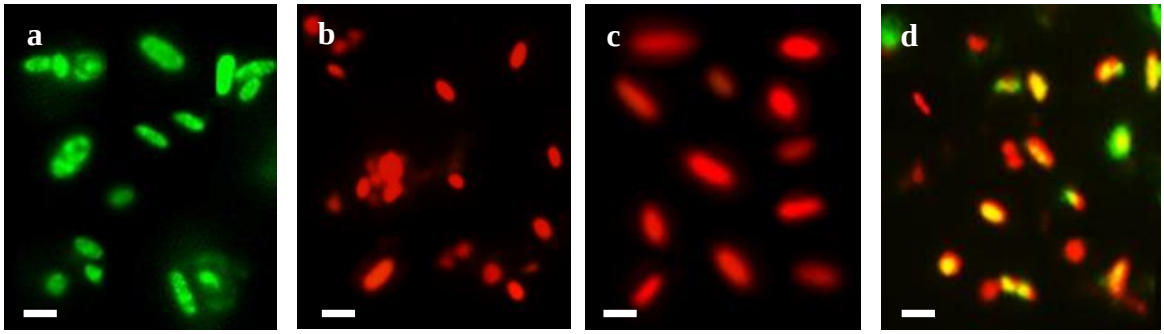


Fig. 2. Overlay of the green and red fluorescence images of *Vibrio fischeri* cells after a contact time of 15 min. a) 2% NaCl solution (control sample), b) 0.1 g/L of $[P_{6,6,6,14}]^+[TFSI]^-$, c) 0.1 g/L of [ZDDP2] and d) 0.1 g/L $[P_{4442}]^+[DEP]^-$. Scale bars: 2.5 μm.

Based on TU data, ZDDP1 and ZDDP2 fell into the category of very toxic (TU between 1 and 100) and extremely toxic (TU > 100), respectively, whereas $[P_{6,6,6,14}]^+[(iC_8)_2PO_2]^-$, $[P_{6,6,6,14}]^+[BEHP]^-$, $[P_{4,4,4,14}]^+[DBS]^-$, $[P_{4442}]^+[DEP]^-$, $[N_{8881}]^+[TFSI]^-$, $[N_{4441}]^+[TFSI]^-$ and $[N_{4441}]^+[DCA]^-$ can be classified as toxic. Therefore, most of the studied ILs had TU values that were lower or much lower than the traditional oil additive ZDDP (see ZDDP1 and ZDDP2 values). This fact, combined with the excellent solubility and tribological performance shown by $[P_{6,6,6,14}]^+[(iC_8)_2PO_2]^-$ and $[P_{6,6,6,14}]^+[BEHP]^-$ in several lubrication studies [25, 42, 45, 48, 54], suggests the possibility of using them as an alternative to this harmful traditional additive. Fig 3. showed the friction results from 60 minutes reciprocating ball on plate tests conducted at a load of 60 N (1.91 GPa of maximum contact pressure), a frequency of 15 Hz, a stroke length of 4 mm and 4 ml of the corresponding lubricant sample. Finally, $[P_{6,6,6,14}]^+[(iC_8)_2PO_2]^-$, $[P_{6,6,6,14}]^+[BEHP]^-$ and ZDDP were employed as additive in a mineral oil (Yubase 4) [54].

