University of Chemistry and Technology Prague
Faculty of Chemical Engineering

Applications of Supramolecular Recognition Principles in the Field of Potentiometric Sensors

Habilitation Thesis

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Declaration:

I conducted the work presented here independently. In the text I provide appropriate citations to the work of other authors.

Dedicated to my parents and the memory of my husband, Otakar Biedermann

I would like to acknowledge the inspiration I received through working and communicating with professor V. Král at University of Chemistry and Technology Prague, who inspired my interest in the application of the supramolecular recognition principle in the field of potentiometric sensors. I am very grateful to my colleagues, who all these years have stood by me in the good times and the bad, who have creatively worked with me and celebrated our results together (Gabriela Broncová, Martin Kronďak, David Sýkora, Pavel Matějka, Magda Vosmanská, Natalia Gospodinova).

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Content

Abbreviations

AFM	Atomic-Force Microscopy
5’-AMP	5′-adenosine monophosphate disodium salt
AMP deaminase	5′-adenylic acid deaminase
AuNPs	Gold nanoparticles
5'CMP	5′-cytidine monophosphate disodium salt
CNT	Carbon nanotubes
CPs	Conductive polymers
EIS	Electrochemical Impedance Spectroscopy
5'GMP	5′-guanosine monophosphate disodium salt
HCC	Hepatocellular carcinoma
HEPES	4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid
HIAA	5-Hydroxyindole-3-acetic acid
HVA	Homovanillic acid
ISEs	Ion-Selective Electrodes
MPBA	Mercaptophenylboronic acid
MRG	Molecular Recognition Group
MWNTs	Multi-walled nanotubes
N-Cbz	N-carbobenzyloxy-protected
OME	Org anic Molecular Evaporation
o-NPOE	*o*-nitrophenyl octyl ether
PANI	polyaniline film
PVC	Poly(vinyl chloride)
QCM	Quartz Crystal Microbalance
SNIM	Scanning Near-Field Infrared Microscopy
SWNTs	Single-Walled Nanotubes
TMs	Tumour markers
5'UMP	5′-uridine monophosphate disodium salt
VMA	Vanillylmandelic acid

We now live in an age of the rapid receipt and processing of information. Properly processed information helps us to make correct decisions and so avoid a number of negative consequences. What information may be important in everyday life? First of all this information is related to our health and our safety. Chemical sensors may be used to provide rapid and valuable information and to improve our standard of living. The concept of electrochemical molecular recognition can be the basis of “real-world” sensor devices. Electrochemical molecular recognition is a rapidly developing area in analytical chemistry combining electrochemistry and supramolecular chemistry. My activities were and are mainly focused on the development of electrochemical, especially potentiometric sensors. As a specialist in the field of potentiometric sensors and member of the Molecular Recognition Group (MRG) at the Department of Analytical Chemistry, University of Chemistry and Technology in Prague, I have actively participated in introducing the principles of supramolecular recognition and supramolecular receptors to the field of potentiometric sensors. My habilitation thesis is focused on selected areas which have been and will be subjects of interest and research within the MRG. The first chapter summarizes the applications of conjugates resulting from the combination of nucleobases and oligopyrrole macrocycles as receptors for potentiometric sensors. The second chapter describes a novel role of chemically generated polymers deposited on the surface of a poly(vinyl chloride) membrane. The layer of chemically generated polymer enables: i) the transfer of hydrophilic anionic species through a phase boundary to be facilitated, ii) the leaching of active components to be limited. The next chapter deals with the problem of attaching supramolecular receptors onto a solid support (the electrode surface). The main aim of this thesis is to show my contribution, the innovation introduced into the development of an electrochemical sensor based on ion-selective electrodes, and to demonstrate their potential to open up new possibilities for applying electrochemical sensors in practice.

1. Traditional potentiometric sensors: State of the art

Today we can see dynamic progress in the field of chemical ion sensors based on molecular recognition. The principles of molecular recognition have been successfully implemented in the field of potentiometric sensors. The formation of the selective potentiometric signal is often the result of a combination of directed multisite hydrogen bonding interactions, specific π-π stacking effects, and generalized electrostatic interactions between the receptor and the target analyte. Because of their selectivity and the use of small, portable, and low-cost instruments, potentiometric sensors have been successfully applied in environmental, food and clinical analysis [1]. Potentiometric sensors are comprised of PVC membrane ion-selective electrodes (ISEs) and all-solid-state electrodes based on conductive polymers (CPs). It may appear otherwise, but the development of a potentiometric sensor is not easy, because there are a number of problems to be solved.

The first problem is to find a receptor capable of selectively complexing the analyte of interest and compatible with a plasticized PVC membrane. Various supramolecular design strategies have been used to improve their specificity in terms of determining the target ion. The most systematic studies to date have been focused on enhancing the potentiometric response of cation-selective electrodes, but relatively little work has been done on anion sensing [2]. The potentiometric response of a PVC membrane ISE with a specific receptor can provide information about the analyte binding/complexing mode and disclose key interactions between the substrate and receptor. From a supramolecular chemist's point-of-view, ISEs are a useful tool for characterizing new supramolecular systems.

The second problem is to optimize the potentiometric parameters (e.g. high sensitivity, low detection limit, long-term life-time). These parameters are the most important for real-life potentiometric sensor applications. The potentiometric properties of ISEs are strongly dependent on the composition of the polymeric membrane [3]. ISE membranes consist of a receptor, lipophilic additive, plasticizer and polymeric matrix. Each component has a specific role. The lipophilic receptor (neutral or charged) governs the molecular recognition and signal generation in the polymer membrane of a potentiometric sensor. The lipophilic additive (i.e. either alkylammonium salts for anion sensing or tetraphenylborates for cation sensing) improves the sensitivity of the ion-selective liquid membrane electrode by reducing lipophilic counterion interference. The polarity of the PVC plasticizer determines the solvation properties of the analyte ion and ion-sensitivity. The polymeric inert matrix, usually PVC, provides the necessary physical properties such as mechanical stability and elasticity.

The recently published ways to improve the parameters of the membrane response are based on adjusting the properties of particular parts of the ISE. The first method is focused on lowering or better yet preventing receptor washout from the membrane to the sample. Washing out is an important factor when small amounts of ionophores are used in the microsensors.

The solutions to overcome the above problems are *i*) covalent linkage of the receptor [4-8] to the polymeric matrix, *ii*) decreasing the total amount of membrane component by using one compound instead of both matrix and plasticizer (e.g. methacrylic-acrylic copolymers and methacrylate copolymers) [5-7, 9-10], *iii*) using the proper amount of lipophilic additive to balance the charge equilibrium in the membrane [11]. Another approach employs the elimination of the ion fluxes through the membrane phase [12]. The main philosophy was based upon optimizing the composition of the inner solution and selecting a membrane less sensitive to concentration gradients (e.g. membranes involving lipophilic microparticles of silica gel [13]).

The promising materials for the construction of potentiometric sensors are CPs with pre-designed functions (so-called smart polymers) [14]. Conducting polymers are known for their ion exchange and redox properties. These features enable CPs to be applied as ion-to-electron transducers in all-solid-state ISEs, where the ion-selective membrane controls selectivity. This approach is well documented in the scientific literature and was used for both macro- and microelectrodes [14]. On the other hand, CPs can be applied in a single potentiometric sensor with its own unique characteristics. In this case, the selectivity is attained either by doping a polymer from solution or by the incorporation of recognition sites directly into the conjugated polymeric chain. Oxidized CPs such as polypyrrole, polythiophene, polyaniline and their derivatives are comprised of a polycationic backbone and a counter ion, which compensates the backbone charge. Therefore, the charge and mobility of counter ions control the ion-exchange properties of CP. Practically, the films electropolymerized in the presence of small anions provide an anionic response, and the films electropolymerized from solution containing bulky anions, which are difficult to exchange, provide a cationic response. In addition, the incorporation of specific recognition sites such as crown ethers, cyclodextrines, calixarenes, or porphyrines makes it possible to improve the selectivity of CPs [14].

Specialized sensors and devices that monitor body fluids for markers of diseases should be part of the current quality of life. Advanced industrialized humanity is calling for help against insidious diseases. The development of special electrochemical sensors may be a step forward in addressing this issue. Therefore, a number of electrochemical laboratories are intensively working on the development of sensors for diagnostics purposes. The promise of potentiometric detection has been demonstrated in the diagnosis of hepatocellular carcinoma (HCC), one of the most common cancers [15]. A sensor array composed of miniaturized metallic sensors and ISEs provided an early monitoring of urinary tumours [16]. Undoubtedly the discovery of novel materials and their application in electrochemical sensors is a driving force in innovation in electrochemical sensors/biosensors [17]. The combination of nanomaterials with electrochemical detection leads to miniaturization and more sensitive detection [18]. The introduction of novel sensing principles expands the range of analytes determined electrochemically [19]. This can be demonstrated on the example of integrating the polyion sensing principle into a simpler setup for point-of-care devices [20]. Electrode surface modification is another promising approach in this field.

2. Supramolecular chemistry and potentiometric molecular recognition

Supramolecular chemists are facing a difficult task to design receptors for the selective recognition of biologically active mono- and polyanions. The search for selective receptors is a common aim that brings together supramolecular and analytical chemists. The task of a supramolecular chemist is to design a selective receptor; that of the analytical chemist is to carry out a complete study of the properties of supramolecular receptors and to find suitable analytical applications for them.

Carrier-based ISEs constitute an established set of analytical tools that provide a suitable means for selectively detecting one or more analytes within a complex mixture. The selectivity of a given sensor is mainly dictated by the complexation specificity of the receptor involved [21]. High selectivity can be achieved by including various multiple binding modes such as H-bonding, π–π stacking, coulombic and hydrophobic interactions. From a more mechanistic perspective, the potentiometric response of membrane-based ISEs containing a specific ligand can be used to provide information about the mode of analyte binding as well as, at least potentially, insights into the relevant analyte–receptor interactions. The construction of ISEs is particularly interesting from the perspective of supramolecular chemistry; such systems could provide a suitable method for characterizing the substrate-binding characteristics of receptors that might not otherwise be accessible to study in the presence of water due to, *e.g.*, poor aqueous solubility and/or overly weak binding affinities. Ion-selective electrodes are also of obvious interest, because they can help to translate the chemistry of new substrate-binding systems into tools that can be used to selectively recognize various target species in the presence of potentially interfering analytes.

The design of anion receptors and the electrochemical, especially potentiometric, detection of anionic species is a particular challenge. The selective binding of anions should take into account their specificity. In contrast to cations, anions have a lower charge to radius ratio, have many different shapes and are more hydrated and pH sensitive [22]. The development of potentiometric anion sensing and the research of novel anion receptors have received increasing attention in our MRG since 1996. Recently, a number of conjugated and nonconjugated oligopyrrolic macrocycles, such as porphyrines, phthalocyanines, expanded porphyrins and calix[4]pyrroles have been synthesized as anion receptors [22, 23]. This chapter discusses the potentiometric results obtained with olygopyrrolic macrocycles as the active components of PVC membranes plasticized with *o*-nitrophenyl octyl ether (o-NPOE).

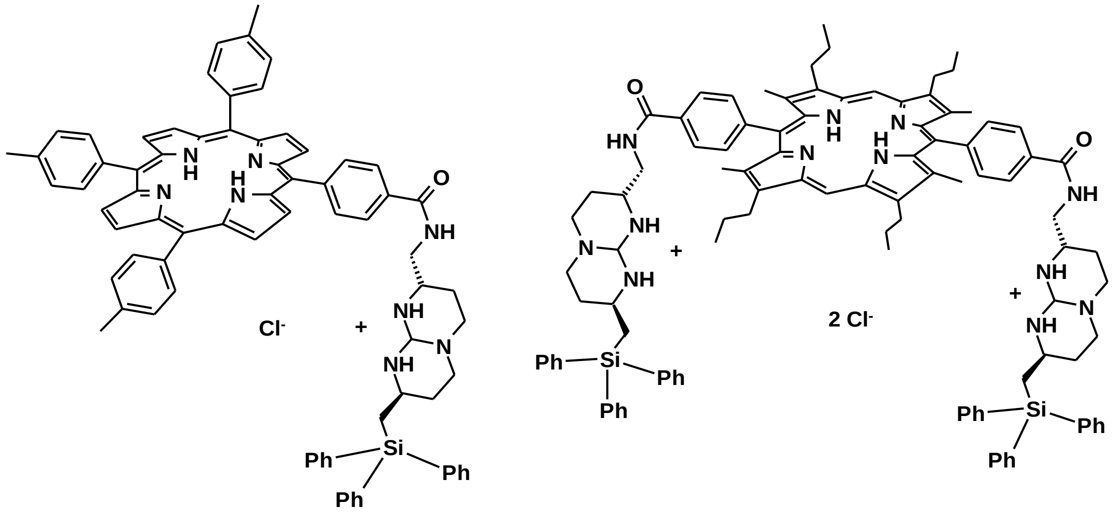
2.1. Oligopyrrolic macrocycles and nucleotide recognition

Complementary base pairing, directed multisite hydrogen bonding interactions, specific (π-π) stacking effects, and electrostatic interactions contribute to the nucleotide recognition observed in biological systems. Incorporating one or more of these recognition motifs into appropriately designed synthetic receptors could culminate in the production of highly specific ISEs more suited for the use in various “real world” bioanalytical applications.

The first nucleotide sensor selective for 5’-adenosine monophosphate (5’-AMP) was produced by Papastathopoulos and Rechnitz [24]. This electrode consisted of a layer of suspended 5′-adenylic acid deaminase (AMP deaminase) and an ammonia-gas-sensing membrane electrode. The 5’-AMP concentration presented in the sample was monitored indirectly and corresponded to the quantity of ammonia produced by the deamination of 5’-AMP.

