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Chapter 20

New Generations of Ionic Liquids Applied to Enzymatic Biocatalysis

Ana P.M. Tavares, Oscar Rodríguez and
Eugénia A. Macedo

Additional information is available at the end of the chapter

1. Introduction

Ionic liquids are salts in a liquid state, combinations of cations and anions that are liquid at temperatures below 100 ºC. Thus, they have been called Room-Temperature Ionic Liquids (RTILs, or just ILs) in order to differentiate them from traditional salts, which melt at much higher temperatures and receive the name of "molten salts". In contrast to conventional organic solvents, ILs usually have extremely low volatility. Indeed, vapor pressures for ILs are scarce in the literature exactly because they are extremely low (< 1 Pa) and have to be obtained at high temperatures (400-500 K) [1]. For this "negligible" vapor pressure, ILs are often said to be "green" solvents when compared to traditional, environmentally harmful volatile organic compounds (VOCs). A big goal in the use of ILs in enzymatic reactions is the replacement of VOCs by ILs. In addition, ILs have other potential advantageous properties such as reasonable thermal stability; ability to dissolve a wide range of organic, inorganic and organometallic compounds; controlled miscibility with organic solvents (which is relevant for applications in biphasic systems) among others. All these properties make them very attractive non-aqueous solvents for biocatalysis. As they have been extensively described, ILs offer new possibilities for the application of solvent engineering to enzymatic reactions. Biocatalysis with ILs as reaction medium was first showed in the beginning of 2000 [2-4]. During the last decade, ILs have fast increased their attention as reaction media for enzymes with some remarkable results [2-4]. The advantage of using ILs in enzymatic biocatalysis, as compared to VOCs, is the enhancement in the solubility of substrates or products without inactivation of the enzymes, high conversion rates and high activity and stability [5]. ILs are also being used as co-solvents in aqueous biocatalytic reactions, since ILs help to

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dissolve nonpolar substrates, while avoiding enzyme inactivation like water-miscible organic solvents, as DMSO or acetonitrile, often do [6].

Another mentioned characteristic of ILs is the possibility of obtaining the desired physicochemical properties by selecting combinations of cations and anions ("tunability"), which makes them "designer solvents". For example, ILs can be produced to be water-miscible, partially miscible or totally immiscible, and can also be synthesized with different viscosities. These interesting properties make them a very important reaction media for enzyme stabilization and reaction. The use of organic solvents in bioprocess presents a number of further problems. The main concerns are the toxicity of the organic solvents to both the process operators and the environment (eco-toxicity), and also the volatile and flammable nature of these solvents, which make them a potential explosion hazard [7]. Thus, ILs have emerged as a potential replacement for organic solvents in biocatalytic processes at both laboratory and industrial scale. The negligible vapor pressure means that they emit no volatile compounds, and also introduces the additional possibility of removal of products by distillation without further contamination by the solvent. It also facilitates the recycling of ILs, decreasing operation costs. All these properties make ILs very important for the stabilization and activation of enzymes; therefore, numerous enzymatic reactions have been investigated in different types of ILs as will be shown in the next sections. Several topics about biocatalysis in ILs will be reviewed: their effect on the activity and stability of enzymes, toxicity of ILs, new generation of ILs and methods to stabilize enzymes will be discussed.

2. Enzymatic activity and stability in ionic liquids

The most important criteria for selecting an enzyme-IL system are the activity and stability of the enzyme within the reaction medium. ILs have been reported to be an appropriated medium to increase the stability and activity of enzymes, as opposed to common organic solvents [8-11]. However, depending on the enzyme nature, the IL can be or not suitable for the reaction [12].

