Transworld Research Network
37/661 (2), Fort P.O.
Trivandrum-695 023
Kerala, India

Recent Advances in Pharmaceutical Sciences II, 2012: 89-113 ISBN: 978-81-7895-569-8
Editors: Diego Muñoz-Torrero, Diego Haro and Joan Vallès

6. Green chemistry: Ecotoxicity and biodegradability of ionic liquids

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Abstract. Green chemistry plays a very important role in the sustainable development, seeking to reduce and prevent pollution at its source, minimizing the hazard and maximizing the efficiency of the chemical processes. Ionic liquids (ILs) are a new generation of chemicals that have a great potential for contributing to the greenness of chemical processes and developing new applications, both being of interest for the pharmaceutical industry. This work deals with the development of ILs as greener alternatives for some of the processes within the frame of green chemistry. It focuses on the environmental impact of the ILs, their ecotoxicity and potential biodegradability, compiling results of different ecotoxicological studies. ILs have the reputation of being "green" chemicals, but not all of them can pass favourably the tests evaluating their environmental effects.

Introduction

Green chemistry can be defined as the design of chemical products and processes that reduce or eliminate the use and generation of hazardous substances, along with the minimization of waste production. Green

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chemistry can be applied across the life cycle of a chemical product, including its design, manufacture, and use. Chemical and pharmaceutical industries are among the main sources of pollution and hazardous waste generation, but they are also responsible for the prevention of any potential danger to the environment.

P.T. Anastas and J. C. Warner are considered to be the promoters of green chemistry. In their book "Green Chemistry Theory and Practice" published in 1998 [1], they formulated the 12 basic principles of green chemistry. The main ideas underlying the principles are to use less chemicals and energy, more secure raw materials, solvents and processes, to achieve energy efficient processes, to minimize waste production and reduce derivatization steps. Green chemistry promotes the use of safe, environment-benign substances, including solvents, whenever possible, the control of waste in real time and the increase of the amount of raw material present in the final product.

Most of the processes that involve the use of chemicals have the potential to cause a negative impact on the environment. For this reason it is essential to eliminate or at least reduce the involved risk to an acceptable level. The simplest form to express the risk is by multiplying hazard and exposure. The traditional way to minimize the risk of chemical processes has been to limit exposure by controlling the so-called circumstantial factors, such as the use, handling, treatment and disposal of chemicals. In contrast, green chemistry seeks to minimize risk by minimizing hazard. It means that the green chemistry shifts control from circumstantial to intrinsic factors, such as the design or selection of chemicals with reduced toxicity and of reaction pathways that eliminate by-products or ensure that they are benign. Green chemistry has become a major component of the science and of the sustainable development in general. Academic interest in green chemistry involves worldwide research aimed at cleaner processing and it has increased significantly in the past few decades.

1. Green chemistry metrics and pharmaceutical industry

A key question is how one can judge whether new processes do indeed have a reduced environmental impact. A series of green chemistry metrics of different categories (mass, energy, safety, ecotoxicity, etc.) can be used in order to evaluate the efficiency and potential environmental impact of the chemical processes [2]. The first one formulated was the Sheldon's Environmental factor (E-factor) [3]. It represents the weight of waste per unit weight of product and has been widely used by chemists. This metric is very simple to understand and use. It emphasizes the waste produced in the

process, instead of in a reaction, thus helping those who try to fulfill one of the twelve principles of green chemistry, namely, to avoid waste production. The E-factor incorporates yield, stoichiometry and solvent utilization and can be used to assess multi-step reactions (step by step or in one calculation).

Another green chemistry metric used is Atom Economy (AE) which gives an idea of how much of the reactants remain in the final product [4, 5]. It is expressed by the ratio (percentage) between the molecular weight of the final product and the molecular weights of the reactants. Its drawback is that, for example, catalysts and solvents are ignored, as they are not incorporated into the final product. AE is focused on the molecular weights and not on the mass or stoichiometric yield.

Glaxo Smithkline (GSK) developed two other metrics [6] one of them being carbon efficiency, which can be calculated by the percentage of total carbon amount in the product compared to that in the reactants. The other one is the reaction mass efficiency, which compares the mass of the final product with the mass of the reactants (in percentage). It takes into account atom economy, the amount of product obtained in a chemical reaction and stoichiometry. Like carbon efficiency, this measure shows the "greenness" of a reaction but not of a process. They do not take into account the global amount of waste produced. These two metrics could describe a process as "very green", but they would not be taking into account any of the solvents used, nor the energy issues.

Another metric is Process Mass Intensity (PMI) [7] that is defined as the ratio between total mass of materials used and mass of product. PMI is similar to the E-factor and it is easy to calculate. It includes all materials used in the synthesis of 1 kg of product (e.g. reagents, solvents, water, etc) not only the waste, as it happens in the E-factor. It is important to take into account that in the life cycle analysis of a pharmaceutical process the waste's contribution is much lower than all the materials used in the process. The pharmaceutical industry, through the American Chemical Society Green Chemistry Institute Pharmaceutical Roundtable, has selected PMI as the key mass-based green metric to know the greenness of processes and uses it to obtain greater efficiency and innovation in the pharmaceutical and fine chemicals industries.

The EcoScale is a recently developed metric tool for evaluation of the effectiveness of a synthesis reaction [8]. It is characterized by its simplicity and general applicability. The EcoScale evaluates the quality of the organic preparation based on yield, cost, safety, conditions and ease of workup/purification. It uses a scale from 0 to 100 with 100 representing the ideal reaction (100% yield and minimal risk for the operator and environmental impact). The EcoScale score is then calculated by lowering the

maximum value of 100 by any applicable penalty points, which take into account both the advantages and disadvantages of specific reagents, set-ups and technologies. By calculating the EcoScale score, a quick assessment of the "greenness" of reaction protocol is obtained, and the areas that need further attention are clearly indicated, which finally can lead to the improvement of reaction conditions. The considered penalty points and its quantification are described by Van Aken et al. [8].

Among all the mentioned metrics, E-factor or PMI seem to be the most adequate to evaluate the “greenness” in the pharmaceutical industry, because they include solvents, catalysts, reaction media and any other substance involved in the processes. E-factor is useful in highlighting the fine chemicals and pharmaceutical industries (Table 1) as the areas in which green chemistry is likely to have its most immediate impact.

