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Electrochemical transistors with ionic liquids for enzymatic sensing

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ABSTRACT

Over the past decade conducting polymer electrodes have played an important role in bio-sensing and actuation. Recent developments in the field of organic electronics have made available a variety of devices that bring unique capabilities at the interface with biology. One example is organic electrochemical transistors (OECTs) that are being developed for a variety of bio-sensing applications, including the detection of ions, and metabolites, such as glucose and lactate.

Room temperature ionic liquids (RTILs) are organic salts, which are liquid at ambient temperature. Their non-volatile character and thermal stability makes them an attractive alternative to conventional organic solvents. Here we report an enzymatic sensor based on an organic electro-chemical transistor with RTIL's as an integral part of its structure and as an immobilization medium for the enzyme and the mediator. Further investigation shows that these platforms can be incorporated into flexible materials such as carbon cloth and can be utilized for bio-sensing. The aim is to incorporate the overall platform in a wearable sensor to improve athlete performance with regards to training. In this manuscript an introduction to ionic liquids (ILs), IL – enzyme mixtures and a combination of these novel materials being used on OECTs are presented.

Keywords: Phosphonium Ionic Liquids, Glucose Oxidase, Enzymes, Electrochemical transistors, OECTS

1. INTRODUCTION

1.1 Organic Salts / Ionic Liquids

Salts are generally regarded to be solid at ambient temperature. The most commonly known salt, NaCl, becomes liquid above 801 °C. Under normal conditions the crystalline structure of NaCl is very stable, however, when heat is applied, each ion gradually vibrates and the salt melts as a result of increased energy. In this situation, the ions have enough energy to escape the attraction of neighboring ions, causing the loss of the crystal lattice order and melting of the substance. The Gibbs free energy of fusion at the melting point (T_m) is given by Equation 1;

$$\Delta G = \Delta H - T_m \Delta S = 0 \quad (1)$$

and hence:

$$T_m = \Delta H / \Delta S \quad (2)$$

One can see that the melting point is thus a subtle balance of the enthalpy change on melting relative to the entropy change in the melting process.

Ionic liquids (ILs) are low melting salts, thus forming liquids that are comprised entirely of cations and anions. According to the current convention, a salt melting below the normal boiling point of water is known as an “ionic liquid” or by one of many synonyms including low / ambient / room temperature molten salt, ionic fluid, liquid organic salt, fused salt, and neoteric solvent^[1]. The first ionic liquid was reported almost a century ago by Walden^[2], who protonated ethylamine with nitric acid to yield ethylammonium nitrate, which has a melting point (T_m) of 14 °C. Recently the most commonly employed IL anions are polyatomic inorganic species. Most common among these is $[\text{PF}_6]^-$, a “workhorse” anion that Wilkes and Zaworotko^[3] paired with imidazolium cations in preparing early water stable hydrophobic ILs. It, and the related $[\text{BF}_4]^-$ ion, are probably the most popular anions used in IL research and the variation in properties between salts (with a common cation) of these species is dramatic. For example, butylmethylimidazolium hexafluorophosphate $[\text{C}_4\text{mim}][\text{PF}_6]$ is immiscible with water, whereas butylmethylimidazolium tetrafluoroborate $[\text{C}_4\text{mim}][\text{BF}_4]$ is water soluble^[4]. This sort of variation in physical properties arising from different anion choice gave rise to Seddon’s description of ILs as “designer solvents”^[5]. The number of potential anion-cation combinations available reputedly equate to one trillion (10^{12}) different ILs^[1]. Ionic liquids have received much attention of late because of their potential application in green chemistry and as a range of novel electrochemical materials. They have indeed become “designer solvents” with many ILs now being designed for a specific application, for example as potential electrolytes for various electrochemical devices^[6-18], including rechargeable lithium cells,^[19, 20] solar cells,^[21-23] actuators^[24-26] and double layer capacitors (DLCs).^[27-29]

1.2 Phosphonium based ILs.

Nitrogen based cations, in particular *N*-methylimidazolium and pyrrolidinium salts have been the subject of many of the publications in the field. A range of phosphonium cation based ionic liquids are also available and have a range of useful properties, but have been much less studied. Early reports regarding phosphonium ILs were published in the 1970’s by Parshall using stannate and germanate salts^[30-35] and Knifton *et. al.*^[36-42] in the 1980s centering on the use of molten tetrabutylphosphonium bromide as an ionic solvent. To some extent the slower uptake of work on phosphonium ILs can be attributed to the difficulty in synthesizing the starting materials, such as tributylphosphine. Although phosphine derivatives have been available on a commercial scale since 1971, it was not until 1990 that tributylphosphine became available on a large scale.^[43] Since then tetrabutylphosphonium chloride and bromide have been produced on a multi-ton scale along with many other trialkylphosphines and their corresponding quaternary phosphonium salts, in particular from Cytec Industries Inc^[43].