A cytosine-pendant triamine was the first nucleotide receptor prepared for a potentiometric sensor that had affinity for the anionic phosphate and the nucleobase [25]. However, the proposed sensor exhibited a non-Nernstian response towards the complementary nucleotide (5’-GMP: –10 mV/decade; $5x10^{-4} - 10^{-2}$ M). In the course of this research, it was found that the recognition of phosphate based on electrostatic interactions is insufficient. There is a need to combine hydrogen bonding with electrostatic interactions to selectively recognize the phosphate group. Therefore, a series of ditopic oligopyrrolic macrocycles was proposed whose nucleotide selectivity will be discussed below.

A set of porphyrin-bicyclic guanidinium conjugates **1** – **2** was proposed for binding nucleotides (Scheme 1). The porphyrin framework is suitable for the nucleobase receptor which exhibits a unique recognition of the substrate through multiple interactions [26]. Guanidine units with bulky lipophilic protecting groups should provide phosphate binding and excellent solubility of the receptor in the o-NPOE plasticized PVC membrane.



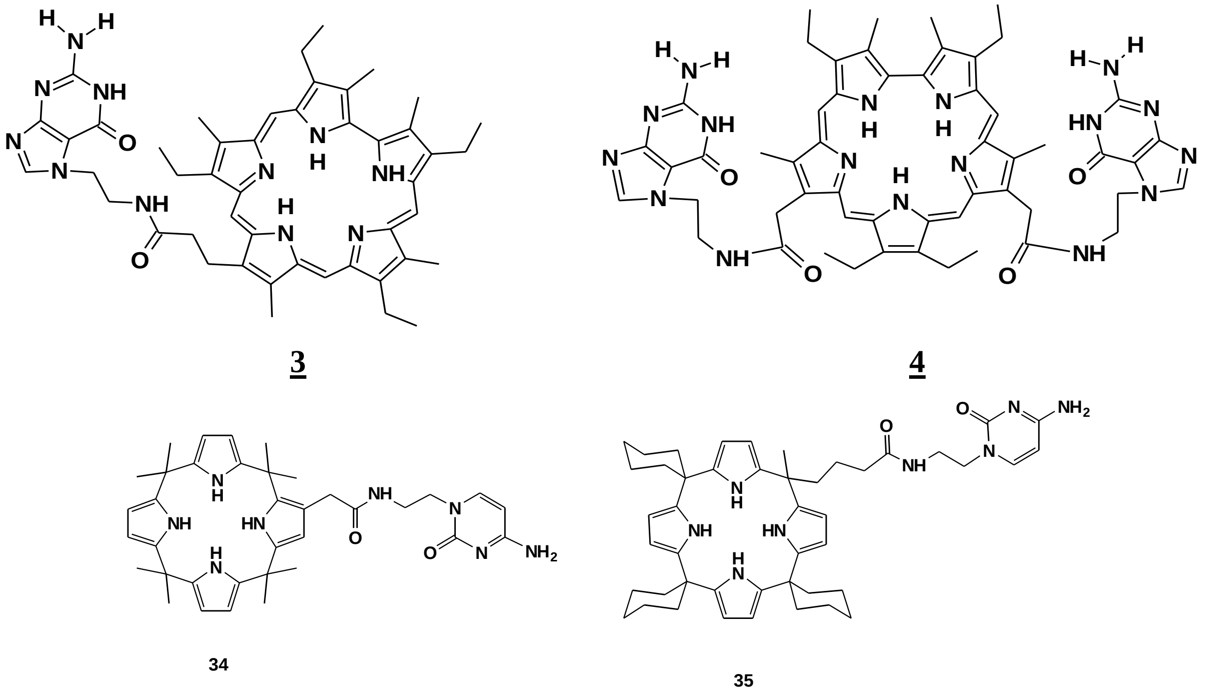
1 **2**

Scheme 1. Porphyrin-bicyclic guanidinium conjugates for recognition of nucleotides.

Table 1 Potentiometric response of o-NPOE plasticized PVC membranes based on **1** and **2** towards the tested nucleotides in 0.1 M HEPES at pH 6.6.

Nucleotide	1		2	
	Sensitivity, mV/decade	**Linear range, mol L^{-1}**	**Sensitivity, mV/decade**	**Linear range, mol L^{-1}**
5'AMP	+36	10^{-3} – 10^{-2}	–41	10^{-3} – 10^{-2}
5'CMP	–14	10^{-3} – 10^{-2}	–34	10^{-4} – 10^{-2}
5'GMP	–17	10^{-3} – 10^{-2}	–23	10^{-6} – 10^{-2}
5'UMP	–9	10^{-4} – 10^{-2}	–27	10^{-4} – 10^{-2}

As can be seen from Table 1, the bis(guanidinium) derivative porphyrin **2** gives a more pronounced potentiometric response towards nucleotides. In this case, there is an obvious effect of the guanidinium moieties serving for phosphate binding. The improvement in both sensitivity and detection limit observed towards nucleotides containing the carbonyl group (5’-CMP, 5’-GMP and 5’-UMP) could be the result of coordination of the nucleobase through the carbonyl group to a porphyrin ring. The experimental findings supported the idea that the ditopic receptor combining the binding sites for both the phosphate and nucleobase motifs are promising for nucleotide recognition. Moreover, the fixed positive charge on the guanidinium groups contributes more to the potentiometric response than the protonation of triamine.



3 **4** **5** **6**

Scheme 2. Guanine-substituted sapphyrins (**3, 4**) and cytosine-substituted calix[4]pyrroles (**5, 6**).

Recently, Sessler´s group has synthesized oligopyrrolic macrocycles, especially sapphyrin and calix[4]pyrrole, capable of binding phosphates using hydrogen bonding and electrostatic interactions [22]. We have used guanine-substituted sapphyrins (**3, 4**) and cytosine-substituted calix[4]pyrroles (**5, 6**) as active components of potentiometric sensors for nucleotide recognition (Scheme 2) [27]. It should be noted that the doubly functionalized guanine derivative, **4**, was a more effective active membrane component than its mono-substituted analogue **3** (Table 2). The binding of 5'CMP is possibly occurring through two kinds of hydrogen bonding interactions, involving both Watson-Crick and Hoogsteen recognition

Table 2. Potentiometric response of o-NPOE plastisized PVC membranes based on mono-(**3**) and bis-guanine-substituted (**4**) sapphyrins towards the tested nucleotides in 0.1 M HEPES at pH 6.6.

Nucleotide	**3**		**4**	
	Sensitivity, mV/decade	**Linear range, mol L^{-1}**	**Sensitivity, mV/decade**	**Linear range, mol L^{-1}**
5'AMP	–32	10^{-6} – 10^{-5}	–42	10^{-6} – 10^{-5}
5'CMP	–17	10^{-5} – 10^{-4}	–59	10^{-6} – 10^{-4}
5'GMP	a	a	–25	10^{-5} – 10^{-3}

[a] No response.

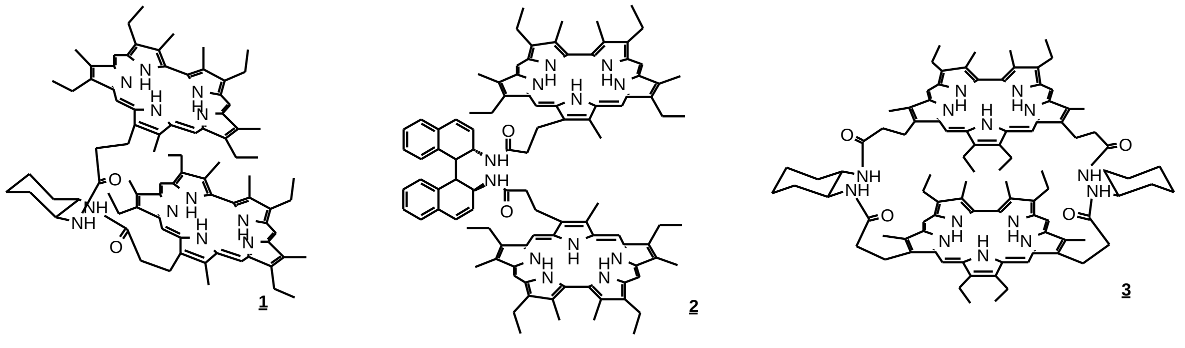
patterns. On the other hand, the selectivity sequence for the *meso*-linked conjugate **5** was more influenced by pH than its *β*-linked congener **6**. The potentiometric selectivity at high pH could help explain the relative lack of for-GMP selectivity observed in the transport experiments involving receptor **5**, where the receiving phase is also kept at high pH. Thus two very different techniques grant us insight into the interactions of nucleobase-oligopyrrole conjugates with nucleotides.

Table 3. Effect of pH on the potentiometric selectivity of o-NPOE plasticized PVC membranes based on *β*- and *meso*-substituted cytosine-functionalized calix[4]pyrroles **5** ($\log k^{\mathrm{sel}}_{5'\text{-UMP}/5'\text{-XMP}}$) and **6** ($\log k^{\mathrm{sel}}_{5'\text{-CMP}/5'\text{-XMP}}$). These membranes contained 50 mol% of TDDMACl.

pH	**5**		**6**	
	5′-AMP	**5′-GMP**	**5′-AMP**	**5′-GMP**
6.6	–0.07	+0.89	+1.53	+2.23
8.5	–0.20	+0.10	–0.68	–1.33

2.2. Sapphyrin dimers and dicarboxylate recognition

Sessler´s group has synthesized the open-chain and cyclic sapphyrin dimers **7**-**8** and **9**, bearing various bisamide spacers (Scheme 3) [28]. Mass spectrometric analysis and transport experiments showed that the new molecules **7**-8 (containing (1*S*,2*S*)-1,2bisamidocyclohexane and (*S*)-2,2´-bisamido-1,1´-binaphthalene chiral auxiliaries, respectively) and **9** complex with N-carbobenzyloxy-protected (*N*-Cbz) aspartate and glutamate. Our aim was to test these molecules as active components of a potentiometric sensor for the determination of *N*-Cbz-aspartate and glutamate. The potentiometric data showed that (*i*) the enantioselectivity of the



7 **8** **9**

Scheme 3. Sapphyrin dimers for recognition of dicarboxylates.

diaminocyclohexane-derived dimers **7** and **9** to glutamate is higher than that of the binaphthalene-containing receptor **8**; (*ii*) the enantioselectivity of both open-chain and cyclic dimers to N-Cbz-aspartate is controlled by the plasticizer polarity (Table 4).

Table 4. Potentiometric selectivity factor ($K_{(+)} / K_{(-)}$) of sapphyrin dilmers **7**-**9** towards enantiomers of dicarboxylates.

Receptor	**Plasticizer**	**Analyte**	**Selectivity factor** $\boldsymbol{K_{(+)} / K_{(-)}}$
7	*o*-NPOE	Glutamate	7.3
8	*o*-NPOE	Glutamate	0.5
9	*o*-NPOE	Glutamate	2.6
8	*o*-NPOE (DOP)	N-Cbz-Aspartate	2.2 (2.6)
9	*o*-NPOE (DOP)	N-Cbz-Aspartate	2.8 (1.6)

2.3 Demand for innovation in the potentiometric determination of anionic species

Interest in the determination of biologically important anions has not diminished over time. However, most anionic species are highly solvated ions, which creates a significant problem for their potentiometric determination, despite the presence of the selective receptor in the membrane phase. It is possible to keep a lipophilic, selective receptor in the membrane phase and to make the membrane phase permeable for the hydrated anion by the depositing a thin hydrophilic film of CP as an outer layer on the surface of a plasticized PVC membrane. Additionally, outer nanoscale films deposited onto ion-selective membranes may help to reduce undesired ion fluxes from the membrane [29], to reduce the interference of large hydrophobic anions [30], and to improve the biocompatibility of the potentiometric sensor [31]. The next chapter will discuss the effect of a polyaniline film (PANI) on the main potentiometric characteristics.

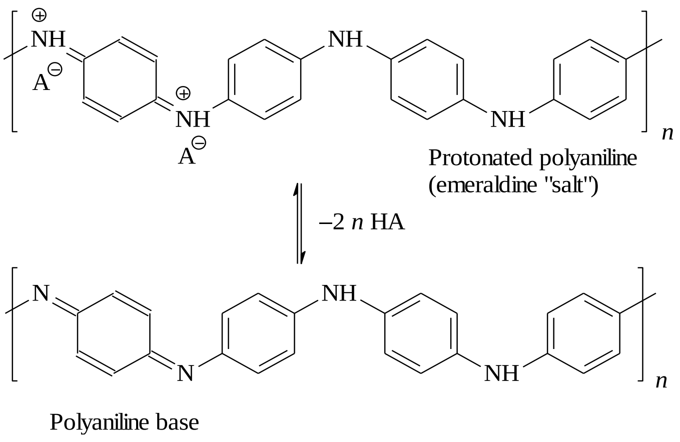
3. Chemically and electrochemically modified electrode surface

3.1. Novel role of chemically generated conducting polymer on the electrode surface

3.1.1. Conducting polymers and potentiometric sensors

Conducting polymers are making a major contribution to development and innovation in the field of sensor technology [32]. A number of groups are searching for lucrative applications for PANI, such as ion-selective sensors [14, 33].

Polyaniline has been used in the development of potentiometric sensors as a solid contact between the transducer and membrane. Due to the pH-dependent PANI (emeraldine) salt–base transition (Scheme 4), the anion incorporated as a dopant into PANI can be exchanged with other anions on the phase boundary between the polymer film and the solution. When used in this way, PANI can be applied as a functional anion-exchanger. Crowley et al. have shown that inkjet- printed films of PANI can open up novel, simple and economical possibilities for applying CPs in the field of sensing [34]. The surface properties of of conducting films, such as their hydrophilicity and surface morphology can improve the properties of various matrices, in particular traditional PVC membranes, and make them more useful in the field of electrochemical sensors.



Scheme 4. Polyaniline (emeraldine) "salt" exists in acidic media and deprotonates to PANI (emeraldine) base in alkaline media. HA is an arbitrary acid.