It is important to emphasize that these green chemistry metrics are an oversimplification and do not allow to know some environmentally important properties, such as the toxicity, the biodegradability, the bioaccumulation and the fate of the chemicals used. However, these factors are often used by chemical and pharmaceutical industries because they are easy to use and provide information that allows the comparison between different processes.

The pharmaceutical industry's constant drive to lower the spending is helping to speed up the adoption of green chemistry because it can also offer significant cost advantages. The savings involve more efficient syntheses that avoid exotic reagents, minimizing energy use, replacing organic solvents, etc. Many pharmaceutical companies are finding innovative ways to reduce their impact on the environment during drug manufacturing. Pfizer [9] is applying the principles of green chemistry in the production of Viagra®, Lyrica®, Lipitor® and Vfend®. For example, in the process of synthesis of Viagra®,

Table 1. Environmental impact as measured by the E-factor [3].

Industry	Product tons per year	E-factor (waste/product ratio by weight)
Oil refining	10^6 - 10^8	~ 0.1
Bulk chemicals	10^4 - 10^6	< 1-5
Fine chemicals	10^2 - 10^3	5-50
Pharmaceuticals	10^0 - 10^3	25->100

the number and quantity of solvents involved has been reduced [10]. The Eli Lilly Company has redesigned the synthesis of one anticonvulsive drug, LY300164. Aventis has developed a new synthesis of hydrocortisone using microorganisms. BHC Company (BASF Corporation) developed an improved efficient method to make ibuprofen using only three steps instead of the former six. In this case, all starting materials are converted to product, reclaimed as by-product or completely recycled in the process. Thus, the generation of wastes has been practically eliminated [11].

All of these examples can be cited as industry models of environmental excellence in chemical processing technology and are implementing the basic principles of green chemistry.

2. Ionic liquids: Properties and applications

In the pharmaceutical industry, organic solvents are a major source of waste because they are used as reaction media or in separation operations and their efficient control can produce a substantial improvement in the environmental impact of a process [2]. The best way to avoid problems with solvents is not to use them, an approach that has been widely exploited in the paints and coatings industries. Most reactions do, however, require a solvent, and a green chemical process must necessarily involve solvents that are environmentally acceptable. One of the new classes of solvents that offer opportunities to move away from traditional chemical processes to new, clean, green technologies are the ionic liquids [12]. BASF designed a new process for scavenging acids in the chemical synthesis of phosphorus compounds using ionic liquids: Basil^{TM} (Biphasic Acid Scavenging Utilizing Ionic Liquids), which offers significant advantages over the conventional system. This is the first large-scale industrial process worldwide that uses ionic liquids, and their developers received the "Innovation Award" of the "European Chemical News" trade journal and the BASF Innovation Award in October 2004.

An ionic liquid (IL) is generally defined as a salt in which the ions are poorly coordinated, with a melting point below 100 °C [13]. Some ILs can be liquid even at room temperature [14] and then they are called room temperature ionic liquids (RTILs). Most of the ILs used up to date have a bulky organic cation (imidazolium[a], pyridinium[b], pyrrolidinium[c], piperidinium[d], ammonium[e], phosphonium[f], etc.), substituted with alkyl chains of different length (from C_1 to C_{22}) and inorganic anions such as halogen (Cl^-, Br^-, I^-), $[N(CN)_2]^-$, $[BF_4]^-$, $[PF_6]^-$, etc. (Fig. 1). New ionic liquids, with linear aliphatic anions are currently being developed [15].

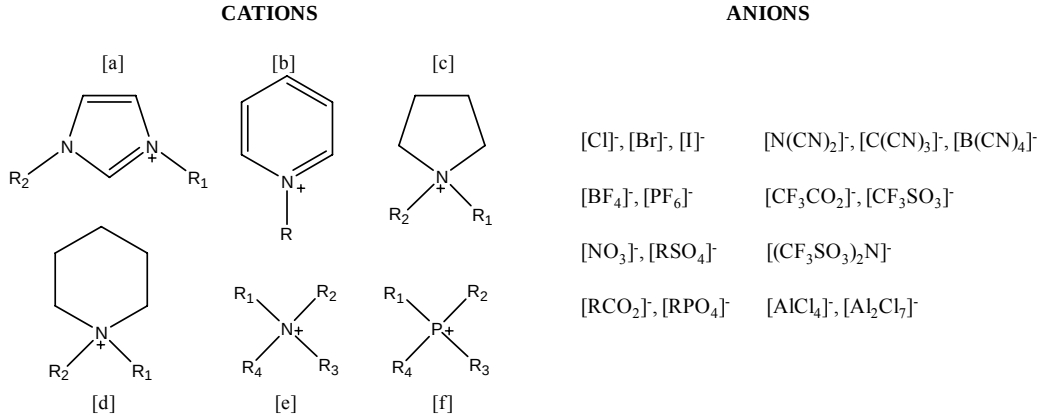

Figure 1. Principal ions present in most ILs.

Ionic liquids (ILs) are not newly discovered compounds, they have been known for almost a century. The first documented observation of ionic liquids by chemists was the so-called 'red oil' formed during Friedel–Crafts reactions in the mid-nineteenth century. The structure of the red oil was later identified by NMR as a stable intermediate composed of a carbocation and a tetrachloroaluminate anion [16, 17]. The earliest example of a room temperature ionic liquid was ethylammonium nitrate ($EtNH_3^+[NO_3]^-$) with a melting point of 12 ºC. It was described by Paul Walden in 1914 and obtained by the neutralization of ethylamine with concentrated nitric acid. One of the first 1,3-dialkylimidazolium RTILs was reported in the early 1980s by Wilkes and co-workers. It was obtained through the mixing of 1-ethyl-3-methylimidazolium chloride with aluminum trichloride [16]. Organo-aluminate ILs have a limited range of applications due to the high reactivity of the chloroaluminate anion towards water [18]. In the early 1990s, Wilkes reported two new ILs such as 1-butyl-3-methylimidazolium tetrafluoroborate and 1-butyl-3-methylimidazolium hexafluorophosphate [16]. The cation 1-ethyl-3-methylimidazolium has been the most widely studied until 2001, and nowadays, 1,3-dialkylimidazolium salts are one of the most popularly used and investigated class of ILs [19]. Research into synthesis and possible applications of new ILs is growing in exponential form. The 20 papers published in 1994 have become more than 2,500 in 2008 [20].

