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Application of Room Temperature Ionic Liquids in Electrochemical Sensors and Biosensors

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1. Introduction

Ionic Liquids (ILs) are a new class of purely ionic, salt-like materials that are liquid at unusually low temperatures. The official definition of ILs uses the boiling point of water as a point of reference: "Ionic Liquids are ionic compounds which are liquid below 100 °C". In particular, salts that are liquids at room temperature are called room-temperature ionic liquids (RTILs). RTILs, also known as organic liquid, molten, or fused salts, are a class of non-molecular ionic solvents with low melting points. The accepted definition of an RTIL is any salt that has a melting point lower than ambient temperature.

Ionic liquids (ILs) can be composed of a large number of cations and anions. Most common RTILs are composed of unsymmetrically substituted nitrogen-containing cations (e.g., imidazolium, pyridinium, pyrrolidinium) with organic or inorganic anions (e.g., Cl⁻, PF₆⁻, BF₄⁻), Fig. 1.

First RTIL was reported by Wilkes et al. in 1982 (Wilkes et al., 1982). It was based on the 1-alkyl-3-methylimidazolium cation. Thereafter, many ILs containing a variety of cations and anions of different sizes have been synthesized for specific applications.

At the same time as ionic liquids become commercially available more and more (more than 350 ILs are now commercially available (Koel, 2009)), they show the interesting perspectives in different fields of researches such as catalysis, materials science, sensors, biosensors and separation technology. Since these techniques are in developing, there is always a need to design and synthesis many new ILs. An estimation predicts the number of possible ILs on order of 10¹⁸ (Koel, 2009).

A variety of cations and anions can be form ILs. The most common classes of ILs are imidazolium, pyridinium, pyrrolidinium, quaternary ammonium, and tetraalkylphosphonium ILs (Fig. 1). Of these, the most popular in experimental laboratory worldwide are undoubtedly 1,3-dialkylimidazolium salts, primarily due to the attractive and suitable physical properties.

The structure of IL and its interaction with the environment is extremely important for evaluating and selecting ILs for special application.

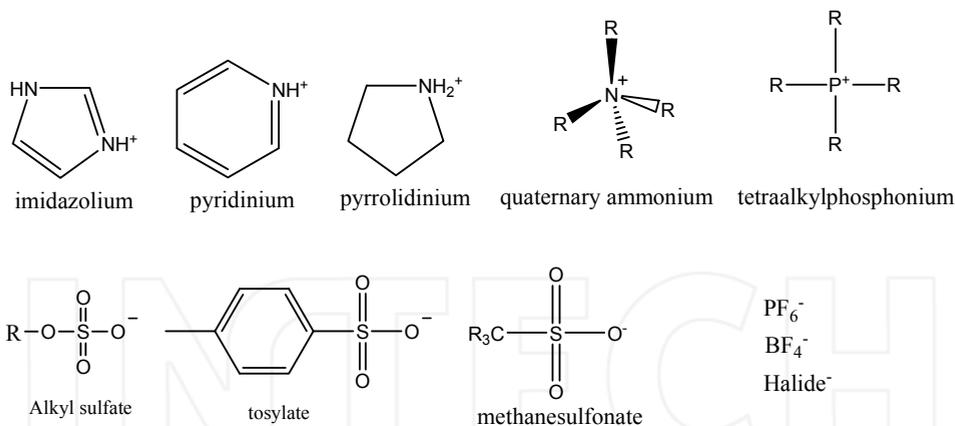


Fig. 1. The most common cations and anions used in construction of ILs

Electrochemical sensors and biosensors are the most rapidly growing class of chemical sensors, in analytical chemistry. Since the 1930s, these devices have found the most practical applicability in different fields due to the low cost, simplicity, portability, and high selectivity. A chemical sensor is a device that provides continuous information about some specific chemical properties of its environment. An ideal chemical sensor provides a certain type of response that is directly related to the quantity of a specific chemical species. Each chemical sensor composed of a transducer which is a device that provides continuous information about its environment, and converts the chemical response into a signal that can be detected by modern instrumentations. The second part which is the most important part of a chemical sensor is a chemically selective material, or the recognition element, which recognizes and differentiates between the response of the analyte and that of its environment. According to the type of signal transduction, chemical sensors can be classified as electrical, optical, mass or thermal sensors. Due to the considerable detectability, simplicity and low cost, electrochemical sensors receive more interest in comparison with optical, mass and thermal sensors which can be applied in a wide range of clinical, industrial, environmental and agricultural analyses.

An electrochemical biosensor is an analytical device which converts a biological response into an electrical signal. The term 'biosensor' is often used to cover sensor devices used to determine the concentration of substances and other parameters of biological process. Transduction of the biological or chemical signal in to the electrical signal can be done by amperometry, potentiometry and conductometry.

ILs due to their interesting properties have recently found various applications in construction of electrochemical sensors and biosensors in order to modify the responses. This chapter summarizes the properties of ILs and discusses their importance in electrochemistry. Then, it review ILs advantageous in construction of electrochemical sensors and biosensors.