3.1.2. Chemically generating polyaniline onto the membrane surface

To the best of our knowledge, the deposition of a thin film of a CP has not previously been considered in the design of ISEs. Among the various techniques employed for PANI synthesis, the chemical oxidative polymerization of aniline has attracted significant interest due to its simplicity and low cost. Polyaniline is typically prepared by the oxidation of aniline with ammonium peroxydisulfate in an aqueous acid medium [35]. Any surface in contact with such a reaction mixture becomes coated with a PANI film of thickness 100–200 nm [14, 36]. We have presented a novel arrangement for the polymeric membranes used in ISEs. The surface of a conventional plasticized PVC membrane was coated with a chemically generated film of PANI.

3.1.3. Influence of surface properties on the deposition of a polyaniline film

Knowing that the PANI film is hydrophilic, it was important to provide a hydrophilic substrate for its deposition. The weight ratio of membrane components usually incorporated into the ion-selective membrane is 1:33:66 (receptor:PVC:plasticizer). The measurements of the contact angles, reflecting the wettability of the membrane surface, indicated that the ratio between the plasticiser and PVC should be 1 to 2 (Figure 1A) to obtain a more hydrophilic surface. Decreasing the content of plasticizer has led to an increase in T_g up to -29.59°C (Figure 1B), but the mechanical properties of membranes with a PVC : NPOE ratio of 2:1 remained appropriate for application in potentiometric sensors [14, 37].

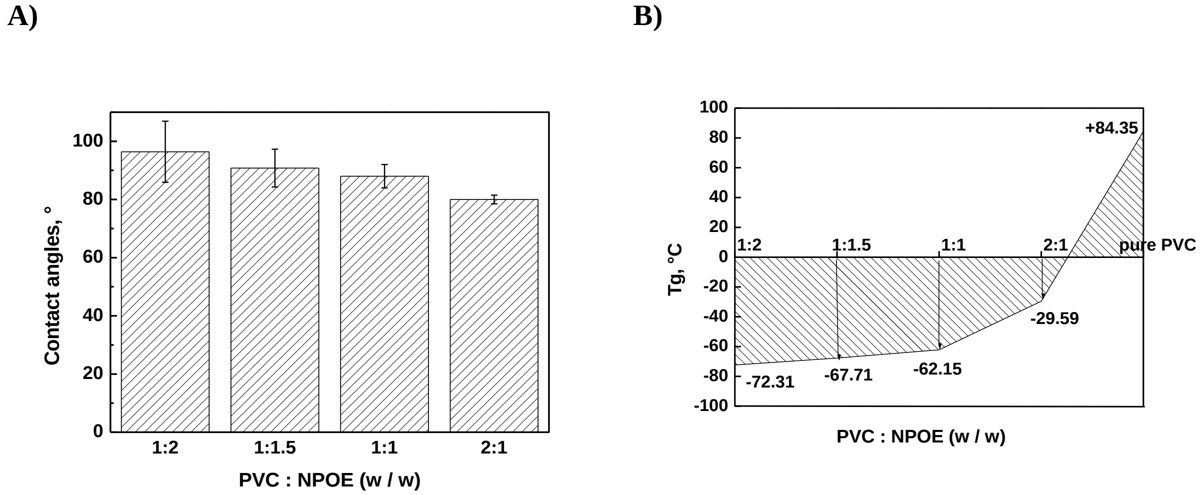


Figure 1. Effect of plasticizer content on (A) water contact angle and (B) glass transition temperature (T_g) of NPOE-plasticized PVC membrane.

The PANI deposition should change the mechanical properties of the ISM. An examination of force curves obtained from AFM force measurements enabled us to distinguish the viscoelastic (corresponding to an uncoated PVC membrane) and rigid (corresponding to PANI) response of the sample. The highest percent of rigid response was obtained for membranes with a minimal plasticizer content (PVC : NPOE 2:1; Table 5). As can be seen from Figure 2, the PANI film had a granular morphology; the size of primary PANI particles was about 30 nm. The UV-vis measurements confirmed that a compact PANI film on the plasticised membrane surface was obtained with the same membranes (Table 5).

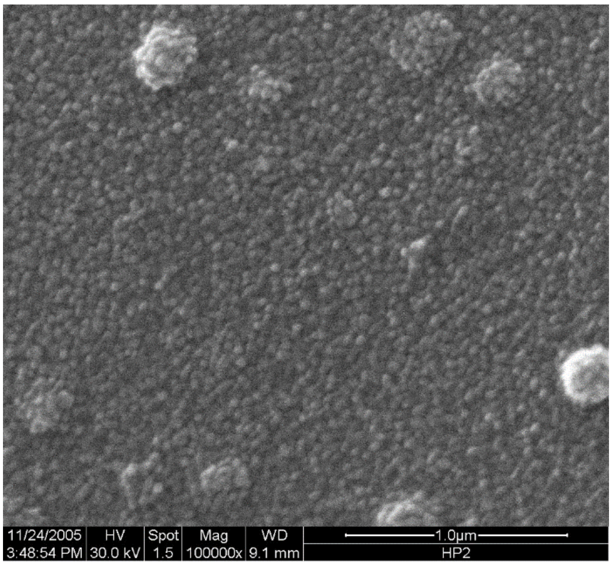


Figure 2. Morphology of PANI film deposited onto NPOE-plasticized PVC membrane with PVC : NPOE ratio of 2:1.

Table 5. Characterization of PANI-modified surfaces of non- and plasticized poly(vinyl chloride) membranes based on tridodecylmethyl ammonium chloride with various ratios of PVC to NPOE using UV-vis and AFM force measurements.

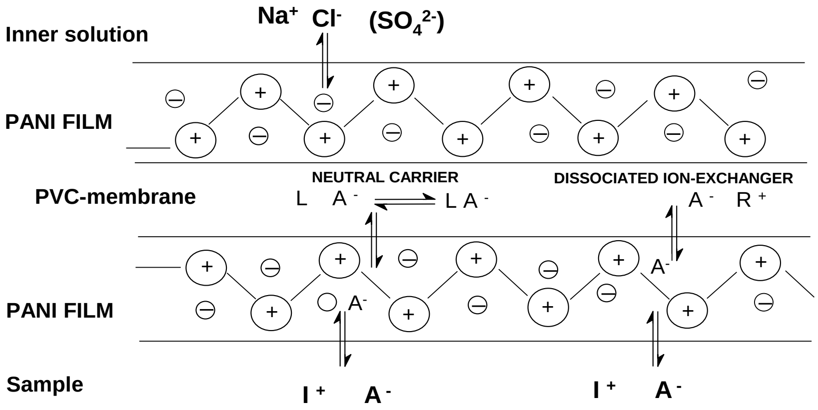
PVC : NPOE (w/w)	1 : 2	1 : 1	2 : 1	PVC [a)]
Viscoelastic sector/ rigid sector	100/0	23/77	2/98	0/100
A (λ=800 nm) (PANI)	0.8	1.2	1.6	2.2

[a)] Data correspond to non-plasticised membrane containing 100% PVC

The experimental findings demonstrated that the adsorption mechanism of the PANI film is the result of the interaction between the hydrated islands present on the substrate surface and the hydrated chains of the deposited polymer (specifically, PANI). Additionally, the deposition of PANI onto the surface of plasticized PVC membranes requires optimizing the membrane composition. Controlling the ratio between the plasticizer and PVC enables the hydrophilic surface to be prepared and a homogeneous PANI film to be deposited through chemical polymerization.

3.1.4. Working mechanism for a polyaniline film chemically generated on a PVC surface membrane

Scheme 5 illustrates the possible function of a PANI film on the surface of conventional PVC membranes in the presence of different kinds of active components, namely, neutral carrier and dissociated ion-exchanger. The sample anions diffuse through the interface into the PANI film and replace the original counter-ions, which are incorporated into PANI by the protonation of nitrogen atoms during polymerization [14, 38]. The permeability of a PANI film for various anionic species may be controlled by both its polycationic nature and hydrophilicity that are controlled by pH. The means of diffusion of an anionic species through a PANI film are open to discussion. The transport of the anions could proceed by an exchange mechanism in which the original counter-ions are replaced by the analysed anions [14, 39]. Alternatively, the anion transport could be coupled with that of a proton, *i.e.* the whole acid molecule diffuses through the PANI film. The further anion transport through the PVC phase conforms to the principles of classical ISEs. The selective potentiometric response is then dictated by a receptor in the PVC membrane.



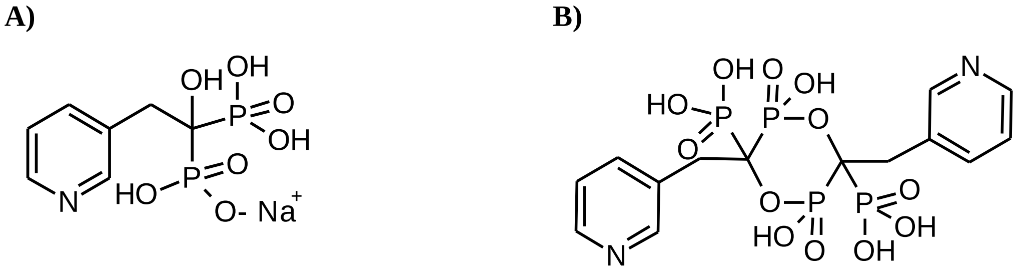
Scheme 5. A PVC membrane surface has been modified by the deposition of a submicrometre film of a conducting polymer, PANI. Membrane selectivity toward anionic species A^- is controlled by the kind of active component in the PVC matrix: a neutral carrier (L) or an anion-exchanger (R^+). Due to its polycation nature, hydrophilicity, and morphology, PANI may be a useful substitute for the conventional quaternary ammonium additive, and can control the anion exchange at the interface between the PVC phase and the sample solution.

Our results suggest that PANI behaves preferentially as an anion-exchanger. The anionic species diffuse from the tested solution through the PANI film into the PVC phase. The fact that the sensitivity of the membranes was especially improved towards highly hydrated anionic species lead us to conclude that the PANI film facilitates the transfer of such species from the sample into the PVC matrix.

3.1.5. Potentiometric sensing based on modified polyaniline membranes

3.1.5.1. Analysis of active pharmaceutical ingredients

Risedronate sodium (Scheme 6A) belongs to a class of drugs called bisphosphonates that are widely used in the treatment and prevention of diseases associated with bone loss. We proposed a very simple and inexpensive potentiometric screening procedure based on the TDDMACl-based membrane coated with a PANI layer for risedronate content verification (Table 6) [40].



Scheme 6. Chemical structures of sodium risedronate (A) and its specific contaminant (B).

Table 6. Comparison of results of risedronate determinations in tablets measured with HPLC and proposed potentiometric methods [40].

Method	**Procedure**	**Risedronate content, mg mL^{-1}**		
HPLC	Calibration plot	0.54	1.01	8.37
Potentiometry	Calibration plot	0.49 ± 0.06 a)	0.99 ± 0.11 a)	7.92 ± 0.95 b)
	Standard addition method	–	1.02 ± 0.06 a)	–

a) n = 4; b) n = 3

The PANI-modified membranes differentiated 0.94 wt % and 3.44 wt % of the contaminant (Scheme 6B) from 1.6 mg mL^{-1} sodium risedronate (Figure 3A). Importantly, the potentiometric signals recorded with three experimental membranes towards the untainted risedronate (0.00 wt %) and contaminated risedronate (3.44 wt %) were reproducible (Figure 3B). To date, there has been only one study devoted to the development of a quantitative potentiometric test for contaminants (namely, oversulphated chondroitin sulphate) in heparin preparations. This detection was based on the differences in charge density for contaminated heparin and for the untainted heparin [41]. Our approach is interesting in that it offers a novel principle for discriminating between analytes based on minor differences in their hydrophilicity.

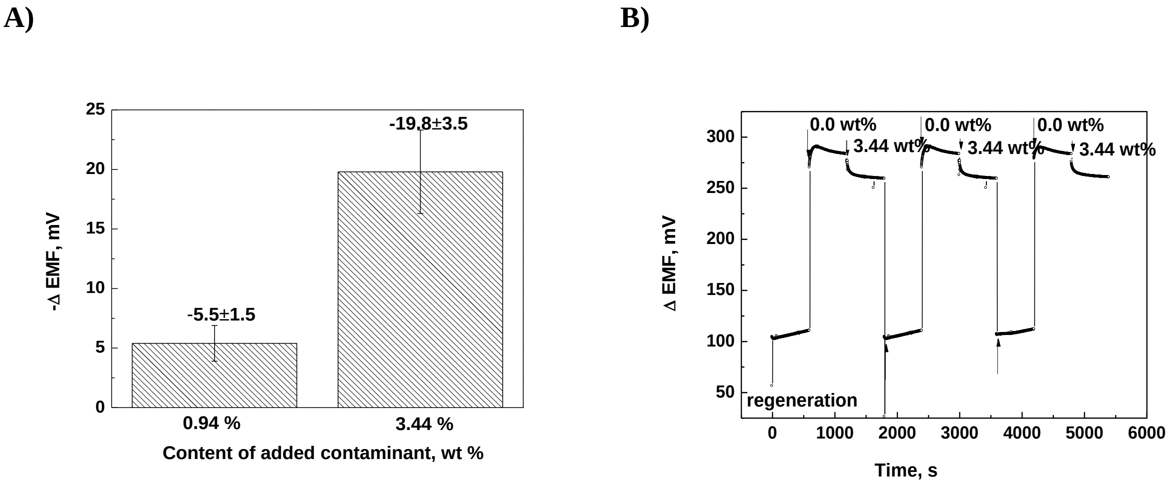
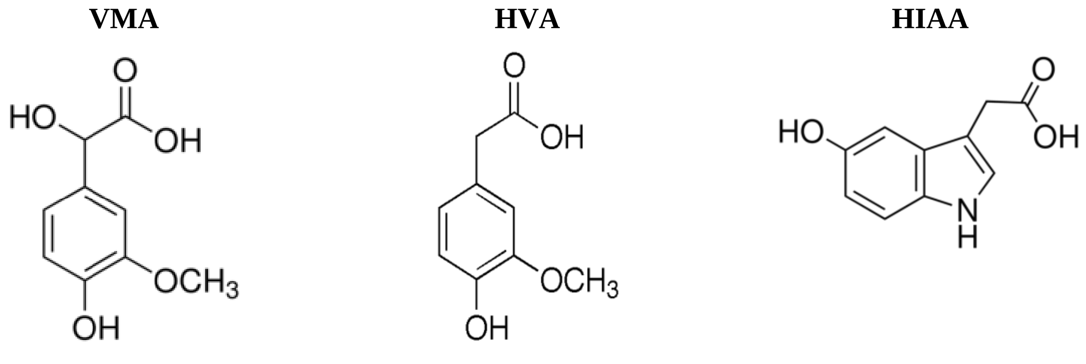


Figure 3.
A) Changes in potentiometric signal observed for TDDMACl-based membranes coated with PANI layer with various ratios of risedronate and contaminant (n = 4). In these experiments, ΔEMF was defined as the difference in the electromotive force measured for untainted risedronate ($\Delta EMF_{untainted\ risedronate}$) and for contaminated risedronate ($\Delta EMF_{contaminated\ risedronate}$). Because the addition of a contaminant led to a decrease in electrode response, the resulting values of ΔE are negative (*i.e.*, $\Delta EMF = \Delta EMF_{untainted\ risedronate} - \Delta EMF_{contaminated\ risedronate}$).
B) Recordings of potentiometric signals observed for three experimental membranes with untainted 1.6 mg mL^{-1} risedronate (0.00 wt %) and contaminated risedronate (3.44 wt %).