The physicochemical properties of the ILs, like all other materials, depend upon the intermolecular and intramolecular forces and, subsequently, upon the structure of the cation and the anion. A significant number of investigations have been conducted for ILs on the relationship between their physicochemical properties and the structure of their cation and anion. [21-24]. In general, ILs have a negligible vapor pressure so they are not volatile and no atmospheric pollution can be expected due to their use. ILs are also

thermally stable so they can be used in chemical processes that require heat input. These and other characteristics (Table 2) such as nonflammability make them useful for many applications, and make that they meet up to the criteria of green chemistry.

There are literally millions of different structures that may be formed by combining different cations and anions and the number of possible combinations is estimated to be as high as 10^{18} [28, 29]. This enormous quantity of possible ILs would permit, based on physicochemical characteristics, to select the most appropriate for a particular purpose. In particular, RTILs are often called "designer solvents" because it is possible to create an IL with a required property. RTILs have been used for several other applications, and their development continues at a considerable rate owing to their peculiar physical and chemical properties such as high thermal and chemical stability, lack of inflammability, low volatility, and tunable solubility in several organic compounds [30]. Due to their low volatility, many of the RTILs have been used as greener alternatives to conventional toxic and volatile organic solvents by taking advantage of their unique properties [31]. This set of properties allows the design of very attractive reaction systems that can solve some of the main drawbacks of currently used methods of synthesis or to obtain new procedures for making various products [32, 33]. Polarity, hydrophilicity/hydrophobicity and other properties of ILs can be adjusted by an appropriate combination of cations and anions [34].

As the unique properties of the ILs were being discovered, there was a rising interest in applying them as a reaction medium in a wide variety of chemical transformations that until recently could only be carried out in organic solvents. The literature describes numerous uses of ILs, some of them being: reaction media for many organic transformations [35], in separations

Table 2. Some physico-chemical characteristics of ionic liquids [25-27].

Decomposition point	150 – 500 ºC
Dielectric constant	Implied ≤ 30
Electrical conductivity at 25 ºC	< 0.6 S m^{-1} (maximum value 11.9)
Flammability	Non-flammables
Melting point	< 100ºC (maximum value 239 ºC)
Temperature range liquid phase	From - 96 to 300 ºC
Thermal conductivity	0.117 – 0.199 W/m/K
Vapor pressure	Negligible
Viscosity	Usually 0.013 – 0.22 Pa.s (maximum value 1.02)

and extractions [36], as electrolytes for electrochemistry [37], in nanotechnology [38], in biotechnology [39], and in engineering processes [40], absorption of gases (CO_2) [13], as catalysts in organic synthesis [41], aldol condensation [42] and organometallic and radical polymerization [43]. Other authors have described ILs specific applications for extraction of active ingredients from medicinal plants [44-46]. Some of the ILs applications are presented in Fig. 2.

The great number of potential ionic liquids and the possible applications makes their classification a very difficult task because different criteria can be used (physical, chemical or structural characteristics, industrial applications, etc.). So, according to the chemical properties, ILs can be divided into protic (PILs) and aprotic (AILs). The distinguishing feature between both is that all PILs have a proton available for hydrogen bonding [47], whereas AILs have not.

However, for some authors, the most useful way of grouping them is based on the properties that have conditioned the evolution of their use. According to Hough et al. [48] the first generation includes ILs for which the accessible physical properties such as decreased vapor pressure and high thermal stability are often unique. Second generation ILs have potential use as functional materials (energetic materials, lubricants, metal ion complexing agents, etc.) which utilize novel tunable physical and chemical property sets. The third and most recent generation of ILs involves biological properties combined with chosen physical and chemical properties. This third generation of ILs with biological properties may be a breakthrough for the pharmaceutical industry because it opens up many possibilities to generate

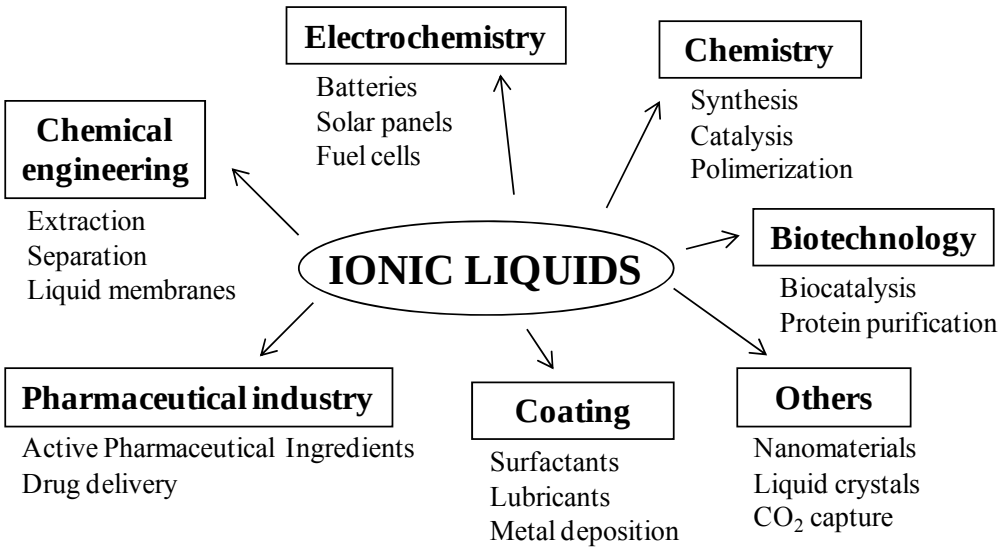

Figure 2. Possible applications of ionic liquids [51].

active pharmaceutical ingredients (APIs) in the form of ionic liquids (IL-APIs). Most of APIs are crystalline salts that present some problems related to dissolution, transport, bioavailability and polymorphism control, which can reduce the pharmacological activity [49, 50]. A possible way to overcome the drawbacks of a drug with an ionic active group is to change the complementary ion for another one able to bring IL physico-chemical properties to the new substance and thus modify its initial pharmacokinetic properties.