2.1. Lipases

Lipases, and *Candida Antarctica* Lipase B (CALB) in particular, are the most studied enzymes in ILs. Most of these reactions in ILs are carried out with, no or low content of water as co-solvent. Therefore, hydrophobic ILs are used and the enzyme activity and stability is dependent on the IL. Several studies in the literature show that enzymes, majority lipases, exhibit greater stability in pure ILs than in traditional organic solvents [13, 14]. A review of Zhao 2005 [15] shows that ILs with larger cations are better for enzyme activity than smaller cations. The reasons for that are the longer hydrophobic alkyl chains in the cation presents less tendency to take away the essential water molecules from the enzyme. In fact, one of the most interest conclusions of Zhao is that hydrophobic ILs maintain lipase activity and stability better than hydrophilic ILs, as the latter will take water molecules away from enzyme structure. According to Diego et al. [16] the enzyme stabilization by water immiscible ILs

(such as $[(CF_3SO_2)_2N]^-$ types) can be explained by a more compact enzyme conformation/confinement formed from the evolution of α-helix to β-sheet secondary structure of the enzyme. On the other hand, hydrophobic ILs may decrease the stability and activity of the enzyme due to: (1) the interaction with the substrates or products, as organic solvents [17]; (2) interaction by electrostatic forces [18] and (3) removing essential water molecules from the enzyme [17]. Lau et al. [19] observed that enzyme activity in ILs was related with the conformation of enzyme; the hydrogen bonding could be the key to understanding the interactions of enzymes and ILs. Another work of Lozano et al. [20] showed that lipase and α-chymotrypsin were strongly stabilized in two ILs ([btma][NTf_2] and [emim][NTf_2]) due to the maintenance of the native structure of the enzymes, as observed by both fluorescence and circular dichroism spectroscopy.

2.2. Cellulases

ILs are also used in the pretreatment of cellulose hydrolysis by cellulase for production of biofuels and other products. However, cellulases can be inactivated in the presence of ILs, even when present at low concentrations. In order to explore these ILs abilities, it is important to find a compatible cellulose-IL system [10]. The IL must solubilize the lignocellulosic biomass and at the same time, keep the enzyme active. It was shown that pretreatment of cellulose with ILs such as [bmim][Cl], [mmim][Cl], and [HEMA] resulted in faster conversion to glucose and thermostability than hydrolysis with cellulose that was not pretreated [22, 23]. A similar behavior was found for cellulases from different sources with imidazolium-based ILs, which enhanced the enzyme and thermal stability [24]. The stability of cellulases from *Penicillium janthinellum* mutants was evaluated in 10-50% (v/v) of [bmim][Cl] and the enzymes were significantly stable in 10% (v/v) of IL [25]. Another work investigated the stability and activity of commercial cellulases in aqueous solutions of 1-ethyl-3-methylimidazolium acetate [emim][OAc]. Cellulases retained 77% of their original activity in 15% and 20% (w/v) of IL and presented an avicel (a model substrate for cellulose) conversion efficiency of 91% [26].

2.3. Oxidoreductases

Several oxidoreductases, such as laccase, peroxidase, chloroperoxidase, D-amino acid oxidase and alcohol dehydrogenases, have been reported as active enzymes in aqueous solution with ILs [27]. When compared to organic solvents, these enzymes are more active and stable in the presence of ILs [27].

Laccases and peroxidases are the most effective enzymes capable to catalyze the degradation of phenolic compounds. Phenolics such as hydroquinone, catechols, guaiacol, and 2,6-dimethoxyphenol are good substrates for these enzymes in either aqueous and non-aqueous media. Recent reports have been addressing the activity and stability of both enzymes in ILs [6, 28-31]. For example, laccase activity and stability was well maintained in the presence of several imidazolium-based ILs [31] such as [C_4mim][Cl], [emim][$MDEGSO_4$], [emim][$EtSO_4$] and [emim][$MeSO_3$] [6], but are inactivated in the presence of [C_{10}mim][Cl] [29]. Peroxidase

was also described to mantain its activitiy and stability in imidazolium-based ILs for concentrations up to 25 % v/v [30].