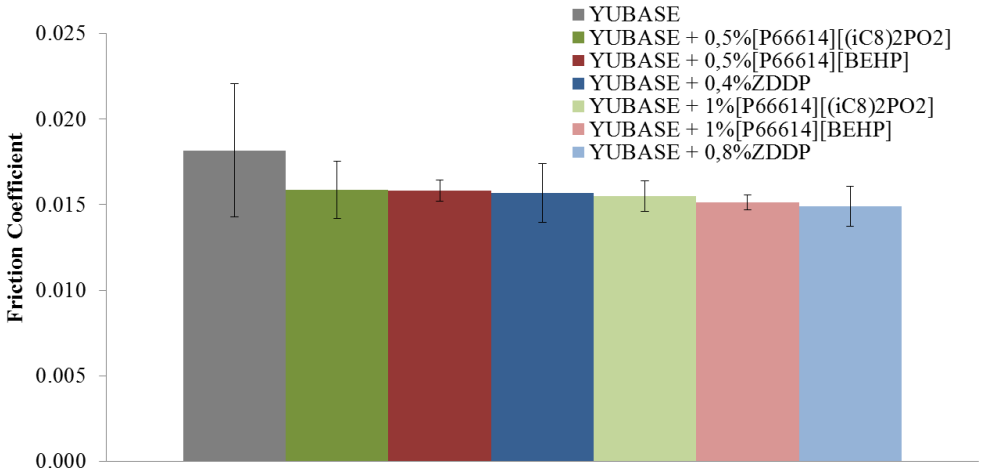


Fig. 3. Mean friction coefficient and deviation of tribological tests made with $[P_{6,6,6,14}]^+[(iC_8)_2PO_2]^-$, $[P_{6,6,6,14}]^+[BEHP]^-$ and ZDDP as additive in Yubase 4 [48].

Regarding the results shown in Fig. 3, the use of similar concentration of phosphorus in the ionic liquids and ZDDP leads to similar friction values, supporting the possibility that these ILs can partially or totally replace ZDDP. Analyzing the antiwear behavior (Table 4), all the mixtures exhibited clear wear reduction properties compared to the neat base oil.

Table 4. Wear results after 60-minutes tests [48].

Lubricants	**60-minutes tests**	
	Wear volume (μm^3) x10^6	Std. Dev.
YUBASE	19.20	2.451
YUBASE + 0.5% $[P_{6,6,6,14}]^+[(iC_8)_2PO_2]^-$	6.018	2.689
YUBASE + 0.5% $[P_{6,6,6,14}]^+[BEHP]^-$	13.58	1.013
YUBASE + 0.4% ZDDP	6.998	1.215
YUBASE + 1% $[P_{6,6,6,14}]^+[(iC_8)_2PO_2]^-$	4.736	1.220
YUBASE + 1% $[P_{6,6,6,14}]^+[BEHP]^-$	8.013	0.607
YUBASE + 0.8% ZDDP	5.339	0.391

In addition, $[P_{6,6,6,14}]^+[(iC_8)_2PO_2]^-$ ionic liquid showed an excellent antiwear behavior, even outperforming the mixtures containing ZDDP. Besides, increasing the amount of ionic liquid and ZDDP in the mixtures improved as expected the antiwear behavior of all samples.

3.1.2. Vibrio fischeri tests: estimation

A basic model was developed for a preliminary estimate of the effect of both ions on bacterial toxicity (in terms of EC_{50}), determining which ones are the most toxic. In order to draw significant conclusions from the model, it is necessary to combine different cation species with several anions and vice versa, obtaining ILs sharing either the cationic or the anionic part. This model is based on the Quantitative Structure-Activity Relationship (QSAR) method [91, 99], which evaluates the effect of structural properties of the ILs on the toxicity against bacteria, mainly *Vibrio Fischeri*. However, our model is different and simpler than the QSAR one, since it is considered that the value of EC_{50} is due to both the anion and cation contributions without analyzing the interactions between them. Thus, a parameter was assigned to each ion present in the ionic liquid, the parameters being: $1/C_i$ for the cations, with "i" ranging from 1 to 5 for $[P_{6,6,6,14}]^+$, $[C_{12}C_1IM]^+$, $[C_{10}C_1C_{10}IM]^+$, $[N_{8881}]^+$ and $[N_{4441}]^+$, respectively and $1/A_j$ for the anions with "j" varying between 1 and 5 for $[(iC_8)_2PO_2]^-$, $[BEHP]^-$, $[TFSI]^-$, $[DCA]^-$ and $[Cl]^-$, respectively.

