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Review Article

Ionic Liquids and their Toxicity on the Enzyme Activity and Stability

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HIGHLIGHTS

- Conventional organic solvents can be replaced by ionic liquids as green solvents.
- Ionic liquids are used as additives, catalysts, or reaction media in industries.
- Advantages and disadvantages of ionic liquids are discussed.
- Potential environmental hazards linked to application of ionic liquids are highlighted.
- The environmental fate needs to be considered in designing safer ionic liquids.

ABSTRACT

Molecular interactions are crucial between the enzyme molecules and the surrounding solution in an enzymatic catalysis. Although aqueous solutions used as conventional enzymatic reaction media, non-aqueous enzymology emerges as a major area of biotechnology research and development. Ionic liquids, as new generation of promising alternatives to traditional organic solvents, possess potential industrial enzymatic applications. Enzymes in ionic liquids present enhanced activity, stability, and selectivity. In addition, the potential of ionic liquids in bio-catalysis is raised by high ability of dissolving a wide variety of substrates and their extensively tunable solvent properties through appropriate modification of the cations and anions. However, despite the bio-friendly nature of ionic liquids for enzymatic reactions, their growing interests increase concerns associated with toxicity and environmental pollution of such compounds. This mini-review presents a brief highlight of the contemporary knowledge of enzymes activity and stability in ionic liquids and the environmental influences regarding the potential risks related to the growing applications of these green solvents.

Introduction

Enzymes catalyze chemical reactions with high specificity and increased rate. These reactions are the biochemical basis of metabolism in all living organisms and provide tremendous opportunities for industry to perform efficient and environmental friendly bio-catalytic conversions (Kirk et al., 2002; Itoh and Hanefeld, 2017). Enzymes have been used for many years in the production of foods and beverages (cheese, sourdough, beer, wine, and vinegar) and also the manufacture of commodities (leather, indigo and linen). Some biocatalysts represent high chemo-, regio-, and enantio-selectivity that make them attractive for production of optically pure chemicals in pharmaceutical industries (Choi et al., 2015). The majority of currently used industrial enzymes are hydrolytic applied for the degradation of various natural substances. Proteases have been remained the dominant enzymes because of

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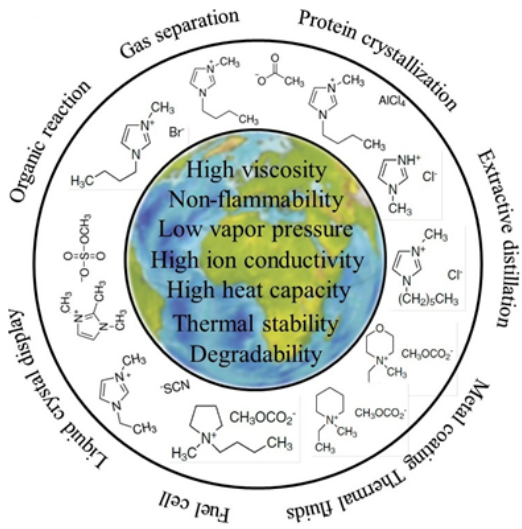


Figure 1. Main properties and applications of ionic liquids.