3.1.5.2. Analysis of tumour markers

In recent years, there has been an increasing demand for mass screening methods for neuroblastoma. Neuroblastoma, a neuroblastic tumour derived from the perimordial neural crest, is most frequently diagnosed in infancy. Neuroblastoma is characterized biochemically by the elevation of urine catecholamine metabolites, particularly homovanillic acid (HVA) and vanillylmandelic acid (VMA) (Scheme 7) [42]. Our studies showed that the PANI-



Scheme 7. The structures of tumour markers determined with the optimized PANI-modified PVC membranes.

modified PVC membranes can be used for the potentiometric detection of three tumour markers (TMs) that serve as a screening test for neuroblastoma.

For infants (0–1 year old) and children (1–13 years old), the level of the monitored TMs is (1.0 – 5.4) × 10^{-5} mol L^{-1} in urine [43]. Experiments were conducted to measure the concentration of TMs added to artificial urine, the composition of which was taken from Laube et al. [44]. The results of the potentiometric determination of TMs summarized in Table 7 indicated that the constituents of the artificial urine sample do not significantly interfere with their detection. These electrodes seemed to provide an alternative device for the direct determination of TMs.

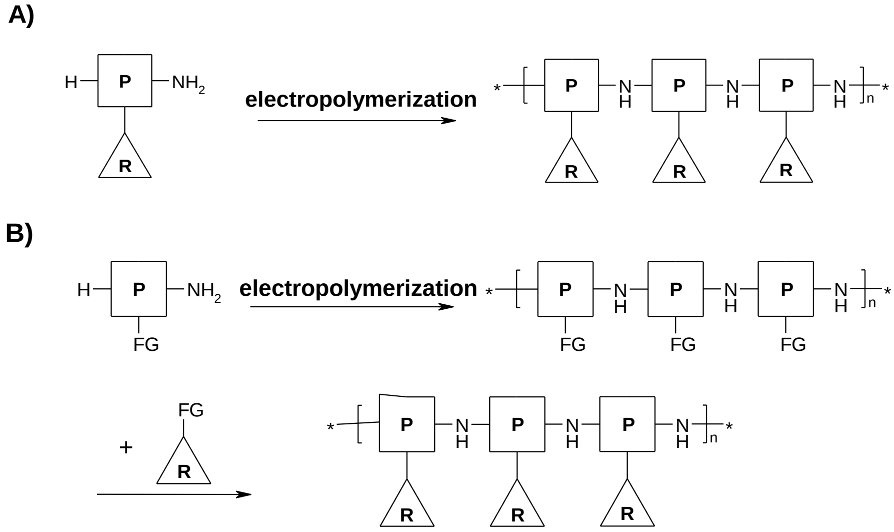
Table 7. Results of potentiometric determination of tumor markers in synthetic urine ($n \geq 6$).

Tumor markers	**Introduced, mol L^{-1}**	**Found, mol L^{-1}**
VMA	5.0×10^{-5}	$(5.2 \pm 0.5) \times 10^{-5}$
HVA	5.0×10^{-5}	$(4.8 \pm 0.7) \times 10^{-5}$
HIAA	5.0×10^{-5}	$(5.5 \pm 0.7) \times 10^{-5}$

3.2. Attaching a supramolecular receptor to a surface for potentiometric sensing

3.2.1. Strategies for attaching a supramolecular receptor onto the electrode surface

Smart CPs represent a new category of materials, which are finding applications in analytical chemistry as materials for sensor construction. These polymers can be generated by electrochemical oxidation using peripherally polymerizable units (e.g. aniline, pyrrole). The CP as sensing membrane is usually electrochemically generated directly on the metal support. The recognition sites are present either primarily in the monomer with its polymerizable unit (Scheme 8A) or are introduced by post-polymer modification (Scheme 8B).

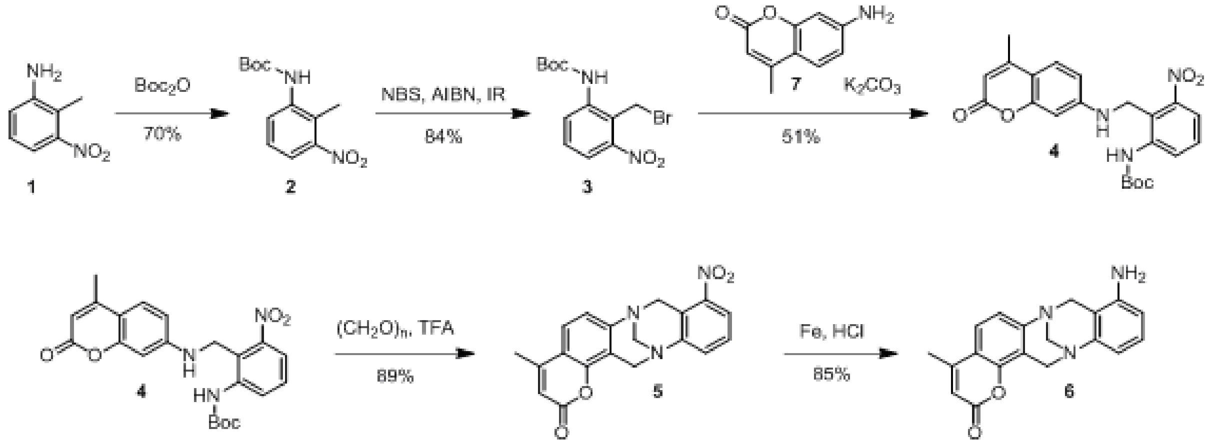


Scheme 8. A) Polymerization of receptor **R** with polymerizable unit **P**. B) Introduction of receptor **R** using chemical post-modification of electrochemically generated polymer bearing protected function group **FG.**

Part of the work of the MRG is directed towards the polymerization of a supramolecular receptor involving an aniline unit. The preparation and analytical application of the polymeric film based on Tröger base derivatives will be described below.

3.2.2. Electrochemical polymerization and characterization of Tröger base derivatives

A Tröger's base is a molecule containing two arenes connected to the *b* and *f* sides of 1,5-methano-1,5-diazocine [45]. Due to its unique structural features (C_2-symmetry and rigid V-shape geometry) and the possibility of functionalization, a Tröger's base is an attractive receptor for the development of sensors. Tröger's base derivatives (Scheme 9) were designed to enable us to deposit them electrochemically on the electrode.



Scheme 9. Stepwise preparation protocol of Tröger base functionalized with amino- and coumarin-units **6**.

The success of the supramolecular design was proven by cyclic voltammetry (Figure 4).

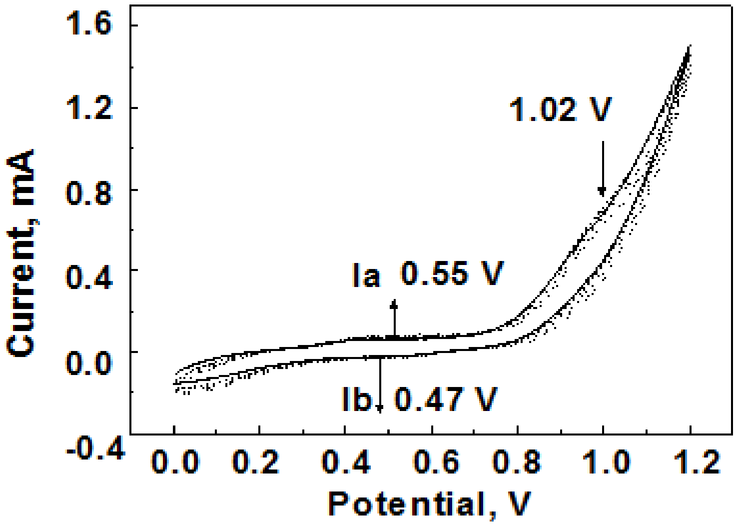


Figure 4. Cyclic voltammogram of a 1x10^{-3} mol L^{-1} solution of **6** in CH_3CN/ 0.2 mol L^{-1} tetrabutylaammonium fluoroborate + 1x10^{-3} mol L^{-1} HCl on a Pt electrode. The electrode potential was swept repeatedly between 0 and 1.2 V vs. Ag/AgCl at a scan rate of 50mV s^{-1}.

Scanning near-field infrared microscopy (SNIM) combined with nanomechanical patterning showed that the polymeric film on the electrode surface is nanostructured and consists of “hills” and “valleys” with a maximal height profile of 0.5 μm (Figure 5A). The amplitude of the infrared signal assigned to the aromatic C–C stretching mode suggested that the adhering film is porous, with grains located at the edges of both “hills” and “valleys” (Figure 5B).

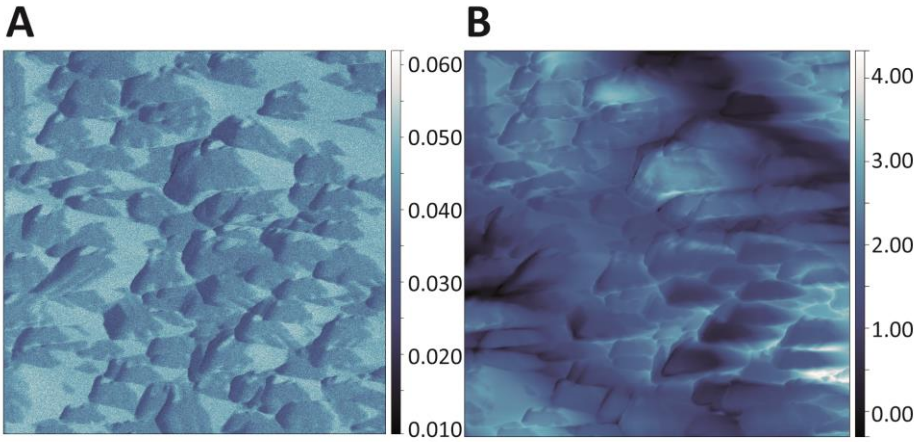


Figure 5. SNIM images 5x5 μm of the Au electrode surface. Mechanical amplitude signal (A) and optical signal (B).

3.2.3. Potentiometric sensing based on electrochemically generated Tröger base derivatives

An electrode based on a polymeric film derived from a Tröger's base gave a potentiometric response of –39 mV/decade towards VMA in the concentration range from 9.9×10^{-6} to to 3.3×10^{-3} mol L^{-1} (Figure 6). The potentiometric response of the prepared electrode towards HVA was insignificant (not shown). Although the tested TMs are structurally closely related (Scheme 7) and differ by the hydroxy group present in VMA, the electrode based on the polymer derived from the Tröger's base enabled the selective detection of VMA in the presence of HVA (log $K^{Pot.}_{VMA, HVA}$= –0.9).

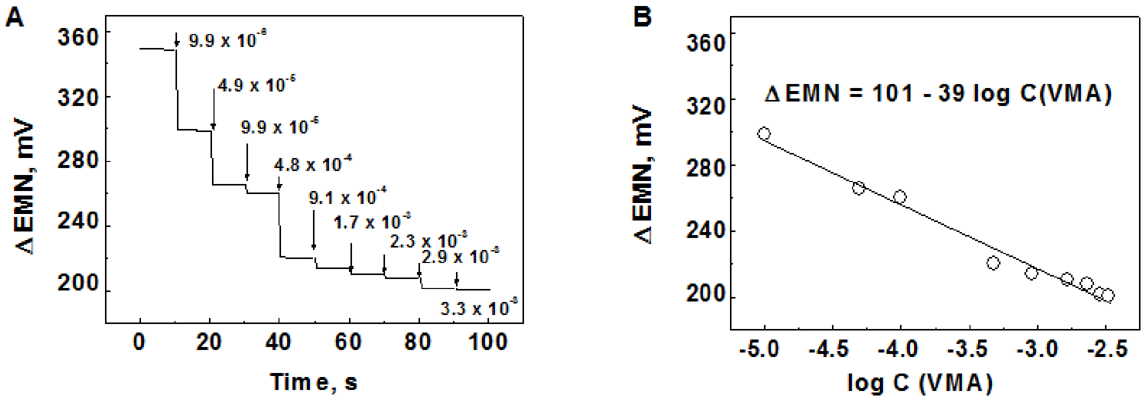


Figure 6. (A) Potentiometric response and (B) calibration graph of an electrode modified with a polymer based on a Tröger's base towards VMA in 0.1 mol L^{-1} phosphate buffer at pH 7.

Thus, we have proposed an innovative detection of neuroblastoma TMs, which is based on specific and selective interaction with a polymer film (prepared from a Tröger’s base**)** and VMA. The detection was purely potentiometric. The geometry of the electrochemically polymerized receptor enabled it to interact specifically with VMA in contrast to HVA.

Since a number of biologically relevant molecules are electroactive, the selectivity of many electrochemical sensors based on electroactivity is limited. Therefore, a promising way to avoid overlapping electrochemical signals and increase selectivity is the development of a potentiometric sensor derived from a recognition receptor selective for the low-molecular-weight target metabolite.

