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Prospects of ionic liquids application in electronic and bioelectronic nose instruments

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Abstract

This paper addresses the problem of application of ionic liquids to chemical sensors and biosensors being an integral part of the electronic and bioelectronic-type instruments. The design and principle of operation of the electronic and bioelectronic noses are compared. Both the benefits and shortcomings of the application of ionic liquids in these type of instruments are described. The prospects of the development and application potential of the electronic and bioelectronic nose instruments containing ionic liquids are presented as well.

key words: ionic liquids, sensors, biosensors, electronic nose, bioelectronic nose, electrochemical analysis

1. Introduction

Ionic liquids (*ILs*) attract increasing attention from researchers from different scientific fields. Unique physico-chemical properties [1,2] and the fact of being classified as 'green' solvents [3,4] significantly increases the number and range of research topics on potential applications of these liquids [5–7]. The characteristics of the ionic liquids with respect to their application in industry are widely covered in the literature. However, some biological properties of ILs, such as their stability, biodegradability, recyclability, toxicity, have not been fully verified

[8,9]. It can also be stated that possibilities of adjustment of the biological and physico-chemical properties *via* modification of a cation or an anion are currently popular topics in the field of ILs [10,11]. General physico-chemical and biological properties of the ionic liquids are presented in Table 1.

Table 1. Selected properties of ionic liquids – ILs [2].

Physicochemical properties	Biological properties
• Liquid state below 100 °C, • Low vapour pressure (100 pPa for [C_4mim][PF_6] [12] as compared with 3 kPa for H_2O at 298 K), • Ability to dissolve both organic and inorganic compounds [13], • High thermal stability (up to 450°C, decomposition: 300–500°C) [14], [15], • Inflammability [16], • Electric conductivity (up to 10 mS cm^{-1}) [17], large electrochemical window (typically 4.5-5 V [18], • Possibility to dampen the surface of some polymers, minerals, metals [19,20], • Possibility to change some properties by varying the anion-cation combination [21,22]: polarity, viscosity, density, electrochemical conductivity, solubility in water, electrochemical durability, sorption properties, melting point, hydrophobicity and hydrophylity, • Diphasic systems possible, • Interionic interactions [23]: cross-correlation terms between cations and anions, polarization effects due to many interactions caused by cations and anions in ILs, • Electrostatic interactions between ions in ILs [24], • Catalytic activity [25], • Lubricating effect, tribological properties [26], • Solubility of gases such as O_2, benzene, ethylene, ethane, nitrous oxide, carbon monoxide etc., which makes them suitable solvents for catalytic hydrogenations, carbonylations, hydroformylation and aerobic oxidation [27],	• Antimicrobial activities and antistatic properties [28], [29], • Activity to some biological materials, including interaction with bacteria, fungi, lipids, proteins, etc. [30], • Pharmacokinetic and pharmacological activity [31], • Ability to penetrate biological membranes [32], • Preservation and conservation of the soft tissues [33], • Ability to modify the surface of some polymers [34] , • Biocatalytic reaction medium [35], • Drug carriers in biological media [36], • Environmental friendly, toxicity of some ILs is still unknown [37,38], • Can be recycled; recovery and recycling of the catalyst are also possible with the ILs [39],

The first compound to be classified as the ionic liquid was [$EtNH_3$][NO_3], discovered in 1914 [40] (Table 2). That year Walden coined the general, currently most popular, definition describing the ionic liquids as *"materials composed of cations and anions, that melt around 100 °C or below as an arbitrary temperature limit"* [41]. The stages of development and application of ILs in different fields of human activity are presented in Table 2.

Table 2. Historical perspective of ionic liquids [42].

Year, inventors/event	Object of invention	Ref.
1888 – Gabriel, Weiner	Ethanolammonium nitrate [$C_2H_8N_2O_4$] was reported	[43]
1914 – Walden	First room temperature Ionic Liquid (RTIL) [$EtNH_3$][NO_3]	[40]
1982 –Wilkes	New class of ILs – dialkylimidazolum chloroaluminates	[44]
1982 – Pacholec, Poole	Use of ethylammonium nitrates as a stationary phase in Gas-Liquid Chromatography	[45]
1983 – Hussey	First major review of Ionic Liquids	[46]
1984 – Magnuson	First study of IL – enzyme systems	[47]
1990s – more targeted approaches for the development of ionic liquids as the promising chemicals solvents		

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1992 – Wilkes, Zaworotko	Second generation of ILs with "neutral" weakly coordinating anions [PF_6^-], [BF_4^-], allowing expanding the field of applications	[48]
1996 – Rogers	Development of ILs as solvent in Liquid Extractrion (LE)	[49]
1996 – Bonhote	First imidazolium IL with [NTf_2] anion	[50]
1998 – The French Petroleum Institute (IFP)	First industrial application of ILs (production of iso-octane from n-butene with Ni as a catalyst dissolved in chloroaluminate ILs)	[51]
1998 – Ohno	First polymeric ionic liquid	[52]
1999 - Welton	Concept of ILs as excellent solvents for a variety of industrially relevant reactions	[53]
1999 – The Queen's University Ionic Liquid Laboratories (QUILL), Belfast, Northern Ireland	First research centre focused of ionic liquids	
2000 – Crete, Greece	NATO advanced research workshop on "Green Industrial Applications of Ionic Liquids"	[54]
2000 – Davis	Concept of task-specific ILs	[55]
2002 – Dai	First IL- based QCM sensor	[56]
2004 – Vo-Thanh	First IL with chiral cation	[57]
2005 – Salzburg, Austria	First International Congress on IL	[58]
2005 – Zhang	One of the first reported IL- based biosensors	[59]

Since the first international conference on ionic liquids as the complying with the principles of green chemistry [3,60] a new approach towards the ionic liquids has developed. Development of sustainable technologies contributed to the expanding application fields of these compounds. Owing to the appropriate combination of the cations and anions it has been possible to design compounds with specific properties tailored to a particular target application presented in literature [6,61–67]. Among the numerous applications of the ionic liquids in different fields of science and technology [68], ILs have also been used to build chemical sensors and improving their operation parameters [69,70]. Due to their unique electrochemical properties [71] and the possibility of modification of their physico-chemical and biological parameters, the ionic liquids have successfully been employed in the design of sensors and biosensors. ILs can be used either as an independent receptor layer or as a supporting element providing additional control over sensitivity and selectivity during interaction with gas phase components. Thanks to their high viscosity ILs can be applied as a binder, a medium between the transducer and a biosensitive material deposited on the surface of the biosensor [72]. Excellent electrochemical properties, both the large electrochemical window [73] and relatively high electrical conductivity [74], make the ionic liquids a valuable material for building and modifying of electrochemical sensors. Moreover, their low volatility, thermal and electrochemical stability mean they can be used under high-vacuum conditions in a safe and controlled way. Owing to the high values of dynamic viscosity coefficients the ionic liquids can be employed as membranes in amperometric sensors, which separate the gas environment from a working electrode. The metrological parameters of the amperometric sensors including sensitivity, selectivity, measurement range, limit of detection

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and quantification or the response time are affected by such physico-chemical properties of the ionic liquids as dynamic viscosity, electrochemical durability range, analyte solubility in ionic liquid, diffusion coefficient of analyte in ionic liquid, hygroscopic properties, water-solubility and electrical conductivity [75].

In recent years, more and more attention is being paid to develop biological olfactory components employed to develop olfactory-based biosensors for the detection and discrimination of specific odorants. By utilizing biosensitive elements, biosensors are able to detect odorants as well as can provide a new platform to investigate the performance of the olfactory system. Excellent properties of smell and taste receptors are generally recognized in the development of biomimetic smell receptors-based biosensors [76]. Achievements in the field of sensors and biosensors for electronics, biology, genetic engineering, biotechnology and nanotechnology allow construction and implementation of devices from an electronic nose family (*EN, artificial nose, odour-sensing system, electronic nose, e-nose)* [77], whose evolution based on implementation of biosensitive materials resulted in bioelectronic noses *(BEN, bioelectronic nose, biomimetic electronic nose, bio- enose, b- enose)* [78]. The heart of electronic nose instruments, which has a direct bearing on the basic operation parameters and application range, is a sensor matrix. Intensive progress towards electronic nose devices has been focused on:

- designing sensors with a high sensitivity, specificity and short response time,
- miniaturization, increasing mobility and cost reduction,
- development of the sensor systems generating signals similar to those of their biological counterparts,
- invention of the electronic systems mimicing human nose and brain.