The values of the parameters $1/C_i$ and $1/A_j$ were obtained from a linear fitting of the EC_{50} values determined using the following equation (Eq. 3) for the ionic liquid formed by cation "C_i" and anion "A_j":

$$EC_{50}(C_iA_j) = 1/C_i + 1/A_j \quad (3)$$

In order to obtain unique solutions for the model parameters, each cation and anion must be present in at least two of the ILs studied. Otherwise, multiple solutions would exist for the parameters of the ions, which appear only in one of the ILs. This is the reason why the cations $[P_{4,4,4,14}]^+$ and $[P_{4442}]^+$ and the anions $[DBS]^-$ and $[DEP]^-$ could not be included in the model. The model fitting was performed with Scientist 3.0 software (MicroMath, Inc.), the values of R^2 being higher than 0.99 in all cases. Table 4 summarizes the information related to C_i and A_j parameters.

Table 5. Values of the model parameters C_i and A_j obtained from *Vibrio fischeri* data.

Cation	**C_i (L/mg)**	**Anion**	**A_j (L/mg)**
$[P_{6,6,6,14}]^+$	$9.94 \cdot 10^{-1}$	$[TFSI]^-$	3.54
$[C_{10}C_1C_{10}IM]^+$	$1.53 \cdot 10^{-1}$	$[DCA]^-$	$2.91 \cdot 10^{-1}$
$[C_{12}C_1IM]^+$	$1.43 \cdot 10^{-1}$	$[Cl]^-$	$1.47 \cdot 10^{-1}$
$[N_{8881}]^+$	$7.93 \cdot 10^{-2}$	$[(iC_8)_2PO_2]^-$	$3.66 \cdot 10^{-2}$
$[N_{4441}]^+$	$5.72 \cdot 10^{-2}$	$[BEHP]^-$	$3.43 \cdot 10^{-2}$

It should be borne in mind that the toxicity of the corresponding cation or anion is directly proportional to the C_i and A_j numerical values. The toxicity of the cationic part decreased as follows: $[P_{6,6,6,14}]^+ > [C_{10}C_1C_{10}IM]^+ >$

$[C_{12}C_1IM]^+ > [N_{1881}]^+ > [N_{4441}]^+$. The $[P_{6,6,6,14}]^+$ cation had the highest toxicity, whereas $[N_{4441}]^+$ exhibited the lowest. This agrees with the findings of other authors, who reported that aromatic cations (such as imidazolium) are more toxic for *Vibrio fischeri* than those bearing ammonium ones [2, 80 87]. Besides, Ventura *et al*. [11] pointed out that the toxicity to *Vibrio fischeri* of the phosphonium family is greater than that of the imidazolium family. Regarding the toxicity of the anion, the trend was as follows: $[TFSI]^- > [DCA]^- > [Cl]^- > [(iC_8)_2PO_2]^- > [BEHP]^-$.

These results are in accordance with those found in the literature, which reported that the substitution of $[Cl]^-$ by $[TFSI]^-$ increased toxicity significantly (up to one hundred times) against *Vibrio fischeri* [88]. Moreover, the $[DCA]^-$ anion was also considered acutely toxic for these marine bacteria [2].

3.1.3. Toxi-ChromoTest tests: calculation

This bacterial colorimetric test is an ecotoxicological assay, which has been verified by the Environmental Protection Agency (USA). This test is becoming a standard tool because of its cost, time effective and sensitivity to low concentrations of toxic substances.