The design of IL-APIs with dual biological activity is a step beyond drug development. This can be achieved by the combination of a cation and an anion (both being APIs) to generate an IL that maintain the pharmacological activities of each drug while improving their pharmacokinetic properties [50, 52]. Hough et al. [48] have synthesized dual IL-APIs, like ranitidine docusate and didecyldimethylammonium ibuprofenate from ranitidine hydrochloride (histamine H_2-receptor antagonist) and sodium docusate (emollient), and didecyldimethylammonium bromide (antibacterial) and sodium ibuprofenate (anti-inflammatory), respectively. The same authors have described synergistic effects in the case of lidocainium docusate, an IL-API prepared by the combination of an analgesic (lidocaine hydrochloride) and an emolient (sodium docusate). Bica et al. [53] have paired salycilate and acetyl salycilate with a set of cations with variable biological activity covering analgesic, local anesthetic, antiarrhythmic, antimicrobial or antibacterial activity to obtain dual functional liquids salts. However although such ILs with aspirin could be prepared, they suffer from limited stability and slowly decompose into the corresponding salycilate ILs when exposed to moisture.

Recently, MEDRx Co. Ltd. (a Japanese pharmaceuticals company) and IL Pharma Inc. (a subsidiary of MEDRx Co., Ltd.) are developing Etodolac Patch (MRX-7EAT) for treatment of pain and inflammation using ILTS® (Ionic Liquid Transdermal System). MRX-7EAT is a pharmaceutical topical patch containing etodolac, a non-steroidal anti-inflammatory drug [54]. The oral etodolac products have been widely known and used to relieve pain and inflammation. However, MRX-7EAT will be the first topical etodolac patch in the world. The results of non-clinical studies in some animals have shown the safety and efficacy of MRX-7EAT, and the results of clinical trials in human have also shown the safety and tolerability.

On the other hand, some considerations should be taken into account when trying to bring new substances to market. The current legislation on chemicals in the European Union [55] called Registration, Evaluation, Authorization, and Restriction of Chemical Substances (REACH) regulates the safety of chemical products, their manufacturing, toxicity, biodegradability, transport and use in the industrial sectors. Even though ILs

have potentially "green" profile, they are basically chemical products, and as such, have to fulfill the REACH criteria, taking into account their possible commercial use. In this sense, a very important aspect that has to be studied in sufficient depth is the potential negative impact of ILs, both on the environment and humans. The findings for the toxicity of ILs must comply with the requests of the REACH.

3. Ecotoxicity of ionic liquids

Even though the ILs are considered as non-volatile and thus cannot contribute to the air pollution, the water solubility of many ionic liquids is not negligible. The potential release of ionic liquids into aquatic and terrestrial environment may lead to water and soil pollution, and related risks. So, it is important to evaluate ecotoxicity, biodegradability, bioaccumulation and environmental fate of these chemicals. Taking into account the possible use and commercialization of ILs, they should pass the REACH evaluation, which recommends that the tests used for the assessment of the potential toxicity of chemicals have to be done in accordance with OECD guidelines. These tests can be divided into three groups: effects on biotic systems, health effects, and degradation and accumulation.

There is a wide range of OECD tests that can be implemented in order to assess the potential ecotoxicity of ILs on biotic systems. Some of the most frequently used up to date are: Freshwater Alga and Cyanobacteria, Growth Inhibition Test [56], *Daphnia* sp. Acute Immobilisation Test [57], *Daphnia magna* Reproduction Test [58], *Lemna* sp. Growth Inhibition Test [59], Earthworm Acute Toxicity Tests [60], Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test [61], Soil Microorganisms: Nitrogen Transformation Test [62], Soil Microorganisms: Carbon Transformation Test [63], Collembolan Reproduction Test in Soil [64]. There are other non OECD methods that are also commonly used to evaluate the ecotoxicity on aquatic organisms: Bioluminiscence inhibition assay in *Vibrio fischeri* [65], snails [66], and zebra mussel [67].

Several properties of ionic liquids and their effects on aquatic organisms have been investigated in different works. Toxicological research studies concerning ionic liquids have been undertaken in the past decade and some of these findings will be summarized below.

Green algae are ecologically relevant organisms that are at the base of the food chain. The algal test has an advantage over tests done with e. g. fish or invertebrates because it measures a population-level response. Algae have short life cycle which makes them ideal for toxicological studies, as they can respond quickly to environmental change [68]. Cho and co-workers [69] have

found that the toxicity of 1-butyl-3-methylimidazolium, 1-butyl-3-methylpyridinium, 1-butyl-1-methylpyrrolidinium, tetrabutylammonium and tetrabutylphosphonium bromides was between two and four orders of magnitude greater than those of the organic solvents examined (methanol, dimethylformamide and 2-propanol). This group was investigating other series of imidazolium based ILs and the conclusion was that the toxicity of ILs increased with the increase in side chain length [70]. Other authors [71] indicate that ILs derived from imidazolium present an acute toxicity to *Daphnia magna* higher than benzene and some organochlorine solvents (tri- and tetra-chloromethane). Latała et al. [72] have examined the effect of imidazolium based ILs on other species of Baltic algae (*Oocystis submarina* and *Cyclotella meneghiniana*). They found that the response of the two species differed dramatically being *Oocystis submarina* more resistant to the toxicity of ILs than the other one, and that the toxicity was reduced in more saline waters. Matzke et al. [73] investigated the effect of imidazolium based ILs with C_4 and C_8 side chains on *Scenedesmus vacuolatus* species. Their findings confirmed other authors conclusions, i.e. that the toxicity strongly depends on the side chain effect.