their extensive uses in the detergent and dairy industries (Sawant and Nagendran, 2014). The second largest group of industrial enzymes is various carbohydrases, primarily amylases, and cellulases applied in starch, textile, detergent, and baking industries (Contesini et al., 2013; Sundarram and Murthy, 2014). Over the past two decades, the employing of biocatalysts for organic synthesis has become an increasingly attractive alternative to conventional chemical approaches. Biocatalysts operate under mild conditions and minimize undesired side-reactions such as decomposition, isomerization, racemization, and rearrangement. Although water is known as the conventional medium for enzymatic reactions and enzymes require a certain level of water in their structures to maintain natural conformation, enzyme applications in organic synthesis are restricted by some disadvantages such as limited water solubility of organic substrates (Carrea and Riva, 2002; Stepankova et al., 2013). Various methods established to raise the solubility of organic compounds in enzyme catalyzed organic reactions such as using surfactants, substitution, and derivatization. Some approaches based on organic solvents were widely adopted to increase the solubility of lipophilic substrates including the use of mixture of water and water-miscible organic solvents, and biphasic systems consist of water and water-immiscible organic solvents. However, most of the solvent market in industry brings environment and health concerns due to their toxicity related to hydrophobicity, as expressed by the logarithm of the partition coefficient of the solvent in octanol and water (Table 1) (Leo et al., 1975; Laane et al., 1987; Quijano et al., 2011). Therefore, development of green technologies devoted to design ionic liquids as a promising alternative for traditional environmentally harmful organic solvents because of their unique properties such as low vapor pressure, ability to dissolve broad range materials, thermal stability, and non-flammability (Fig. 1). The technological utility of enzymes can be enhanced greatly by their use in ionic liquids rather than conventional organic solvents or their natural aqueous reaction media. Advantages of applying ionic liquids over the use of organic solvents as reaction medium for bio-catalysis also include their high ability of dissolving a wide variety of substrates, especially those highly polar ones, and their widely tunable solvent properties through appropriate modification of the cations and anions (Moniruzzaman et al., 2010; Jafari et al., 2017). This mini-review presents a brief overview of physical and chemical properties of ionic liquids, their effects on enzyme performance such as activity, stability, and selectivity, applications in bio-catalysis, environmental influences concerned the potential risks associated to the growing uses of ionic liquids, and highlights developing anxieties.

Aqueous solution as the traditional environment of proteins and enzymes

Water is often considered as the best solvent for enzymatic reactions. Interactions between an enzyme molecule and the surrounding water (hydration) are of crucial significance for enzymatic catalysis. Water act as a lubricant or plasticizer that allows enzymes to exhibit the conformational mobility required for optimal catalysis. For example, hydrophobic interactions that result from the peculiar structuring of water near hydrophobic amino acids provide thermodynamic stability to folded

Table 1. Log *P* of commonly used organic solvents (Leo et al., 1975; Laane et al., 1987; Quijano et al., 2011).

No.	Solvent	Log *P*	No.	Solvent	Log *P*	No.	Solvent	Log *P*
1	Dimethylsulfoxide	-1.3	31	Methylbutylamine	1.2	61	Pentylacetate	2.2
2	Dioxane	-1.1	32	Propylacetate	1.2	62	Isobutylene	2.3
3	N,N-Dimethylformamide	-1.0	33	Ethylchloride	1.3	63	Propane	2.3
4	Methanol	-0.76	34	Pentanol	1.3	64	Dimethylphthalate	2.3
5	Acetonitrile	-0.33	35	Hexanone	1.3	65	Octanone	2.4
6	Ethanol	-0.24	36	Benzylformate	1.3	66	Heptanol	2.4
7	Acetone	-0.23	37	Phenylethanol	1.4	67	Toluene	2.5
8	Acetic acid	-0.23	38	Cyclohexanol	1.5	68	Ethylbenzoate	2.6
9	Ethoxyethanol	-0.22	39	1,2-Dichloroethane	1.5	69	Ethoxybenzene	2.6
10	2-Propanole	0.07	40	Methylcyclohexanone	1.5	70	Dibutylamine	2.7
11	Dimethyl ether	0.10	41	Phenol	1.5	71	Pentylpropionate	2.7
12	Methylacetate	0.16	42	*m*-Phthalic acid	1.5	72	Chlorobenzene	2.8
13	Propanol	0.28	43	Triethylamine	1.6	73	Cyclohexene	2.8
14	Propionic acid	0.29	44	Benzylacetate	1.6	74	Octanol	2.9
15	Butanone	0.29	45	Butylacetate	1.7	75	Nonanone	2.9
16	Trifluoroacetic acid	0.36	46	Chloropropane	1.8	76	Dibutylether	2.9
17	Hydroxybenzylethanol	0.40	47	Acetophenone	1.8	77	Butane	2.9
18	Tetrahydrofuran	0.49	48	Hexanol	1.8	78	Styrene	3.0
19	Tetrahydrofuran	0.52	49	Nitrobenzene	1.8	79	Tetrachloromethane	3.0
20	Diethylamine	0.64	50	Heptanone	1.8	80	Pentane	3.0
21	Ethylacetate	0.68	51	Benzoic acid	1.9	81	Cyclopentane	3.0
22	Pyridine	0.71	52	Dipropylether	1.9	82	Ethylbenzene	3.1
23	Butanol	0.8	53	Hexanoic acid	1.9	83	Xylene	3.1
24	Pentanone	0.8	54	Chloroform	2.0	84	Neopentane	3.1
25	Butyric acid	0.81	55	1,3-Butadiene	2.0	85	Cyclohexane	3.2
26	Diethylether	0.85	56	Benzene	2.0	86	Benzophenone	3.2
27	Benzylethanol	0.9	57	Methylcyclohexanol	2.0	87	Propoxybenzene	3.2
28	Cyclohexanone	0.96	58	Methoxybenzene	2.1	88	Diethylphthalate	3.3
29	Methylpropionate	0.97	59	Methylbenzoate	2.2	89	Pentane	3.4
30	Dihydroxybenzene	1.0	60	Propylbutylamine	2.2	90	Nonanol	3.4
91	Decanone	3.4	101	*p*-Cymene	4.1	111	Undecane	6.1
92	Hexane	3.5	102	Pentylbenzoate	4.2	112	Dipentylphthalate	6.5
93	Propylbenzene	3.6	103	Diphenylether	4.3	113	Dodecane	6.6
94	Butylbenzoate	3.7	104	Octane	4.5	114	Dihexylphthalate	7.5
95	Methylcyclohexane	3.7	105	Undecanol	4.5	115	Tetradecane	7.6
96	Ethyloctanoate	3.8	106	Ethyldecanoate	4.9	116	Hexadecane	8.8
97	Dipentylether	3.9	107	Dodecanol	5.0	117	Dioctylphthalate	9.6
98	Benzylbenzoate	3.9	108	Nonane	5.1	118	Butyloleate	9.8
99	Decanol	4.0	109	Dibutylphthalate	5.4	119	Didecylphthalate	11.7
100	Heptane	4.0	110	Decane	5.6	120	Dilaurylphthalate	13.7