An increase in knowledge on the mechanisms governing odour perception in the biological olfactory systems [79–82] is accompanied by significant progress in the field of odour biosensors [83,84]. It is expected that the research on new materials, compatible with different coatings and development of suitable mediums involved in immobilization processes can lead to new prototypes of the devices from the bioelectronic nose group intended for odour analysis. Significant part of the development of sensor is appropriate selection of immobilization medium. Different aspects of the ionic liquids have been dynamically

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investigated during recent years [85,86]. In this article the authors present the prospects as well as the achievements in the field of chemical sensors and biosensors whose integral part are the ionic liquids, and which are utilized in the design of electronic nose instruments. Moreover, the development potentials of electronic and bioelectronic nose devices are discussed.

2. Comparison of design and principle of operation of electronic and bioelectronic noses

The human ability to sense thousands of different odours is determined by a complex structure of olfactory epithelium covering the nasal cavity, where several millions of olfactory neurons are present. The tips of their modified dendrites are equipped with tiny spikes, which detect odorous substances. Smell impulses are detected by cilia located in the olfactory epithelium. The results of the latest research on odour perception mechanisms at a gene and protein level [87,88] reveal that owing to spatial segregation of neurons and axons each odorant excites a defined set of olfactory glomeruli. Human genes coding particular receptor proteins are responsible for interception of odorous substances [89]. The rhinencephalon is considered to be the site responsible for odour discrimination [90]. Differences in the structure of receptor proteins determine interaction with different odorants. After reaching the rhinencephalon, the signal is converted in such a way that it is recognized as odour. The impulses from the same olfactory receptors reaching the different olfactory cortex regions allow simultaneous and diversified processing of the same sensory impulses. Cortex neurons integrate odour information that makes odour sensing possible [91–94].

The principle of operation of electronic and bioelectronic noses is based on the olfactory systems present in their human counterparts. The mechanisms of odorous substances identification implemented in the electronic analogues closely resemble those of human nose [95]. Excitation of the sensor matrix sensitive elements in particular types of odour detection and identification systems leads to the generation of specific signals. Hence, it provides a convenient approach to signal processing and data analysis, which enables a generation of unique responses *(odour fingerprints*) for particular odour samples [96,97]. The identification of a given gas sample is possible owing to the excitation of the entire sensor matrix constituting the electronic nose. However, each sensor is excited to a different extent. As a result, a characteristic set of responses from particular sensors is acquired. Analysis of the obtained response vector allows the gas sample classification into a particular reference vector

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set recognized earlier. In the case of the human, this role is played by brain, which compares the nerve impulses obtained from the olfactory bulb with the memory-stored database of odours encountered during the lifetime. With the electronic nose, the obtained vector is assigned to the reference sets present in a computer database using appropriate data processing methodologies, namely statistical-chemometric algorithms [98], [99].

In some aspects, the bioelectronic nose differs from conventional electronic noses [100] where the active material is made of chemically sensitive components with a broad response range. Application of the electronic noses encounters certain problems associated with a high cost of equipment, identification of odours at concentration levels higher than those of the biological counterpart and complicated calibration [101]. A limitation of the electronic nose instruments is the inability to reach a biological sense of smell sensitivity. To overcome this limitation, sensitive elements, inspired by the biological sense of smell, are successfully implemented in construction of the devices belonging to a bioelectronic noses. Fast operation, non-invasive sample analysis and simple measurement make the electronic nose instruments a real alternative to other popular methods of odour analysis [102–105]. Moreover, the possibility of a more precise imitation of the human sense of smell *via* implementation of highly selective and sensitive sensors utilized in the bioelectronic noses can significantly expand the application spectrum of the devices from the electronic senses group [106]. The sensory part of the bio-enose can be built either from the olfactory receptors (*OR*) of the olfactory epithelium or from the cells exhibiting expression of the olfactory receptors proteins. The sensitive element made of the biomaterial of that type is directly connected, using a suitable medium, with the sensor intended for odour identification and conversion of the biological signal into an analytically suitable signal – typically an electrical or optical one [107]. Sensors used in these types of noses are composed of two elements – primary and secondary converters. The first one is built from the olfactory receptors cells, for instance, whereas the other (transductor) is a non-biological device. Signal generation occurs as a result of contact between the odorous substance and the olfactory receptor followed by a number of reactions resulting in generation of an action potential in the receptor and axon depolarization. The signal is then directed to the non-biological element where it is converted into an electrical signal [78,108]. A comparison of the structure and operation principle for electronic and bioelectronic noses is illustrated in Figure 1.

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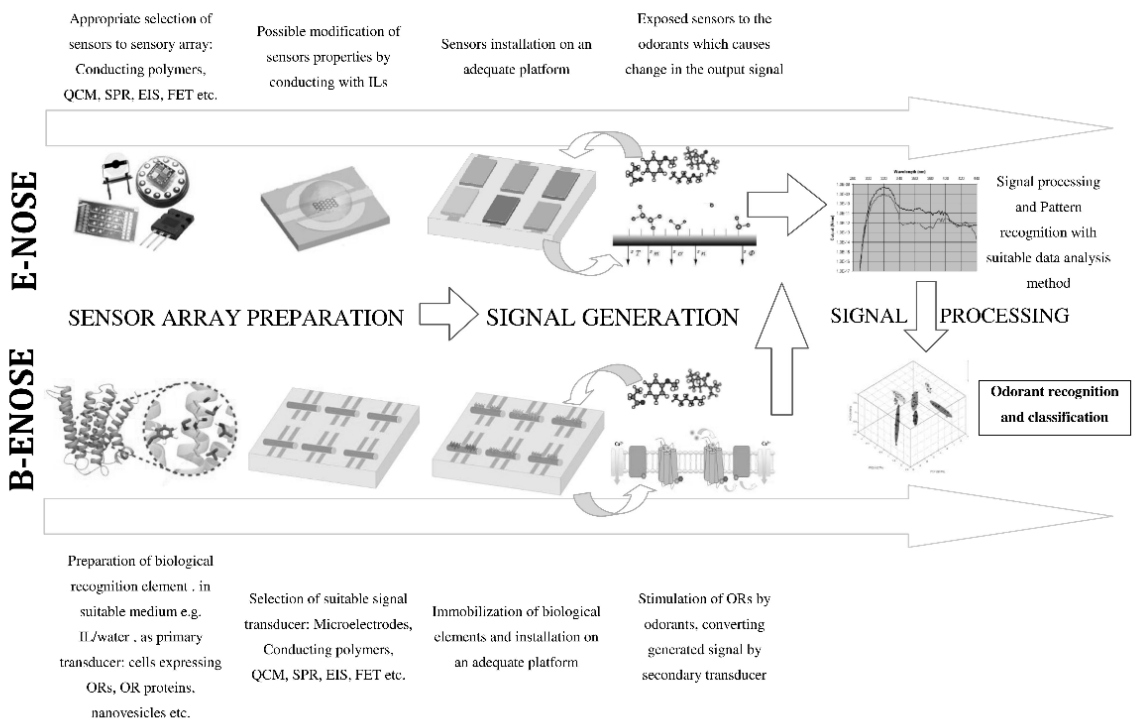

Fig. 1. Comparison of the structure and principle of operation of electronic and bioelectronic nose.

3. Ionic liquids as engineering materials for sensors

Despite numerous literature reports and performed investigations confirming unique properties of the ionic liquids, including their crucial feature, the possibility of modification of their properties by selection of appropriate cations and anions, they have not found a wide application in chemical sensors employed in the electronic nose instruments. On the other hand, there are a number of ionic liquid applications to chemical sensors or stationary phases of GC columns, which are not the components of electronic noses [109]. There is a type of the electronic nose instrument based on ultra-fast gas chromatography combining advantages of gas chromatography and electronic nose. In this case the surface area of a single chromatographic peak (or peak height) is treated as a signal from a single chemical sensor. The ionic liquids found permanent applications as chemically sensitive materials in the electronic nose instruments only in the case of gravimetric sensors. It seems that such situation are due to the MOS-type sensors, which are still successfully employed in the electronic nose devices. These sensors are characterized by a low price and a relatively acceptable metrological parameters. Another aspect, which makes the ionic liquids so popular

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owing to single sensors is the fact that addition of the ionic liquid to chemical sensors often results in improvement of the metrological parameters. However, from a global perspective, this benefit is not significant enough to trigger mass utilization of the ionic liquids in the electronic nose instruments [110]. The cost of the ionic liquids is also relatively high as compared to that of the remaining components of the electronic nose. These factors together with the fact, that at room temperature, most of the ionic liquids are in a liquid state, which restricts the functionality of the chemical sensor containing the liquid, are the reasons that the ionic liquids have not been widely employed in electronic nose design, although they offer potentialities towards such application [111]. Based on literature [112–120] it can be stated that the ionic liquids have successfully been utilized in:

- conducting polymers (CP) sensors,
- amperometric sensors,
- optical sensors,
- capillary columns as polar stationary phases.