Ecotoxicological literature on ILs toxicity to invertebrates mainly focuses on the use of *Daphnia magna* as a test organism because it is an important link between microbial and higher trophic levels. The results of all studies noted the link between toxicity and alkyl chain length of the tested ILs containing imidazolium, pyridinium or quaternary ammonium as cations

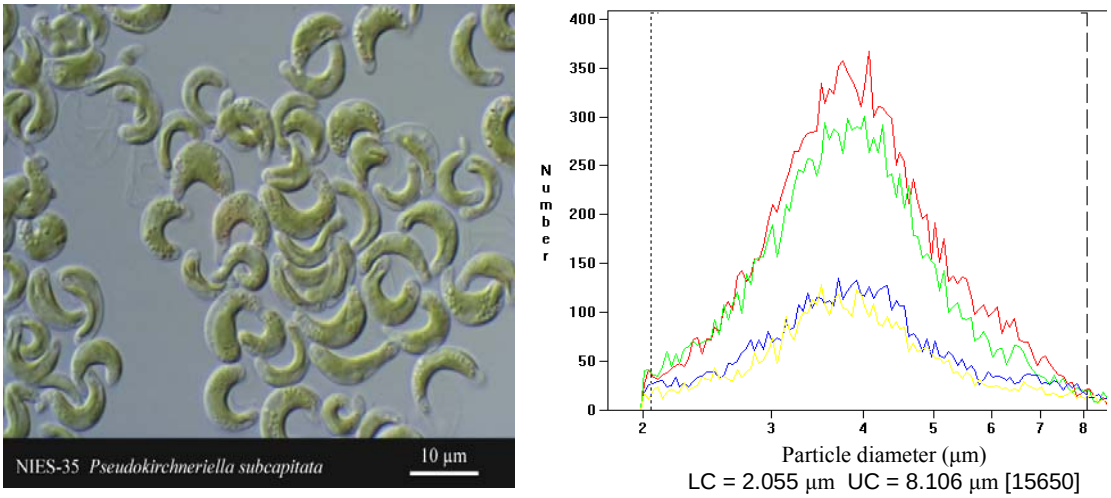

Figure 3. Morphology of *Pseudokirchneriella subcapitata* (photo from National Institute for Environmental Studies of Japan) [74] and growth algae inhibition curves showing the ecotoxicity of 100 mgL^{-1} of 1-butyl-3-methyl imidazolium (blue and yellow lines) in comparison with a control (red and green lines). Unpublished results from the authors.

[71,75-78]. Similar results were obtained in other studies on the snail *Physa acuta* with imidazolium and pyridinium based ILs [79] and also on *Dreissena polymorpha* (zebra mussel) [80]. In these tests a positive relationship between alkyl chain length and toxicity of ILs was also demonstrated, as well as the lower sensitiveness of *Physa acuta* than *Daphnia magna* to ILs.

Zebrafish (*Danio rerio*) plays an important role in ecotoxicology as an important model vertebrate. Concerning toxicity of ILs to the zebrafish, Pretti et al. [81] evaluated the toxicity of commonly used (imidazolium and pyridinium ILs) and new AMMOENG® ILs (quaternary ammonium derivates). The results of this study revealed that ILs may cause a completely different effect on fish than expected according to their chemical structures. Imidazolium, pyridinium and pyrrolidinium could be regarded as non-highly lethal towards zebrafish. On the other hand, some ammonium salts showed LC_{50} remarkably lower than that of organic solvents and tertiary amines. They also demonstrated that fish species are less sensitive to ILs toxicity compared to other species belonging to lower trophic levels.

The duckweed, *Lemna minor*, is a common aquatic plant that has been frequently a focus of the investigation of phytotoxicity of ILs [73, 82-84]. In general, 1-alkyl-3-methylimidazolium compounds with longer alkyl chains were more toxic to *Lemna minor* than those with short alkyl chain lengths. Imidazolium and pyridinium cations with butyl groups had similar EC_{50} (the concentrations that produced a 50% reduction in root growth) while the equivalent ammonium cation had a much higher EC_{50} and thus proved to be less toxic.

Microtox® is a standardised toxicity test system which is based on the reduction of the bioluminescence of the marine bacteria *Vibrio fischeri* when exposed to a range of concentrations of the tested chemical. This is a rapid, sensitive, reproducible, ecologically relevant and cost effective test. It is recognised and used throughout the world as a standard test for aquatic toxicity testing. Docherty and Kulpa [85] investigated the toxicity of imidazolium and pyridinium ILs and found that the hydrophobicity, which corresponds to the increasing alkyl chain length of the IL cation induces rising toxicity. These findings are consistent with those from other authors [73, 86]. Couling and co-workers [75] have expanded the range of investigated ILs and noted that the quaternary ammonium compounds were less toxic than the imidazolium and pyridinium analogues. In comparison to some commonly used industrial solvents such as phenol, toluene and benzene, long chain (> C_4) ILs are more toxic. The values of EC_{50} for the most frequently used organic solvents for the Microtox® test are presented in the Table 3.

Table 3. Acute toxicity of organic solvents to *Vibrio fischeri* [87].

Solvent	**log EC_{50} (μM)**
Methanol	3.50
Acetonitrile	2.77
Acetone	2.52
Benzene	2.03
Phenol	1.49

Generally speaking, all of the aquatic toxicity tests showed that the head group (cationic part of the molecule) was responsible for the toxicity of the ILs. In most cases there was no influence of the anionic part of the ILs molecule to the toxicity. Only the anion bis[(trifluoromethyl)sulfonyl]imide $[(CF_3SO_2)_2N]^-$ showed higher toxicity than others. The side chains on the head groups were proven to have a very strong influence on the toxicity. The longer and more branched the side chain is, the more toxic is the ionic liquid. Most toxic ionic liquids have an alkyl chain with more than eight carbons. These results point to the fact that some ILs may be more toxic than the commonly used organic solvents, and not as green as expected.

In Table 4 the toxicity of ILs to different levels of biological complexity is presented.

Table 4. Toxicity of different ILs expressed as logEC_{50} (μM): 1-butyl-3-methylimidazolium (C_4mim), 1-hexyl-3-methylimidazolium (C_6mim), 1-octyl-3-methylimidazolium (C_8mim), 1-butylpyridinium (C_4py), 1-butyl-3-methylpyridinium (C_4mpy), 1-butyl-3-methylpyrrolidinium (C_4mpyr), 1-butyl -3-methylpiperidinium (C_4mpip). N.A. non available.