2. General properties of ionic liquids

In general, ILs have some unique properties, such as a low vapor pressure, good thermal stability, high polarity, tunable viscosity and an ability to dissolve many compounds, a wide

electrochemical window, the ability to dissolve many compounds high conductivity, high heat capacity and solvents available to control reactions. Although they have wide range of polarity and hydrogen-bonding ability, they are liquid from 180 K to 600 K.

The physical and chemical properties of ILs depend mostly on the nature and size of both their cation and anion constituents. The main structural factors of the cation are symmetry, charge density, number of carbon atoms in the alkane substituent and its flexibility, the rotational symmetry of the head ring, the cyclic and branched structures, and the functional tail group. Similar structural factors have an influence on the properties of anions, including charge delocalization either by a large volume of the central atom, or by the presence of the perfluoroalkyl chain.

ILs are mostly denser than water with values ranging from 1 for typical ILs to 2.3 g cm^{-3} for fluorinated ILs. Density strongly depends on the size of the ring in the cation, on the length of the alkyl chain in the cation, on the symmetry of ions and on the interaction forces between the cation and the anion. The ILs with aromatic head ring, in general, present greater densities than pyridinium head ring ILs and than do imidazolium ring ILs. By increasing the symmetry of the cations, density will increase. ILs with functional group reveals higher densities than those of alkyl chains.

Transport properties play an important role in chemical reactions, electrochemistry, and liquid-liquid extraction. This concerns mainly the viscosity of ILs and their solutions with molecular solvents. Compared with typical organic solvents, ILs are much more viscous. Viscosity of ILs, typically at the level of 10-500 cP at room temperature, is much higher than water ($\eta(\text{H}_2\text{O}) = 0.89 \text{ cP}$ at 298.15 K) and aqueous solutions. The viscosity of ILs is determined by van der Waals forces and hydrogen bonded structures. Also, electrostatic forces can affect viscosity. The viscosities of the same class of ILs (with the anion held constant) increase as the alkyl group is lengthened. Increasing the alkyl chain length from butyl to octyl of a series of 1-alkyl-3-methylimidazolium cations, increases the hydrophobicity and the viscosity of the IL, while densities decrease. This is due to the stronger van der Waals forces between cations, leading to an increase in the energy required for molecular motion (Endress & Abedin, 2006). It is expected that the replacement of the alkyl chain by a hydroxyl functional group would increase the viscosity by increasing the H-bonding. As previously reported by Okoturo and Van der Noot viscosity temperature dependence in ILs is more complicated than in most molecular solvents. Most of ILs do not follow the typical Arrhenius behaviour. Most temperature studies fit the viscosity values into the Vogel-Tammann-Fulcher (VTF) equation, which adds an additional adjustable parameter (glass transition temperature) to the exponential term. In general, all ILs show a significant decrease in viscosity as the temperature increases (Okoturo and Van der Noot, 2004).

As a type of substances, ILs have been defined to have melting points (m.p.) below 373 K, however, most of them are liquid at room temperature. A typical ILs, e.g., 1-ethyl-3-methylimidazolium ethylsulfate has m.p. of $<-20 \text{ }^\circ\text{C}$) while a typical inorganic salt e.g. NaCl has m.p. of about $801 \text{ }^\circ\text{C}$. In this IL, the charge of the cation as well as the charge of the anion is distributed over a larger volume of the molecule by resonance. As a consequence, the solidification of the IL will occurred at lower temperature. However, in some long aliphatic side chains, a glass transition is observed instead of a melting point. In general, salts with a halogen anion, reveal to a higher melting temperature which are known as the precursors of ILs. Both, cations and anions have influence on the lowering melting points of ILs. Typically, the increase in anion size and its asymmetric substitution leads to a decrease in the melting

point. Also, the size and symmetry of the cation have an important impact on the melting points. For the longer alkane chain as C_{10} , the melting point increases. The short chain alkyl substituents in 1,3-dialkylimidazolium salts decreases the melting temperature.

Most of ILs have a glass transition temperature about 200 K (Domanska 2009). It is very interesting that also for the glass transition temperatures the structure of cations is important. However, the changes of glass transition temperatures with changing the length of the alkyl chain are much smaller than the melting temperatures. Common ILs are thermally stable up to 700 K. Thermal stability is limited by the same factors that contribute to the melting temperature. Branching the alkyl chain decreases the thermal stability of imidazolium ILs. Thermal stability increases with increasing anion charge density if the cationic charge density is also high.

Many synthetic reactions are faster in RTILs, and this saves time and or energy (Anderson et al., 2006). Compare to traditional organic solvents, a few volatile organic compounds are produced due to the little vapour pressure of ILs. The potential for explosions is minimized in RTIL because many RTILs have little or no flammability and no flash point. In many cases, products can be extracted from the RTIL after reaction, and the RTIL can be recovered and recycled.

3. The importance of ionic liquids in electrochemistry

Among the most important characteristics of ionic liquids, ionic conductivity, the width of the electrochemical potential window, viscosity, hydrophobicity and non-volatility cause the use of these solvents in electrochemical devices. In general, an ideal electrolyte should have high ionic conductivity ($>10^{-4}$ S/cm), fast ion mobility during redox reactions ($>10^{-14}$ m²/V.s), large electrochemical potential windows (>1 V), and low volatility. RTILs exhibit many of these properties and characteristics.