Those types of sensors and GC columns are characterized below, together with a description of ionic liquid influence on their operation parameters. In the case of the conducting polymers sensors, their sensitivity to particular components of a gas mixture depends on a gas matrix-polymer partition coefficient. The ionic liquid as a component of the polymer layer, provides not only selective sorption with respect to a given analyte but also offers modification of electrical conductivity of the polymer matrix. An additional aspect of application of the ionic liquids is the ability of the sensor to operate at higher temperatures and lowering the sensitivity of the sensor's signal to relative humidity of a gas sample. In the amperometric sensors the ionic liquids were used as an internal electrolyte, which enabling measurement of chemical compounds that could not be determined as electrolytes in aqueous solutions of acids or bases due to a too narrow electrochemical window ensuring electrochemical durability of a particular internal electrolyte of the sensor. Another advantage of ionic liquids application in the amperometric sensor is the ability to treat them as a membrane separating a gas sample from the surface of a working electrode thanks, to high values of the dynamic viscosity coefficient. In the optical sensors the sensitivity depends on the type of dye or dye mixture applied as well as on the type of the polymer matrix containing a fluorescent dye. The properties, including polarity, hydrophobicity, porosity and the tendency towards expansion,

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of a supporting polymer containing the fluorescent dye in its matrix have a significant impact on the sensor response. Employing the ionic liquids in the optical sensors, enabled varying of the physico-chemical characteristics of the polymer matrix, mainly its polarity and sorption properties, thus increasing the sensor sensitivity. The application of the ionic liquids as stationary phases in the capillary columns affected their selectivity with respect to separated polar compounds, improved efficiency of their separation and most of all increased their thermal durability and ensured a high column coating performance. Table 4 presents the information on application of the ionic liquids and their function in the aforementioned sensors and capillary GC columns.

Table 3. Application and function of ionic liquids in CP-type, amperometric, optical sensors and capillary GC columns.

Sensor type	Function of the ionic liquid in the sensor	References
Amperometric	Electrolyte,	[121–127]
Optical	Immobilizing the dye in the matrix,	[128,129]
	Solvent in a polymer network,	[130]
Conducting polymers sensors	Modification of conductivity of polymer matrix,	[131–133]
Capillary GC columns	Stationary phase	[118,119,134,135]

3.1. Techniques of immobilization of ionic liquids on the surface of sensors

Attempts at developing of new materials and transducers for the gas sensors operating at high temperatures as well as in normal conditions are aimed at optimization of operation parameters of the sensors. Synthesized IL thin films provide additional control over the selectivity and sensitivity of interaction between components of a gas mixture and the receptor layer. Maintenance of high activity of the sensitive layer on the sensor's surface is a fundamental point of every immobilization procedure. The ionic liquid can either be immobilized on a solid carrier, remain in contact with the sensitive layer or particular sensitive elements can be dissolved in IL. The term immobilization usually refers to the way of immobilization of defined units on a separate solid component or that constituting an integral part of the substrate. Immobilization on or in a solid carrier involves a reaction of the immobilized material or its precursor (for instance by formation of a covalent or ionic bond)

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with the substrate. The material or its precursor can be immobilized on the substrate by absorption, adsorption, chelation, hydrogen bond, entrapment or other techniques utilized for immobilization of chemical compounds [139,140]. The immobilization method should enable the most effective interaction of a newly formed sensitive layer with the analytes. Immobilized material can be localized in a close vicinity of the sensor's inlet, electrode or working electrode to improve operation parameters. Application of the ionic liquids in electronic devices requires specific design and preparation of a special, leak-tight micro-chamber for IL storage. Such approach was employed by Stetter et al. [125,141] for construction of the amperometric sensors. Another approach to design the gas sensors containing the ionic liquids is application of solid polymer electrolytes (SPE) where the ionic liquid is immobilized in a polymer matrix [116,142–145]. Solid electrolytes of the polymer-ionic liquid type exhibiting high ionic conductivity can be obtained through *in situ* photo-polymerization [146]. This method allows obtaining solid polymer electrolytes as a result of the reaction occurring at room temperature in a relatively short time (about 10 minutes). An appropriate selection of the substrates can yield polymer electrolytes with a high ionic conductivity of the order of 10^{-3} - $2 \cdot 10^{-2}$ S/cm, which in some cases exceeds that of the ionic liquid applied. The approach utilizing SPE allows the development of fully printed, elastic sensors, for example on PET substrate, additionally characterized by a lower cost of production. Kuberský et al. [145] proposed a procedure of immobilization of five different ILs, the first stage of which was conditioning of SPE with appropriate ionic liquid at 70 °C for 3 minutes, resulting in lowering the IL viscosity. Then 15 mg of SPE was developed *via* casting against a ceramic substrate and the layer formed was placed onto a heating plate (120 °C, 3.5 min). The final stage was deposition on a carbon nanotubes substrate using an aerosol jet printing (AJP) technology. Morphology and structure of the SPE layer, which depend mainly on PVDF [poly(vinylidene fluoride)] crystalline phases, can be described using scanning electron microscope and X-ray diffraction analysis.

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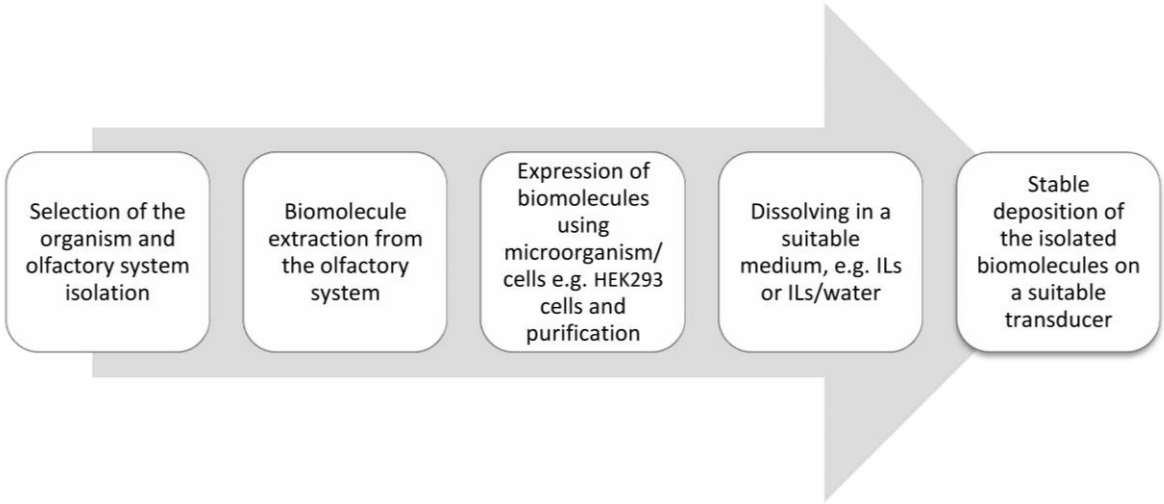

Fig. 2. Scheme of ORs fabrication and immobilization process [86], [42].

The application of ionic liquids as electrolytes and/or solvents creates stimulates the development of sensors composed of modified electrodes. The surface of such an electrode is covered with a material or a biomaterial capable to generate a chemical or biological signal, which can be converted by the electrode. Properties of the surface-modified electrodes are closely connected with physico-chemical properties of the immobilized molecules and with the immobilization technique applied. ILs, as solvents with low volatility, are combined with many materials intended for construction of the modified electrodes. This fact has a positive influence on metrological parameters of the gas sensors. An improvement of operation parameters of the sensor can be observed even when ILs are used as neutral solvents for immobilized materials [86]. Their low melting points and a weak tendency to crystallization cause that, depending on requirements and destination, ILs can be employed as modifying agents for the gas sensors. Among different types of electrode modifications to be implemented in the gas sensors, the most popular are those with IL drops or with IL films. Preparation of the sensitive surface of the electrode relies on direct deposition of IL [147] or a diluted IL solution in a solvent of high volatility [148]. Each procedure offers a specific character of deposition. Thin liquid film geometry is recommendable for those sensors, whose surface is built from a porous Teflon membrane. There is also a possibility to elaborate the IL-modified electrodes *in situ* trough direct adsorption of an IL aqueous solution [149]. ILs are envisaged to be useful for investigation of electrochemical properties of water-miscible

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ILs in aqueous media provided the same counteranion is used as the supporting electrolyte [149]. An appropriate selection of the electrode and the ionic liquid is important in this case to obtain a stable deposition of liquid upon contact with the aqueous solution. Studies on IL-modified electrodes started from coating their surface with the IL drops or films. The current trend is focused on modification of the IL-based electrodes with organic or inorganic polymers, nanoparticles, nanotubes and other micro- or nanocomponents. Broad potentialities of electrode modification range from deposition of simple, plasticized IL films to multi-component composites [150]. Direct modification of the electrodes, especially with imidazole ionic liquids, can be performed to get functionality associated with the immobilization techniques used. Different types of functionality encompass self-assemblies, covalent bonds present on the electrode's surface, which can be used for development of polymer based ionic liquids (PILs) and for the functionalization of conducting components of polymer films. In a prepared electrode, the counterions are electrostatically attracted by positively charged functionalities and can be exchanged by immobilization on the electrode's surface. Such tiny modifications are implemented to achieve desired properties of the electrode [151,152]. High viscosity of the ionic liquids and of hydrophobic ionic liquids is utilized to increase efficiency of the IL-based carbon paste electrodes (ILCPE) [153–155]. As opposed to classic CPEs, the binder consists of charged particles and ensures ionic conductivity. ILCPEs are prepared by grinding and mixing of graphite particles with the ionic liquid and placing them in the cavity of a polymer or glass tube.