Ionic liquid	*Vibrio fischeri*	*P. subcapitata*	*Lemna minor*	*Daphnia magna*
[C_4mim]Cl	2.95 [85]	2.34 [73]	2.82 [73]	1.93 [75]
[C_4mim]Br	3.07 [86]	3.46 [88]	N.A.	1.57 [75]
[C_4mim][BF_4]	3.55 [86]	N.A.	2.49 [82]	1.68 [75]
[C_4mim][PF_6]	3.07 [89]	2.20 [78]	N.A.	1.85 [75]
[C_6mim]Br	1.42 [75]	2.57 [70]	N.A.	0.78 [75]
[C_6mim][BF_4]	3.18 [86]	N.A.	N.A.	N.A.
[C_8mim]Cl	1.19 [89]	1.46 [78]	N.A	N.A
[C_8mim][BF_4]	1.41 [86]	N.A.	N.A	N.A
[C_4py]Cl	3.18 [84]	N.A.	2.32 [84]	N.A.
[C_4mpy]Br	2.12 [85]	3.46 [88]	N.A.	1.76 [75]
[C_4mpyr]Cl	>4.30 [84]	3.67 [88]	N.A.	N.A.
[C_4mpip]Br	4.27 [84]	3.27 [84]	0.47 [84]	N.A.

Peric et al. (unpublished results) have studied the toxic effect on aquatic organisms of a new family of water soluble PILs which are composed of ammonium substituted organic salts. The cationic moiety is mono-, di- or tri-hydroxiethylammonium while the anionic moiety is an alkylcarboxylate ($\leq C_5$). The results show no toxicity to aquatic organisms, with EC_{50} values being between 460 and 2600 mg L^{-1} for the tests of aquatic toxicity (Microtox® test and green algae growth inhibition test). According to the EU regulation [90] they have no toxic effects on aquatic organisms (all of the EC_{50} are above the limit value of 100 mg L^{-1}). Within this group of PILs the toxicity increases with the increase of complexity of the molecule. Comparing the EC_{50} values obtained for the aprotic ILs it can be seen that the new PILs are less toxic than the AILs studied up to date.

Studies on the effects of the ILs on soil and sediment organisms are very limited or still missing so far. Terrestrial organisms such as the spring tail *Folsomia candida*, a soil invertebrate [73], the earthworm *Eisenia foetida* [91] or *Caenorhabditis elegans* (a soil roundworm) [92] have been tested. In this last case, the authors suggest the use of *Caenorhabditis elegans* as a model organism for inexpensively and quickly exploring toxicological effects of 1-alkyl-3-methylimidazolium chloride ILs.

Concerning higher plants, Matzke et al. [73] investigated the toxicity of ILs derived from imidazolium with different anions to wheat (*Triticum aestivum*) and cress (*Lepidium sativum*). The side chain effect was once again confirmed, with slightly diverse patterns of toxicity depending on the anion used. Matzke et al. [93] investigated the influence of different clay minerals and clay concentrations on the toxicity of the anionic moieties of imidazolium based ILs towards wheat plants. The obtained data showed that 1-butyl-3-methylimidazolium bis[(trifluoromethyl)sulfonyl]imide appeared to be the most toxic, independently of the type of clay added to a reference soil. The toxicity of different ILs with the same cationic moiety (1-butyl-3-methylimidazolium chloride, tetrafluoroborate and hydrogen sulfate) was mainly dependent on the cation and the observed effects varied according to the added clay type and clay concentration to the reference soil. An increase of smectite clay content resulted in less inhibitory effects of these ILs. Studzinska and Buszewski [94] have proved that hazardous effects of imidazolium ILs are closely connected with organic matter content in soil. Soil with more organic carbon was observed to sorb IL cations more extensively than soil with little or no organic matter; hence, the more fertile the soil, the lower probability of hazardous effect of ILs to plants.

Wang and co-workers [95] conducted a study on the effect of 1-butyl-3-methylimidazolium tetrafluoroborate on wheat seedlings. The increase of this IL concentration in soil showed a significant negative effect both on

germination and roots and shoot length of the wheat plants. At low concentrations, 1-butyl-3-methylimidazolium tetrafluoroborate did not inhibit, and even promoted wheat seedling growth. At high concentrations, this IL inhibited wheat seedling growth significantly and decreased chlorophyll content, thereby reducing photosynthesis and plant growth. In another research, the phytotoxicity tests of chiral ILs containing (-)-nopyl derivatives were carried out in a plant house using spring barley (*Hordeum vulgare*) which is a monocotyledonous plant, and a common radish (*Raphanus sativus* L. subvar. *radicula* Pers.) which is a dicotyledonous plant [96]. According to the obtained results, increasing the concentration of ILs resulted in a systematic decrease in the crop fresh weight of total sprouts and the crop fresh weight per plant, both for spring barley and for common radish. It could also be noted that barley was more resistant than the radish and tolerated higher concentrations of ILs in soil.

A study conducted by Peric et al. [97] evaluated the toxicity of three protic ionic liquids: hydroxyethylammonium formate, dihydroxyethylammonium propionate and trihydroxyethylammonium pentanoate to terrestrial organisms by performing different bioassays with plants (onion, grass, and radish) and soil microorganisms involved in the most important biogeochemical cycles (carbon and nitrogen mineralization). The PILs analyzed in the study showed no toxicity, with EC_{50}s above 1000 mg kg^{-1} in all assays except for *Raphanus sativus* plant test with trihydroxyethylammonium pentanoate (EC_{50} = 826 mg kg^{-1}) (Fig. 4). Within the group of terrestrial organisms, the higher plants (the three plant species tested) have shown to be more sensitive to the presence of PILs than the soil microbiota, with *Raphanus sativus* being the most sensitive to the presence of PILs. From the results it can be deduced that, in general, the compounds with more complex molecular structure have a greater tendency to cause inhibition in the organisms tested than the compounds with the smaller molecule and simpler structure. The three analyzed PILs seem to be non toxic in the terms

Figure 4. Effect of trihydroxyethylammonium pentanoate on germination and growth of *Raphanus sativus* plants [97].

of chronic toxicity for plants and C and N cycles. Comparing the results from this test with those obtained by other authors for the group of aprotic imidazolium based ionic liquids [73, 95, 98], it was observed that the analyzed PILs are less toxic than the AILs. Even though the plant species used in the test were not the same as those used by other authors, the values of EC_{50} for the AILs were generally one order of magnitude lower than EC_{50} for the tested PILs.