Alcohol dehydrogenases are enzymes that catalyze the reduction of ketones. Due to the vast field of aplication of alcohol dehydrogenases, the study of this enzyme in ILs is promising. A recent work presented the effect of 10 different ILs (with either imidazolium or ammonium cations) on the enzyme stability. Improved storage stabilities and improved enzyme activities were found in the most promising, ammonium-based, AMMOENG™ 101 IL [32]. Later, the same group [33] proved the feasibility of continuous production using the previously recommended IL, combined with product separation using a membrane bioreactor (the so-called process integration). Hussain and co-workers [34] showed that the use of 10% (v/v) [bmp][NTf_2] facilitated the conversion of ketone to the chiral alcohol. Dabirmanesh et al. [21], showed the influence of different imidazolium based ILs on the structure and stability of alcohol dehydrogenase and the results exhibited that the ILs could affected the enzyme stability, but not the tertiary structure, suggesting that the enzyme was reversibly inhibited.

There are only few reports investigating the enzyme activity of D-amino acid oxidase in ILs. This enzyme catalyzes the deamination of various d-amino acids into imino acids. The activity and stability of free and immobilized d-amino acid oxidase in five imidazolium ILs were evaluated, and the most promising ILs were [bmim][BF_4] and [mmim][$MMPO_4$]. Total conversion of substrate in presence of 20% [mmim][$MMPO_4$] was obtained [35].

3. Factors affecting enzymes in ionic liquids

The section before showed the stabilization and activation of enzymes in ILs. However, it is also very important to understand the factors affecting the enzymes activity and stability in IL media. It has been reported that enzyme reactions in ILs can be affected by several factors such as the water activity, pH, excipients and impurities [36]. Several properties of ILs have also been related to the activity and stability of enzymes. The most important include: polarity, hydrogen-bonding capacity, viscosity, kosmotropicity/chaotropicity and hydrophobicity, among others. It is clear from this set of properties that the type and strength of interactions ILs can establish with enzyme molecules will certainly influence their 3D structure. Such influence may produce or not changes in enzyme activity.

A few works have related the ILs polarity with the activity of enzymes. Lozano and coworkers [37] observed that in less polar IL, lower activities of α-chymotrypsin were obtained. The same behavior was obtained for lipase, the enzyme activity increased with the increase in IL polarity during the acetylation of racemic 1-phenylethanol with vinyl acetate [38] and for the synthesis of methylglucose fatty acid esters [39].

The negative effect of hydrogen-bonding on the enzyme activity in the presence of ILs can be associated with the anions effect and their action as hydrogen-bonding acceptors for the protein (lipase) [40]. Another work suggested a similar reasoning for the effect of anions: the decrease of lipase activity in [bmim][lactate] was caused by secondary structure changes of

the protein, due to hydrogen-bonding interactions between lactate anions and peptide chains [19]. However, due to the limited number of ILs and enzymes investigated, deeper studies are required for a better understanding of this interaction.

As the majority of ILs are viscous fluids, the mass transfer limitations should be considered when the reaction is rapid and the IL is relatively viscous. Many enzymatic reactions in pure ILs can be heterogeneous due to the low solubility of the enzymes in ILs. Some studies have reported that the activity of enzymes is dependent on the IL viscosity: Bose et al. [23] attributed the lower activity of cellulase to the high IL ([HEMA]) viscosity. Lozano et al. [37] indicated that the activity of α-chymotrypsin was dependent on the IL viscosity, and thus higher enzyme activities were observed in less viscous ILs. On the other hand, the work of Zhao et al. [41] suggested that IL viscosity was not directly related to the lipase activity, but mass transfer limitations. The high viscosity may reduce the reaction rate, however the IL structure was responsible for lipase stabilization. So the author concludes that IL viscosity could influence the enzymatic reaction rates, however it is not the principal factor for the enzyme stabilization. Basso et al. [42] suggested that in the reactions for amide synthesis by immobilized penicillin G amidase, the high viscosities of the ILs did not affect the initial rates. Concluding, the effect of IL viscosity can affect the reaction rate, but this behavior is not the same for all enzymatic reactions in ILs, specially when reaction rates are measured in equilibrium instead of kinetics [42].