As compared to classic CPEs, the IL-based carbon electrodes exhibit much higher conductivity owing to enlarged active surface capable of electron transfer and/or owing to changes in morphology, enhanced analyte solubility and additional interface between liquid/liquid phase [149]. Due to their outstanding characteristics, ILCEPs have been used for a highly selective and specific analysis of gases [156]. Figure 3 presents the possibilities of the modified electrodes integration with the ionic liquids.

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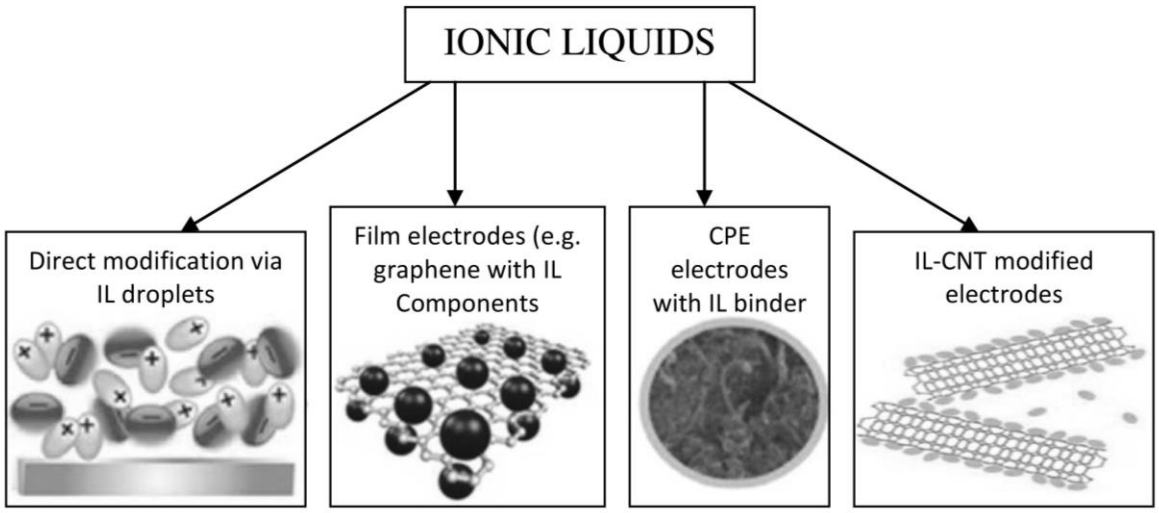

Fig. 3. Schematic presentation of commonly employed electrode modification protocols utilizing ILs as one of the components.

One of the first examples of construction of ethylene electrochemical sensor based on a thin IL film was reported of Zevenbergen et al. [157]. The sensor response time and amplitude depended on the thickness of the ionic liquid layer (tris(pentafluoroethyl)trifluorophosphate ([HMIM][FAP]), the relative humidity and applied potential in agreement with a theoretical model based on diffusion. The detection limit of that type of sensor was estimated at a level of 760 ppb and the linear range extended up to 10 ppm, which enabled to detection of ethylene in fruit quality monitoring. However, practical application of ILs in the gas sensors is limited due to problems associated with immobilization of the liquid in micro-devices. One of the solutions to this problem is utilization of ionic liquid-based polymers (ILPs). ILs in the form of gel induced by polymers, commonly termed *ion gels*, offer excellent conductivity and mechanical durability [158]. The ion gels, similarly to polymers, enable modification of mechanical properties; additionally they exhibit physico-chemical properties inherent to ionic liquids and were elaborated as the substitutes for solid-state polyelectrolytes [159]. Carvalho et al. [160] proposed to use an *Ion Jelly*® (IJ) ionic liquid, classified as a hydrogel, as a component of the sensor. IJ is the electrolyte of a flexible structure that stems from combination of a gelating agent (e.g. gelatine) with the ionic liquid. It allows formation of the gel characterized by versatile properties and high conductivity, which can be formed into various shapes using different techniques, thus expanding the field of application over numerous surfaces (blocks, films, fibres, microparticles) [161], [162]. Moreover, IJs exhibit high conductivity of the order of

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10^{-3} S cm^{-1} and a high thermal stability, up to 180 °C. The main advantages of IJs utilized in design of the electronic nose instruments include:

- fast response to volatile compounds and short recovery time,
- good repeatability,
- high sensibility,
- low cost and ease of preparation,
- no heater requirement, low power consumption,
- portability.

The ion jellies are used in commercial devices as electrochromic windows [159,163]. They can be successfully utilized in construction of the gas sensors matrixes of the electronic nose instruments. It was also shown that IJs were a suitable material for biosensors, *e.g.* for a glucose biosensor [164]. IJs provide biocompatible environment for the enzymes, such as oxi-reductases (including HRP, glucose and others), employed for construction of electrochemical biosensors, maintaining high activity of immobilized biological materials. The cyclic voltammetry technique confirmed a direct signal transfer between deposited biomaterial and a modified electrode, this providing a potential to construct odour biosensors [165]. Schematic representation of the gas sensor with IJ as a sensitive component is displayed in Figure 4.

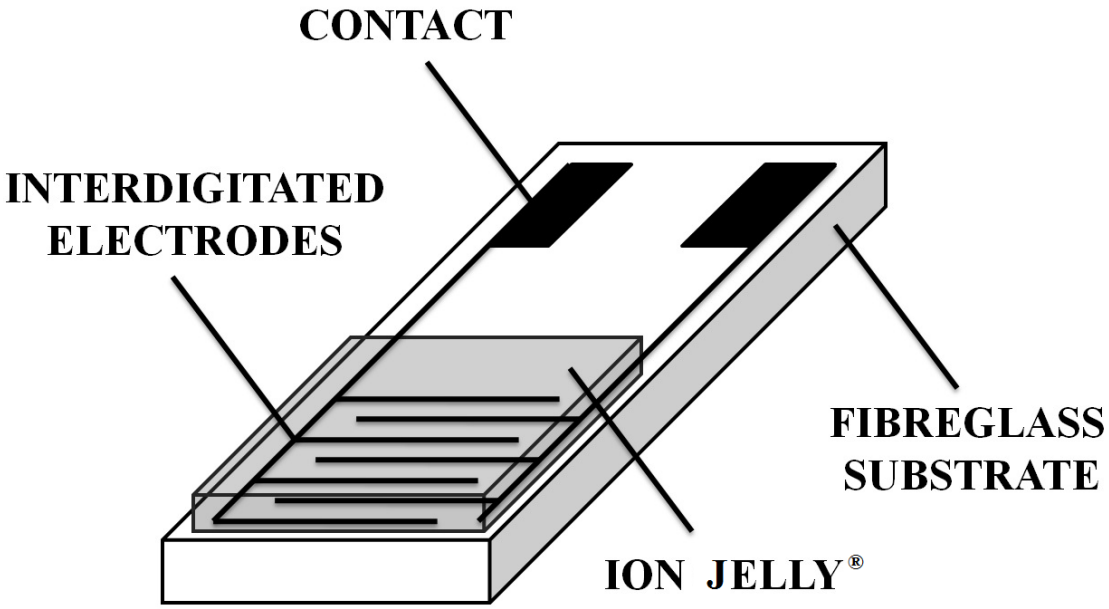

Fig. 4. Ion Jelly® gas sensor [160].