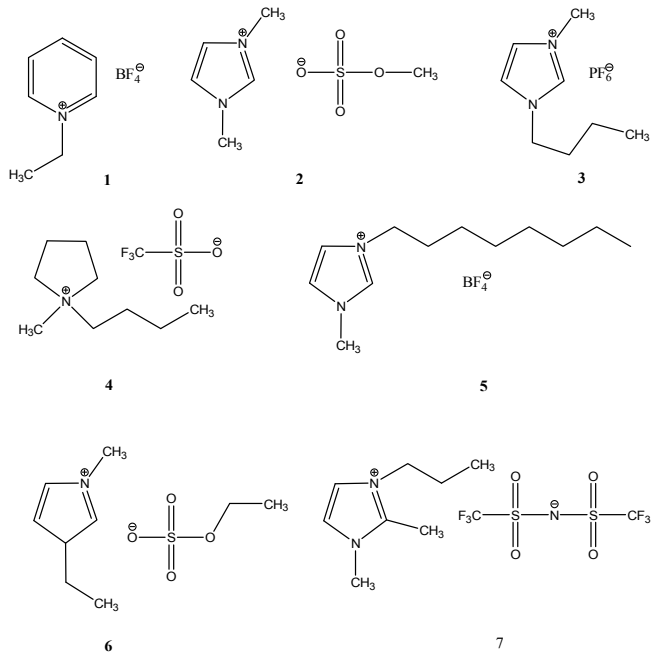


Figure 2. Chemical structures of some ionic liquids. Each one composed of two parts including a cation and an anion. (1)1-ethylpyridinium trifluoroacetate, (2)1,3-dimethylimidazolium methyl sulfate, (3)1-butyl-3-methylimidazolium hexafluorophosphate, (4)1-butyl-1-methylpyrrolidinium trifluoromethanesulfonate, (5)1-methyl-3-octylimidazolium tetrafluoroborate, (6)1-Ethyl-3-methylimidazolium ethyl sulfate, (7)1, 2-dimethyl-3-propylimidazolium bis(trifluoromethylsulfonyl)imide.

protein structures in aqueous solution. In addition, to function of water as solvent, these molecules can mediate enzymatic catalysis either directly by participating in the reaction or indirectly through providing a solvation medium for reactants, transition state, and products. Enzyme catalysis extremely depends on the pH of aqueous solution. However, the flexibility of the enzyme is caused by water and results in high catalytic activity. Elimination of major amount of water from the surroundings of the enzyme can cause pronounced destabilization compared to the situation in aqueous solution.