The results related to the toxicity (EC_{50}) of the modified *E. coli* bacteria after 90 min of incubation are shown in Table 5. The degree of enzyme inhibition caused by the IL solutions followed this trend: $[P_{6,6,6,14}]^+[TFSI]^- > [C_{10}C_1C_{10}IM]^+[TFSI]^- = [C_{12}C_1IM]^+[TFSI]^- > [P_{6,6,6,14}]^+[DCA]^- > [P_{6,6,6,14}]^+[Cl]^- > [N_{8881}]^+[TFSI]^- > [N_{4441}]^+[TFSI]^- > [P_{4,4,4,14}]^+[DBS]^- > [N_{4441}]^+[DCA]^- > [P_{6,6,6,14}]^+[BEHP]^- > [P_{6,6,6,14}]^+[(iC_8)_2PO_2]^- > [P_{4442}]^+[DEP]^-$. As can be seen from the EC_{50} values of $[P_{66614}]$ cation-based ILs, the nature of the anion affected their toxicity largely. Thus, the EC_{50} of $[P_{6,6,6,14}]^+[TFSI]^-$ and $[P_{6,6,6,14}]^+[DCA]^-$ ILs were around 4 and 2.5 times lower than that of $[P_{6,6,6,14}]^+[Cl]^-$ IL, respectively. Similar behaviour was found by other authors, who reported that the replacement of halides (Cl^- or Br^-) by other anions, such as $[TFSI]^-$ or $[DCA]^-$, resulted in a reduction in bacterial activity [100].

Considering imidazolium ILs, the values of EC_{50} were identical for $[C_{10}C_1C_{10}IM]^+[TFSI]^-$ and $[C_{12}C_1IM]^+[TFSI]^-$. This indicated the existence of a "cutoff" effect, in which, once a certain length is reached, a further increase in the chain has no further impact on toxicity [2, 83, 88]. This effect was reported in research studies using highly lipophilic compounds [89] and the imidazolium-based ILs analysed here can be included within this group, since they are poorly soluble in an aqueous medium (Table 2). In this case, ZDDP1 and

ZDDP2 were also found to be the most toxic samples. Again, this may be principally due to the presence in their composition of zinc, which is very toxic for *E. coli*, even at low concentrations [101]. Toxic units (TU) were summarized in Table 6.

Table 6. EC_{50} and TU values for *E. coli* after 90 min of incubation at 37 ºC.

IL sample	EC_{50} (mg/L)[a]	TU[b]
$[P_{6,6,6,14}]^+[(iC_8)_2PO_2]^-$	33.1 ± 0.5	3.02
$[P_{6,6,6,14}]^+[BEHP]^-$	26.0 ± 0.3	3.85
$[P_{6,6,6,14}]^+[TFSI]^-$	1.6 ± 0.2	62.50
$[P_{6,6,6,14}]^+[DCA]^-$	4.0 ± 0.3	25.00
$[P_{6,6,6,14}]^+[Cl]^-$	6.2 ± 0.5	16.13
$[P_{4,4,4,14}]^+[DBS]^-$	11.6 ± 0.4	8.62
$[P_{4442}]^+[DEP]^-$	39.1 ± 0.6	2.56
$[C_{12}C_1IM]^+[TFSI]^-$	3.1 ± 0.7	32.25
$[C_{10}C_1C_{10}IM]^+[TFSI]^-$	3.1 ± 0.5	32.11
$[N_{8881}]^+[TFSI]^-$	6.5 ± 0.3	15.38
$[N_{4441}]^+[TFSI]^-$	10.4 ± 0.4	9.62
$[N_{4441}]^+[DCA]^-$	12.7 ± 0.8	7.87
$[P_{6,6,6,14}]^+[(iC_8)_2PO_2]^-$	0.9 ± 0.2	111.11
$[P_{6,6,6,14}]^+[BEHP]^-$	0.8 ± 0.2	125.00

[a] **EC_{50} classification for aquatic life**: non-toxic: EC_{50} > 100 mg/L; harmful (acute 3): EC_{50}: 10 - 100 mg/L; toxic (acute 2): EC_{50} : 1 - 10 mg/L; and very toxic (acute 1): EC_{50} < 1 mg/L.