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4. Ionic liquids-based sensors

The Unique properties of the ionic liquids: bifunctionality (organic/inorganic, solvent/electrolyte), diphasic character (solid/liquid) qualify them to a wide range of applications, including gas sensors transducers. The selection of an apprioprate ionic liquid and the transducers for an electronic nose matrix is directly associated with characteristics of the ILs. Due to the weaker analyte-solid/liquid interactions, it is justified to utilize ILs for construction of the acoustic transducers. Bifunctionality of ILs can be utilized in designing membrane-free electrochemical sensors. An appriopriate selection of transducer with a high efficiency of gas analysis in particular application is determined by synergistic combination of properties of a selected transducer (e.g. transducing or response mechanism) with the physico -chemical characteristics of the receptor material. These aspects and the appropriate method of sensor production have direct bearing on fundamental metrological parameters of the sensor, such as sensitivity, stability, application range, *etc*.

4.1. Mass sensing transducers

The ionic liquid-based chemical sensors being the components of electronic nose belong to the class of gravimetric sensors, namely surface acoustic wave sensor (SAW) and quartz crystal microbalance piezoelectric sensor (QCM).

The sensors utilizing surface acoustic wave (SAW) are built from a piezoelectric substrate, on which there are two electrodes (transmitting and receiving ones) [166]. A chemically sensitive membrane, typically made of polymers, lipids, Langmuir-Blodgett films or self-assembled monolayers, is placed between the electrodes. Schematic design of the surface acoustic wave sensor is shown in Figure 5.

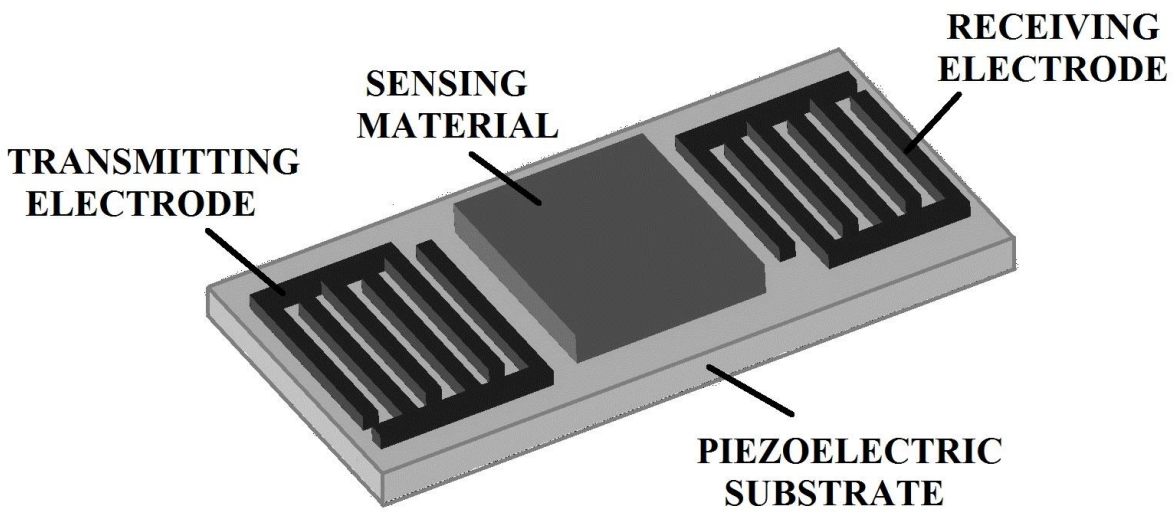

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Fig. 5. Scheme of SAW-type sensor.

An Alternating electrical signal is applied to the transmitting electrode generating surface acoustic wave propagating on the piezoelectric surface with a frequency between 100 and 1000 MHz [167,168]. A change of membrane mass caused by the sorption of chemical compounds from the environment results in alteration of the frequency of acoustic wave propagation. This change is recorded as the sensor signal. Sensitivity of the SAW sensors depends primarily on the type of chemically sensitive membrane. Such layers can be composed of different types of substances, that had earlier been employed as GC columns packing. A wide selection of these layers expands the spectrum of odorous compounds that can be identified. This means that the analysis of mixtures of odorous compounds requires more than one SAW-type instrument. The ionic liquids have the biggest impact on sorption properties as well as hydrophilicity and hydrophobicity of applied membranes. A selection of appropriate ionic liquids contributes to an increase in sorption selectivity with respect to sample components. An additional advantage of utilization of the ionic liquids in this type of sensors is lowering of signal sensitivity to variations of temperature during sensor operation.

The sensors based on quartz crystal microbalance operate on a similar principle as that of the SAW-type sensors. AC voltage applied to one of the electrodes generates acoustic wave of the frequency range of 10-30 MHz [169–171]. With the QCM-type sensors the wave does not propagate on the surface but inside a quartz crystal. The chemically sensitive membrane is placed on the surface of the quartz crystal (Figure 6). Volatile chemical compounds are adsorbed on the membrane and build up mass of the sensor. The increase in mass modulates the frequency of crystal oscillation, which is recorded as the sensor signal.

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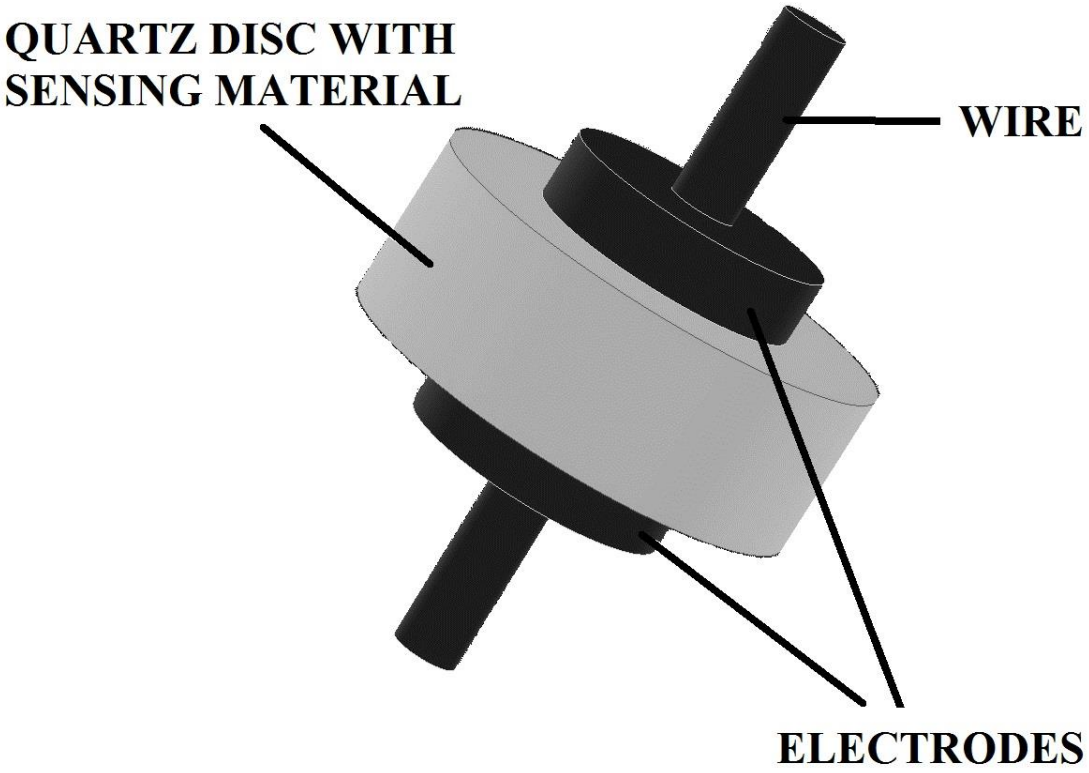

Fig. 6. Scheme of quartz crystal microbalance.

The sensitivity depends primarily on the operation frequency and the surface area of the membrane. It was shown that an increase in the frequency and a decrease in the surface area of the membrane contributed to an increase in sensor sensitivity. Similar to the SAW-type sensors, dominating aspects associated with application of the ionic liquids in the QCM-type sensors are their sorption characteristics and the possibility of selective measurement of particular component of a gas sample. Electrochemical quartz microbalance (EQCM) belongs to efficient analytical tools, in which the ionic liquid can be used as both the electrolyte and the receptor layer of a sensor. Table 5 presents the information about application of the ionic liquids in the SAW-type and the QCM-type sensors, which are the components of the electronic nose instruments.

Table 4. Application of ionic liquids in SAW-type and QCM-type sensors as the components of electronic nose instruments.