Proteins and enzymes in organic solvents

Enzymes are being increasingly exploited for asymmetric synthetic transformations and optically pure pharmaceuticals. The use of enzymes is restricted to their natural aqueous reaction media by some vital considerations such as insolubility of substrates in water, unwanted side reactions, and degradation of organic reagents in aqueous media (Klibanov, 2001). In addition, thermodynamic equilibria of some processes are unfavorable in water, and product recovery is sometimes difficult from this medium. In principle, most of these problems might be overcome by switching from water to organic solvents as the reaction media (Klibanov, 1997). At first sight, this substitution would seem impossible in the light of the conventional idea that enzymes lose their native structure and thus catalytic activity in organic solvents. Enzymes are very rigid in the absence of water which acts as a molecular lubricant. Although in aqueous-organic mixtures protein molecules have both proclivity to denature and sufficient conformational flexibility to do so, in dry solvents their drive to unfold is greater but the pliability necessary to proceed is lacking (Stepankova et al., 2013). As a result, various crystalline enzymes essentially retain their native structures even in anhydrous organic solvents. The nature of the organic solvent and amount of water in the reaction medium are two fundamental factors that determine the behavior of enzymes in water-restricted environments. Water is absolutely essential for enzymatic activity. Indeed, it participates in all noncovalent interactions maintaining the protein in its native conformation and plays a crucial role in enzyme dynamics (Sheldon and van Pelt, 2013). Organic solvents affect an enzymatic reaction by influencing the distribution of water between an enzyme and a reaction medium. In addition, an organic solvent may directly interact with the enzyme and change the native catalytically active conformation leading to decrease of enzyme activity. Partitioning of substrates and products between the active site of an enzyme

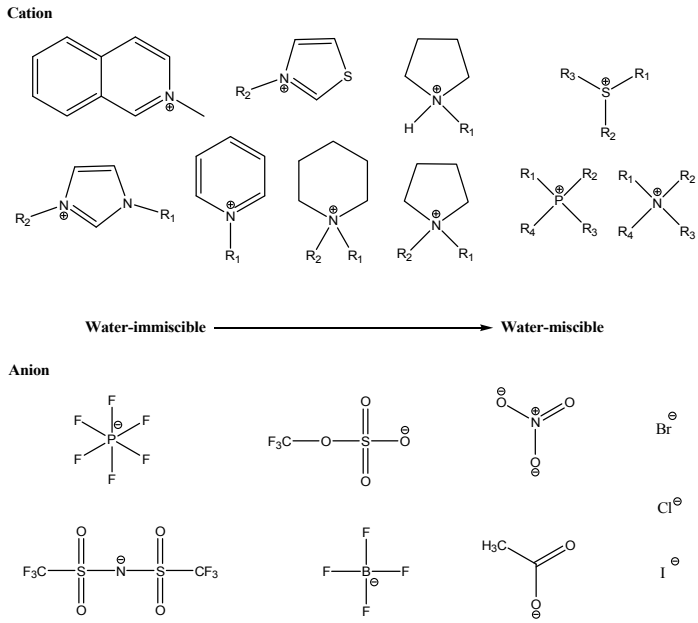


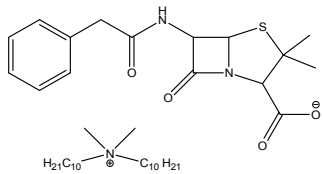
Figure 3. Most commonly used cations and anions for synthesis of ionic liquids.

and medium may influence a number of kinetic and thermodynamic parameters of the process (Jesionowski et al., 2014). The resulting effect of an organic medium on an enzymatic process is most often a combination of the above factors. The ability of organic solvents to affect and often enhance the potential application of enzymes offers strategies for creating improved biocatalysts that sit alongside such techniques as site-directed mutagenesis, phage display, directed evolution, and the production of catalytic antibodies.