Non-flammability, high ionic conductivity, electrochemical and thermal stability of ILs are a unique properties for used as an electrolyte in electrochemical devices like in batteries, capacitors, fuel cells, photovoltaics, actuators, and electrochemical sensors (Wei & Ivaska, 2008).

Most properties of ILs relevant to electrochemistry are centrally based on the following three properties: conductivity, viscosity, and electrochemical potential windows.

3.1 Large electrochemical window

One of the very important properties of ILs is their wide electrochemical potential window, which is a measure for their electrochemical stability against oxidation and reduction processes. In fact, the electrochemical potential window is a voltage range between which the electrolyte is not oxidized or reduced. This value, on the one hand, characterizes the electrochemical stability of ionic liquids, i.e., the limits of the window correspond to the start and the end of the electrochemical decomposition of the involved ions. On the other hand, the width of the electrochemical window governs the range of potentials available for the electrochemical processes not affecting the solvent.

The electrochemical potential window is sensitive to impurities. Halides are oxidized much easier than organic anions. In organic anions the negative charge is delocalized over larger volume. Thus, contamination with halides cause to lower electrochemical stabilities.

3.2 Ionic conductivity

The conductivity of an ionic liquid mainly depends on the mobility of its cation because in general the diffusion coefficients of ILs cations are higher than anions. Ionic liquids based on imidazolium and pyridinium cations have the highest ionic conductivity (~ 1 and 10^{-1} S/m, respectively) (Every et al., 2000). Typical RTILs have conductivities of $>10^{-2}$ S/cm which are often useless as electrolyte. This can be problematic because IL electrolyte ions also migrate along the potential gradient. However, ILs possessing a zwitterionic structure in which the cation and anion are not expected to migrate with the potential gradient are useful in construction of an electrochemical cells. They showed much lower ionic conductivities in the range of 10^{-5} – 10^{-7} S/cm. (Wilkes et al., 1982; Anderson et al., 2006). The electrochemically most stable materials having comparable small conductivities like N-butyl-N-methylpyrrolidinium bis(trifluoromethylsulfonyl)imide, triethylsulphonium bis(trifluoromethylsulfonyl)imide, and N-methyl-N-trioctylammonium bis(trifluoromethylsulfonyl)imide. These materials are good electrolytes for use in batteries, fuel cells, metal deposition, and electrochemical synthesis of nano-particles.

The ILs showing the highest conductivities, e.g. 1-ethyl-3-methylimidazolium thiocyanate and dicyanamide exhibit the lowest electrochemical stabilities. Nevertheless, these materials are good candidates for use in any application where a high conductivity combined with thermal stability and non-volatility is necessary.

When conductivity and electrochemical stability are both required in an application, e.g., in supercapacitors, sensors and biosensors, imidazolium-based ILs with stable anions e.g., tetrafluoroborate or trifluoromethylsulfonate are applied. Table 1 summarized the electrochemical conductivity of some common ionic liquids which can be suitable for electrochemical sensors and biosensors.

Entry	Name	Electrical conductivity (S/m) In 25 °C *
1	1-hexyl-3-methylimidazolium bis[(trifluoromethyl)sulfonyl]imide	0.26
2	1-butyl-3-methylimidazolium bis[(trifluoromethyl)sulfonyl]imide [C ₄ mim] [NTf ₂]	0.40
3	1,3-dimethylimidazolium bis[(trifluoromethyl)sulfonyl]imide	0.74
4	1-butyl-1-methylpyrrolidinium bis[(trifluoromethyl)sulfonyl]imide	0.22
5	1-butyl-3-methylimidazolium hexafluorophosphate; [BMIM][PF ₆]	0.14
6	1-butyl-3-methylimidazolium tetrafluoroborate ([BMIM][BF ₄])	0.35
7	1-butyl-3-methylimidazolium methylsulfate	0.21
8	1-butyl-3-methylimidazolium trifluoroacetate	0.31

*Data are according to "Ionic Liquids Database - (ILThermo); <http://ilthermo.boulder.nist.gov/ILThermo/pureprp.uix.do>".

Table 1. Electrical conductivity of some ILs which can be used in construction of electrochemical sensors and biosensors

As it can be seen from Table 1, effect of ILs cation on electrical conductivity of the ILs is more than its anion. Nos. 3 to 5 have the same cations but the difference in the electrical conductivity are not too much. While, Nos. 1 to 4 the difference is more.

3.3 Hydrophobicity

Miscibility with water is often understood by hydrophobicity. The hydrophobicity mainly depends on the composition of ILs. From the point of view of hydrophobicity (solubility in water), ILs can be divided into two groups. Water-immiscible or hydrophobic IL like 1-butyl-3-methylimidazolium hexafluorophosphate ([BMIM][PF₆]) and 1-decyl-3-methylimidazolium bis(trifluoro-methylsulfonyl)imide ([DMIM][Tf₂N]) and water-miscible or hydrophilic ILs such as [BMIM][BF₄] (Wei & Ivaska, 2008). The first group due to the immiscibility with water are a good candidate for using in construction of electrochemical sensors and biosensors because these electrochemical devices contact water for a long period of operation. The second group are unstable in aqueous solutions and are not suitable for using in devices which are in contact with water.