Function of the ionic liquid as components in the sensor	Analyte/matrix	Reference

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of electronic nose		
	VOC's	[56]
	THF, cyclohexane, iso-octane, methanol, toluene, acetonitrile	[168]
	Ethanol, heptane, benzene, dichloromethane	[172]
	Benzene, ethanol, dichloromethane, heptane	[173]
	Ethyl acetate	[174]
Chemically sensitive membrane	Toluene, ethanol, acetone, dichloromethane, benzene, toluene, hexane	[175]
	Nitroaromatic compounds	[176]
	Toluene, ethanol, acetone, dichloromethane	[177]
	Toluene	[178]
	Methane, nitromethane, 1-ethyl-2-nitrobenzene	[179]

4.2. Multi-transduction systems

Despite the easy commercial accessibility and possibility of synthesis of the ionic liquids and their composites, some concepts of the gas sensors arose involving a complicated methodology of the gas analysis. The gas sensors systems can be built using available transducers and methodology in a way that allows quantitative measurement of several gases in a gas mixture, in spite of the fact that it also contains interfering substances. Initial metrological parameters of the sensor can vary due to:

- presence of analytes and interferants above calibrated detection levels,
- variations of temperature and humidity,
- contamination and poisoning of the receptor surface of the sensor,
- mechanical damage,
- aging of measuring material of the sensor.

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Facing these problems requires a search for new materials for the construction of sensors or devices composed of chemical sensors matrix. It can be noticed that the application of multimodal transduction systems has gained increasing popularity [150,180]. As the examples of such design solutions are the following combinations:

- electrochemical-QCM [181,182],
- FET-QCM [183],
- optical-QCM [184],
- cantilever-capacitor-calorimeter [185,186],
- opto-electronic [187,188],
- multiparametric detection with the same transduction principle [189,190],
- FET-temperature programmed sensors [191].

A limitation of these types of combinations is the selection of a suitable sensitive material, compatible with particular transducers. The ionic liquids, thanks to their properties, are one of the materials, which can be used in such applications [181]. An example of multimodal transduction system is shown in Figure 7.

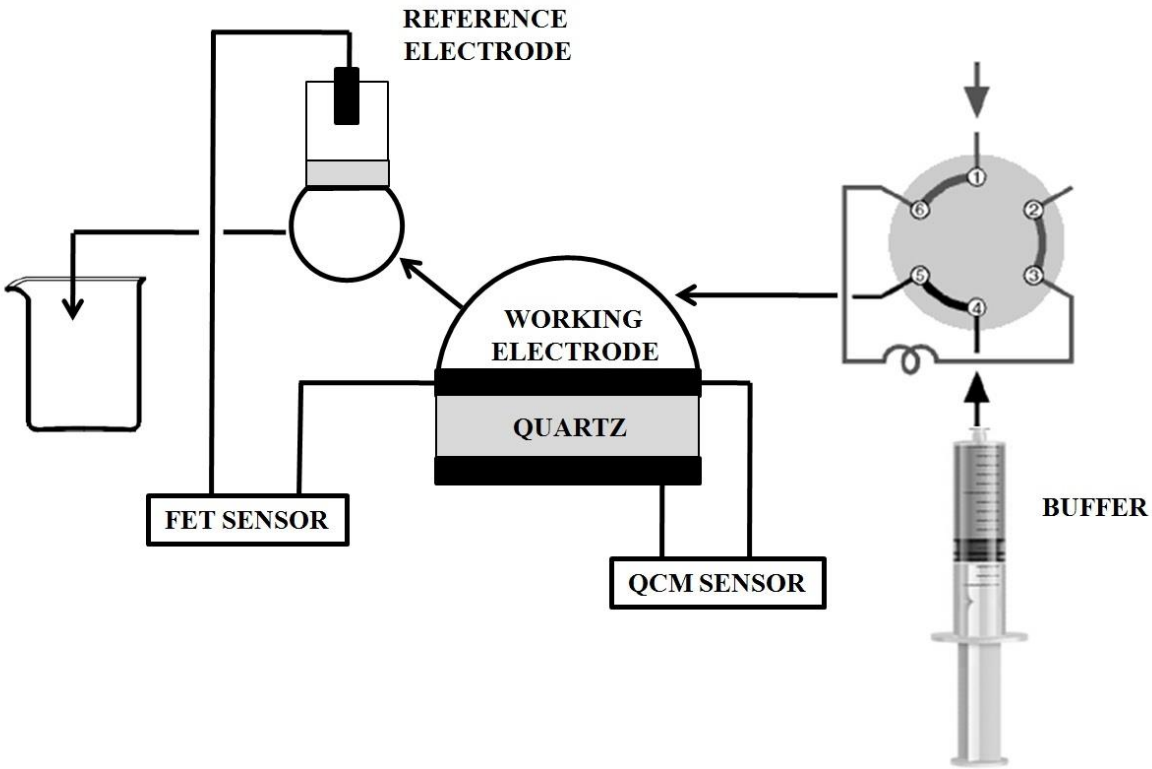

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Fig. 7. Example of multimodal transduction system for gas sensor development. An integrated setup of QCM and FET sensors [183].

5. Ionic liquids-based biosensors

Tracing the development of electrolytes' application in design of the biological sensors shows that water has been the main component for a long time. However, its low thermal stability, volatility, narrow range of operation temperature significantly limited development of the biosensors [72]. The ionic liquids were introduced in order to overcome these shortages [73]. High compatibility between the ionic liquids and biomaterials [192–195] can result in enhanced activity of some biomolecules. Appriopriate composition of the receptor layer, composed of the biomaterial, and appropriate selection of the ionic liquid leads to an improvement of utility properties of the biosensor [196,197]. Development of biosensors technology is aimed at approaching the sensitivity range of the biological counterparts, where odorous substances can be detected at the nanomole level (or lower) [198–200]. The concept, capable of meeting those these expectations, hinges on employing biological components originating from olfactory systems [201,202]. Utilizing ILs as binding agents in the biosensors, enabling efficient immobilization of the biosensitive materials on the sensor's surface. Sensor design should account for a fundamental relation between viscosity and mass transport in solution [72]. Electrochemical biosensors can be used for qualitative and quantitative analysis of a wide spectrum of analytes. Depending on its destination the receptor layer of the biosensor can be built from different types of biosensitive elements (enzymes, nucleic acids, antibodies, microorganisms, receptors, *etc*.). An integral element of the biosensor is a converter part, in which a signal due to interaction of the biological material with an analyte is changed into electrical signal. ILs, as the medium for biological materials, are successfully utilized in electrochemical biosensors, mainly thanks to a relatively good biocompatibility with some biomaterials applied [136–138].

The stability of protein in ionic solution depends on maintenance of an equilibrium between protein/ion and ion/ion interactions. Optical methods belong to the most useful biophysical techniques enabling investigation of the structure and behaviour of proteins in such media as ILs or ILs/water. They provide information about the ILs influence on the higher-order structure and aggregation of proteins. The application of the non-invasive small-angle neutron scattering (SANS) method enables investigation of the size of proteins and detection of protein aggregates. However, it is not sufficient for detecting subtle changes in secondary and tertiary protein structures [203,204]. Fluorescent measurements, utilizing

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tryptophan light absorption at 340 nm, are used to determine the extent of protein folding [205,206]. When the protein-containing tryptophan in a hydrophobic core undergoes denaturation, one can notice a red shift in the emission spectrum generated by tryptophan exposure to polar environment [207]. According to Fujita *et al.* [208] aqueous solutions of some ILs provide promising media regarding protein dissolution and they do not cause structural changes. Only a small amount of water must be added to preserve basic properties of the ionic liquids [209]. Imidazole ionic liquids are one of the most popular ILs, which are applied in combination with proteins [210]. The influence of the ionic liquid in particular amino acids in peptide/protein sequence can be assessed with NMR analysis or via molecular simulations [211]. These techniques can be also useful in explanation of the mechanism of denaturation and destabilization of proteins upon the ionic liquids impact.

Selection of a suitable converter body is of utmost importance for designing a particular biosensor. The most common converter types include potentiometric, amperometric and optical biosensors. It can be noticed that two of the converters were also displayed in Table 4 as the sensors with ionic liquids. Amperometric detection is widely utilized in sensors for the measurement of oxygen concentration. The literature provides a number of papers describing application of the amperometric sensors for the detection of oxygen, which contain the ionic liquid as the internal electrolyte or where it plays a double role as the internal electrolyte and the membrane [121,212–216].

In the case of the receptor layer of sensors, the ionic liquids are generally hardly compatible with the biomolecules and there is a significant difficulty in maintaining a proper structure of the molecules after dissolution in ILs. That is why studies on biocompatibility of ILs as solution media have gradually been switched towards application of ionic liquid/water mixtures instead of pure ionic liquids. The investigations focus on maintenance of a desired structure and biological activity of the biomolecules in the presence of small amounts of ILs in a buffer. Decreasing concentration of the ionic liquid in a solution usually results in reduction of unique characteristics of ILs [110]–[112]. The mechanisms of interaction between ILs and the biomolecules still need explanation.