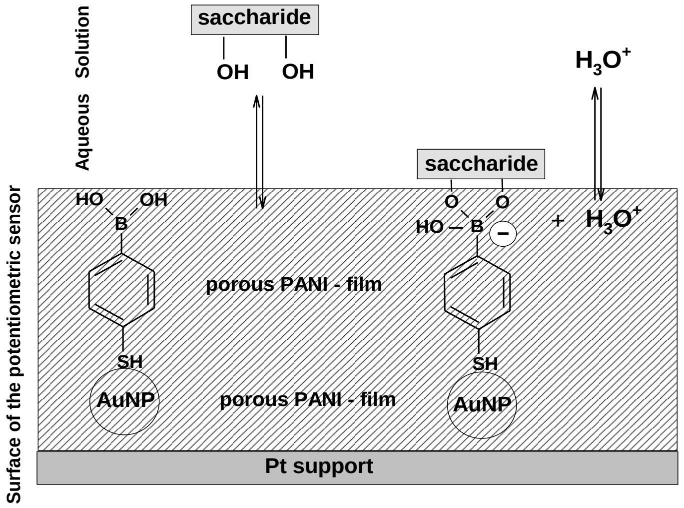
4. New approaches to immobilizing receptors

4.1. Nanomaterials

Nowadays, nanotechnology offers many kinds of nanomaterials for the immobilization of biomolecules and supramolecular receptors [45, 46]. The unique chemical and physical properties of nanomaterials, especially gold nanoparticles (AuNPs), make them suitable for designing sensitive and selective electrochemical sensors [19]. The immobilization of AuNPs onto the electrode surface is an extremely important challenge. There are several strategies for integrating AuNPs into sensing systems for the development of electrochemical sensors with high sensitivity [46, 47]. A simple strategy consists of the direct electrodeposition of nanoparticles onto the electrode surface. Another useful strategy includes the use of the LBL technique in which controlled multilayer films of modified AuNPs are attached onto the electrode surface through electrostatic and covalent interactions. The superstructure formed in this way provides an ideal matrix for the construction of various biosensors. Another useful strategy is the incorporation of nanomaterials into composite electrode matrices.

Recently, the excellent conducting capability of AuNPs, has been successfully applied for amperometric and voltammetric electrochemical biosensors [46, 48]. It should be emphasized that to date, most systematic studies on the application of nanomaterials for potentiometric detection were focused on the determination of heavy metals [49]. G. Jágerszki et al. demonstrated the potentiometric detection of Ag^+ ions with a nanomolar detection limit using an ionophore immobilized on inert AuNPs [50]. X.G. Li´s group has shown that a significant improvement in the detection limit of a potentiometric sensor for Pb^{2+} ions can be achieved by immobilizing copolyaniline nanoparticles as the ionophore in a vinyl resin matrix [51]. Attachment of the receptors onto the AuNP and immobilization in the polymeric matrix help to prevent the leaching of the active component from the membrane. Moreover, attaching the receptor onto AuNP helps to overcome the main obstacle for a receptor that should be either soluble in or compatible with tetrahydrofuran [3].

Our group has proposed a concept for the analytical application of AuNPs modified with mercaptophenylboronic acid (MPBA-AuNPs) as a receptor of saccharides [52]. The main idea was to embed MPBA-AuNPs during the electropolymerization of aniline into a polymeric matrix which should serve as a transducer of the potentiometric signal. The determination of saccharides, in particular glucose, was based on the determination of released protons as products of the reaction between mercaptophenylboronic acid and saccharides (Scheme 10). Differential pulse voltammetry showed that approximately 33% of the MPBA- AuNPs present in the polymerization mixture were built into the PANI polymeric film. The absorption and reflectivity spectra confirmed the recognition process occurring between mercaptophenylboronic acid and glucose inside the pH-sensitive polymeric matrix. A potentiometric response of MPBA-AuNP-modified PANI electrodes towards D-glucose in the concentration range from 0.31 to 33 mM with a sensitivity of +47 mV/decade was verified. No interference from important biological interferents such as uric acid and dopamine was observed with MPBA-AuNP-modified PANI electrodes during the determination of D-glucose (Table 8).



Scheme 10. Complexation of diols with phenylboronic acid and formation of potentiometric response.

Table 8. Determination of glucose in the presence of 1.10^{-4} M uric acid and 1.10^{-4} M dopamine using electrode based on PANI film modified with MPBA-AuNPs.

Sample	**Introduced, mM**	**Found, mM**
Glucose	6.6	6.6 ± 0.6 [a)]
Glucose + Dopamine	6.6	6.5 ± 0.9
Glucose + Uric Acid	6.6	6.7 ± 1.3

[a)] Mean ± standard deviation (($n \leq 4$).

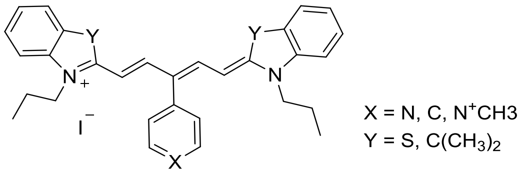
Table 9 gives a comparison of the detection range of our newly proposed sensor, with glucose sensors already described in the literature. The concentration of glucose which is usually detected is 5mM, but it is over 10 mM for diabetic patients. Taking this into account, we can conclude that the potentiometric sensor developed in this work may be useful for detecting millimolar concentrations of glucose.

Table 9. Comparison of detection range of phenylboronic acid-based sugar sensors.

Sensor system	**Receptor**	**Detection range, mM**	**Reference**
Potentiometry	4-mercaptophenylboronic acid	0.31–33.0	[53]
Potentiometry	Phenylboronic acid-substituted polyaniline-coated electrode	3.4–40.8	[54, 55]
Voltammetry	4-mercaptophenylboronic acid	30–300	[56]
Voltammetry	Dithiobis(4-butyrylamino-m-phenylboronic acid)	3–300	[56]
Fluorometry	Chalcone fluorescent dye	1–50	[57]

4.2. Nanocomposites

The electrical contact between the recognizing site immobilized onto the membrane and the electrode surface is an extremely important challenge in the field of sensors. Potentiometric sensors with solid-supported membranes, which have been well-known for a long time, can be such an example. In order to stabilize the potentiometric signal, a hydrophobic conducting polymer such as poly(3-octylthiophene) is placed between the membrane and metal. Amemiya et al have demonstrated that a solid-supported ion-selective electrode can be successfully applied as a voltammetric sensor for the detection of biomedically important polyions such as heparin [58]. However, it should be kept in mind that a conducting polymer is a polymer with redox properties affected by the environment and other redox-active components. This problem can be solved by introducing novel nanocomposite materials. The nanocomposite may be a material combining a polymer with nanomaterials. Recently, nanocomposites based on polyaniline, polypyrrole with entrapped carbon nanotubes (CNT), single-walled (SWNTs) and multi-walled nanotubes (MWNTs) have gained significant popularity due to the ease of preparation through copolymerization via a chemical or electrochemical approach [59]. A novel nanocomposite was proposed by our group. The proposed nanocomposite consists of a mixture of AuNPs and receptors dispersed in an organic solvent (methanol or tetrahydrofuran) that are physically adsorbed onto the electrode surface and covered with a non-conducting polymeric matrix to prevent the leaching of the active component. Polymetinium salts differing in lipophilicity were used as receptors. Recent spectroscopic studies showed that polymetinium salts exhibited a high affinity and selectivity for biologically important anionic analytes [60]. The structure framework of polymetinium salts (Scheme 11) includes cationic charge for coulombic interaction and hydrophobic units for increasing lipophilicity. Thus it is reasonable to assume that polymetinium salts will be interacting with anionic species and compatible with the PVC matrix. The mixture consisting of AuNPs and polymetinium salts was deposited onto the carbon electrode surface by evaporating away the solvent, and was then coated with a thin layer of NPOE-plasticized PVC. This electrode was used to detect heparin (Figure 7). Importantly, the presence of



Scheme 11. Polymethinum salt derivatives proposed for heparin binding.

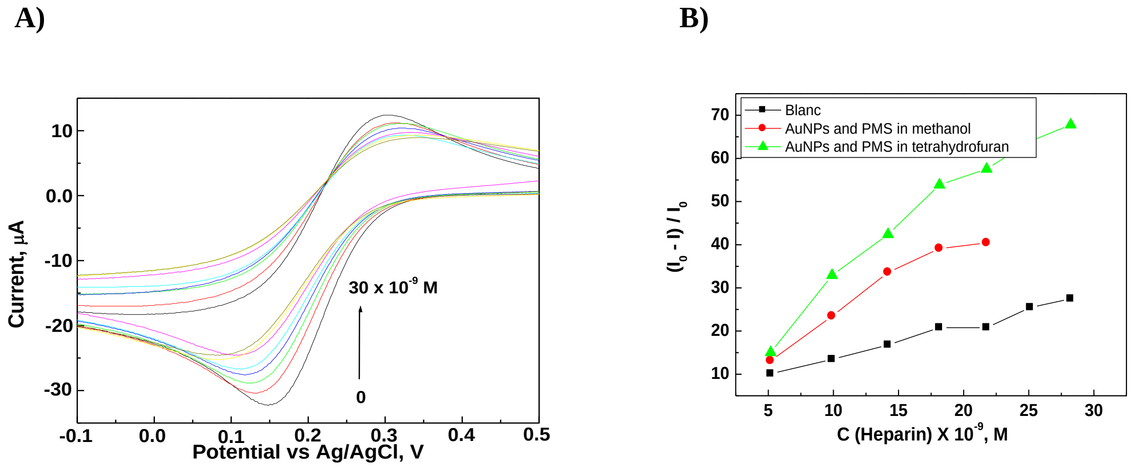


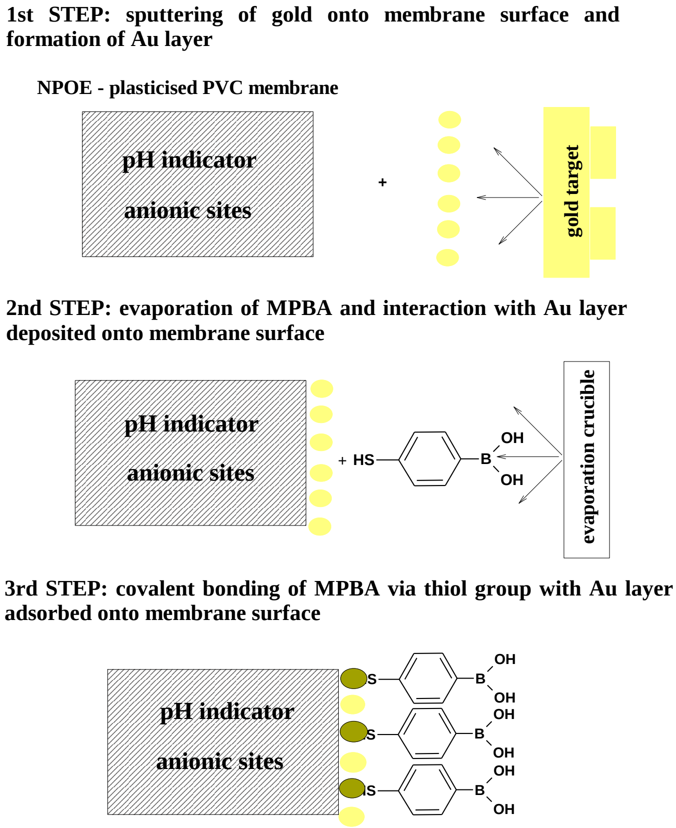
Figure 7. A) Cyclic voltammograms obtained with the polymetinium salt/AuNPs/plasticized PVC-modified carbon electrode in 10^{-3} mol L^{-1} $K_3Fe(CN)_6$ after the addition of different concentrations of

heparin. B) Effect of organic solvent used for dispersing polymetinium salt/AuNPs on electrode response. Experimental conditions: 0.10 mol L^{-1} phosphate-buffer at pH 7.0, scan rate 50 mV s^{-1}.

AuNPs amplified the voltammetric response (not shown). The voltammetric response toward heparin was increased using tetrahydrofuran as organic solvent, which is compatible with a PVC matrix. It should be noted that this research direction is still in progress in our group. However, introducing nanocomposites is a promising option for the development of various kinds of electrochemical sensors including voltammetric, amperometric, and impedimetric ones. Combining the experience of specialists in such fields as supramolecular design, nanotechnology, and electrochemistry may lead to electrochemical sensors with unique selectivity patterns.

4.3. Organic Molecular Evaporation Technique

The preparation of the majority of potentiometric sensors based on a PVC membrane requires the receptor to be soluble in tetrahydrofuran. This restrictive condition for membrane technology has remained over the last few years despite the progress made in the development of receptors. To overcome this obstacle and to expand the possibility for interdisciplinary cooperation between supramolecular and electrochemical chemists, we have proposed a new design for a potentiometric sensor based on a plasticized PVC membrane [61]. Our approach was based on a smart multilayer arrangement where the synthetic receptor is immobilized onto gold sputtered onto the surface of a PVC membrane which is in direct contact with the sample. This approach requires the *a priori* deposition of gold onto the non-conducting support (namely, PVC-membrane) and then deposition of the receptor (bearing a thiol group) onto the gold-coated surface with the subsequent formation of a covalent S-Au bond. Considering recent work on the deposition of uniform and adherent films, first the gold layer was sputtered on the PVC membrane and then the Organic Molecular Evaporation (OME) technique was used to deposit the receptor possessing a thiol group (Scheme 12). Taking into account the technological aspects of sensor preparation, it is clear that sputtering is a conventional, "mature" technique for the deposition of thin metal layers, even when the metals have to be deposited onto PVC sensor membranes [62]. In contrast to sputtering, the OME technique is relatively new. The OME technique is applicable for the evaporation of non-polymeric and non-polymerizing organic substances, which (when appropriately heated) produce sufficient vapour pressure and do not decompose. This enables us to obtain high-quality thin films and structures which can find applications in chemical sensors, biosensors, biochips, etc. [63]. Our concept for applying the aboveOME techniques for the preparation of sensing layers has not been used so far. Utilizing both of these technologies, we are able to uniformly deposit nanostructured gold together with ensuring its excellent adherence to the substrate. The gold surface is then uniformly covered with the receptor with no possible contaminants. Both sputtering and OME are suitable for the uniform coverage of large areas of membranes. The continuous quartz crystal microbalance (QCM) monitoring of layer growth ensures a precise control of the layer thickness and a very good reproducibility of the deposition process.