Variations of the four substituents on the phosphonium cation along with the multitude of available anions represent an enormous number of possible salts as shown in the review written by Fraser *et. al.*^[44] These include salts with the traditional halide anions such as trihexyl(tetradecyl)phosphonium chloride and bromide (CYPHOS® IL 101 and 102) which are liquid at room temperature and have glass transition temperatures as low as -65°C^[45]. Salts containing other anions such as tosylate, dicyanamide, methylsulfate diethylphosphate, phosphinate, bistriflamide ($[\text{NTf}_2]^-$), tetrafluoroborate and carboxylates are also available. Of course, not all such phosphonium salts are liquid at room temperature, but by careful selection of R and R’ as well as the appropriate anion, there are many phosphonium salts that can be prepared that are in fact liquid at room temperature and many more which fall within the broader general definition of ionic liquids ($T_m < 100$ °C).

Reasons why one might consider a phosphonium ionic liquid in an industrial process include availability and cost. Phosphonium salts can meet both of these demands as they are already manufactured on a multi-ton scale.^[43] In comparison to the nitrogen based ILs, the higher thermal stability of phosphonium based ILs is useful in processes which operate at greater than 100 °C.^[46] A good example where phosphonium salts out-perform their ammonium counterparts is the biphasic conversion of aromatic chlorides to fluorides using potassium fluoride at temperatures exceeding 130 °C.^[47] Other advantages of phosphonium based ILs as compared to their imidazolium cation analogues is that the C2 proton of the latter tends to make them slightly acidic, which can lead to carbene formation.^[48] Alkylphosphonium salts are generally less dense than water, which can be beneficial in product work-up steps that involve decanting aqueous

layers which contain inorganic salt by-products. For these reasons phosphonium ILs are now appearing in applications as solvents,^[49-52] phase transfer catalysts,^[52-54] electrochemical applications^[55], exfoliating montmorillonite clays^[56-62], catalysts in epoxy curing^[63] and high temperature polycarbonate reactions.^[64, 65] Other very useful reviews of the field of phosphonium based ILs include those by Zhou *et al.*^[43] and Clyburne *et al.*^[66]

Commercial based phosphonium salts have been available for many years, halide salts being the most popular^[43]. Historically these compounds have been used as biocides^[67, 68] and phase transfer catalysts^[69-71]. Ever growing interest in phosphonium ILs led to Bradaric *et al.*^[43] closely examining a range of potential ILs for industrial production. In doing so they synthesized a range of phosphonium based salts that were liquid at or near room temperature. Trihexyl(tetradecyl)phosphonium chloride ([P_{6,6,6,14}][Cl]) has been a starting material for the synthesis of numerous phosphonium based ionic liquids by anion exchange reactions^[72]. Furthermore, [P_{6,6,6,14}][Cl] has been a commercial product for Cytec long before the term 'ionic liquids' achieved the prominence it currently enjoys^[43]. The ion exchange reactions involving phosphonium based ILs generally fall into two categories (as shown in Equations 3 and 4).



Where R, R' = alkyl; X = halogen; M = alkali metal; A = anion such as phosphinate, carboxylate, tetrafluoroborate, hexafluorophosphate^[43]. Ionic liquids containing the anions shown in Fig 1 can be synthesized by one or the other of the routes shown in Eq 3 and Eq 4

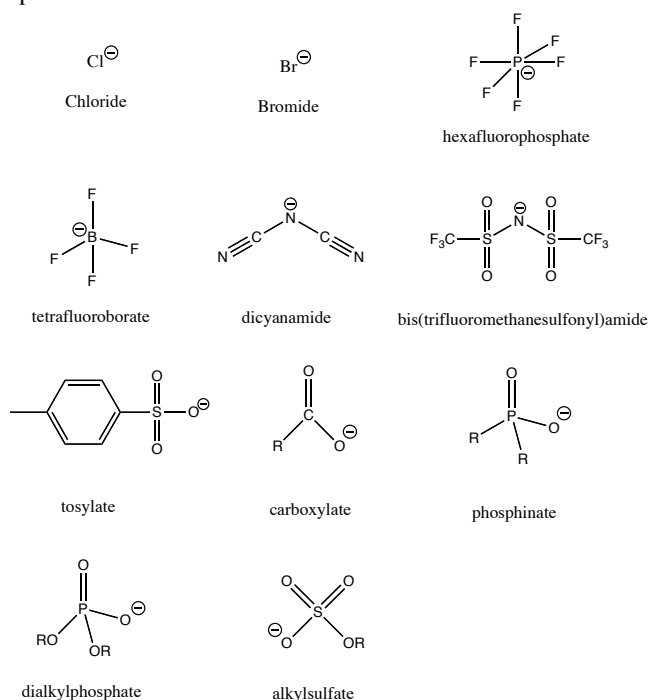


Figure 1: Examples of anions that can be paired with Tetraalkylphosphonium cations to produce ionic liquids.