Although the investigation of the phytotoxicity of ILs and their effect on soil has not been intensive, the available data can give a boost for the environmental scientists to start dealing more with the potential impact of ILs towards plants and soil.

4. Biodegradability of ionic liquids

Some methods for the determination of biodegradability in water and soil are described in the Section 3 (Degradation and accumulation) of the OECD guidelines for the testing of chemicals. The most widely used, that permit the screening of chemicals for ready biodegradability in an aerobic aqueous medium, are included in the Test No. 301: Ready Biodegradability [99]. The methods are: the dissolved organic carbon (DOC) Die-Away test, the CO_2 Evolution test (Modified Sturm Test), the MITI (Ministry of International Trade and Industry, Japan) test, the Closed Bottle test, the Modified OECD Screening Test and the Manometric Respirometry Test. One of the most frequently used parameters for the assessment of biodegradation in aqueous medium is biodegradation percentage, which represents the ratio beetwen the biological oxygen demand (BOD) and the Theoretical Oxygen Demand (ThOD) calculated from the chemical formula of the compound. The pass level for ready biodegradability is 60% of ThOD for respirometric methods, reached within 28 days. Compounds reaching or exceeding the pass level after more than 28 days (the usual duration of the tests) are not to be considered as readily biodegradable. The biodegradability in soil can be assessed by means of the test No. 307 Aerobic and anaerobic transformation in soil [100] and it is also expressed as the biodegradation percentage. Other methods as test No. 217, Soil microorganisms: carbon transformation test [63] and ASTM D 5988-96 have been used by different authors to evaluate indirectly the biodegradation process [101].

The biodegradation potential of ILs in aqueous media has been addressed in some works. Wells and Coombe [78] investigated the biodegradability of ammonium, imidazolium, phosphonium and pyridinium compounds by measuring BOD. They observed that the cations with short side chains (C_4) were not biodegradable. A strong inhibitory potential to the inoculum used in

the test was observed for series with longer side chains (C_{12}, C_{16} and C_{18}), indicating the toxicity of these ILs towards the microorganisms used. Docherty and co-workers [102] examined the biodegradability of *N*-methylimidazolium and 3-methylpyridinium compounds substituted with butyl, hexyl and octyl side chains and bromide as the anion. A dependency between biodegradability and the side chain length was found in the DOC Die-Away tests and in tests monitoring the changes in the total dissolved nitrogen concentration. Another IL, 1-octylpyridinium bromide, met the OECD criterion for being classified as readily biodegradable, whereas 1-hexylpyridinium bromide exhibited a decreased degradation rate. Compared to the pyridinium ILs the mineralisation of the imidazolium ILs was lower. The 1-methyl-3-octylimidazolium cation showed significant degradation rates, but those were not high enough for a classification as readily biodegradable. For the pyridinium and imidazolium head groups carrying a butyl side chain no significant biodegradation was observable. In another study, Gathergood et al. [103] found that the influence of anion was important only in a case of the octyl sulfate anion, which proved to be considerably more biodegradable than the other commonly used anions. The introduction of an ester group in the side chain of the 1,3-dialkylimidazolium cation led to biodegradation values very close to the pass level of the Closed Bottle test.

Stolte et al. [104] investigated the biodegradation of different *N*-substituted imidazoles, imidazolium, pyridinium and 4-dimethylaminopyridinium compounds bearing various alkyl side chains. They found a significant biodegradation for ecotoxicologically unfavourable compounds carrying long alkyl side chains (C_6 and C_8). In contrast for ecotoxicologically more recommendable imidazolium ILs with short alkyl ($\leq C_6$) and short functionalised side chains, no biological degradation could be

Figure 5. Manometric respirometers used to determine carbon mineralization and indirectly the biodegradation of chemicals in water and soil (Photo from the authors).

found. The introduction of different functional groups into the side chain moiety, thus offering a higher chemical reactivity, did not lead to the expected improvement of the biological degradation potential. After an incubation period of 24 days for the 1-methyl-3-octylimidazolium cation different biological transformation products carrying hydroxyl, carbonyl and carboxyl groups were identified. Furthermore, shortened side chain moieties were identified indicating the degradation of the octyl side chain via β-oxidation.

Gathergood and co-workers [105] intended to design, synthesize and evaluate biodegradable ILs containing ester or amide groups in the alkyl side chain. The introduction of a group susceptible to enzymatic hydrolysis greatly improves the biodegradation, compared with the commonly used dialkylimidazolium ILs, 1-butyl-3-methylimidazolium tetrafluoroborate and hexafluorophosphate. For the 3-methyl-1-alkyloxycarbonylmethylimidazolium bromide series, the greatest biodegradation was observed when alkyl side chain had 4 or more carbons. The corresponding amide analogs proved to be poorly biodegradable. In the next phase of their investigation [106] they tried to establish the influence of the anionic moiety on the biodegradability of ILs. Different C_4mim cations combined with Br^-, $[BF_4]^-$, $[PF_6]^-$, $[N(CN)_2]^-$, $[(CF_3SO_2)_2N]^-$ and octylsulfate as the counter ion were analyzed using the Sturm and Closed-Bottle test protocols. No compound showed significant degree of biodegradation with the exception of the IL containing octylsulfate anion which had higher levels of biodegradability. The same group did a further research toward the discovery of biodegradable ILs [103]. The aims of the study were to incorporate additional structural modifications in order to improve biodegradability. They incorporated 2-methyl group into the molecule of imidazolium ILs to provide an additional site for metabolism, parting from a fact that 2-methylimidazole is significantly more biodegradable than imidazole. The incorporation of a methyl group in the 2-position of imidazolium cation had no significant effect on biodegradability. The commonly used 1-butyl-3-methylimidazolium core showed negligible levels of degradation. Part of this team continued their research by trying to design, synthesize and evaluate biodegradable pyridinium ILs. Harjani et al. [107] prepared ILs bearing an ester side chain moiety, using either pyridine or nicotinic acid. These ILs showed high levels of biodegradation under aerobic conditions and can be classified as ‘readily biodegradable’. In contrast, pyridinium ILs with alkyl side chains showed significantly lower levels of biodegradability in the same test.