1st STEP: sputtering of gold onto membrane surface and formation of Au layer

2nd STEP: evaporation of MPBA and interaction with Au layer deposited onto membrane surface

3rd STEP: covalent bonding of MPBA via thiol group with Au layer adsorbed onto membrane surface

Scheme 12. Representation of the modification process of pH-sensitive transducer for potentiometric detection of neutral saccharides.

To verify the practical applicability of the proposed design, we selected MPBA evaporated onto an Au-coated PVC membrane as the receptor (recognizing sites), glucose as the analyte and a pH-indicator incorporated into the PVC matrix as the potentiometric transducer. The formation of a potentiometric response towards saccharides is similar to that described in Scheme 1. Moreover, from Electrochemical Impedance Spectroscopy (EIS) measurements, we found that the membrane capacitance is also a quantity which is able to provide valuable analytical information. This approach is currently being tested with the potentiometric detection of heparin.

The literature data and experimental findings obtained within our group enable us to conclude that although great progress has been made in the field of sensors based on functionalized AuNPs, new techniques and approaches are highly desirable.

5. Conclusion and outlook

The development of a potentiometric sensor involves a wide range of tasks. The key area of special interest is new receptors, which have been and will be central to the development of potentiometric sensors. Innovative approaches to receptor immobilization and the introduction of nanomaterials enable the development of selective and sensitive potentiometric sensors suitable for point-of-care and in-field testing applications. In conclusion it can be said that novel potentiometric sensors with high selectivity and low detection limits are needed and are still of interest. The interdisciplinary activity in potentiometric sensor research will open up avenues for the construction of portable, easy-to-use and low-cost analytical devices.

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Phosphoryl Containing Podands as Carriers in Plasticized Membrane Electrodes Selective to Quaternary Ammonium Surfactants

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Abstract. Open chain polyethers with phosphoryl-containing terminal groups have been studied as carriers in plasticized membrane electrodes that are selective towards cationic surfactants. Host–guest complexation has also been studied by means of batch extraction and bulk membrane transport experiments. For the various guests, the bulk membrane (chloroform) transport rate decreases in the series tetraalkyl ammonium > alkyl pyridinium > alkyl ammonium, while the series of solvent extraction efficiency is exactly opposite; an explanation for this behaviour is proposed. The performance of electrode membranes follows the series for bulk membrane transport. Among the ISE membranes of various composition, one containing the longest (seven oxygen atoms) podand, *o*-nitrophenyl octyl ether (plasticizer), and sodium tetraphenyl borate (anionic additive) performs the best. The slope of the electrode function equals 58.5 mV/decade, the detection limit for dodecyltrimethylammonium is 3.2×10^{-6} M. ISE response time is 5–10 s, the working pH range is 2–11 and lifetime is at least 6 months. The electrode selectivity is significantly better than that of conventional ion-association and crown ether based electrodes.

Key words: phosphoryl podands, quaternary ammonium compounds, surfactants, ion-selective electrodes.

1. Introduction

Neutral carriers are widely used in plasticized membrane ion-selective electrodes (ISE) for the determination of organic compounds. Some recent examples are ISEs for primary amines [1–3], amino acid esters [1, 4–6], and guanidine [7]. The chemical mechanisms which are utilized in these electrodes are based on host–guest complexation, i.e., non-covalent association of carrier with analyte. The association is typically driven by hydrogen bonding and charge–dipole interactions and, in principle, may provide high binding efficiency and selectivity towards the substrate of proper size, shape, and functionality [8–10]. This justifies the growing interest of analytical chemists in host–guest complexation.

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Quaternary ammonium compounds represent a particularly important though challenging target for the development of 'host–guest based' electrodes. Due to the widespread use of these compounds (as cationic surfactants, phase-transfer catalysts, food and cosmetics additives, etc.), there exists a growing need for fast and reliable methods to analyze them. Selective electrodes provide a unique combination of selectivity, simplicity, cheapness, and ability to determine the target compounds in the required concentration range. However, only a few reports have been published on such ISEs based on host–guest complexation [11, 12]. The main reason is in the difficulty in choosing appropriate carriers (hosts).

As is well known in host–guest complex chemistry, the more protons on the nitrogen of an ammonium guest, the better it will be complexed by classic crown ether hosts. Unfortunately, quaternary ammonium and related *N*-alkyl pyridinium guests lack protons and are not capable of any hydrogen bonding. Not surprisingly, it was reported that the introduction of dibenzo-18-crown-6, DB18C6, into the plasticized membrane of an ISE for detecting dodecylpyridinium cation does not lead to improved electrode performance [12].

We suggest that the use of long and flexible open-chain polyethers, podands, will lead to better results. One may expect that many oxygen atoms, which are combined into a very flexible framework, should 'encircle' the guest and provide multiple ion–dipole interactions. Though a single interaction of that type is much weaker than a hydrogen bond, the complexation may still be efficient if the number of interactions is high. This leads to a choice of 'polydentate' hosts with minimal steric constraints, podands. We also propose the use of podands with electron-enriched terminal atoms for further strengthening of the binding. Our previous experience has revealed that podands containing electron-rich phosphoryl groups are eminently effective carriers in various ISEs [13, 14].

Here we report on the successful use of such podands as carriers in cationic surfactant selective electrodes. We also present the data for extraction and bulk membrane transport, which explains the details of host–guest complexation, which underlie analytical applications.

2. Experimental

2.1. CHEMICALS

The structures of the hosts and guests used in the present study are shown in Chart 1.

2.1.1. *Podands*

All the structures of the synthesized compounds were verified by elemental analysis and ^1H and ^{31}P NMR spectra (recorded on a Bruker CXP-200 spectrometer in $CDCl_3$ with tetramethylsilane as internal and 85% H_3PO_4 as external references). Melting points (uncorrected) were measured on a Boetius PHMK-05 instrument.

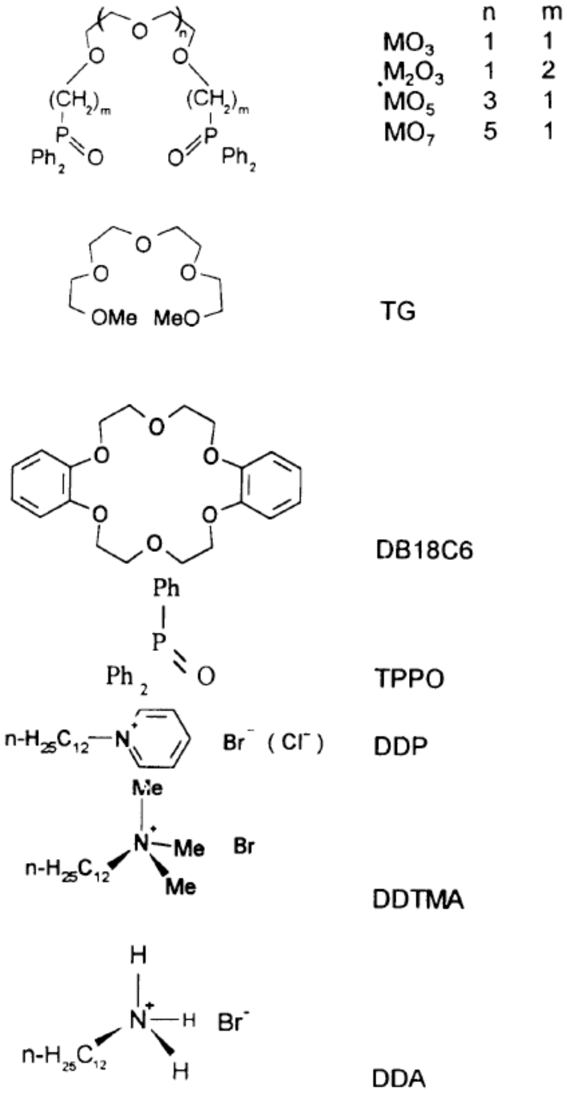

Chart 1. The hosts and guests studied in this work.

1,11-Bis(diphenylphosphinyl)-3,6,9-trioxaundecane (M_2O_3) was synthesized as described previously [15].

1,9-Bis(diphenylphosphinyl)-2,5,8-trioxanonane(MO_3). A mixture containing 15.0 g (64.0 mmol) of diphenylphosphinylmethanol [16], 10.52 g (32.0 mmol) anhydrous caesium carbonate, 12.8 g (32.0 mmol) ethylene glycol di-*p*-tosylate and 80 mL anhydrous dioxane was heated and stirred at 100 °C for 12 h under dry argon. After the reaction the mixture was diluted with 350 mL of water and extracted into $CHCl_3$ (4 × 50 ml). The organic layer was separated, washed with dilute (1 : 1) hydrochloric acid (3 × 30 mL), water (3 × 40 mL) and evaporated under reduced pressure; after addition of diethyl ether a crude solid was obtained. Column chromatographic purification ($CHCl_3$—EtOH (20 : 1) on SiO_2 yields 12.8 g (75%) as white crystals, m.p. 102–104 °C (acetone–ether) [17]. *Found:* C 67.40, 67.45; H 5.20, 5.25; P 11.70, 11.85. $C_{30}H_{32}O_5P_2$ *Calculated:* C 67.40, H 5.10, P 11.60. ^1H NMR ($CDCl_3$ (δ, ppm): 3.50 (m, 4H, $2OCH_2$); 3.69 (m, 4H, $2OCH_2$); 4.30 (d, 4H, 3JHP3D6 Hz, $CH_2P(O)$); 7.44 (m, 12H, Ar—H); 7.86 (m, 8H, Ar—H). ^{31}P NMR ($CDCl_3$ (δ, ppm): 27.76.

1,15-Bis(diphenylphosphinyl)-2,5,8,11,14-pentaoxapentadecane(MO_5). 1,15-Bis(diphenylphosphinyl)-2,5,8,11,14-pentaoxapentadecane(MO_5) was obtained analogously to MO_3 from 15.00 g (64.0 mmol) of diphenylphosphinylmethanol, 10.52 g (32.0 mmol) anhydrous caesium carbonate, and 8.49 g (26.63 mmol) 1,17-dichloro-3,6,9,11,14-pentaoxaheptadecane in 40 mL anhydrous dioxane. After the reaction, the mixture was diluted with 250 mL of water and extracted into $CHCl_3$ (4 × 50 mL). The organic layer was separated, washed with dilute (1 : 1) hydrochloric acid (3 × 30 mL), water (3 × 40 mL) and evaporated under reduced pressure. Column chromatographic purification ($CHCl_3$–EtOH (20 : 1) on SiO_2 yields 13.93 g (70%) as an oil. *Found:* C 65.60, 65.55; H 6.50, 5.45; P 10.10, 10.15. $C_{34}H_{40}O_7P_2$ *Calculated:* C 65.60, H 6.47, P 9.95. ^1H NMR ($CDCl_3$ (δ, ppm): 3.40–3.74 (m, 16H, $8OCH_2$); 4.34 (d, 4H 3JHP=3D6 Hz, $CH_2P(O)$); 7.41 (m, 12H, Ar—H); 7.80 (m, 8H, Ar—H). ^{31}P NMR ($CDCl_3$ (δ, ppm): 27.97.

1,21-Bis(diphenylphosphinyl)-2,5,8,11,14,17,20-heptaoxaheneicosane(MO_7). 1,21-Bis(diphenylphosphinyl)2,5,8,11,14,17,20-heptaoxaheneicosane(MO_7) was obtained analogously to MO_5 from 12.36 g (53.27 mmol) of diphenylphosphinylmethanol, 10.52 g (32.0 mmol) anhydrous caesium carbonate, 7.39 g (32.0 mmol) 1,11-dichloro-3,6,9-trioxaundecane in 40 mL anhydrous dioxane. After the reaction, the mixture was diluted with 250 mL of water and extracted into $CHCl_3$ (4 × 50 mL). The organic layer was separated, washed with dilute (1 : 1) hydrochloric acid (3 × 30 mL), water (3 × 40 mL) and evaporated under reduced pressure. Column chromatographic purification ($CHCl_3$—EtOH (20 : 1) on SiO_2 yields 13.13 g (77%) as oil. *Found:* C 64.10, 64.25; H 6.60, 5.75; P 8.70, 8.75. $C_{38}H_{48}O_9P_2$ *Calculated:* C 64.21, H 6.80, P 8.72. ^1H NMR ($CDCl_3$ (δ, ppm):

3.47–3.75 (m, 24H, 12OCH$_2$); 4.35 (d, 4H, 3JHP=3D6 Hz, CH$_2$P(O)); 7.48 (m, 12H, Ar—H); 7.85 (m, 8H, Ar—H). ^{31}P NMR (CDCl$_3$ (δ, ppm): 27.93.

2.1.2. *Other reagents*

Commercially available DB18C6 (Cherkassy, Ukraine) was used without further purification; tetraglym (TG) was from Merck. We also used alkyltrimethylammonium and alkyl pyridinium bromides (chlorides); dioctyl phthalate (DOP) from Aldrich; *o*-nitrophenyl octyl ether (*o*-NPOE) from Sigma; triphenylphosphineoxide (TPPO), sodium tetraphenyl borate (NaTPB) and poly(vinyl chloride) (PVC) C-70 (Russia). Tetrahydrofuran (THF) was distilled before use. The other chemicals were ‘chemically pure’ grade. The water solutions were prepared with bidistillate.

2.2. MEMBRANE TRANSPORT

A U-shaped vessel containing 5 mL of both aqueous source (1.0×10^{-2} M guest) and receiving phases, separated with 10 mL of liquid membrane (10 mL of CHCl$_3$, 5.0×10^{-3} M host or blank) was used for measuring cation transport rates. The organic phase was vigorously stirred with a magnetic stirrer and an air stream was passed through the water phase. A custom-made film membrane electrode and an Ag/AgBr electrode were used for monitoring the guest content in the receiving phase. Transport rate was calculated as the slope of linear part of the graph of guest content, M, in receiving phase vs. time (through a linear least-squares approximation).