The series of phosphonium phosphinates are of particular interest. Bis(2,4,4-trimethylpentyl)phosphinic acid, better known as CYANEX 272, is a well-known and popular solvent for the extraction of cobalt from nickel in both sulfate and chloride media^[73, 74], and is currently used to produce more than half of the western world's cobalt^[75-77]. Ionic

liquids containing the bis(2,4,4-trimethylpentyl)phosphinate anion are thus of interest not only for the usual reasons, particularly for solvent extraction applications^[43].

1.3 Applications of phosphonium based ILs

Environmental pressure to reduce waste and re-use materials has stimulated the development of “Green” chemistry^[78]. Recent reviews have covered these emerging fields^[79, 80] and it is apparent that one of the most difficult areas to make more environmentally friendly is solution phase chemistry^[52]. Solvents play key roles in chemical reactions; they serve to homogenize and mix reactants, and act as a heat sink for exothermic processes. Solutions of ethylmethylimidazolium tetrafluoroborate support reactions such as alkene oligomerizations, alkylations^[81] and acylations^[82]. Ramnial *et al.*^[52] have reported that imidazolium based ILs are unsuitable for reactions involving either active metals (i.e., Na or K) or in reactions that involve strong bases (i.e. Grignards, organolithiums, and amides) since these reagents react with the imidazolium-based solvents^[52]. For instance, imidazolium ions react quantitatively with potassium metal to produce imidazol-2-ylidenes (N-heterocyclic carbenes, NHCs)^[83], and treatment of imidazolium ions with bases, such as lithium di-iso-propylamide or potassium tert-butoxide, is the standard method for generation of NHCs^[84]. Even with weaker bases, such as NR₃, Aggarwal showed that during the Baylis–Hillman reaction in an imidazolium-based ionic liquid, the low reported yields were the result of addition of the deprotonated imidazolium cation to an aldehyde^[85]. Ramnial *et al.*^[52] found that NHCs are persistent in phosphonium based ILs^[83], such as [P_{6,6,6,14}][Cl]^[43]. NHCs are highly basic (pK_a = 22–24)^[86, 87] and the authors were surprised that deprotonation of the [P_{6,6,6,14}][Cl] to produce a phosphorane did not occur. This led the authors to study whether stronger bases would be persistent and reactive in phosphonium based ILs. Indeed what was found was that [P_{6,6,6,14}][Cl] was capable of supporting reactions involving strong bases such as Grignard reagents. The reactions between the Grignard reagent and added reactants proceed cleanly, and there was no observed reaction between the IL and the strongly basic reagents^[52]. The high thermal capacity of [P_{6,6,6,14}][Cl] limits the need to cool the samples for reaction. Use of certain phosphonium ILs also facilitates product separation due to the triphasic nature of water, ionic liquid and hexane combinations. The identification of this phase behavior opens up the possibility of limiting the use of ethereal solvents in this class of reactions thus allowing for a general “Greening” of Grignard chemistry^[52].

1.4 Bio compatible ILs

It has been pointed out that the use of the term “green” to describe IL chemistry is something that should be done with care^[88]. Rogers and coworkers have offered excellent discussion on the topic, covering the use of fluorosulfonate anions, the most commonly used in IL work^[89]. The most commonly used, non-toxic, organic anions are acetate and lactate^[89, 90]. However, carboxylates are basic, readily engage in hydrogen bonding, and are strongly coordinating towards transition metal ions^[91]. Such attributes are not typical of the fluorosulfonate anions on which so many IL compositions are based. These properties are likely to be useful in some circumstances and detrimental in others^[91]. In an attempt to steer away from fluorosulfonate anions, a communication by Carter *et al.*^[91] has opened up a new field in the ionic liquid world. For the first time the use of common sweeteners such as saccharin and acesulfamate were applied in the formation of new ionic liquids.^[91] These anions, in their alkali metal salt form, are widely used not only in foodstuffs but also non-nutritive sweeteners.^[92] More significantly, when incorporated into ILs, these anions exhibit behavior that, in several regards, more closely resembles those of certain fluorosulfonate anions than those of common carboxylates. Fig 2 shows similar motifs in the [NTf₂]⁻, [Sacc]⁻ and [Ace]⁻ anions.