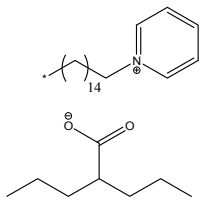
Structure and features of ionic liquids

In general, the ″ionic liquids″ stands for liquids composed of an asymmetric cation and an anion of weak coordination properties that are usually molten below 100 °C (Fig. 2). Ionic liquids have diverse range of physical and chemical properties depending on their cations and anions such as polarity, hydrophobicity, and viscosity which play important roles in affecting the activity and stability of proteins (Fig. 3) (Sivapragasam et al., 2016; Rezaei et al., 2017a). Ionic liquids are considered as highly polar solvents based on their ionic nature and strong intermolecular forces between anion and cation molecules cause high viscosity which affect stirring, mixing, and pumping operations (Liszka et al., 2016, Rezaei et al., 2017b). As ion liquids are designed solvents composed of at least two anionic and cationic components, the solvents can be designed to show a specific set of properties (Kaar, 2017). Ethylammonium nitrate as the first low melting salt (12 °C) was synthesized in 1914 and low melting point ionic liquids were proposed as medium of reaction or catalysts (Fry et al., 1985; Boon et al., 1986). In addition, ionic liquids with unique corresponding change in physical properties by structural modifications of cation or anion leads to design optimized solvent system for specific processes. Based on the chemical structure of ionic liquids, cations are usually variously substituted bulky organic molecules containing a positively charged nitrogen, sulfur, or phosphor atom and inorganic or organic species such as halides, tetrafluoroborate, hexafluorophosphate, bis(trifluoromethylsulfonyl) imide, acetate, and dicyanamide (Pham et al., 2010).

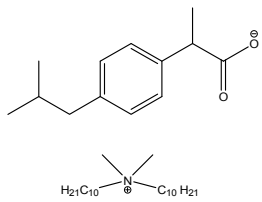
While the fabrication of biopolymer based composites suffers from their difficulty of dissolving due to highly crystalline nature of such compounds, ionic liquids with a synthetic flexibility by varying the combinations of cation and anion possess great potential to dissolve biopolymers such as cellulose, chitin, chitosan, silk, and gelatin. Biopolymer containing composites prepared by using ionic liquid mixtures are applied for the various biomedical applications such as implantable devices, tissue engineering scaffolds, drug delivery, and wound dressing. Recently, enzymatic transformations of amino acid derivatives have benefited from the use of ionic liquids as catalyst or reaction media to increase the yield, enantiomeric excess, or stability and recycling of the enzyme (Mogharabi and Faramarzi, 2014). For example,



Didecyldimethylammonium penicilline G



Hexadecylpyridinium valproate



Didecyldimethylammonium ibuprofen

Figure 4. Chemical structure of some ionic liquids containing a bioactive part, such as antibiotics, antiepileptics, and non-steroidal anti-inflammatory substances.

the ionic liquid 1-ethylpyridinium trifluoroacetate (1) applied as a catalyst for the esterification of amino acids. Rogers et al. (2007) patented a method for the preparation of active pharmaceutical including antibiotic, non-steroidal anti-inflammatory agents, and an antiepileptic agent as ionic liquids (Fig. 4). Ionic liquids possess different industrial applications such as catalysis, extraction, organic synthesis, dissolution, batteries, thermoresponsive materials, pharmaceutical products, nuclear industry, and food industry; which may produce enormous amount of ionic liquid containing wastes leading to accumulation in soil or reach to groundwater with potential threats to the human health, aquatic environment, and eco-systems (Zhao et al., 2007; Bubalo et al., 2014).

Unlike traditional solvents, which can be described as molecular liquids, ionic liquids are composed of ions. Ionic liquids have low melting points and remain as liquids within a broad range of temperature. One of the most special properties for ionic liquids is their high polarity. On the normalized polarity scale setting tetramethylsilane at 0.0 and water at 1.0, the polarity of common ionic liquids normally falls in the range of 0.6–0.7, similar to that of lower alcohols and formamide (Carmichael and Seddon, 2000). A correlation is observed between the decrease in both the chain length of the alkyl substituents on the imidazolium ring of the cation and the anion size with an increase in polarity (Dzyuba and Bartsch, 2002). Because of the high polarity, ionic liquids present an ideal reaction media for chemical and biochemical reactions due to their ability to dissolve a wide range of different substances including polar and nonpolar organic, inorganic, and polymeric compounds. Despite of their high polarity, most of ionic liquids are hydrophobic and can dissolve up to 1% of water, and the presence of water may affect the physical properties of the ionic liquids. Ionic liquids present a higher viscosity with longer alkyl chains on the cation and a larger anion size (Aki et al., 2001). One obvious advantage of using ionic liquids over the use of normal organic solvents is that the physical and chemical properties of the ionic liquids, including their polarity, hydrophobicity, viscosity, and solvent miscibility, can be finely tuned by altering the cation, anion, and attached substituents. Manipulating the solvent properties allow to design an ionic liquid for specific reaction conditions, such as to increase the substrate solubility, to modify the enzyme selectivity, or to tailor the reaction rate.