The kosmotropicity/chaotropicity (Hofmeister series) is related with the effect of water structure (and thus, protein salting in/out). There are reports in the literature that try to correlate the ion kosmotropicity with the enzyme behavior in aqueous solutions of ILs [43-48]. The reviews by Zhao et al. [15] and by Yang [49] discuss the probable mechanisms of Hofmeister effects of ILs. Kosmotropic anions (PO_4^{3-}, CO_3^{2-}, SO_4^{2-}, …) and chaotropic cations (Cs^+, Rb^+, K^+, NH^{4+}, …) stabilize enzymes, while chaotropic anions (NO_3^-, I^-, BF_4^-, PF_6^-,...) and kosmotropic cations ($(C_4H_9)N^+$, $(C_3H_7)_4N^+$, $(C_2H_5)_4N^+$,…) destabilize it [15].

Attending to the solubility of ILs in water, they can be divided into hydrophobic (water immiscible) and hydrophilic (water miscible). Most often, water miscibility depends on the ILs anions rather than the cations [50]. The hydrophobicity in ILs is generally determined by the log *P* scale, based on the partition coefficient of ILs between 1-octanol and water [51]. The stablility of enzymes can also be related to the log *P*. Usually, enzymes are more stable in solvents with a larger log *P* (>3) [106]. Many works from literature have reported that for lipases, activity increases with the increase in the IL hydrophobicity [13, 51-55]. Nevertheless, this conclusion is in contradiction with the polarity effect mentioned at the beggining of the section (more polar ILs promote enzyme stability). In our opinion, the vast pool of ILs and enzymes, and the large differences in their chemical structure, make it very difficult to extract general trends and conclusions. Just as an example, several authors [37] have proved that hydrophobic ILs (thus, less polar) maintain better immobilized lipase activity in the pure IL (low water content). At the same time, our group has shown that laccase and peroxidase activities are best maintained in more polar (less hydrophobic, more hydrophilic) ILs, when used in aqueous solutions [6; 29]. Both statements are correct, because the reaction conditions are completely different: very low water content in the former study, and water

excess in the latter. Thus, the IL affinity for water will be dramatic at low water content, but not when there is plenty of water.

4. Green aspects of ionic liquids

The interest in the development of biocatalytic processes in ILs media is desired to obtain green technologies and unconventional properties to replace organic solvents (namely VOCs). ILs appear free of many problems associated with the use of VOCs due to their non-volatility, non-flammable character and both high thermal and chemical stability. However, the use of certain ILs raises some concerns regarding environmental impact, attending to their potential toxicity and biodegradability. As the use of ILs has been increasing in different fields from biology to electrochemistry, the assessment of their environmental, health and safety impact is highly required. In recent years, environmental aspects related to ILs have been strongly addressed, stating that many ILs commonly used cannot be regarded as 'green solvents'. In general, ILs used in biocatalysis have not been designed for biocompatibility and harmless. There are some recent reports showing that the ecotoxicity of alkylmethylimidazolium cations (the most used in biocatalysis) is undesirable, and ecotoxicity increases with the length of alkyl chains in cation [56-58]. Thus, for future applications it is necessary to improve the green aspects of ILs. These improvements are currently going on. The best examples are the choline-derived cations (which are based on food grade choline chloride) or imidazolium derivatives designed for biodegradability (*e.g.*, adding ether groups in the alkyl side chains) [59], and ILs based on amino acids [60-61]. It is expected that much improved and green ILs will become available soon. Currently, three different generations of ILs can be identified, as described below.

4.1. First generation of ionic liquids

The first IL known was ethylammonium nitrate, reported in 1914 by Walden [62], but attracted little interest. The first generation of ILs with widespread utilization was mainly composed of cations like dialkylimidazolium and alkylpyridinium derivatives, and anions like chloroaluminate and other metal halides which have been described as toxic and non-biodegradable [57]. The most common anions are chloroaluminate or other metal halide anions that react with water and thus are not suitable for biotransformations. This generation of ILs was also oxygen-sensitive [63] and can only be handled under inert-gas atmosphere due to the hygroscopic nature of $AlCl_3$ [64]. In the 1980s, Wilkes et al. started the extensive research on first generation ILs [65]. However, due to these limitations, the progress in their use was limited. For this reason, research was directed towards the synthesis of air- and water-stable ILs, the second generation of ILs.