2.3. ELECTROCHEMICAL MEASUREMENTS

Electrode membranes were fabricated as described previously [14]. They contained poly(vinyl chloride), PVC (66.7–95 mg), plasticising solvent mediator (93–133.3 mg) and a carrier (10 mg) either alone or in a mixture with a lipophilic additive (NaTPB, 4 mg). Some control membranes were of PVC and plasticising solvent mediator alone or with lipophilic additive (10 mg). Membrane discs were assembled into electrodes using DDTMA (2.5×10^{-4} M) as the inner filling solution.

All potentiometric measurements were made with the following electrochemical cell (at 22 ± 1 °C):

Ag | AgCl || internal solution | membrane || sample solution || saturated KCl solution | AgCl | Ag

The reference electrode was silver chloride electrode EVL-1M3 (Russia). Responses were recorded with an I-130 ionometer (Russia); pH was monitored using a glass electrode ESL-63-07 (Russia). Electrodes were calibrated by spiking test solutions at 22 ± 1 °C with a standard solution of DDTMA after conditioning in DDTMA solution (2.5×10^{-5} M) for 24 h before use.

Selectivity coefficients $K_{i,j}^{\mathrm{pot}}$ (i = primary ion, j = interfering ion) were determined by the separate solution method using the following relationship [18]:

$$-\log K_{i/j}^{\mathrm{pot}} = \frac{(E_1 - E_2)}{S}$$

where E_1 and E_2 are the response potentials of the alkyltrimethylammonium electrode for $[i]$ and $[j]$ at 1×10^{-3} M, respectively, and S is the slope of the electrode function (mV/decade).

2.4. SOLVENT EXTRACTION

The aqueous solution of the guest ($V = 5$ mL, $C = 5 \times 10^{-4}$ M) was contacted in a separating funnel with a chloroform solution of the host ($V = 5$ mL, $C = 1 \times 10^{-3}$ M) for the time required to establish equilibrium. After separating, the aqueous layer was analyzed for surfactant with either a custom-made film membrane electrode or the Ag/AgBr electrode.

After calculating guest distribution coefficients, D, equilibrium constants were derived from their concentration dependencies. The following system was considered:

(1) Extraction facilitated by host–guest complexation

$$\mathrm{Guest}^+_{(\mathrm{aq})} + X^-_{(\mathrm{aq})} + \mathrm{Host}_{\mathrm{org})}\text{ÍÍ}\ (\mathrm{Host\text{–}Guest})^+ X^-_{(\mathrm{org})} \quad K_{\mathrm{ex}};$$

(2) ‘Blank’ extraction with pure solvent (measured in a separate run)

$$\mathrm{Guest}^+_{(\mathrm{aq})} + X^-_{(\mathrm{aq})} = \mathrm{Guest}^+ X^-_{(\mathrm{org})} \quad K_{\mathrm{ex}}^{\mathrm{Guest}X};$$

(3) Host–guest association in organic phase

$$\mathrm{Guest}^+ X^-_{(\mathrm{org})} + \mathrm{Host}_{(\mathrm{org})}\text{ÍÍÍÍ}\ (\mathrm{Host\text{–}Guest})^+ X^-_{(\mathrm{org})} \quad K_{\mathrm{ass}}.$$

(We neglected the distribution of the host into water.)

Evidently,

$$\log K_{\mathrm{ex}} = \log D - \log[X^-]_{(\mathrm{aq})} - \log[\mathrm{Host}]_{(\mathrm{org})}$$

$$\log K_{\mathrm{ass}} = \log K_{\mathrm{ex}} - \log K_{\mathrm{ex}}^{\mathrm{Guest}X}.$$

Table I. Bulk membrane transport of dodecyltrimethylammonium. Chloroform; $C_{\text{host}} = 5 \times 10^{-3}$ M, $C_{\text{guest}} = 1 \times 10^{-2}$ M.

Host	Transport rate (μmol/h)
Pure solvent	0.8 ± 0.1
MO_3	2.6 ± 0.1
M_2O_3	6.7 ± 0.8
MO_5	12.0 ± 1.0
MO_7	13.0 ± 1.0
DB18C6	5.4 ± 0.2
TG	3.5 ± 0.4
TPPO	2.9 ± 0.1

3. Results and Discussion

3.1. BULK MEMBRANE TRANSPORT

Without the host, DTMA is transported across a bulk chloroform membrane at a low rate. All the hosts studied significantly increase the rate of transport, as illustrated in Table I.

Phosphoryl podands, which do have many more potential binding centres (oxygen atoms of two terminal phosphoryl groups and oxyethylene units), are more effective ionophores than 'unidentate' TPPO, which is an analogue of the podand's terminal functionality. Performance of the conventional 'non-phosphoryl' podand tetraglym, TG, is comparable to that of TPPO. Macrocyclic 'non-phosphoryl' polyether DB18C6 is a better carrier than TG. However, a combination of high 'denticity' and electron-enriched phosphoryl groups leads to the best performance.

The ionophoric activity of phosphoryl containing podands depends crucially on the flexibility of the molecule and the number of oxygen atoms. The rate of transport of DDTMA with small and rigid MO_3 is lower than that with simple polyether TG having the same number of oxygens; it is also slightly lower than the rate of transport with TPPO. However, replacing a single methylene by the more flexible ethylene at the bridge 'polyether chain-phosphoryl ends' dramatically increases transport efficiency. Podand M_2O_3 is at least twice as effective an ionophore as MO_3, TG or TPPO. Actually, it is even a better carrier than the macrocycle DB18C6. We may conclude that the expected increase of a host's performance through adding phosphoryl ends to a polyether chain is a fact. As expected, too, the increase depends crucially on the placement of phosphoryl groups and the flexibility of the whole molecule. Increasing the number of oxyethylene units (length of the polyether chain) leads to a significant increase of ionophoric activity (MO_5

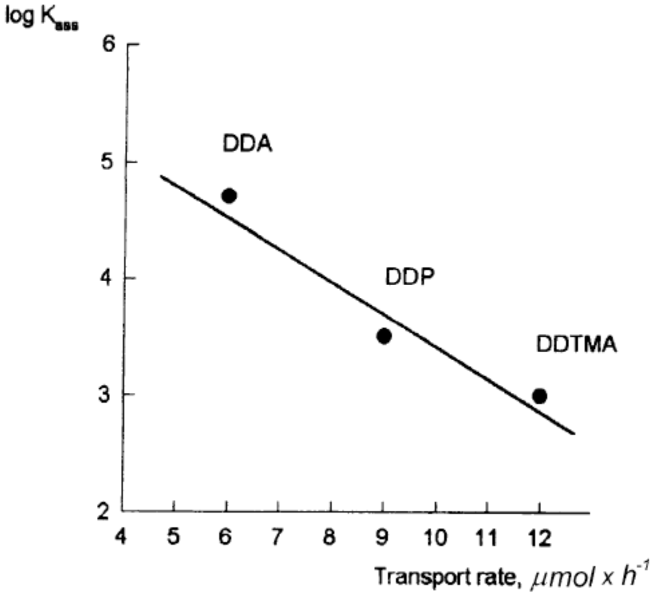

Figure 1. The correlation between transport rate and log K_{ass}.

vs. MO_3, M_2O_3) followed by saturation. The longest podand MO_7 performs only slightly better than MO_5 (Table I).

We assumed that the force underlying host–guest complexation is an ion–dipole interaction between the podand's oxygens and the guest's cationic centre. The more oxygens present, the stronger is the interaction. The 'saturation' pattern may be related to increased steric clashing in the complexes. The practical conclusion is that the best carriers for tetraalkyl ammonium are podands comprising a long polyether chain with phosphoryl terminal groups.

Another point of practical interest is the host's selectivity with respect to various guests. For the podands, we observed an exact inverse of the common series, which is typical of crown ethers [12]: tetraalkyl ammonium is transported better than alkyl pyridinium, and alkyl pyridinium better than monoalkyl ammonium (with the same number of carbons at the guest's *N*-alkyls; data for host MO_5 are presented as an example, Figure 1). This is very interesting behaviour, since monoalkyl ammonium usually interacts with polyethers more strongly due to its ability to form hydrogen bonds. In order to reveal the nature of this unusual selectivity, we studied the solvent extraction of various guests.

Table II. Extraction with various hosts. Chloroform; $C_{\text{host}} = 1 \times 10^{-3}$ M, $C_{\text{guest}} = 5 \times 10^{-4}$ M; pH 2–9.

	DB18C6	TG	MO_5	MO_7		
	DDTMA	DDTMA	DDTMA	DDP	DDA	DDTMA
Equilibrium attainment time, min	10	5	5	15	30	5
Recovery, %	40	30	50	76	90	72
log K_{ex}	6.3 ± 0.1	5.9 ± 0.1	6.6 ± 0.1	6.5 ± 0.1	7.3 ± 0.1	7.2 ± 0.1
log K_{ass}	2.7 ± 0.1	2.3 ± 0.1	3.0 ± 0.1	3.4 ± 0.1	4.7 ± 0.1	3.6 ± 0.1

3.2. SOLVENT EXTRACTION

Our extraction study immediately indicated that the unusual selectivity series is of purely 'transport origin' and is not related to the selectivity of host–guest complexation *per se*. Both extraction and association constants (1 : 1 host–guest complexation confirmed with bilogarithmic plots) for MO_5 shows that the podand does favour monoalkyl ammonium and alkyl pyridinium (Table II), as is usual. We must conclude that the affinity of RNH_3^+ and R—Py^+ guests towards the host is too strong, which results in slow release at membrane phase interface (back extraction) thus depressing membrane transport (some examples of similar behaviour have been reported earlier [19, 20]).

As seen from Tables I and II, the other extraction and membrane transport data are in direct correlation.

3.3. PLASTICIZED MEMBRANE ELECTRODES

Podands MO_5 and MO_7, as well as reference macrocycle DB18C6, were used as carriers in *o*-NPOE plasticized membrane electrodes. For the sake of comparison, a conventional ion-association tetraphenyl borate membrane was also studied.

The main features of the electrodes are presented in Table III. As can be seen, they are reversible with respect to $DDTMA^+$ cation over a wide pH range, 2–11. The upper limit of analyte concentration is determined by the critical micellar concentration of the surfactant.

In general, the performance of all electrodes is comparable. However, podand-based ISEs gain advantages in terms of limit of detection, response time and, in particular, in reproducibility (Table III). The improvement of electrode selectivity is more drastic (Figure 2). It is worth noting that the conventional ion-association membrane has worse selectivity than all neutral-carrier membranes.

Interestingly, even the DB18C6-based membrane provides better selectivity in the presence of alkali metal ions. This may seem strange, since DB18C6 is well

Table III. Electrochemical properties of membranes plasticized by *o*-NPOE and based on neutral carriers in the solutions of dodecyltrimethylammonium.

Carrier	Slope (mV/decade)	Response time (s)	Linear range (M)	Limit of detection (M)
NaTPB	58.8 ± 2.2	10	5.0×10^{-5}–1.4×10^{-5}	1.4×10^{-5}
DB18C6	55.7 ± 6.6	20	5.0×10^{-5}–1.4×10^{-2}	1.3×10^{-5}
MO5	54.3 ± 0.7	20	2.5×10^{-5}–1.4×10^{-2}	6.3×10^{-6}
MO7	53.0 ± 1.0	15	1.0×10^{-5}–1.4×10^{-2}	5.0×10^{-6}
MO7*	58.5 ± 0.3	5	5.0×10^{-6}–1.4×10^{-2}	3.2×10^{-6}
MO7**	55.2 ± 6.9	20	5.0×10^{-5}–1.4×10^{-2}	2.5×10^{-5}

* Lipophilic additive.
** DOP.

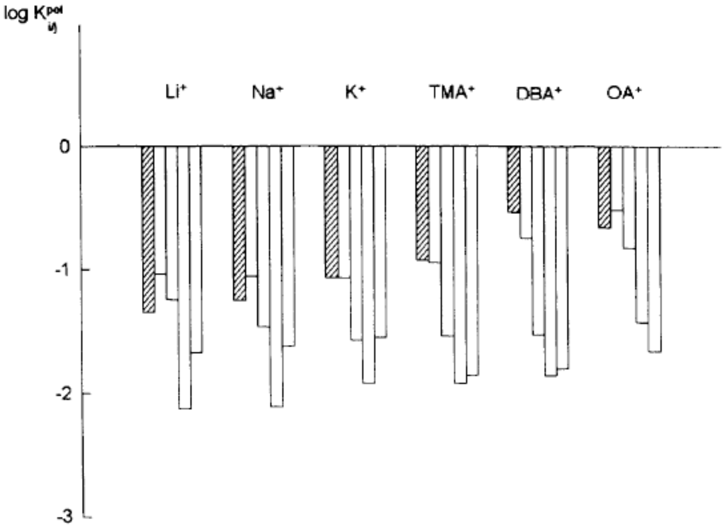

Figure 2. The influence of the host structure on potentiometric selectivity of ISEs reversible to dodecyltrimethylammonium (the bars on chart correspond to hosts: no-carrier, NaTPB, DB18C6, MO5, MO7, left to right; TMA^+-trimethylammonium, DBA^+-dibutylammonium, OA^+-octylammonium).

Table IV. The influence of homologous surfactants ($C_nH_{2n+1}N(CH_3)_3Br$) to response of MO_7-based ISE.