[b] **TU classification:** non-toxic: TU < 1; toxic: TU: 1 - 10; very toxic: TU: 10 - 100 and extremely toxic: TU > 100

Considering the GHS classification, the $[P_{6,6,6,14}]^+[TFSI]^-$,$[P_{6,6,6,14}]^+[DCA]^-$,$[P_{6,6,6,14}]^+[Cl]^-$, $[C_{12}C_1IM]^+[TFSI]^-$,$[C_{10}C_1C_{10}IM]^+[TFSI]^-$ and $[N_{8881}]^+[TFSI]^-$ ILs are toxic for the aquatic environment (EC_{50} values between 1-10 mg/L), whereas ZDDP1 and ZDDP2, with EC_{50} values < 1 mg/L, can be considered as very toxic. The other ILs studied can be included in the category of harmful (EC_{50} varied from 10 to 100 mg/L). Taking into account TU data, ZDDP1 and ZDDP2, can be considered as extremely toxic compounds, whereas $[P_{6,6,6,14}]^+[(iC_8)_2PO_2]^-$,$[P_{6,6,6,14}]^+[BEHP]^-$, $[P_{4,4,4,14}]^+[DBS]^-$, $[P_{4442}]^+[DEP]^-$, $[N_{4441}]^+[TFSI]^-$ and $[N_{4441}]^+[DCA]^-$can be categorised as toxic. On viewing these results, the ILs proved to be again either less or much less toxic to *E. coli* than the traditional lubricant additive ZDDP.

3.1.4. Toxi-ChromoTest tests: estimation

As with the *Vibrio fischeri*, a basic model was established to allow preliminary evaluation of the influence of both ions on ionic liquid toxicity. The data related to the values of model parameters C_i and A_j are set out in Table 7.

Table 7. Model parameters C_i and A_j obtained from *E. coli* data.

Cation	**C_i (L/mg)**	**Anion**	**A_j (L/mg)**
$[P_{6,6,6,14}]^+$	$6.23 \cdot 10^{-1}$	$[TFSI]^-$	$6.07 \cdot 10^{4}$
$[C_{10}C_1C_{10}IM]^+$	$3.23 \cdot 10^{-1}$	$[DCA]^-$	$4.20 \cdot 10^{-1}$
$[C_{12}C_1IM]^+$	$3.23 \cdot 10^{-1}$	$[Cl]^-$	$2.18 \cdot 10^{-1}$
$[N_{8881}]^+$	$1.54 \cdot 10^{-1}$	$[BEHP]^-$	$4.10 \cdot 10^{-2}$
$[N_{4441}]^+$	$9.65 \cdot 10^{-2}$	$[(iC_8)_2PO_2]^-$	$3.18 \cdot 10^{-2}$

This table shows that the toxicity of the cationic part to *E. coli* descended as follows: $[P_{6,6,6,14}]^+ > [C_{10}C_1C_{10}IM]^+ = [C_{12}C_1IM]^+ > [N_{8881}]^+ > [N_{4441}]^+$. Although the trend is very similar to that obtained in the bioluminescence tests, measuring toxicity to *Vibrio fischeri*, the values of C_i for the $[P_{6,6,6,14}]^+$ cation were lower (approximately 1.6 times), and for the rest of the ILs the values were higher (around 2 times). This indicates that imidazolium and quaternary ammonium-based ILs were more toxic for *Vibrio fischeri* than for *E. coli*.

With respect to the toxicity of the anion, the trend was as follows: $[TFSI]^- > [DCA]^- > [Cl]^- > [BEHP]^- > [(iC_8)_2PO_2]^-$. The value of A_j was significantly higher for $[TFSI]^-$ than for the rest of the anions considered. These results are in agreement with those found by Bräutigam *et al.* [102], where the *E. coli* was deeply affected by the exposure to ILs based on $[TFSI]^-$ anion. The rapid biosorption (adsorption plus bioaccumulation) of $[Cl]^-$ can explain its greater toxicity in comparison to $[BEHP]^-$ and $[(iC_8)_2PO_2]^-$ [2]. Besides, the parameters A_j for *E. coli* were higher than those estimated for *Vibrio fischeri*, which suggests that *E. coli* is more sensitive to the toxic stress produced by the ILs.