The miscibility of ILs in water is strongly dependent on their anions (Wei & Ivaska, 2008). Cl⁻, Br⁻, I⁻, NO₃⁻, CH₃COO⁻ and CF₃COO⁻ are anions that make the ILs miscible with water. ILs composed of anions such as PF₆⁻ and Tf₂N⁻ are in immiscible with water. Miscibility of water of ILs based on anions such as BF₄⁻ and CF₃SO₃⁻ is dependent on the structure of the cations, even though they in general are miscible with water. The miscibility will decrease with the increase in the cation chain length which is due to the increased surface activity of the longer chain cations (Fitchett et al. 2005a,b; Wei & Ivaska, 2008).

3.4 Viscosity

Since ILs have much higher viscosities than normal electrochemical supporting electrolytes, they have an effect on the diffusion coefficients of species. A previous reported study compares the diffusion coefficients of a neutral molecule and the radical cation produced after an electrochemical reaction in an IL and acetonitrile. The diffusion coefficient of the radical cation was consistently about half that of the neutral molecule. In contrast, in acetonitrile, the ratio of the diffusion coefficients was nearly 60% higher, an indication that viscosity and charge have considerable effects on the transport of diffusing species in IL solutions. Table 2 listed viscosity of some common ILs suitable for the electrochemistry.

Entry	Name	Viscosity (p) In 25 °C *
1	1,2-dimethyl-3-propylimidazolium tetrafluoroborate	0.377
2	1-butyl-3-methylimidazolium bis[(trifluoromethyl)sulfonyl]imide [C ₄ mim] [NTf ₂]	0.069
3	1,3-dimethylimidazolium bis[(trifluoromethyl)sulfonyl]imide	0.047
4	1-butyl-1-methylpyrrolidinium bis[(trifluoromethyl)sulfonyl]imide	0.074
6	1-octyl-3-methylimidazolium bis[(trifluoromethyl)sulfonyl]imide	0.096
5	1-octyl-3-methylimidazolium tetrafluoroborate; [C ₈ mim] [BF ₄]	0.439
7	1-butyl-3-methylimidazolium methylsulfate	0.21
8	1-butylpyridinium tetrafluoroborate	0.163

*Data are according to "Ionic Liquids Database - (ILThermo); <http://ilthermo.boulder.nist.gov/ILThermo/pureprp.uix.do>".

Table 2. The viscosity of some ILs suitable for electrochemistry

4. Ionic liquids used in electrochemical sensors

Some studies revealed that ILs can be useful as electrolytes in batteries, electrochemical cells, and electroplating. However, they have been recently used also in construction of electrochemical sensors and biosensors. An electrochemical sensor is an analytical device which converts a chemical response into an electrical signal. Most of the sensors which are used in electrochemical measurements and have ability to modify with ionic liquids are ion selective electrodes based on polymeric membrane, carbon paste electrodes, and all solid state electrodes.

4.1 Ion selective liquid membrane sensors

Ion selective electrodes (ISEs) have been developed over four decades as sensitive, inexpensive and handy electrochemical sensors to selectively determine the concentration (activity) of ions in aqueous media. Typical ion selective membrane sensors are composed of a hydrophobic plasticized polymeric membranes or films that are doped with one ionophore in addition to a lipophilic ion-exchanger that plays an important role to the sensor response. The membrane matrix should acts as a solvent of low viscosity for all active sensing materials in the membrane. Therefore, it is required to use a plasticizer that can reduce the glass transition temperature of the polymer to below room temperature and increase the elasticity of the polymeric membrane and helps providing mechanical stability. Plasticizer also can improve the solubility of the sensing materials in the membrane (Peng et al., 2008).

According to the mentioned above, ILs can be excellent materials to prepare ISEs membranes because they have polymer plasticizing ability and ionic nature. Hence, they can be used as an ionic additive and plasticizer at the same time.

One of the key factors which helps the ions extract to the liquid membrane is a plasticizer. It is well known that the selectivity and working concentration range of the membrane sensors are affected by the nature and amount of the plasticizer used. This is due to the influence of the plasticizer on the dielectric constant of the membrane phase, the mobility of the ionophore molecules. Dielectric constant of the plasticizer can affect the selectivity manner of the ion selective membrane electrode. For example, for the extraction of polar ions, a high polar plasticizer or solvent mediator is required, and in contrast, for extraction of a lipophilic cation a low polarity plasticizer is better. Sometimes, very polar plasticizers lead to the extraction of the polar interfering ions which may have negative effects on the selectivity behaviour of the sensor, thus, a low polarity solvent mediator is more suitable. A variety of dielectric constant and polarity of the ILs offers a various number of plasticizer choices. However, it should be noted that hydrophobic (immiscible) ionic liquids are suitable for use in liquid membrane.

Recently, tetraphenylborate derivatives are used as cationic additives and lipophilic tetraalkylammonium salts are applied as anion exchangers. The hydrophilic counter ions of these lipophilic additives are exchanged with the primary ion when the ISE is conditioned in specific aqueous solution.