Ionic liquids from amino acids

Ionic liquids as polar aprotic solvents are suitable solvents for dissolving very polar molecules such as amino acids, small peptides, and proteins. Most of these alternative solvents are immiscible with water and various reactions involving amino acid derivatives are performed successfully in ionic liquid containing media (Chen et al., 2008). In addition, amino acids represent a powerful class of starting materials for synthesis of new chiral ionic liquids because of their availability in both enantiomeric forms at a reasonable cost. Although asymmetric synthesis and using chiral starting materials are known as two main strategies to prepare chiral ionic liquids but using chiral substrates is the most convenient approach (Kirchhecker and Esposito, 2016). Several groups have selected this methodology to construct the chiral anion, the chiral cation, or both in the ionic liquid. However, chiral ionic liquids are constructed without modification of the amino acid residue, with modification of the side chain and preservation of the amino acid moiety, with alteration of one function (acidic or basic), or with polyfunctional modification of both amine and acid functions (Plaquevent et al., 2008). Amino acids are used to design chiral anions or chiral cations by deprotonation of the carboxylic acid or protonation of the amino group using a suitable Bronsted base or acid, respectively. The required properties of amino acid derived chiral ionic liquids such as the viscosity or the melting point

are defined by protection of functions in the amino acid derivatives (Plaquevent et al., 2008). Fukumoto et al. (2005) reported the synthesis of ionic liquids from a series of twenty common amino acids. They showed that it is possible to predict the glass transition temperature, the ionic conductivity, and the miscibility with organic solvents based on the structure of the side chains on the component ions (Fukumoto et al. 2005). Fukumoto and Ohno (2006) reported the synthesis of hydrophobic ionic liquids composed of tetrabutylphosphonium cation and chiral anions derived from alanine and isoleucine modified with trifluoromethane sulfonyl groups using a simple straightforward method. Natural amino acids, readily available at low cost, offer an interesting molecular diversity to provide new opportunities for the design and synthesis of chiral ionic liquids.

Functionality and stability of proteins in ionic liquids

The tremendous potential of room temperature ionic liquids is well recognized as an alternative to environmentally harmful ordinary organic solvents. The use of enzymes in ionic liquids presents some advantages such as high conversion rates, high selectivity, and improved enzyme stability and activity (Rantwijk and Sheldon, 2007). Several factors seem to be responsible for the enzyme activity and stability including ionic character, polarity, hydrogen bonding, basicity, and anion nucleophilicity (Kragl et al., 2002). However, there is no simple correlation between a parameter and the enzyme activity. Therefore, the current understanding of the influence of ionic liquids on enzyme activity is still in its infancy (Park and Kazlauskas, 2003). Enzymes showed high stabilities in hydrophobic ionic liquids in a number of applications that is explained by a lesser tendency of hydrophobic solvents to take away the essential water from the enzyme surface. Hydrophilic dissolved Ionic liquids in aqueous media are dissociated into anions and cations and the ions stabilize enzymes according to Hofmeister series. Generally, kosmotropic anions and chaotropic cations stabilize proteins while chaotropic anions and kosmotropic cations lead to their destabilization (Moniruzzaman et al., 2010; Naushad et al., 2012). β-Galactosidase from *Bacillus circulans* catalyzes the synthesis of N-acetyllactosamine starting from lactose and N-acetylglucosamine in a transglycoslyation reaction. The addition of 25% v/v of 1,3-dimethylimidazolium methyl sulfate (2) as a water miscible ionic liquid increased the secondary hydrolysis of the product (Kaftzik et al., 2002). The stability of the esterase from *Bacillus stearothermophilus* at 40 °C was considerably increased in the presence of ionic liquids 1-butyl-3-methylimidazolium hexafluorophosphate (3) and 1-butyl-3-methylimidazolium tetrafluoroborate for the enzymatic transesterification of 1-phenylethanol (Persson and Bornscheuer, 2003). Four ionic liquids containing dialkylimidazolium cations and perfluorinated and bis(trifluoromethylsulfonyl) amide anions were used as reaction media for butyl butyrate synthesis catalyzed by *Candida antarctica* lipase B. Lipase activity enhanced in ionic liquids in comparison with two organic solvents hexane and 1-butanol (Lozano et al., 2001).