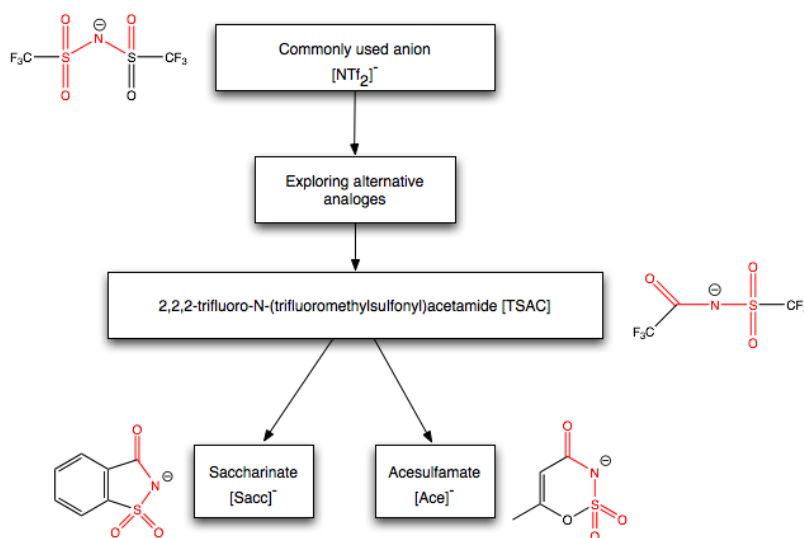


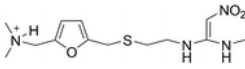
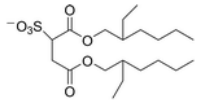
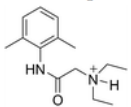
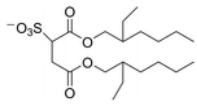
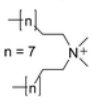
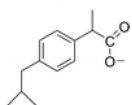
Figure 2: Structural similarities of saccharinate ($[\text{Sacc}]^-$), acesulfamate ($[\text{Ace}]^-$) and $[\text{TSAC}]^-$ anions compared to that of the widely used Bis(trifluoromethylsulfonyl)imide ($[\text{NTf}_2]^-$) anion^[91].

From Fig 2 the key properties to note about the saccharinate and acesulfamate anions are: they are both non-fluorous sulfamides and have well-established toxicological profiles^[92]. A resultant patent has been published with regard to the preparation of anionic-sweetener-based ionic liquids as non-volatile reaction media for a host of chemical reactions, separations in the gas phase, for altering dissolution rates and as heat storage media^[93].

Further attempts to move away from non-fluorous anions have led Fukumoto *et al.*^[94] to prepare ILs from naturally occurring amino acids with much success. Using conventional metathesis reactions in the preparation of these ILs was unsuccessful due to the amino acids coordinating to the transition metal ions^[95]. To overcome this, the authors used methods which involved preparing imidazolium hydroxide to neutralize a series of target amino acids^[94]. Among several onium cations that were tested, the 1-ethyl-3-methylimidazolium cation $[\text{C}_2\text{mim}]^+$ exhibited an excellent ability to form RTILs with selected amino acids. Using the neutralization method for the preparation of the ILs was favorable as all of the resulting amino acid ionic liquids obtained were transparent, nearly colorless liquids at room temperature. The authors comment that many of the ILs prepared were liquid at room temperature due to the increase of the van der Waals forces between alkyl side chains of the amino acid anion with the $[\text{C}_2\text{mim}]^+$ cation.

Further developments in IL synthesis by Rogers and coworkers have investigated an area largely overlooked by the ionic liquid field; “Drug Ionic liquids”. Many of the known Active Pharmaceutical Ingredients (APIs) are salts, therefore offering the opportunity to form materials with increased performance such as controlled solubility (hydrophobic, hydrophilic), bioavailability, elimination of polymorphism and new delivery options (*e.g.* slow release or the IL-API as a solvent)^[96, 97]. The cations and anions are commonly chosen on the basis of low symmetry and charge delocalization (found in many typical APIs)^[97]. Even nitrogen-containing heterocycles, so commonly used in ILs today, are frequently found in APIs or API precursors^[98]. Care must be taken when choosing appropriate IL-forming ion pairs. Many of the important APIs are not permanent ions, but rather are protonated or deprotonated to form the commonly used salts; thus, suitable pK_a differences need to be considered^[99]. Ionic liquids synthesized using APIs have included lidocaine hydrochloride (a pain reliever) with sodium docusate (an emollient) and didodecyldimethylammonium bromide (an antibacterial agent) with sodium ibuprofen (an anti-inflammatory)^[97]. As can be seen in one example the cation holds the active ingredient, whereas in the other, the anion plays the active ingredient.

Table 1: Examples of Ionic liquids synthesized from API starting materials^[97]. Reproduced by permission of The Royal Society of Chemistry (RSC) for the Centre National de la Recherche Scientifique (CNRS) and the RSC.