The incorporation of lipophilic ILs in the ion-selective membrane electrode as an ion-exchanger diminishes the ohmic resistance and enhances the response behaviour and selectivity manner and also, in the case of the poor extraction capability, increases the sensitivity of the membrane electrodes.

In 2005, Coll et al. (Coll et al., 2005) reported using the hydrophobic IL ([BMIM][PF₆]) to prepare the poly(vinyl chloride) (PVC) membrane. A high selective response to sulfate anion was observed. They showed that ILs can be used as ionic additives in conventional ISE membranes. The ionophore used in this electrode polyazacycloalkane.

In 2006, Shvedene et al. (Shvedene et al., 2006) have used two ILs in different polymer membranes both as the plasticizer and the ion-exchange additive. The compounds 1-butyl-2,3-dimethylimidazolium bis(trifluoro-methylsulfonyl)imide ([BDMIM][Tf₂N]) and dodecylethyldiphenylphosphonium bis(trifluoro-methylsulfonyl)imide ([DEDPP][Tf₂N]) were used to plasticize the PVC and poly(methyl methacrylate) (PMMA) membranes, respectively. Good and stable response to relatively hydrophobic cations and anions were obtained with the proposed membrane compositions.

In 2008, Nishi et al. showed that hydrophobic RTILs can be used as a non-volatile ionic medium for ion-selective liquid membrane sensors. They used a hydrophobic RTIL, trioctylmethylammonium bis(nonafluorobutylsulfonyl)imide ([TOMA⁺][C₄C₄N⁻]) in a K⁺ ion selective liquid membrane sensor (Nishi et al., 2008). The phase-boundary potential at the interface between an aqueous KCl solution and ILs, shows the Nernstian response to K⁺. Dicyclohexano-18-crown-6 (DCH₁₈C₆) was used as an ionophore in the membrane. The complex formation constant of K⁺ with DCH₁₈C₆ in ([TOMA⁺][C₄C₄N⁻]) is estimated to be on the order of 10⁹ from the upper detection limit using a partition equilibrium model in the presence of a neutral ionophore.

In 2008, Peng et al. (Peng et al., 2008) also used RTILs as both ion-exchanger and plasticizer for PVC-based ion-selective membranes. 1-Methyl-3-octylimidazolium chloride (MOImCl) and trihexyltetradecylphosphonium chloride (THTDPCI) can plasticize PVC to form flexible ion-sensing membranes. PVC-MOImCl membrane without additional ionophore and ion-exchanger demonstrated Nernstian response to sulfate ion with slope of 29.1 mV/decade in the concentration range of 10⁻⁵ to 10⁻¹ M. PVC-MOImCl-based electrode have fast response time within 10 s and wide pH independent range of 3–10. PVC-THTDPCI membrane exhibited stable and Nernstian response to different anions and the selectivity followed the Hofmeister series.

4.2 Carbon Paste Electrodes (CPEs)

Most of potentiometric carbon paste electrodes reported are based on incorporation of a sensing material into the carbon paste. The carbon paste usually consists of graphite powder dispersed in a non-conductive mineral oil such as paraffin.

Incorporation of mineral oil gives CPEs some disadvantages. Mineral oil is not component-fixed since it is derived from refining of petroleum and processing of crude oil. As a result, contaminants or matrix components may unpredictably effect on detection and analysis. In addition, the mechanical stability of CPEs rests somewhere between that of liquid membrane electrodes and solid state electrodes.

Carbon paste electrodes (CPEs) have attracted attention as ion selective electrodes mainly due to their improved renewability, stable response, and low ohmic resistance when compared to membrane electrodes (Javanbakht et al. 2007; Norouzi et al., 2010; Ganjali et al., 2009a, 2010).

Recently, RTILs have been widely used in construction of carbon paste electrodes (CPEs). These sensors are, in turn, called carbon ionic liquid electrodes (CILEs) (Safavi et al., 2007; Ganjali et al. 2009b,c; Faridbod et al. 2010). RTILs are a good choice as binders in carbon

paste electrodes due to their chemical stability, low vapor pressure, low toxicity, low melting temperature, high ionic conductivity and good electrochemical and thermal stability (Maleki et al., 2006). Using room temperature ionic liquids instead of paraffin oil in the carbon paste yields more efficient extraction of ions with high charge density into the carbon paste surface. This is due to the much higher dielectric constant of the ionic liquids as binder compared to paraffin oil (Ganjali et al. 2009b,c; Faridbod et al. 2010).

Due to its mechanical strength, the IL CPE could be applied as an effective flow-through detector in flowing streams, and since a mixture of IL and graphite is easily moldable, the fabrication of different electrode geometries is completely feasible. The favorable electrochemical response, high reversibility, sensitivity, and selectivity observed for these electrodes toward molecules together with its resistance to electrode fouling make it an excellent candidate for the construction of a new generation of sensors.

Recently, carbon nanotubes (CNTs) have also been added to the carbon paste (Ganjali et al. 2009b,c; Faridbod et al. 2010). CNTs have very interesting physicochemical properties, such as an ordered structure with a high aspect ratio, ultra-light weight, high mechanical strength, high electrical conductivity, high thermal conductivity, metallic or semi-metallic behavior and high surface area (Ajayan 1999). The combination of these characteristics makes CNTs unique materials with the potential for diverse applications.