Toxicity of ionic liquids

Although ionic liquids with unique properties show extensive technical and commercial potentials and generally are considered as nontoxic solvents, some recent researches have reported their toxicity to microbes, aquatic organisms, and mammalian cell lines, even higher than that of traditional organic solvents (Table 2). Preliminary toxicological investigations of ionic liquids have shown inhibitory effects by increasing the length of alkyl chain substituted in the pyridinium, imidazolium, and quaternary ammonium salts to several bacteria such as *Bacillus cereus*, *B. subtilis*, *E. coli*, *Pichia pastoris*, *Pseudomonas fluorescens*, *Saccharomyces cerevisiae*, *Staphylococcus aureus*, and *Vibro fischeri* (Docherty et al., 2005). Antibacterial and anticorrosion impacts of 1-butyl-1-methylpyrrolidinium trifluoromethanesulfonate (4) were used a dual function inhibitor for the planktonic bacterial population growth and the corrosion of steel in 3.5% NaCl solution (El-Shamy et al., 2015). Chronic toxicity effects of this compound on the microalga, *Scenedesmus quadricauda* showed inhibition of both esterase activity and chlorophyll fluorescence biosynthesis as a major mechanism of toxicity. In addition, cell density was also decreased by culturing using ionic liquid which was clearly concentration-dependent (Deng et al., 2015). Marine mussel *Mytilus galloprovincialis* exposed to various concentrations of two commonly used imidazolium ionic liquids, 1-methyl-3-octylimidazolium tetrafluoroborate (5) and 1-butyl-3-methylimidazolium tetrafluoroborate (3) revealed oxidative and genotoxic effects related to the alkyl chain length and the lipophilicity of ionic liquids (Tsarpali et al., 2015). The ionic liquid 1-octyl-3-methylimidazolium hexafluorophosphate inhibited activities of catalase and superoxide dismutase, decreased levels of reactive oxygen species (ROS), and caused DNA damage in zebrafish (*Danio rerio*) (Du et al., 2012). Comparison of synergistic toxicity of heavy metals including Cd (II), Ni (II), Cu (II), and Zn (II) and six ionic liquids on photobacterium Q67 indicated greater toxicity of ionic liquids (Ge et al., 2014). Enzymatic inhibition assays provides a model for the assessment of adverse effect of ionic liquids on human health; acetylcholinesterase and carboxylesterases known as valuable biomarkers of toxicity assessment (Fig. 5). The quantification of the inhibitory effects of ionic liquids by calculating the IC_{50} (inhibitor concentration

Table 2. Toxicity (IC_{50}, μmol L^{-1}) of some commercially available ionic liquids on different biological systems.

Biological system		Ionic liquids							Reference
		1-Butyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide	1-Butyl-3-methylimidazolium bromide	1-Butyl-3-methylimidazolium chloride	1-Butyl-3-methylimidazolium tetrafluoroborate	1-Ethyl-3-methylimidazolium chloride	1-Ethyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide	1-Hexyl-3-methylimidazolium chloride	
Bacteria	*E. coli*	2000	-	207500	15700	261800	-	-	Łuczak et al., 2010
	V. fischeri	339	3359	2500	3500	21000	844	-	Matzke et al., 2007; Steudte et al., 2012
	Enterococcus sp.	-	-	207500	15600	261800	-	-	Łuczak et al., 2010
	S. aureus	-	-	207500	15700	-	-	-	Łuczak et al., 2010
Fungi	*C. tropicalis*	-	-	207500	62500	261800	-	15600	Łuczak et al., 2010
	C. albicans	2000	-	-	-	-	-	-	Łuczak et al., 2010
	S. cerevisiae	-	-	207500	31250	261800	-	15600	Łuczak et al., 2010
Algae	*S. capricornutum*	63	2138	2884	-	600	170	-	Steudte et al., 2012; Cho et al., 2008
	C. vulgaris	-	-	1026	-	6330	-	-	Latała et al., 2009a; Latała et al., 2009b
	B. paxillifer	-	-	6.48	-	34.4	-	-	Latała et al., 2009b
Enzymes	*Acetylcholinesterase*	90	80	81	540	120	110	-	Matzke et al., 2007; Arning et al., 2008
	Luciferase	-	-	123000	-	150000	-	-	Ge et al., 2010
Invertebrates	*D. Magna*	45	70	85	53	-	230	-	Yu et al., 2009
	D. polymorpha	-	5887	-	-	-	-	-	Costello et al., 2009
	P. acuta	-	1045	-	-	-	-	-	Bernot et al., 2005
Cell cultures	*CaCo-2*	-	-	28690	6026	-	-	-	García-Lorenzo et al., 2008
	IPC-81	500	2692	3600	1318	-	-	-	Ranke et al., 2007; Stepnowski et al., 2004
	Hela	1170	2750	12300	4550	-	-	-	Stepnowski et al., 2004