4.2. Second generation of ionic liquids

After one decade the second generation of ILs [66] appeared. The water- and oxygen-reactive anions were replaced by halides (Cl^-, Br^-, I^-) or anions such as BF_4^-, PF_6^- and $C_6H_5CO_2$, which are stable to water and air. Cations such as dialkylimidazolium or alkylpyridinium were maintained, and ammonium and phosphonium were added. These ILs present interesting properties such as lower melting points, different solubilities in classic organic solvents, viscosities, etc. Due to these properties, the second generation attracted a great interest in various fields, and research in ILs experienced an important boost from the 1990's. The first reports of biocatalysis with ILs were published in the beginning of 2000´s [2, 4, 38, 67]. One of the disadvantages of these ILs is the high cost. According to Gorke et al. [66], the high costs are related to starting materials (namely fluorinated components) and purification of final product required in the preparation. The most important disadvantage of the second generation is the toxicity, which in general is similar to those of chlorinated and aromatic solvents [56]. However, this second generation of ILs attracted the attention of the wide scientific community and has been providing interesting and novel applications in differents areas. This generation of ILs is the most studied and a great number of applications in biocatalysis have been published. The activity, stability, kinetic and thermal stability of different enzymes such as oxidases, lipases or cellulases has been studied, and sinthesis of various products has been carried out.

4.3. Third generation of ionic liquids

The third generation of ILs (advanced ILs) is based on more hydrophobic and stable anions such as $[(CF_3SO_2)_2N^-]$, sugars, amino or organic acids, alkylsulfates, or alkylphosphates and cations such as choline. The cations and/or anions used are biodegradable, readily available, and present lower toxicities. Besides, a new class of solvent systems, called deep eutectic solvents (DES), is more hydrophilic than the second generation, and in general is water-miscible [66]. DES are mixtures of salts (in general they are not liquids at room temperature) such as choline chloride, and uncharged hydrogen bond donors such as amines, amides, alcohols, carboxylic acids, urea, or glycerol [28]. A typical example is the choline chloride/urea mixture, which produces a DES with a melting point of 12°C at concentrations around 50% [66]

The advantages of the third generation are: lower costs (similar to organic solvents), simple to prepare, biodegradable, do not require purification, the purity of the starting materials determines the final purity and uses anions and cations with low toxicity. As this generation is recent, few works have been published [68, 69]. The transesterification of ethyl valerate with 1-butanol, showed good activity in DES, and in choline chloride: glycine the activity was similar to activity in toluene for all lipases [69]. The third generation will reach the commercial level soon [70].

5. Methods for stabilization of enzymes in ionic liquids

Stabilization of enzymes in ILs is one of the keys for the development of more efficient biocatalytical processes for industrial, environmental, or biomedical applications. As discussed in previous sections, stabilization of enzymes in ILs is one of the keys for the development of more efficient biocatalytic processes for industrial, environmental, or biomedical applications. The use of enzymes in ILs presents different advantages when compared to conventional organic solvents. On the other hand, in some cases the application of enzymes can be limited by the low solubility, activity or stability in ILs. The improvement of enzyme functionality is crucial for large-scale applications in order to be economically viable. The methods to stabilize and activate enzymes in ILs can be divided into two different strategies: the modification of enzymes and/or the modification of the solvent (ILs). The modification of enzymes includes lyophilization (to change the morphology of the solid enzyme), chemical modification (for the chemical addition of functionalities into the enzyme biomolecule) and immobilization in a suitable support. The second strategy includes the modification of the IL reaction media, such as IL coating, additives or use of microemulsions with ILs. These methodologies have been used with promissory results [5].

5.1. Modification of solvent media

In order to avoid the enzyme insolubility, some works have reported the introduction of functional groups in IL structure such as hydroxyl, ether, and amide (which present high affinity for enzymes) [19]. For enzymes that are active in pure solvents, such as lipases, the most hydrophilic ILs can remove enzyme-bound water molecules that are essential to maintain protein structure and active function. In such case, these ILs (hydrophilic) are not adequate.