Our research group, recently used [bmim]BF₄ in order to modify the response of an erbium carbon paste potentiometric electrode (Faridbod et al., 2010). The general procedure to prepare the carbon paste electrode was as follows: Different amounts of the ionophore [5-(dimethylamino) naphthalene-1-sulfonyl-4-phenylsemicarbazide] (NSP), along with an appropriate amount of graphite powder, ionic liquid and MWCNTs were thoroughly mixed. The resulting mixture was transferred into a glass tube with 5mm i.d. and a height of 3 cm. After homogenization of the mixture, the paste was carefully packed into the tube tip to avoid possible air gaps, which often enhance the electrode resistance. A copper wire was inserted into the opposite end of the CPE to establish electrical contact. The external surface of the carbon paste was smoothed with soft paper. A new surface was produced by scraping out the old surface and replacing the new carbon paste. The electrode was finally conditioned for 48 h by soaking it in a 1.0×10^{-3} mol L⁻¹ Er(NO₃)₃ solution. Using RTILs instead of paraffin oil in the carbon paste yield more efficient extraction of Er(III) (which is a cation with high charge density) into the CPE. This is due to the much higher dielectric constant of the RTIL binder when compared to paraffin oil. Using MWCNTs in the carbon paste improves the conductivity and, therefore, conversion of the chemical signal to an electrical signal. Carbon nanotubes have many properties that make them ideal as components in electrical circuits, including their unique dimensions and their unusual current conduction mechanism. The performance of the Er(III) carbon paste sensor can be greatly improved by using RTIL instead of mineral oil (paraffin), and also by using MWCNTs as enhanced signal transducers. The modified CPEs show better potentiometric response than unmodified CPEs in terms of sensitivity, Nernstian slope, linear range, and response stability. These are important characteristics of ion selective electrodes. The proposed sensor exhibits a long lifetime (about three months). The best performance for the modified sensor was obtained with an electrode composition of 20% [bmim]BF₄, 20% NSP, 45% graphite powder and 15% MWCNT. This particular sensor formulation exhibits a Nernstian response (19.8 ± 0.3 mV/decade⁻¹) toward Er(III) ions in the range of 1.0×10^{-7} to 1.0×10^{-1} mol L⁻¹ with a detection limit of 5.0×10^{-8} mol L⁻¹. The proposed modified Er(III) sensor can be used over the pH range from 3.5 to 9.0.

In addition to potentiometric measurements, carbon paste modified with ILs have more been used in other electrochemical sensors, particularly voltammetric sensor. For the first time, in 2005, Liu et al. introduced using imidazolium salt functionalized by polyelectrolyte in carbon paste electrodes (Liu et al., 2005). In voltammetric carbon paste sensors, as well as advantages mentioned in potentiometric carbon paste sensors, addition of traces of the ILs increased the electrocatalytic activity of the electrode. Maleki et al. used N-octylpyridinium hexafluorophosphate (OPFP) as a binder in a carbon paste electrode (Maleki et al., 2006). This type of electrode has lower ohmic resistance than CPE and gives very reproducible and sensitive voltammetric results. The electrocatalytic activity of the ILs 1-octyl-3-methylimidazolium hexafluorophosphate [OMIM][PF₆] in carbon paste electrode was investigated by using the redox probe Fe(CN)₆^{3-/4-} (Maleki et al., 2007). Trace amount of chloride has been measured by linear sweep, square wave and cathodic stripping voltammetry using [BMIM][BF₄], [BMIM][Tf₂N] and [BMIM][PF₆] (Villagran et al., 2004). In another report a carbon paste base ILs was used in a flow-injection system with a voltammetric detector (Shen et al. 2007). The electroactive compounds to be determined can diffuse better from the eluent into the thin layer of the ionic liquid on the surface of a carbon paste electrode as a working electrode.

4.3 All solid state sensors

All-solid-state sensors are a kind of potentiometric sensors which are more durable and can be miniaturized. The potential of the sensor to be miniaturized is an additional requirement that can give a lot of new applications to this device. Solid state ISEs are based on conducting polymers as the transduction layer in the electrode construction. Although the fabrication of all-solid-state sensor, which do not require any internal filling solutions, is one way to achieve durable sensors, the design of a proper solid contact between the ion-selective membrane and the electronic conductor is a difficult challenge in the way of obtaining reliable all solid-state electrodes.

Application of ILs in construction of all-solid-state sensor has recently been reported by Maminska et al. (Maminska et al., 2006). They used 1-dodecyl-3-methylimidazolium chloride [DMIM][Cl] in PVC membranes. In this way, the electrode showed good potential stability and reproducibility. In 2007, Kakiuchi et al. have also introduced a new solid-state reference electrode (Kakiuchi et al., 2007). A new type of Ag/AgCl reference electrode is consists of a Ag/AgCl electrode coated with a AgCl-saturated with a hydrophobic ionic liquid ionic liquid, 1-methyl-3-octylimidazolium bis(trifluoromethylsulfonyl)imide ([C₈mim⁺][C₁C₁N⁻]), instead of the internal aqueous solution. The [C₈mim⁺][C₁C₁N⁻] phase plays dual roles, that is, as a medium dissolving AgCl and an ionic-liquid-type salt bridge upon contact with an aqueous solution. This new class of reference electrodes opens the way for a variety of miniaturized and solid-state reference electrodes.