3.2. Biodegradability evaluation

3.2.1. Biodegradability estimation

Biodegradability under different conditions changes with the structure of the compound. Generally, an aromatic substituent (electron scavenger) increases the persistence of the chemical, possibly because enzymes find it harder to react with. However, functional groups such as carboxylic and amine among others, which are electron donors, usually produce a rise in the degree of biodegradability of the compound. The model called "Qualitative

Substructure Model" has been developed by Niemi *et al*. [103] using a database of Biological Oxygen Demand (BOD) converted to average lifetimes. This means that a theoretical biological oxygen demand of 50% or higher obtained over a period of five days has a half-life ($t_{1/2}$, time necessary for 50% of the concentration to be eliminated) of five days, and that a theoretical BOD of 25%, obtained over ten days, has a half-life of 20 days. Table 8 shows the results obtained.

Table 8. Estimation of the biodegradability through the qualitative model based on the substructure.

Ionic liquid	Functional Group	Estimated biodegradability ($t_{1/2}$)	Classification
$[P_{6,6,6,14}]^+[(iC_8)_2PO_2]^-$	Highly branched structures	>100	Persistent
$[P_{6,6,6,14}]^+[BEHP]^-$	Highly branched structures	>100	Persistent
$[P_{6,6,6,14}]^+[TFSI]^-$	Two halogen substitutions on an unbranched, noncyclic structure Highly branched structures	>100	Persistent
$[P_{4,4,4,14}]^+[DBS]^-$	Benzene ring with >2 substitutions Highly branched structures	>100	Persistent
$[P_{4442}]^+[DEP]^-$	-	-	Not applicable
$[C_{12}C_1IM]^+[TFSI]^-$	Two halogen substitutions on an unbranched, noncyclic structure Highly branched structures	>100	Persistent
$[N_{4441}]^+[TFSI]^-$	Two halogen substitutions on an unbranched, noncyclic structure Highly branched structures	>100	Persistent
$[N_{8881}]^+[TFSI]^-$	Two halogen substitutions on an unbranched, noncyclic structure Highly branched structures	>100	Persistent
$[C_{10}C_1C_{10}IM]^+[TFSI]^-$	Two halogen substitutions on an unbranched, noncyclic structure Highly branched structures	>100	Persistent
$[P_{6,6,6,14}]^+[DCA]^-$	Highly branched structures	>100	Persistent
$[N_{4441}]^+[DCA]^-$	-	-	Not applicable
$[P_{6,6,6,14}]^+[Cl]^-$	Highly branched structures	>100	Persistent

ZDDP1	Highly branched structures	>100	Persistent
ZDDP2	Highly branched structures	>100	Persistent

Using the above-mentioned model, the structural features associated with chemicals should be identified as biodegradable or persistent. It is important to notice that if the chemical does not have any biodegradable structure, the compound is classified as persistent. On the contrary, if the substance has essentially a biodegradable structure, in absence of any non-biodegradable element, the substance is classified as biodegradable. Therefore, the biodegradable classification is only used in absence of persistent elements. For those ILs whose chemical structures contain more than one functional group (two halogen substitutions, highly branched, etc), the estimated biodegradability ($t_{1/2}$) is calculated by considering the most restrictive group. Chemical compounds that do not have any of these mentioned descriptors are considered as unclassifiable.

In view of the results obtained, it is not an easy task to extract interesting conclusions from the application of this model. Most of the ILs and ZDDPs here studied are highly branched, which implies a low biodegradability and high persistence ($t_{1/2}$>100 days). In addition, nothing can be said about $[P_{4442}]^+[DEP]^-$ and $[N_{4441}]^+[DCA]^-$ because they do not fit into any of the possible criteria. However, they could be expected to be more biodegradable than the rest of the samples, due to the absence of heterocycles, fluorine and large branches [1, 82].