A fundamental parameter in the practical application of the biosensors is their stability resulting from the ability of the obtained receptor protein to maintain its three-dimensional structure on the biosensor's surface. It is expected that the ionic liquids can be employed in the matrixes of bioelectronic devices [221]. Implementation of ILs in this type of devices should significantly affect the conductivity and lifetime of proteins of the olfactory receptors [30]. The range of ionic liquids application as components of the biosensors increases owing

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to stability of their properties and flexibility in their selection *via* varying their ionic composition.

5.1. Application of ionic liquids in bioelectronic nose instruments

The application potentialities of the ionic liquids in odour biosensors used for construction of the bioelectronic nose instruments are still studied experimentally. There is a distinct trend towards immobilization of biological components of olfactory systems on the FET-type semiconductor sensors, which can employ the ionic liquids as a medium containing the olfactory receptors. A field gating effect utilizing different electrolytes (including ILs) was noticed during immobilization of carbon nanotubes on the surface of the field-effect transistors [222,223]. Development of the bioelectronic nose devices based on the FET-type biosensors should take into account the following:

- uniformity of transducers in the sensor matrix,
- type of olfactory receptors,
- appropriate selection of the liquid matrix enabling stable interaction between the odorants and olfactory receptors.

Kwon et al. [224] proposed a liquid-ion system to utilize potential of hORs coupled with graphene micropatters (GMs) as the secondary transducer. Such a strategy enables effective and close contact between the odorant molecules and a biomarker-conjugated transducer, leading to enhancement of the signal. A comparison of the FET-type sensor and the biosensor based on the ionic liquids is presented in Figure 8.

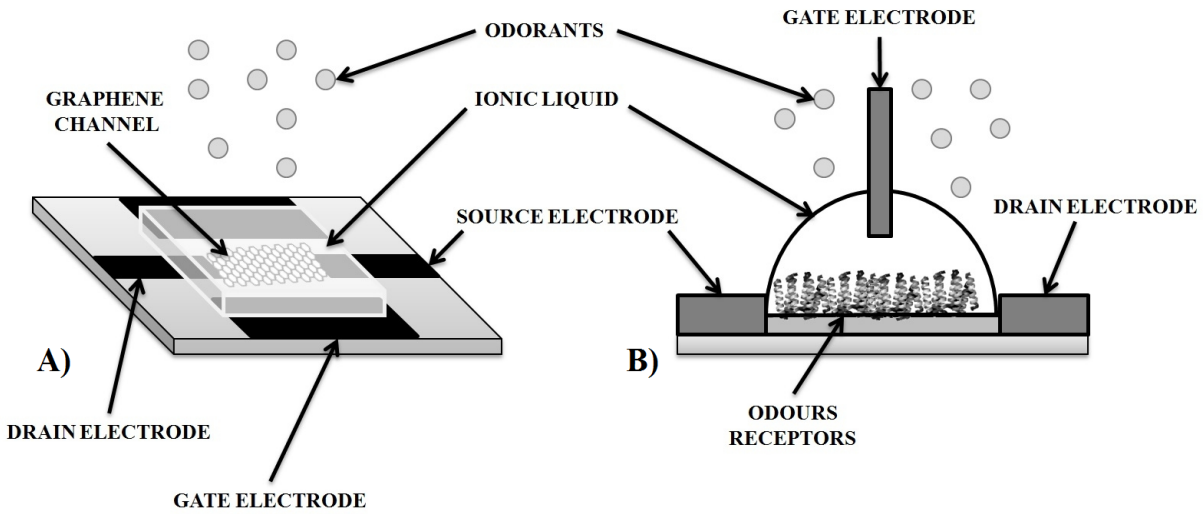

Fig. 8. Comparison of different FET-type sensors. A) Gas sensor based on a field-effect transistor with graphene channel and an ionic liquid gate [225]. B) Graphene-based, liquid-ion gated FET B-nose.

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A carbon nanotube/ionic liquid (CNT/IL) approach is gaining popularity in design of the receptor layer of the electrochemical biosensors owing to:

- retaining inherent mechanical, electrical, and thermal properties of CNT (*e.g.*, large surface area, favourable electroconductive nanowires, etc.),
- superior solubilizing and conductivity properties of ILs (*e.g.* large potential window, ion conductor, etc.),
- adequate interaction between CNT and ILs.

The direct adsorption has routinely been used for immobilization of the biomolecules on the sensor's layer. A limitation in preparation of CNT/IL *via* direct mixing of CNT with appropriate IL is a high background current, which limits monitoring of faradaic current. This increased capacitive charging current can be reduced using a steady-state linear-sweep voltammetry at the rotating disk electrodes, as suggested by the Compton group [226].

6. Development prospects of electronic nose instruments based on ionic liquids

The ionic liquids are probably one of the most intensively investigated chemical compounds during this decade. It is justified by unique physico-chemical properties of the ionic liquids, which enable their application in many scientific fields. The performance of biosensors depends on their components, among which the matrix material, specifically the layer between the recognition layer of biomolecule and transducer, play a crucial role in defining the stability, sensitivity and shelf life of a biosensor. Despite that, the search still continues for new construction and equipment solutions for design of the electrochemical gas sensors, in which the ionic liquids could significantly improve the metrological parameters [227]. The possibility of tailoring properties of ILs to desired applications creates big opportunities as far as construction of gas sensors and biosensors is concerned. The emergence of nanotechnology has opened new horizons for electrochemical biosensors. It is believed that highly sensitive and selective biosensors can be realized through the integration of biomolecules and nanomaterial-based sensor platforms. Over the last fifteen years, the sensitivity and performance of sensors are being improved by using nanomaterials such as nanotubes (NTs), nanoparticles (NPs), nanorods, nanofibers or nanoporous materials due to

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high surface area and in vivo biosensing compatibility [228–230]. Carbonaceous materials and nanomaterials providing an improvement of the detecting systems, but are also representing a very attractive and favourable biosensing platform. Nanoscale structures offer many unique features and show great promise for faster response and higher sensitivity at the device interface than planar sensor configurations. Their nanometer dimensions, being in the scale of the target analyte, show an increased sensing surface and strong binding properties, thus allowing a higher sensitivity. As well, use of ILs modified nanomaterials exhibits improved sensing properties instead of using them separately [231]. The term NTs is used to describe structures made from a wide range of materials including carbon, silica, titanium dioxide, boron nitride *etc.* Many of these structures have unique properties suitable for biosensing. The enormous promise of NTs as biosensing elements is their potential to develop systems where direct electron transfer between biomaterial and electrodes is possible [232]. Carbon nanotubes (CNTs) are in principle graphene sheets wrapped into a hollow cylinder with the ends capped or open. CNTs have individual structure with unique mechanical and electronic properties. The electrochemical properties of CNTs have been revealed, and their application toward electrochemical sensors and biosensors has gained a great interest nowadays [233]. Promising concept in development in chemical modification and funcionalization methods of CNTs with ILs is to solubilise and disperse CNTs in water [234]. As CNTs has low dispersity, thus ILs can be used to increase the stability and dispersity of CNTs also show merits in electrochemical measurements due to its high ionic conductivity and promotes mass diffusion [235]. A variety of biosensors based on CNTs have been developed [236–238]. Amongst CNTs, two varieties have been found, which be different in the arrangement of their graphene cylinders, *i.e.*, single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs).

Literature reports recommend CNTs as one of the most efficient sensor in comparison to other nanomaterials for sensing. Owing unique 3-D structure of CNTs lead to high loading of biosensing material onto the surface and increase the efficiency for electrocatalysis. In addition, CNTs have ultra sensitivity due to large length to diameter ratio, which enhances the electron transfer rate between the molecules and transducer. CNTs have excellent biosensing potential, however, still there are some challenges like, synthesis of pure, defect free, and cost-effective CNTs. Overcoming these challenges, can reveal full potential of CNTs for sensor applications. Even though, big progress has been accomplished by using graphene (GR) as sensors material, GR sheets tend to stack together through the π-π interactions [240].

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Unique properties of GR make this nanomaterial to be very attractive for sensing applications [241,242]. The biggest challenge of GR 2D nanomaterials is to alleviate the aggregation of GR layers and increase the surface area of GR [138]. More efforts are being applied in improving the electrochemical activity of GR-based electrochemical sensors [242]. In order to further boost the activity of GR with suitable composite *e.g.* CNT, conductive ionic IL can be applied [243]. The combination of CNTs/GR and IL may bring out new possibilities connected with properties and endow GR with high electrocatalytic activity. Owing to synergetic effects of conductive functionalised GR, MCNT, and biocompatible IL, the direct electron transfer between DA (co to za skrót?) and electrode surface can be efficiently achieved. The sensitivity of the presented sensor along with its improved selectivity shows potential use of this type of composites in electrochemical sensors.