There is another report in 2009 (Chernyshov et al. 2009) in which ILs that melt slightly above room temperature (and may be called low-melting ionic solids, LMISs) were used as sensing materials for the detection of ions in aqueous solutions. In this work, Chernyshov et al. used a simple method to prepare solid-contact ion-sensitive electrodes based on the consecutive melting and further solidification of the LMISs. Indeed, instead of using plasticizer, ion-exchanger polymeric matrix, they used LMIS in the potentiometric sensor. A potentiometric response toward a number of anions and the possibility of altering the selectivity by incorporating additional ionophores into the LMIS matrix was studied in this work.

5. Ionic liquids used in electrochemical biosensors

An electrochemical biosensor is an analytical device which converts a biological response into an electrical signal. In general, it is difficult to exchange the electron between an enzyme and solid surface of the electrodes directly. This is because of the inaccessibility of its redox center and loss of bioactivity of the enzyme due to the conformational changes by adsorption on the electrode surface. ILs have shown good compatibility with biomolecules and enzymes, and even whole cells. Thus, ILs can be used in electrochemical biosensors typically as both binder and conductor. Common ILs advantages observed when they incorporate into the biosensors include higher conductivity, good biocatalytic ability (Park, S. & Kazlauskas, 2003), long-term stability (including stability at high temperature), superior sensitivity, improved linearity, better selectivity, and the ability to fabricate third-generation biosensors with direct (without using mediator) electron transfer between protein and electrode.

ILs have a wide electrochemical potential window. The difference between the potentials of their anodic (E_a) and cathodic (E_c) decomposition is usually greater than 3 V (Ohno, 2005), while for aqueous electrolytes is about 1.2 V. Because of this remarkable property of ionic liquids, they find wide use in electrochemical biosensors.

Recently, some authors have reported increased stability of enzymes in ILs compared with stability in some organic solvents (Lozano et al., 2001; Laszlo, et al. 2002; Park, S. & Kazlauskas, 2003; Persson & Bornscheuer, 2003). ILs were also found to act as agents to stabilize proteins effectively at high temperatures (Baker et al., 2004). [BMIM][BF₄], [BMIM][PF₆], [OMIM][PF₆], 1-(2-hydroxyethyl)-3-methylimidazolium tetrafluoroborate [HEMIM][BF₄], and Butylpyridinium hexafluorophosphate ([BP][PF₆]) was widely used in construction of electrochemical biosensors due to their very good biocompatibility.

Most investigations to date have focused on the amperometrically determined biocatalytic activity of common enzymes such as glucose oxidase (GOx), horseradish peroxidase (HRP), or various other heme proteins (e.g., Hb, Mb) incorporated into electrodes. Laszlo and Compton have also reported the catalysis of hemin activated by an electron acceptor in IL solutions and it was found that the activity of hemin increased with the enhanced amount of IL in the methanol-IL system (Laszlo & Compton, 2002). Dramatic enhanced activity and thermal stability of horseradish peroxide (HRP) were obtained when it was immobilized it in the [BMIM][BF₄] based sol-gel matrix (Liu et al. 2005a). Direct electrochemical response of HRP (Liu et al. 2005b), myoglobin (Ding et al., 2007) and Hb (Sun et al., 2007) have been observed on IL modified electrodes. Direct electrochemical reduction of hemin has been studied by cyclic voltammetry and chronocoulometry in the ILs, [BMIM][PF₆] and [OMIM][PF₆] (Compton & Laszlo, 2002).

Nafion films have been used widely in construction of electrochemical biosensors. Nafion, due to its easy fabrication, good electrical conductivity, high chemical stability and good biocompatibility, has been used as a protective coating material for enzyme immobilization. Mixture of nafion with ILs can improved the coating ability and stability. Nafion-[BMIM][PF₆] composite film has been reported to help immobilization of enzyme HRP on the glassy carbon electrode (Chen et al. 2007). Paraffin can also be replaced by [BMIM][PF₆] as binder in H₂O₂ and nitrite carbon paste biosensor (Sun et al., 2007).

Yu et al also reported that the water-miscible imidazolium-based ILs can interact with glassy carbon electrode and form molecular films on the electrode surface (Yu et al. 2005). Various approaches have been investigated to use ionic liquids with carbon nanotube in

biosensors. One interesting approach involves the use of multiwall carbon nanotube-IL (MWCN-IL) modified glassy carbon electrodes (GCEs). In this approach, MWCNs were thoroughly mixed with the ILs, by grinding them together in a mortar to create a gel-like paste which was then applied to the surface of a cleaned GC electrode. Using a platinum wire and a saturated calomel electrode as auxiliary and reference electrodes, respectively, cyclic voltammograms (CVs) can be measured. An immediate advantage of the MWCN-IL is a larger peak current with smaller peak separations, an indication of faster electron transport to the electrode surface.