Biological properties	Cation source	Anion source	IL
Histamine H ₂ -receptor antagonist, emollient	Ranitidine hydrochloride Function: histamine H ₂ -receptor antagonist 	Sodium docusate Function: emollient 	Ranitidine docusate Form: dark red gel Melting point: liquid at RT T (glass transition): -12 °C T (liquid-liquid transition): 29 °C
Pain reliever, emollient	Lidocaine hydrochloride Function: pain reliever 	Sodium docusate Function: emollient 	Lidocaine docusate Form: colorless gel Melting point: liquid at RT T (glass transition): -29 °C T (liquid-liquid transition): 78 °C
Antibacterial, anti-inflammatory	Didecyltrimethylammonium bromide Function: antibacterial 	Sodium ibuprofen Function: anti-inflammatory 	Didecyltrimethylammonium ibuprofen Form: yellow liquid Melting point: liquid at RT T (glass transition): -73 °C T (liquid-liquid transition): 69 °C

The advantages of drug ionic liquid designs are that ILs can not only provide the solution to the problems often faced by the solid drug, such as limited shelf life, but can also introduce new treatment or delivery options which are not available through use of solid APIs or traditional approaches (such as iontophoresis). Shown in Fig 3 is an excellent representation of the evolution of ionic liquid research, from tunable properties through to “drug ILs”^[97].

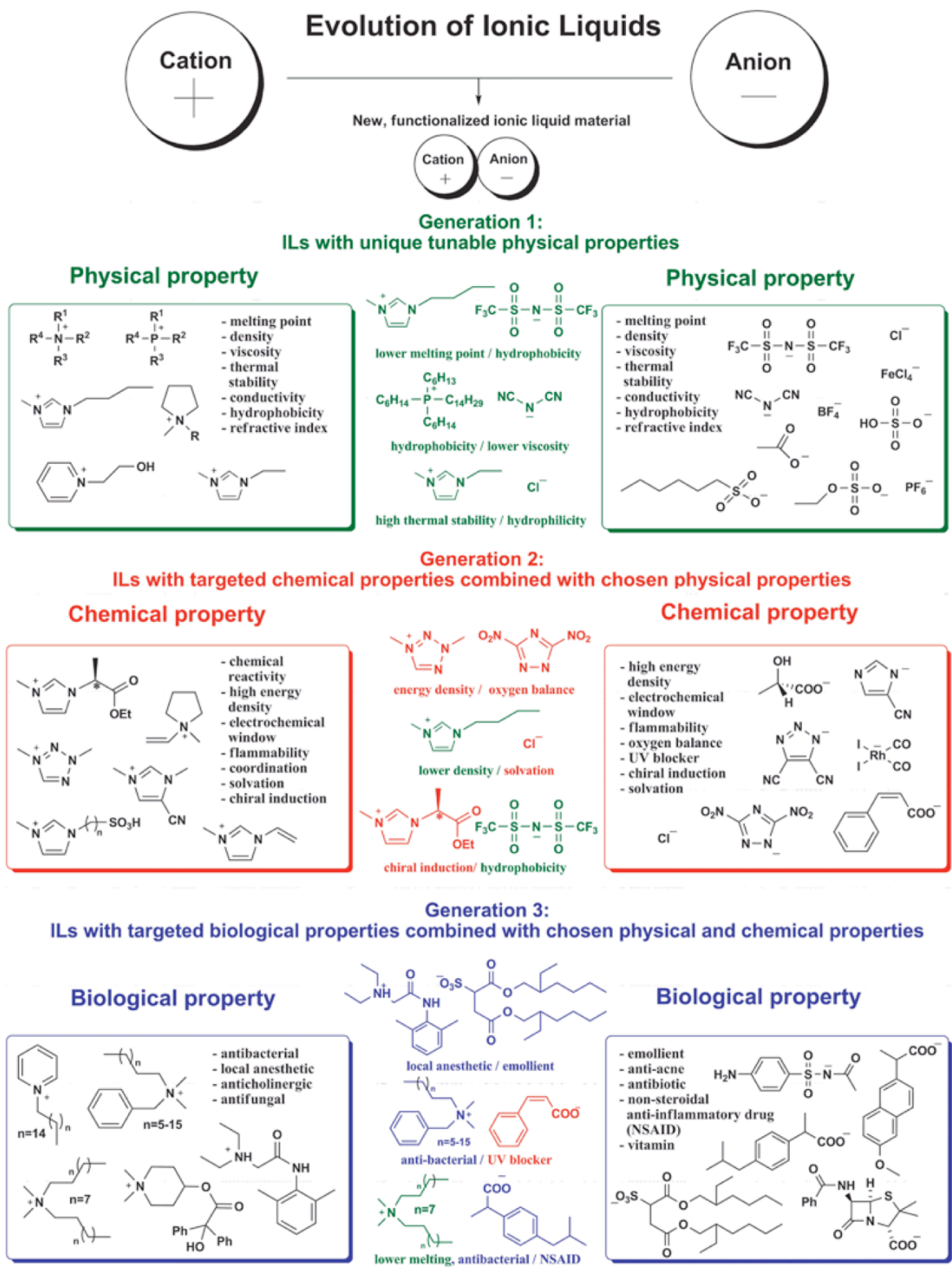


Figure 3: Evolution of Ionic liquids from a scientific perspective^[97]. Reproduced by permission of The Royal Society of Chemistry (RSC) for the Centre National de la Recherche Scientifique (CNRS) and the RSC.