Studies on the electrochemical and biosensing reveal that sensors modified by a composite film containing nanomaterials as well as IL provide better metrological parameters. It has also been observed that for biosensing, IL can gain increasing attention because biosensing material remain active and stable in this medium [244]. ILs-based composite nanomaterials provide a good ability to promote the development of highly selective, sensitive and reproducible sensors to be used in electronic nose instruments. Besides that, further research is required to improve the reusability of these ILs/nanomaterial-based sensors through the development of advanced techniques including the simplification of the immobilization method and the enhancement of the components' stability. Future efforts will also aim at guiding and tailoring the synthesis of novel materials for meeting specific electrochemical biosensing applications. The unique properties of nanomaterials suggest that future interdisciplinary research is likely to lead to a new generation of electrochemical biosensors. Researchers are now focus on understanding the various biomolecule–transducer interactions using suitable nanomaterials. Moreover, further characterization of nanostructured materials and their properties in mediums such as ILs is essential to advance the field of electrochemical biosensors. The most important challenge of nanomaterials based biosensor is the real application, such as analysis of targets in real samples. The main issue is the real sample matrix influence, such as plasma or blood. Real samples always contain the mixture of molecules and ions that would generate the nonspecific signal. Generally, most reports were performed with pure analytes without matrix effect evaluation. For that reason, researchers should also make more effort on exploring the novel biosensor designs with high selectivity in complex conditions. What is more, generally reported nanomaterial-based electrochemical

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biosensors were invented and fabricated at lab scale and are unsuitable for commercial production with good reproducibility and low cost. It is expected that future innovative research on nanomaterials coupled with other major technological advance, such as lab-on-chip, lateral-flow, 3D printing, techniques for the development of next-generation biosensors.

Although, the application of ILs in electrochemical biosensors is begun about half a decade before, we have observed a remarkable progress in the development in this field. However, many fundamental challenges related to the realization of highly stable and reliable continuous biomolecule monitoring sensors need to be resolved. Therefore, fundamental understanding of the underlying material chemistry, biochemistry, electrochemistry, surface chemistry and technological advances are needed in order to enhance the capabilities and enhance the functionality, reliability, and portability of IL-based biosensors to bring the biosensors in real applications. Researches on biosensing concepts employing ILs coupled with technological innovations, can open possibilities to widespread applications of IL-based electrochemical biosensors. Bioelectronic nose instruments has a great potential in many applications and is expected to accelerate odour classification and standardization. In particular, a multiplexed bioelectronic nose can provide complex odour information using pattern recognition techniques, and could even reproduce odours via an integrated olfactory display system. It is expected that the research on new materials for construction of the sensing and transducing parts of the sensors can lead to new prototypes of the devices from the electronic nose group intended for odour analysis [224].

Despite the fact that the main efforts are directed towards developing electrochemical biosensors based on nanomaterials, it is the authors' opinion that the research in the following areas will continue:

- The manufacture of conducting polymer sensors (CP) in which the ionic liquid is immobilised in a polymer matrix. The use of ionic liquid shall not only increase the sorption of a given analyte, but also improve the conductive properties of the polymer matrix.
- The manufacture of ionic gels the use of which, like polymers, gives the possibility to modify certain mechanical and physicochemical properties of ionic liquids, in particular of the electrical conductivity. Ionic liquids would be a substitute for semiconducting polyelectrolytes.

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- The manufacture of multi-transduction systems, in which the ionic liquid would be a component of a two- or multi-transducer circuit (e.g. electrochemical-QCM, FET-QCM, optical-QCM), either as an electrolyte or as a receptor layer.
- The immobilization of biological elements on the transducer's surface. The ionic liquid would as a binding medium between the transducer's base and the biological material.
- The improvement of metrological parameters of secondary transducers by using ionic liquids in biosensors, as already implemented in QCM, FET and CP sensors.

The main prospective directions of the development of ionic liquids used as components of sensors and biosensors constituting an integral part of electronic and bioelectronic noses are schematically indicated in Fig. 9.

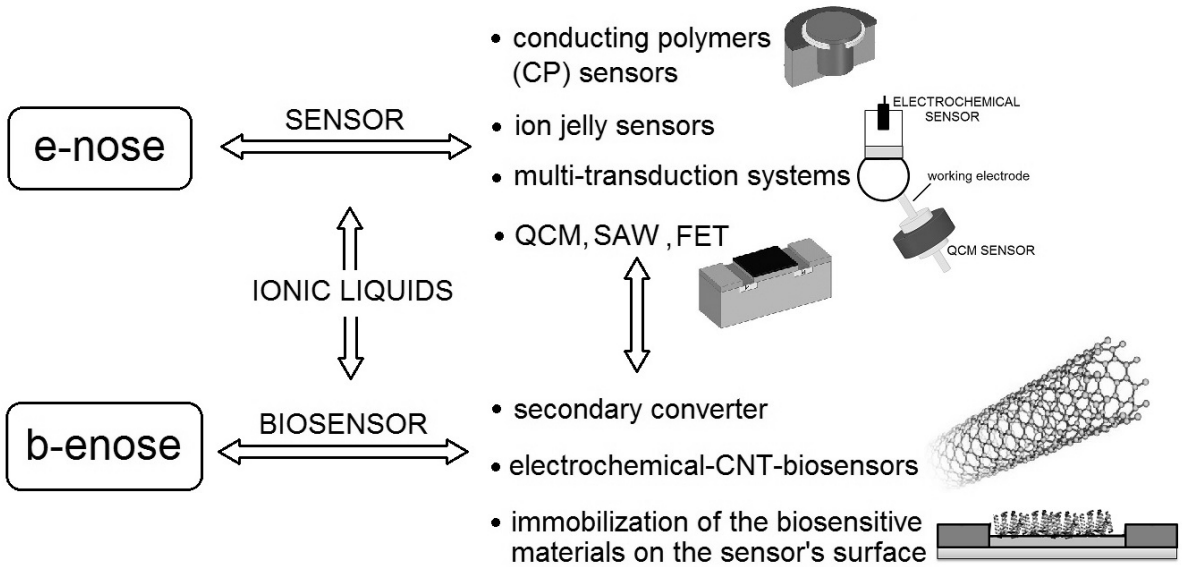

Fig. 9. A schematic diagram of the development of electronic and bioelectronic noses based on

7. Summary

The key aspect impeding the progress in the ionic liquids-based sensors seems to be a lack of their testing in real operation conditions and a lack of prototype device implementation. Acceleration of the commercialization process requires cooperation of research teams from various scientific fields. Some issues connected with electrochemistry, biochemistry, surface interactions on ionic liquids' surface remain still unexplained. An example can be the presence of contaminants and water excess in the ionic liquids serving as electrolytes, which can have a negative influence on electrochemical properties. Contaminants in ILs can trigger complicated interactions between the analyte molecules and the resulting negative impact is

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not easy to estimate. Similarly, water influence due to hygroscopicity and its overly high content in biosensor application can lead to a decrease in the electrochemical reaction rate. It is known that the ion transport rate is important in the electrochemical reaction. A high viscosity of ILs can inhibit the transport rate of the molecules to an electroactive surface, which deteriorates the response time. The way of aggregating of the substances dissolved in ILs, such as that ogbiomolecules, is not fully explained, either. Significant interest in research on the ionic liquids must be effectively sustained to focus on the stability and recycling potential of ILs. An important factor influencing biosensor development is also mass transport of the biological materials immobilized on heterogenic substrates. Despite this, the high catalytic activity of the ionic liquids and a simple procedure of preparation for immobilization stimulates the development of highly selective, sensitive odour sensors and biosensors, which can be used in the electronic nose instruments. One of the advantages of ILs is that they can be employed both for an immobilization of the biological materials on the sensor's surface and as direct transducers between the receptor part and the second-order transducer.

The progress in biochemistry, chemistry, nanotechnology and electronics is indispensable for improving potentialities, reliability, mobility and functionality of biosensors based on the ionic liquids. An increasing interest in the potential of the ionic liquids can result in a substantial progress in the odour biosensors. The development of techniques for the production of suitable materials and immobilization will make it ready to implement and commercialize biosensors based on different biological materials in many practical applications. The rapid progress in the field of sensors and growing nanostructures-based electrochemical sensors will have a remarkable influence on the development of new biosensing interfaces for resolving our future clinical diagnostics and consideration biological processes at a single molecule level. It is expected that the forthcoming decade will bring progress in the field of the biosensors designated for the bioelectronics noses, which will contribute to the development of odour analysis. Commercialization of such devices and their commercial availability as an alternative for the classic methods of odour analysis, could become a real perspective soon. A combination of the ionic liquids with gas sensors has t potential to revolutionize the fields where specific and sensitive analysis of odorants is a necessity.

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