In 2010, a nano-composite material consisting of amine functionalized multi-walled carbon nanotubes and a room temperature ionic-liquid, 1-butyl-3-methylimidazolium tetrafluoroborate, was reported to be used in construction of a novel catalase based biosensor for the measurement of hydrogen peroxide. The modified electrode exhibited a quasi-reversible cyclic voltammogram corresponding to the Fe(II)/Fe(III) redox couple in the heme prosthetic group of catalase with a formal potential of -460 mV in 0.1 M phosphate buffer solution at pH=7.0. The nano-composite film showed an obvious promotion of the direct electron transfer between catalase and the underlying electrode. The apparent charge transfer rate constant and transfer coefficient for electron transfer between the electrode surface and enzyme were reported as 2.23 s⁻¹ and 0.45, respectively. The immobilized catalase exhibited a relatively high sensitivity (4.9 nA/nM) toward hydrogen peroxide (Rahimi et al., 2010).

Table 3, summarized some important ILs which are immiscible in water and they have been widely used in construction of electrochemical sensors and biosensors.

6. Conclusion

In this chapter, besides a brief discussion of the properties of ILs, the application of ILs in electrochemical sensors and biosensors are reviewed. ILs are liquids with non-volatility, high ion conductivity, the ability to dissolve many compounds, thermal stability, high viscosity, high polarity, and low vapor pressure. Ionic liquids have many applications, such as powerful solvents and electrically conducting electrolytes. Nowadays, electrochemical sensors and biosensors can be powerful tools for analysis of different species. Due to the some special characterization of ionic liquids such as wide potential windows (a voltage range between which the electrolyte is not oxidized or reduced.) and high electrical conductivity, hydrophobicity and the insolubility in water, the extraction and plasticizing ability, they are used in construction of electrochemical sensors and biosensors. They can be applied for improvement and modification of the composite materials in an electrochemical sensor or biosensor. Using room temperature ionic liquids instead of paraffin oil in the carbon paste electrodes yields more efficient extraction of ions with high charge density into the carbon paste surface. This is due to the much higher dielectric constant of the ionic liquids as binder compared to paraffin oil. Also, in the case of voltammetric measurements, the electroactive compounds to be determined can better diffuse from the eluent into the thin layer of the ionic liquid on the surface of a carbon paste electrode. In addition, ILs can be used in electrochemical biosensors typically as both binder and conductor. Common ILs advantages observed when they incorporate into the biosensors include higher conductivity, good biocatalytic ability, long-term stability, superior sensitivity, improved linearity, better selectivity, and the ability to direct (without using mediator) electron transfer between protein and electrode.

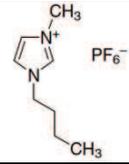
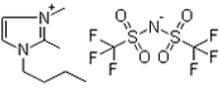
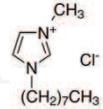
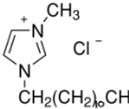
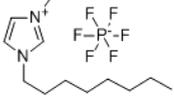
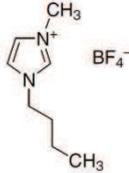
Name	Structure	M.P. (°C)	Viscosity (cP) in 20°C	Application	Reference
1-butyl-3-methylimidazolium hexafluorophosphate ([BMIM][PF ₆])		11	272.7	ISE	Coll et al., 2005
1-butyl-2,3-dimethylimidazolium bis(trifluoromethylsulfonyl)imide ([BDMIM][Tf ₂ N])		-15	-	ISE	Shvedene et al., 2006
1-Methyl-3-octylimidazolium chloride (MOImCl)		0	-	ISE	Peng et al., 2008
1-dodecyl-3-methylimidazolium chloride [DMIM][Cl]		102	-	All-solid-state	Maminska et al., 2006
1-octyl-3-methylimidazolium hexafluorophosphate [OMIM][PF ₆]		-	807.1	Voltammetric carbon paste sensor	Maleki et al., 2007
1-butyl-3-methylimidazolium tetrafluoroborate ([BMIM][BF ₄])		-71	99.9	1-CPE 2- Biosensor	Ganjali et al., 2009b,c; Faridbod et al., 2010; Rahimi et al., 2010

Table 3. Some common ILs which are immiscible in water and can be used in electrochemical sensors and biosensors

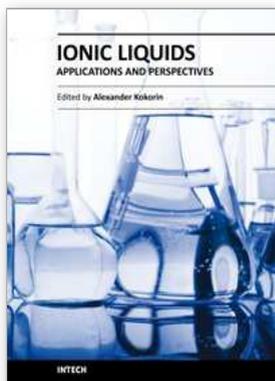
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This book is the second in the series of publications in this field by this publisher, and contains a number of latest research developments on ionic liquids (ILs). This promising new area has received a lot of attention during the last 20 years. Readers will find 30 chapters collected in 6 sections on recent applications of ILs in polymer sciences, material chemistry, catalysis, nanotechnology, biotechnology and electrochemical applications. The authors of each chapter are scientists and technologists from different countries with strong expertise in their respective fields. You will be able to perceive a trend analysis and examine recent developments in different areas of ILs chemistry and technologies. The book should help in systematization of knowledges in ILs science, creation of new approaches in this field and further promotion of ILs technologies for the future.

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