1.5 Stabilization / activating of enzymes in ionic liquids.

Ionic liquids offer, in some cases, a number of advantages over other types of organic solvents, including, in specific cases and applications, higher thermal stability, lower viscosities and wider electrochemical windows. It has been well documented that enzyme performance in an IL is affected by several parameters including water activity, pH and impurities^[100]. Other important factors that play a role in enzyme stability / activity include IL polarity, hydrogen bond basicity and nucleophilicity of anions, ion kosmotropicity and viscosity. Although outside the scope of this discussion, these areas have been discussed in an excellent review on the topic by Zhao^[101]. Abe *et al.*^[102] recently synthesized a number of phosphonium salts that have an alkyl ether group present. The phosphonium salts moiety is commonly found in living creatures, and it was hypothesized that this family of ILs have good affinity with enzyme proteins and may provide a good environment for enzymes.

Methods to stabilize and activate enzymes in ILs can be classified in two ways. The first method involves enzyme immobilization (on solid supports, sol-gels) via physical or covalent attachment to Polyethylene glycol (PEG) for example. The second approach includes water-in-IL microemulsions, IL coating, the use of additives in ILs and specifically designed ILs whose ions are enzyme compatible^[101] (such as those found in section 1.4).

The most employed method for enzyme stabilization and activation in ILs is the use of immobilized entities instead of free forms^[101]. These immobilized methods generally fall into three categories: binding to a solid carrier, sol-gel encapsulation, and protein cross-linking^[103, 104]. Immobilization techniques are carried out on enzymes as they are the most straightforward of the methods. A new area in enzyme immobilization is the incorporation of carbon nanotubes^[105]. The high surface area and unique nanoscopic dimensions of carbon nanotubes enable high protein loading and low mass-transfer resistance. Jia *et al.*^[106] prepared novel biosensors consisting of thin films of polyethylenimine-functionalized IL containing carbon nanotubes and gold nanoparticles with glucose oxidase. The “cocktail” of IL, nanoparticles and carbon nanotubes showed good electrochemical response to glucose and high enzyme compatibility^[106]. Further to this a disposable biosensor was constructed and reported by Ding *et al.*^[107] The composite materials were based on N-butylpyridinium hexafluorophosphate, sodium alginate, and graphite; after optimization, the newly developed biosensor could detect H₂O₂ in a linear calibration range of 1.0 to 6.0 $\mu\text{mol L}^{-1}$ with a detection limit of 0.5 $\mu\text{mol L}^{-1}$ ^[107].

1.6 OECTs with ionic liquids for enzymatic sensing.

Organic electrochemical transistors (OECTs) provide an exciting alternative to field-effect transistors. A typical configuration of an OECT utilizes an electrolyte as an integral part of the device structure: they consist of a conducting polymer film (channel) brought in contact with an electrolyte, such as ionic liquids. A gate electrode is inserted in the latter, while source and drain electrodes measure the current that flows through the channel (drain current, I_d). The application of a bias at the gate (gate voltage, V_g) causes ions from the electrolyte to enter the polymer film and dedope it, thereby decreasing the drain current^[108].

Early OECT fabrication used varying types of conducting polymers, for example: polyaniline^[109, 110], polycarbazole^[111], polythiophene, and their derivatives^[112, 113]. However limitations were also faced when using these devices as bio-sensors. For example, polypyrrole when exposed to hydrogen peroxide (H₂O₂) undergoes an irreversible conductivity change limiting its use with enzymes such as glucose oxidase (GOx) that generate H₂O₂, during interaction with suitable analytes^[114]. Polyaniline loses its electrochemical activity at a pH higher than 5, limiting the sensing capability of polyaniline-based OECTs in physiological fluid (pH \sim 7.3)^[115]. Although attempts have been made to overcome this limitation by modifying polyaniline with high molecular counter ions such as poly(vinyl sulfonate) or poly(styrene sulfonate)^[115, 116], the development of a more durable conducting polymer was required^[117].

Zhu *et al.*^[118] have since then demonstrated that OECTs based on the commercially available conducting polymer, poly(3,4-ethylenedioxythiophene) doped with poly(styrene sulfonic acid) (PEDOT:PSS), is capable of sensing glucose in a neutral pH buffer solution^[118]. The OECT setup proposed by Zhu *et al.*^[118] showed that when the current modulation in the PEDOT:PSS channel, induced by the application of a gate voltage on a platinum (Pt) wire electrode was dramatically increased when both glucose and GOx were present in phosphate buffer solution (PBS). These result

indicates that PEDOT: PSS has good stability both in neutral pH and in the presence of H₂O₂, and the limitations that result from the use of polypyrrole and polyaniline maybe overcome^[117].