3.2.2. Biodegradability calculation

Biodegradation can be defined as the assimilation of chemical compounds by microbes [83]. A quantitative description of the tendency of lubricant additives to undergo biodegradation is given in Table 9, which shows the biodegradability index (BOD_5/COD) of the different aqueous solutions containing the ILs and the traditional lubricant additive. In all the studied ILs, it was found values of $\frac{BOD_5}{COD} < 0.3$, indicating that these compounds are poorly biodegradable [104-106]. Therefore, their carbon content is not a suitable carbon source for the microorganisms [107]. This fact can be explained due to the presence in the chemical structure of different elements such as halides, heterocycles, quaternary carbon atoms and high alkyl branching [108, 109]. In addition, literature research showed that rising the length of carbon side chains (until C10 - C11) should enhance biodegradation due to the presence of additional oxidizable carbons [110]. This may be the reason why the ILs

here studied showed a BOD_5/COD ratio slightly higher than those reported by Oliveira *et al*. [83], in which this ratio varied between 0.01 and 0.02 for short side chain (C_4) ILs. This fact makes complicated to establish a balance between biodegradability and toxicity with ionic liquids [1]. However, in view of the lower BOD_5 values obtained with $[TFSI]^-$ anion, the presence of halides seems to affect biodegradability more significantly than the length of carbon side chains.

Table 9. Biodegradability index (BOD_5/COD) of the studied additives ILs and ZDDPs.

IL sample	**COD (mg O_2/L)**	**BOD_5 (mg O_2/L)**	**BOD_5/COD**
$[P_{6,6,6,14}]^+[(iC_8)_2PO_2]^-$	824 ± 3	54.5 ± 0.3	0.070 ± 0.010
$[P_{6,6,6,14}]^+[BEHP]^-$	758 ± 4	52.3 ± 0.2	0.070 ± 0.010
$[P_{6,6,6,14}]^+[TFSI]^-$	605 ± 1	32.7 ± 0.3	0.050 ± 0.010
$[P_{6,6,6,14}]^+[DCA]^-$	832 ± 2	51.6 ± 0.2	0.060 ± 0.007
$[P_{6,6,6,14}]^+[Cl]^-$	824 ± 2	53.9 ± 0.4	0.065 ± 0.009
$[P_{4,4,4,14}]^+[DBS]^-$	768 ± 3	58.1 ± 0.5	0.080 ± 0.010
$[P_{4442}]^+[DEP]^-$	646 ± 5	46.4 ± 0.4	0.070 ± 0.020
$[C_{12}C_1IM]^+[TFSI]^-$	472 ± 2	29.8 ± 0.5	0.060 ± 0.020
$[C_{10}C_1C_{10}IM]^+[TFSI]^-$	549 ± 5	36.7 ± 0.2	0.070 ± 0.010
$[N_{8881}]^+[TFSI]^-$	290 ± 4	20.0 ± 0.1	0.060 ± 0.020
$[N_{4441}]^+[TFSI]^-$	463 ± 2	31.0 ± 0.1	0.067 ± 0.009
$[N_{4441}]^+[DCA]^-$	864 ± 3	66.5 ± 0.5	0.080 ± 0.010
$[P_{6,6,6,14}]^+[(iC_8)_2PO_2]^-$	478 ± 2	20.5 ± 0.1	0.043 ± 0.009
$[P_{6,6,6,14}]^+[BEHP]^-$	596 ± 2	16.0 ± 0.1	0.027 ± 0.009

The traditional lubricant additive ZDDP (ZDDP1 and ZDDP2) were also poorly biodegradable, with BOD_5/COD values lower than those observed in the ILs. These additives have very large molecular weights, which mean that they are not easily reachable to the microbes involved in degradation processes [111]. Various substituted imidazolium, phosphonium and quaternary ammonium derivatives have been screened for their biodegradability and, in general, they also exhibited low levels of biodegradation [77, 89, 110, 112-115]. Besides, it should be noted that most studies related to the evaluation of biodegradation have concluded that ionic liquids are compounds with low biodegradability [116, 117].