required to cause 50% of inhibition) demonstrated that the cetylpyridinium group is a very toxic cation (Costa et al., 2014). Recently, the molecular and biochemical mechanism of ionic liquids cytotoxicity investigated on the human hepatocellular carcinoma (HepG2) cells by using 1-methyl-3-octylimidazolium bromide. The evaluation of cell viability, oxidative stress, apoptosis, caspase activity, and apoptosis-related gene expression

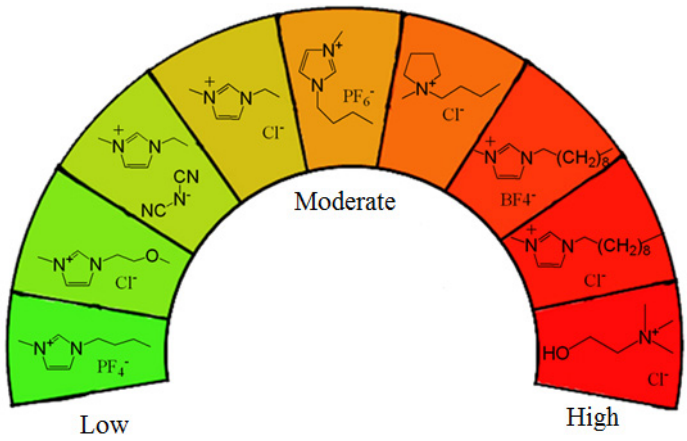


Figure 5. Relative toxicity of ionic liquids toward acetylcholinestrase.

in ionic liquid exposed HepG2 cells showed that their viability was reduced by a decrease in the concentration of ionic liquid. In addition, biochemical assays demonstrated that exposure of 1-methyl-3-octylimidazolium bromide induces apoptosis, causes overproduction of ROS, inhibits catalase and superoxide dismutase, decreases glutathione content, and increase the cellular malondialdehyde level of HepG2 cells. Caspase-3, caspase-8, and caspase-9 were activated in HepG2 cells and showed an important function in the initiation and execution apoptosis of HepG2 cells (Li et al., 2015). While functionalization of ionic liquids with natural amino acids applied as a convenient method to decrease their toxicity, Egorova et al. (2015) reported that exposure to 1-butyl-3-methylimidazolium and 1-butyl-3-methylimidazolium chloride can induces apoptosis in NIH/3T3 cells. Because of the relative stability features of ionic liquids, their adverse effects on the environmental health becomes very important and it is suggested that chemical researchers should cooperate by toxicologists to develop green solvents with the purpose of perfecting the bio-renewable and bio-degradable of the new generation of ionic liquids.

Conclusion

From the studies done so far, ionic liquids having hydrophobic nature, less viscosity, kosmotropic anion, and chaotropic cation usually enhances the activity and stability of enzymes. However, a general correlation could not be established because of many contradictory results. Overall, the information in present review could be helpful for the researchers to choose or design compatible ionic liquids to serve as solvent for enzymatic reaction and protein preservation. Furthermore, information regarding structural and conformational dynamics of proteins could be useful for engineering and scientific communities to understand how ionic liquids enhance the stability and activity of enzymes.

Competing Interests

The authors declared no conflict of interest.

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