Another strategy is the addition of water in IL (co-solvent), but the enzyme may present low catalytic activity due to a changed conformation in ILs [71]. Several researchers have reported enzymatic reactions, especially for oxidative enzymes, in hydrophilic ILs with a high concentration of water (in the range 5 – 50%) and promissory results have been found [6, 29-31].

Water-in-IL microemulsions, or reverse micelles, have been used as a very efficient technique for solubilizing enzymes in hydrophobic ILs. The advantage of this approach is that the enzyme is protected of the contact with the solvent by a layer of water and surfactant molecules. As an example, the use of water-in-IL microemulsions was reported by Moniruzzaman et al. [50] as a new medium for dissolving various enzymes and proteins. Additionally, several authors have reported the use of different microemulsions systems with good results for enzyme stability [72-74].

5.2. Modification of the enzyme

The most common methodology for enzyme modification is immobilization. It is well known that immobilization of enzymes presents excellent advantages for biocatalysis, namely in the recovery of the enzyme for reutilization, product separation and recovery from the reaction media, application in continuous systems, and for enzyme stabilization. Indeed, enzyme immobilization increases thermal and operational stability of the biocatalysts compared to the free enzyme.

The use of immobilized enzymes in IL media has been reported by many research groups, using different methods of immobilization and supports. The most frequently used enzyme immobilization techniques are: physical adsorption, covalent attachment, entrapment in polymeric matrixes and cross-linking of enzyme molecules. For lipases, it was found that reaction rates in ILs were comparable or higher than in organic solvents and also immobilized lipase was more active than its free form [75-79]. The same behavior was found for proteinase [80], papain [81] and for heme-containing proteins [82].

The chemical modification of enzymes with poly(ethylene glycol) (PEG) is a well-known method (the so-called PEGylation) for enzyme stabilization in denaturing environments. PEG presents both hydrophilic and hydrophobic properties, so the modified enzymes can increase their solubility in some ILs [83]. Turner et al. [84] also reported higher activity of PEGylated cellulase than free cellulase in IL solutions.

Another method for activating and stabilizing enzymes in non-aqueous media is co-lyophilization of the enzyme. Maruyama et al. [85] lyophilized lipase with poly(ethylene glycol) (PEG) to prepare PEG–lipase complexes, finding that the activity of lipase in ILs increased more than 14-fold. Wang and Mei [86] also lyophilized lipase with cyclodextrins, and the activity of lipase in ILs ([bmim][PF_6] and [bmim][BF_4]) was improved.

6. Applications of ionic liquids in biocatalysis

The use of ILs as solvents or co-solvents for reaction media of enzymes is well recognized in biocatalysis. Examples available in the open literature include: polymerizations, biosensors, production of biofuels, synthesis of sugar- and ester-derivatives, among many others.

A large number of examples of the use of ILs for the enzymatic production of esters by lipase have been published [87]. The common esters synthetized in ILs are aliphatic and aromatic esters, for applications in polymers, biodiesel, and in the perfume, flavour and pharmaceutical industries. The synthesis of a wide range of aliphatic organic esters was carried out by transesterification from vinyl esters and alcohols and catalyzed by lipase in different 1,3-dialkylimidazolium ILs [88, 89]. Aromatic esters have also been synthetized with lipase in two ILs, [bmim][PF_6] and [bmim][BF_4] [90]. The esterification of 2-substituted-propanoic acids with 1-butanol was catalyzed by lipase in ILs [bmim][PF_6] and [omim][PF_6] [91]. Yuan et al. [92] studied the enantioselective esterification of menthol with propionic anhydride using lipase in [bmim][PF_6] and [bmim][BF_4]. The resolution of (R,S)-ibuprofen by es-

terification with lipases in the same ILs is another interesting example [93]. The aliphatic polyester synthesis by lipase, also in [bmim][PF_6], was reported by Nara et al. [94]. Later, the enzymatic preparation of polyesters by ring-opening polymerization and by polycondensation with lipase in [bmim][Tf_2N], [bmim][PF_6] and [bmim][BF_4] was also investigated [95]. According to these authors, the use of ILs could be an advantage in the polymerization of highly polar monomers with low solubility in organic solvents.