4. Conclusions

In the ILs studied here, both the cationic and anionic parts exerted a significant effect on their toxicity to bacteria, although this was mainly determined by the cation. The $[P_{6,6,6,14}]^+[TFSI]^-$ was found to be the most toxic IL for both *Vibrio fischeri* and *E. coli*, the EC_{50} values being 1.3 ± 0.6 mg/L and 1.6 ± 0.2 mg/L, respectively, whereas the $[P_{4442}]^+[DEP]^-$ IL was the least toxic, with values of 55.2 ± 0.7 mg/L and 39.1 ± 0.6 mg/L, respectively. This can be explained by the higher side chain length of the cation and the presence of fluorine in the anion. It should be noted that *E. coli* was more sensitive to the stress caused by the ILs, since the EC_{50} values were generally lower than those obtained with *Vibrio fischeri*. The EC_{50} values of the traditional lubricant additive (ZDDP1 and ZDDP2) were generally lower than 1.0 ± 0.2 mg/L for both *Vibrio fischeri* and *E. coli*, showing the high toxicity of these compounds. The presence of zinc in their composition is most likely to be the main factor behind such toxicity.

In addition, all the ILs were poorly biodegradable, showing BOD_5/COD values in the range of 0.05 ± 0.01 to 0.08 ± 0.01. This is due to the presence in their chemical structure of halides, heterocycles, alkyl chain branching or/and quaternary carbon that hinders biodegradability. ZDDP1 and ZDDP2 were also found to be poorly biodegradable with values between 0.043 ± 0.009 and 0.027 ± 0.02, respectively.

The excellent tribological properties as a lubricant additive shown by $[P_{6,6,6,14}]^+[(iC_8)_2PO_2]^-$ and $[P_{6,6,6,14}]^+[BEHP]^-$ in previous research, combined with their having better environmental properties than the traditional lubricant additive ZDDP, make these two ILs interesting candidates as partial or total replacements for this traditional additive. In this sense, the ease of combining different kinds of cations and anions in order to synthesize new ILs may also allow highly biodegradable ionic liquids with low toxicity to be found in the near future.

Abbreviations

$[P_{6,6,6,14}]^+[(iC_8)_2PO_2]^-$	Trihexyltetradecylphosphonium bis(2,4,4-trimethylpentyl) phosphinate
$[P_{6,6,6,14}]^+[BEHP]^-$	Trihexyltetradecylphosphonium bis(2-ethylhexyl)phosphate
$[P_{6,6,6,14}]^+[TFSI]^-$	Trihexyltetradecylphosphonium bis(trifluoromethylsulfonyl) imide
$[P_{6,6,6,14}]^+[DCA]^-$	Trihexyltetradecylphosphonium dicyanamide
$[P_{6,6,6,14}]^+[Cl]^-$	Trihexyltetradecylphosphonium chloride

$[P_{4,4,4,14}]^+[DBS]^-$	Tributyltetradecylphosphonium dodecylbenzenesulfonate
$[P_{4442}]^+[DEP]^-$	Tributylethylphosphonium diethylphosphate
$[C_{12}C_1IM]^+[TFSI]^-$	1-Dodecyl-3-methylimidazolium bis(trifluoromethylsulfonyl) imide
$[C_{10}C_1C_{10}IM]^+[TFSI]^-$	1,3-Didecyl-2-methylimidazolium bis(trifluoromethylsulfonyl) imide
$[N_{8881}]^+[TFSI]^-$	Methyl-trioctylammonium bis(trifluoromethylsulfonyl)imide
$[N_{4441}]^+[TFSI]^-$	Tributylmethylammonium bis(trifluoromethylsulfonyl) imide
$[N_{4441}]^+[DCA]^-$	Tributylmethylammonium dicyanamide
ZDDP1	Phosphorodithioic acid, mixed O,O-bis (1,3-dimethylbutyl and iso-Pr) esters, zinc salts
ZDDP2	Phosphorodithioic acid, mixed O,O-bis (diisooctyl) esters, zinc salts
ILs	Ionic liquids

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