2. RESULTS & DISCUSSION

2.1 Enzymatic OECT sensor.

An enzymatic sensor based on an OECT that employs a RTIL as an integral part of its structure has been described recently^[119]. The authors reported that patterning the RTIL over the active area of the OECT enables the RTIL to act as an electrolyte and a reservoir for the enzyme. When the solution containing the analyte is added to the device, it mixes with the RTIL. The analyte, the enzyme, and the mediator are then allowed to interact and the OECT transduces this interaction. An important requirement for the RTIL is that it wets the PEDOT:PSS film, thus allowing the enzyme and the mediator to be patterned over the active area of the device. Moreover, the RTIL should be miscible with the aqueous solution that carries the analyte (PBS). The RTIL triisobutyl-(methyl)-phosphonium tosylate ([P_{1,4,4,4}][Tos], Fig. 4a, supplied by Cytec Industries) satisfies these requirements, as the Tos anion gives it a rather hydrophilic character. Previous studies have also shown [P_{1,4,4,4}][Tos] to be a biocompatible medium for glucose consumption by bacteria^[120]. The layout of the device is shown in Fig. 4b. Two parallel stripes of PEDOT : PSS, with widths of 100 mm and 1 mm, respectively, were patterned on a glass support using photolithography. Contact pads at the end of the stripes allowed facile electrical connection to the source-measure units. The wide stripe was used as the transistor's channel and the narrow one as the gate electrode, as it has been shown that for enzymatic sensing the area of the channel must be larger than that of the gate electrode^[121]. A monolayer of (tridecafluoro-1,1,2,2-tetrahydrooctyl) trichlorosilane (FOTS) was patterned on the surface of the device leaving uncovered only a small area of the channel and of the gate electrode. These areas of PEDOT:PSS which were left uncovered by FOTS served as hydrophilic "virtual wells"^[122] and were shown to be effective in confining the RTIL (and the glucose solution, when it was added) over the centre of the device.

The experiments involved placing a small amount of [P_{1,4,4,4}][Tos] that included the enzyme glucose oxidase and the mediator ferrocene [bis(n5-cyclopentadienyl)iron] on the centre of the device and allowing it to be accommodated in the hydrophilic virtual wells. Subsequently, 50 μl of glucose solution in PBS were added to the device and allowed to mix with the RTIL solution, as seen in Fig. 4c. Fig 4d shows the incorporation of the OECT / IL electrolyte mixture into a common plaster, illustrating the versatility of the material.

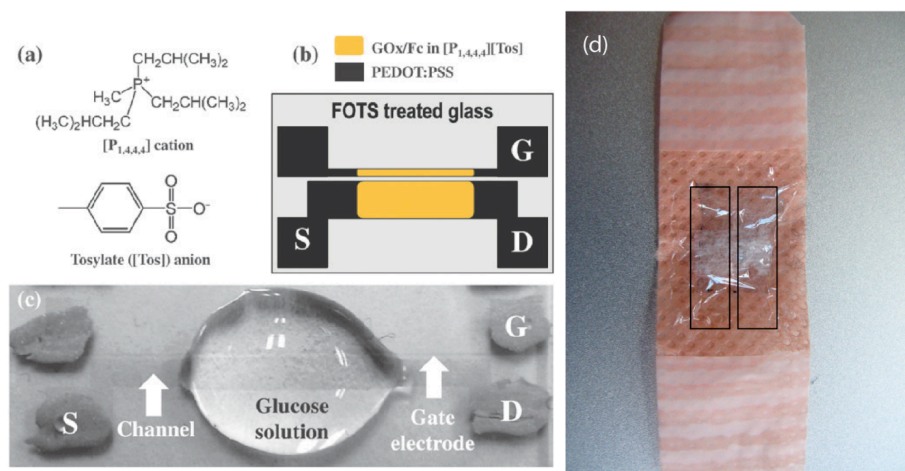


Figure 4: (a) Chemical structure of [P_{1,4,4,4}][Tos]. (b) Layout of the OECT, indicating the area where the RTIL was confined. (c) Photo- graph of the OECT with a drop of glucose solution added. The balls at the pads are made of silver paste^[119]. Reproduced by permission of The Royal Society of Chemistry. (d) Incorporation of the OECT into a flexible material (plaster).

Fig. 5a shows the transient response of the drain current of an OECT for different concentrations of glucose solution, upon the application of a 0.4 V pulse at the gate electrode with a duration of 3 minutes. The drain voltage was 0.2 V. The data show the characteristic decrease of drain current upon gating,^[123] which has been understood on the basis of the reactions shown in Fig. 6.