The production of biofuels, such as biodiesel (fatty acid methyl esters) has been also investigated in ILs through the transesterification of a triglyceride with methanol. Biodiesel is a renewable and environmentally-friendly fuel. Several ILs have been utilized for biodiesel production. Most often, the synthesis of biodiesel by enzymatic reactions in ILs is based on a short-chain 1,3-dialkylimidazolium cation, such as [bmim][PF_6] or [bmim][NTf_2], and the reaction is carried out in a biphasic system with lipase and using an adequate substrate (e.g., soybean oil) [96]. For homogeneous one-phase systems, imidazolium ILs with long alkyl chains such as [C_{16}mim][NTf_2] and [C_{18}mim][NTf_2] have been used [97,98]. These long chain, lipophilic ILs create a nonaqueous system suitable for oil transesterification. Ha et al. [99] studied the biodiesel production using immobilized lipase in 23 ILs. Among the ILs tested, it was found that highest biodiesel production yield was obtained in [emim][TfO]. But it is important to highlight that several works have been published for biodiesel production by lipases [100-102].

In recent years, a significant number of publications have showed the direct electron-transfer reaction between redox proteins or enzymes and IL-based composite electrodes. Biosensors are small devices which convert the biological recognition event into an electrical signal, so it can be used for selective analysis [103]. Several composite electrodes based on ILs have been prepared. Many of them can be found in a recent review by Shiddiky and Torriero[104], such as: hemoglobin biosensor; myoglobin and cytochrome c biosensors; catalase biosensors; glucose oxidase biosensors; horseradish peroxidase biosensors.

Sugar-based compounds are widely used in pharmaceuticals, cosmetics, detergents and food. A recent review by Galonde et al. [105] shows the synthesis of glycosylated compounds in ILs.

7. Conclusion

Ionic liquids have demonstrated to be suitable solvents for enzymatic reactions. They can be beneficial regarding to activity, (enantio)selectivity and stability of enzymes. The use of enzymes in ILs opens new possibilities for non-aqueous enzymology with high efficiency in several areas. Here, it was shown that a large variety of enzymes tolerate ILs or aqueous-IL mixtures as reaction medium. Moreover, the development of green and biodegradable ILs is reinforcing enzymatic applications of ILs, as stated in this work. Indeed, it is expected to become a standard in biotransformations, thus contributing to a greener chemical and biochemical industries.

Nomenclature

tris-(2-hydroxyethyl)-methylammonium methylsulfate [HEMA]

Cations:

[mmim] = 1,3-dimethylimidazolium

[emim] = 1-ethyl-3-methylimidazolium

[bmim] or [C_4mim] = 1-butyl-3-methylimidazolium

[bmp] = butylmethylpyrrolidinium

[btma] = butyl-trimethylammonium

[omim] = 1-octyl-3-methylimidazolium

[C_{10}mim] = 1-decyl-3-methylimidazolium

[C_{16}mim] = 1-hexadecyl-3-methylimidazolium

[C_{18}mim] = 1-octadecyl-3-methyl- imidazolium

Anions

[OAc] = acetate

[MDEGSO_4] = 1-ethyl-3-methylimidazolium 2-(2-methoxyethoxy)

[MeSO_3] = methanesulfonate

[TfO] = trifluoromethanesulfonate

[BF_4] = tetrafluoroborate

[MMPO_4] = dimethylphosphate

[Cl] = chloride

[EtSO_4] = ethyl sulfate

[$(CF_3SO_2)_2N^-$] = bis(trifluoromethylsulfonyl)amide

[MeSO_4] = methyl sulfate

[PF_6] = hexafluorophosphate

[NTf_2] = bis(trifluoromethylsulfonyl)imide

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Author details

Ana P.M. Tavares*, Oscar Rodríguez and Eugénia A. Macedo

LSRE - Laboratory of Separation and Reaction Engineering - Associate Laboratory LSRE/LCM, Faculdade de Engenharia, Universidade do Porto, Porto, Portugal

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