The fact that only the long side chains in ionic liquids improved biodegradability creates a conflict of aims between minimizing the toxicity and maximizing the biodegradability. The issue of biodegradability seems to be a problem in the development of environmentally safer ionic liquids.

Peric et al. (unpublished results) have studied the biodegradability in water of three ILs from the new family of PILs, derived from substituted ammonium salts (hydroxyethylammonium formate, diydroxyethylammonium propionate, and trihydroxyethylammonium pentanoate). Almost all of the analyzed PILs showed to be readily biodegradable with biodegradation rates of 57 to 86% (expressed in terms of theoretical oxygen demand) in a 28 days period. These results can be due to the fact that these PILs do not show toxicity towards microorganisms and also to their non cyclic simple structure. Even though they have cations and anions composed of short alkyl chains ($\leq C_5$), these chains have hydroxyl and carboxyl functional groups which can increase the biodegradability rate, in accordance with the findings of other authors related to ester and amide functional groups [105].

Like in the case of the terrestrial ecotoxicity, the data on the biodegradability in soil is scarce. Kumar et al. [108] investigated the fate of 1-butyl-3-methylimidazolium tetrafluoroborate when in contact with soil-microorganisms, wastewater microorganisms, *Pseudomonas putida* and *E. coli*. Although 1-butyl-3-methylimidazolium tetrafluoroborate was indicated to be recalcitrant in Sturm and Closed-Bottle test assays as mentioned above, it was observed in this study that *P. putida* was able to break down 1-butyl-3-methylimidazolium tetrafluoroborate after 15 days of incubation.

The aerobic biodegradation processes of ionic liquids in soil were monitored for the first time by Modelli et al. [101] , working with four ionic liquids obtained from the 1-butyl-3-methylimidazolium and 1-methoxyethyl-3-methylimidazolium cations combined with the tetrafluoroborate and dicyanamide counter anions, by measuring the total production of CO_2 over six months, according to ASTM D 5988-96. The results indicate that the biodegradability rate ranges between 17 and 52% for 1-butyl-3-methylimidazolium and between 0.1 and 3.6% for 1-methoxyethyl-3-methylimidazolium, with dicyanamide and tetrafluoroborate anions respectively. In both cases the biodegradation rate did not exceed 10% in 28 days of the test duration.

Peric et al. (unpublished results) have performed other type of studies of biodegradability of ILs in soil, according to test No. 217, Soil microorganisms: Carbon transformation [63], using three ILs of the new family of PILs, (hydroxyethylammonium formate, dihydroxyethylammonium propionate, and trihydroxyethylammonium pentanoate). The results indicate that the biodegradation rate ranges between 60 and 90%, with the most complex ILs showing the lowest biodegradation rate. The ILs from the new family of PILs are clearly less toxic than the ILs derived from imidazolium, pyridinium, pyrrolidium, etc.; and also less toxic than the conventional solvents, with a notable rate of biodegradation in water and soil. This can be a path to follow

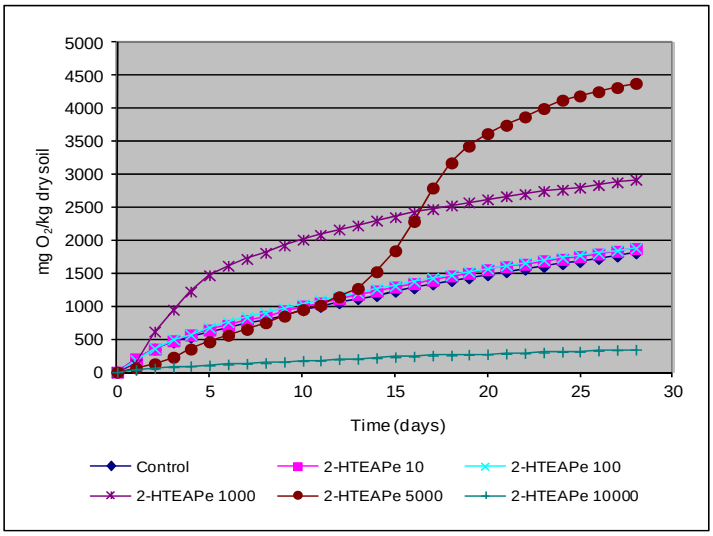

Figure 6. Cumulative consumed oxygen of different concentrations of trihydroxyethylammonium pentanoate (2-HTEAPe) compared to the control soil, according to OCDE test 217 [63], to observe inhibitory effect (10,000 mg kg^{-1}) and the biodegradation (1,000/5,000 mg kg^{-1}) of IL. Data from the authors.

regarding a synthesis of really green ILs, formed by a pair of organic ions, with simple (short and lineal) structure and functional groups which facilitate biodegradation.

5. Conclusion

The large number of possible ILs structures and their unique physical, chemical and biological properties make it feasible to consider them as an opportunity to contribute to the greenness in the various fields in which they can be applied. Recent studies show that not all ILs synthesized to date are as green as expected. Some of the cyclic AILs, especially the ones derived from imidazolium and pyridinium with long side chain substituents, have proven to be even more toxic for aquatic organisms than the classical organic solvents that they are aiming to replace. However, the constant synthesis of new ILs presents an opportunity for the design of compounds which can comply with the most demanding technical requisites while presenting low levels of ecotoxicity and high biodegradability. A good example of this is the new familiy of PILs based on ammonium substituted organic salts.

Acknowledgements

The authors wish to express their appreciation to Dr. Miguel Iglesias and Dr. Rafael González Olmos and their research group from University of Santiago de Compostela and University of Girona for providing samples of

ionic liquids synthesized and characterized by them. B. Peric would like to thank the Spanish Ministry of Foreign Affairs and Cooperation and the Spanish Agency for International Cooperation for Development (Grant MAEC-AECID II.A).

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