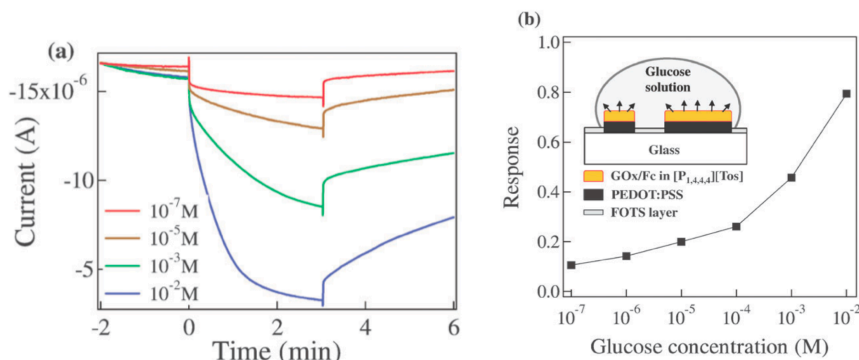


Figure 5: (a) Transient response of the drain current of an OECT upon application of a gate voltage of 0.4 V and duration of 3 min. The drain voltage was 0.2 V. (b) Current modulation (represented as the dimensionless quantity $\Delta I/I$) of the OECT as a function of glucose concentration. Inset shows the concept of device operation, and the arrows indicate the dissolution of the RTIL carrying the enzyme and the mediator into the analyte solution^[119]. Reproduced by permission of The Royal Society of Chemistry.

As glucose in the solution is oxidized, the enzyme (GOx) itself is reduced, and cycles back with the help of the Fc/ferricenium ion (Fc^+) couple, which shuttles electrons to the gate electrode (Fig. 6a). For example, for 10⁻² M of glucose, this cascade of reactions causes a current of 8×10^{-8} A to flow to the gate electrode. At the same time, cations from the solution enter the PEDOT:PSS channel and dedope it (Fig. 6b),^[124] thereby decreasing the drain current to a degree that depends on glucose concentration^[123]. Due to the amplification inherent in the OECT, the change in the drain current is much larger than the gate current itself (for 10⁻² M of glucose the drain current changes by 1.2×10^{-5} A, as shown in Fig. 5a). Fig. 5b shows the response of the OECT, in terms of change in drain current ($\Delta I/I$), to glucose concentration. The detection range is shown to be at least from 10⁻⁷ to 10⁻² M, and covers the clinical glucose level in human saliva (0.008–0.21 mM), suggesting that this device could be used as a glucose detector for monitoring glucose both in blood (2–30 mM) and in saliva^[125]. It should be noted that in order to avoid fouling and dilution effects, a new device was used for each glucose solution that was measured (each data point in Fig. 5b was taken from a different device). This is consistent with the mode of operation of single-use sensors, which is particularly suitable to organic electronic devices as they can be produced using low-cost techniques. The device-to-device reproducibility was found to be better than 10%. It is important to note that, contrary to Fc, which dissolved in [P_{1,4,4,4}][Tos], GOx was present in a dispersed state in [P_{1,4,4,4}][Tos], and it dissolved only when the glucose solution was added to the OECT. It is well known that the dissolution of enzymes in a RTIL can result in a change of the secondary and higher enzyme structure and causes the loss of enzyme activity^[126]. Therefore, a heterogeneous state in which GOx is dispersed in the RTIL can protect it from denaturation and help maintain its activity. In the same context, dispersion rather than dissolution can be used as a way to enhance the long-term stability of biosensors. Although we did not investigate this matter in any depth, we tested an OECT stored at ambient temperature 30 days after its fabrication. When a 10⁻² M glucose solution was added the response was the same (~ 0.8) as that of a freshly fabricated device.

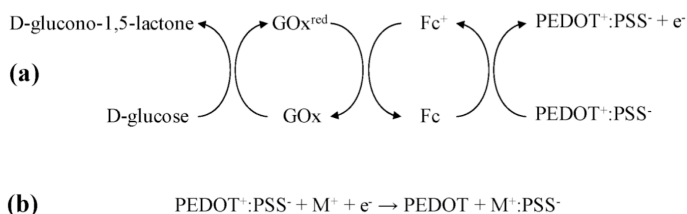


Figure 6: Reactions at the gate electrode (a) and at the channel (b) of the OECT^[119].

2.2 Concluding remarks.

Phosphonium ionic liquids clearly offer, in some cases, a number of advantages over other types of ionic liquids, including, in specific cases and applications, higher thermal stability. The integration of some commercially available phosphonium based ionic liquids onto electrochemical transistors has been shown by Yang *et al*^[119]. The ionic liquid was confined on the surface of the transistor using a photolithographically patterned hydrophobic monolayer, which defined hydrophilic virtual wells. An enzyme and a mediator were immobilized in the ionic liquid and, when the aqueous solution which carried the analyte was added, they dissolved in it. The enzyme was in a dispersed state in the ionic liquid, which may prove to be a good strategy for improving long-term storage. Using the glucose/ glucose oxidase pair as a model, it was demonstrated that the glucose analyte detection fell in the region of 10^{-7} to 10^{-2} M concentration range.

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