	$n = 10$	$n = 12$	$n = 14$	$n = 16$
Slope (mV/decade)	49.9 ± 0.3	53 ± 1	57 ± 1	59.2 ± 0.8
Linear range (M)	5.0×10^{-5}– 6.0×10^{-2}	1.0×10^{-5}– 1.4×10^{-2}	1.0×10^{-5}– 3.6×10^{-3}	5.0×10^{-6}– 9.0×10^{-4}
Limit of detection (M)	1.0×10^{-5}	5.0×10^{-6}	3.5×10^{-6}	2.5×10^{-6}
Response time (s)	30	15	10	5–10

known for its ability to bind these cations, particularly potassium; it is actually used as the membrane-active component in K^+-selective electrodes [21]. Interestingly too, the series of alkali metal interfering influence for our $DDTMA^+$-selective crown ether electrode is an exact inverse: $Li^+ > Na^+ > K^+$. We suggest that this may be attributed to different ligand preorganization. Indeed, since the $DDTMA^+$-selective membrane was conditioned in DDTMA solutions for at least 4 days prior to our experiments, one may expect that the carrier in the membrane phase (or, at least, near the interface) should exist as a complex with this cation. Obviously, the crown ether in such a complex should have a conformation which is different from both those of free DB18C6 and DB18C6 complexed with potassium (encapsulating mode of complexation). This may lead to an anomalously low affinity for alkali metals, in general, and to unusual selectivity in particular.

The podand-based membranes demonstrate the best potentiometric selectivity. The interfering influence of both metal and primary ammonium cations is lower than those for ion-association and DB18C6-membranes. One should note that literature data (alkali metals [22]) and our previous reports (primary ammonium selective electrodes [13, 14]) indicate that podands can be used in electrodes for both these kinds of analytes. However, this is true for shorter podands, while the use of longer MO_5 and MO_7 leads to weaker interaction with these ions and a low interfering influence.

A particularly important question is the influence of interfering surfactants. As expected, podand-based membranes do respond to other ammonium surfactants (Table IV). Moreover, they may be used for the determination of DDTMA homologues. The longer the alkyl chain, the better is the performance of the corresponding electrode. Naturally, this is related to the higher hydrophobicity of the host–guest complex. The interfering influence increases in the series:

$$RNMe_3^+ < RPy^+ < RNH_3^+,$$

which reflects both the guest's ability to form hydrogen bonds and the steric accessibility of its cationic charge.

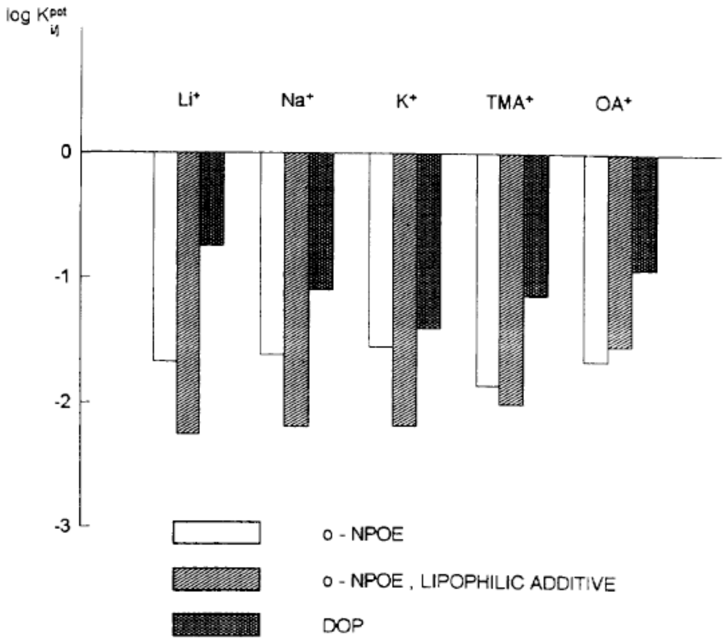

Figure 3. The potentiometric selectivity of MO7-based membranes in solution of dodecyltrimethylammonium vs. nature of plasticizer and liphophilic additive.

The nature of the plasticizer used strongly influences electrode performance. Use of DOP rather than *o*-NPOE results in a narrower linear range and reduced selectivity (Table III, Figure 3). Conversely, use of a lipophilic anionic additive leads to a broadening of the electrode's working range and an improvement of selectivity (Table III, Figure 3). In summary, the best electrodes for DDTMA$^+$ determination are those based on phosphoryl podands, *o*-NPOE plasticizer and, preferably, a specially introduced lipophilic additive.

The electrodes developed were successfully used for the determination of alkyl ammonium surfactants in environmental samples and in cosmetics ($S_r \leq 0.04$), as well as for the evaluation of surfactant critical concentration of micelle formation. The application to the detection in FIA/HPLC and to the study of the interaction of water-soluble polymers with cationic surfactants is reported elsewhere [23, 24].

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Molecular Recognition at an Organic−Aqueous Interface: Heterocalixarenes as Anion Binding Agents in Liquid Polymeric Membrane Ion-Selective Electrodes

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Abstract: Poly(vinyl chloride) (PVC)-derived ion-selective electrodes (ISEs) have been prepared from a neutral anion binding receptor, *meso*-octamethylcalix[4]pyrrole (**1**). Analogous systems were also prepared from the novel pyridine-containing analogues of **1**, namely dichlorocalix[2]pyrrole[2]pyridine (**2**) and tetrachlorocalix-[4]pyridine (**3**). At lower pH values (i.e., 3.5 and 5.5), ISEs derived from **1** display strong anionic (negative slope) responses toward Br^-, Cl^-, and $H_2PO_4^-$ and, to a much lesser extent, F^-. By contrast, at high pH (i.e., pH 9.0) ISEs derived from **1** not only display cationic (positive slope) responses toward chloride and bromide anions but also selectivities (i.e., $Br^- < Cl^- < OH^- \approx F^- < HPO_4^{2-}$) that are non-Hofmeister in nature. This is considered consistent with the PVC-supported receptor **1** behaving as a direct anion binding agent at low pH but acting, at least in part, as an hydroxide-complexing receptor at higher pH. For the ISEs based on **2** and **3**, no special non-Hofmeister selectivity is observed at pH 9.0. However, at lower pH values both increased anionic responses and improved selectivities for hydrophilic anions (e.g., F^- and $H_2PO_4^-$) are observed. These observations are rationalized in terms of protonation effects involving the pyridine-containing receptors from which these ISEs are derived. For all the receptors discussed in this paper, the addition of tridodecylmethylammonium chloride (TDDMA), a known lipophilic additive, serves to increase the magnitude of the ISE response but only at the price of greatly reduced anion selectivity.

Introduction

Calix[4]pyrrole (**1**), dichlorocalix[2]pyrrole[2]pyridine (**2**), and tetrachlorocalix[4]pyridine (**3**) (Figure 1) constitute a matched set of macrocyclic analogues that, under conditions where the pyridine subunits remain unprotonated, should act as relatively strong, intermediate, and weak anion binding agents, respectively.[1] Recent solution-phase studies, carried out in dichloromethane-d_2, have served to confirm that calix[4]-pyrrole **1**, although a neutral entity, does indeed act as an effective anion receptor, showing selectivities for $F^- > Cl^- > H_2PO_4^-$.[2] On the basis of solid-state structural studies and low-temperature ^{19}F NMR spectroscopic analyses, this recognition behavior is currently rationalized in terms of an ability to stabilize nonisotropic pyrrole NH-anion hydrogen bonds.[3] Since systems **2** and **3**, in their neutral forms, should be far less effective hydrogen bond donors than **1**, appropriate intercomparisons among the matched set of heterocalixarene analogues defined by **1**−**3** could provide important further support for this critical mechanistic proposal. Toward this end, we have prepared poly(vinyl chloride) (PVC)-derived ion selective electrodes (ISEs) containing receptors **1**−**3** and wish to report here that the calixpyrrole-based system **1** shows strong, non-Hofmeister anion-selective responses under conditions where the dichlorocalix[2]pyrrole[2]pyridine and tetrachlorocalix[4]pyridine-based systems do not.

Carrier-based ISEs constitute an established set of analytical tools that in optimal cases can provide a convenient means of detecting selectively one or more analytes within a complex mixture. In point of fact, it is primarily the stability constant of the ion−carrier complex that dictates the operational selectivity of a given sensor.[4] From a more mechanistic perspective, the potentiometric response of membrane-based ISEs containing a specific ligand can be used to provide information about the mode of analyte binding as well as, at least potentially, molecular insights into the details of the relevant substrate−receptor interactions. Not surprisingly, therefore, the construction of ISEs is particularly interesting from the perspective of the supramolecular chemist; such systems could provide a convenient method for characterizing, under interfacial organic−aqueous conditions, the substrate binding characteristics of receptors that might not otherwise be amenable to study in the presence of water due to, e.g., poor aqueous solubility and/or overly weak binding affinities. ISEs are also of obvious interest because they can help translate the chemistry of new substrate binding systems into tools that can be used to recognize selectively various targeted species in the presence of potentially interfering analytes. In the specific case of anion recognition,

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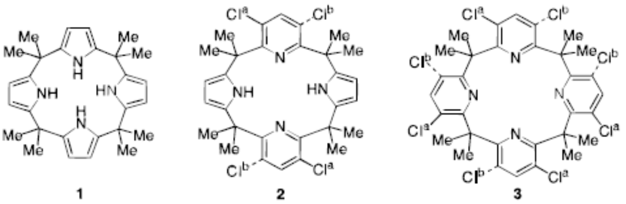

1 **2** **3**

Figure 1. Structures of investigated receptors **1**−**3**. Receptors **2** and **3** were obtained and studied as a mixture of isomers. Thus, in these structures each of the individual pyridine subunits bears a single chlorine substituents at either the 3 or 5 positions (Cl^a or Cl^b), but not both.

this approach has been explored extensively by Umezawa, Meyerhoff, Simon, Reinhoudt, and others using a range of receptors including protonated sapphyrins[5] and protonated polyamines[5] as well as a variety of Lewis acidic systems such as metalloporphyrins[6,7,9−12] uranylsalenophenes,[8] metallocenes,[13] other organometallic derivatives,[14,15] and fluorinated compounds.[16] Not yet studied as potential sensory elements, however, are neutral, nonaromatic anion-binding agents such as calix[4]pyrrole **1** and its analogues. ISEs based on these newer materials are the subject of this paper.

Experimental Section

Reagents. High molecular weight poly(vinyl chloride) (PVC), 2-nitrophenyl octyl ether (*o*-NPOE), tridodecylmethylammonium chloride (TDDMA), and tetrahydrofuran (THF; stored over 3 Å molecular sieves) were purchased from Fluka Chemika A.G. (Germany). The sodium salts of fluoride, chloride, bromide, and di- and hydrophosphates were purchased from Lachema s.p. (Brno, Czech Republic). All reagents were of the highest grade commercially available and used without further purification. Distilled water was used to prepare standard solutions.

Table 1. Composition of Membranes Based on Receptors **1**−**3** and TDDMA

		composition, wt %			
membrane	active component	active component	cationic additive	plasticizer	PVC
■	**1**−**3**	1.0		66.0	33.0
●	**1**−**3**	1.0	0.2	65.9	32.9
control experiment	TDDMA	0.2		66.5	33.3

Syntheses of *meso*-octamethylcalix[4]pyrrole (**1**), dichlorocalix[2]pyrrole[2]pyridine (**2**), and tetrachlorocalix[4]pyridine (**3**) have been reported previously.[1,2]

Membranes and Cell Assembly. PVC-membranes based on **1**−**3** were made by adding the appropriate receptor to a mixture of PVC and plasiticizer (*o*-NPOE) both in the presence and absence of an ancillary lipophilic additive, tridodecylmethylammonium chloride (TDDMA). The resultant mixtures, made up as per Table 1, were then taken up in THF (ca. 0.7 mL per 100 mg of resultant mixture), poured into a metallic tube, and allowed to dry for 24 h. A circle (diameter, 12 mm; thickness, 0.15 mm) was cut out from the resulting polymeric disk. Membranes obtained in this way were then fixed on a polymeric mounting ring (inner diameter: 8 mm) and mounted on a liquid membrane-type ISE body obtained from Crytur, Monokrystaly s.p. (Turnov, Czech Republic). Control electrodes, containing just TDDMA, were also prepared using an analogous approach. The cell assembly for potentiometric measurements was as follows:

$$\mathrm{Hg/Hg_2Cl_2,\ KCl\ (satd)|3\ M\ KCl|test\ solution|}$$
$$\mathrm{modified\ PVC\ membrane|0.01\ M\ KCl|Ag/AgCl}$$

EMF Measurements. Potentiometric measurements were made using a digital voltammeter, model M1T330, obtained from Metra Blansko s.p. (Czech Republic). The reference electrode was a Hg/Hg_2Cl_2, KCl (satd) electrode obtained from Crytur, Monokrystaly s.p. (Turnov, Czech Republic). The pH was monitored using glass electrode Type 01-29 B (Labio Prague, Czech Republic) on a pH-Meter type OP-205/1 (Budapest, Hungary). Before each set of measurements, the electrodes were soaked in water and adjusted to the relevant experimental pH by means of added NaOH or H_2SO_4, in the absence of analyte (for at least 5−10 min and sometimes overnight). All potentiometric measurements were carried out at ambient temperature.

Analyte solutions were prepared by diluting stock solutions with water adjusted to the experimentally desired pH by means of added NaOH or H_2SO_4. Calibration curves were constructed by plotting the observed potential vs the logarithm of the concentration of the substrate present in the analyte solution. Key parameters, namely sensitivity (S, mV/decade) and linear working range (M), were then deduced and

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Table 2. Potentiometric Sensitivity toward Anions of PVC-Membranes Based on **1**−**3** as a Function of pH and Lipophilic Additive,TDDMA[a]

		pH = 3.5		pH = 5.5		pH = 9.0	
anion	active component	■ S, mV/decade (linear range, M)	● S, mV/decade (linear range, M)	■ S, mV/decade (linear range, M)	● S, mV/decade (linear range, M)	■ S, mV/decade (linear range, M)	● S, mV/decade (linear range, M)
F^-	**1**	$-4\ (10^{-3}-10^{-1})$	$-34\ (10^{-3}-10^{-1})$	$-7\ (10^{-3}-10^{-1})$	$-28\ (10^{-2}-10^{-1})$	$-4\ (10^{-4}-10^{-1})$	$-3\ (10^{-4}-10^{-1})$
	2	$-28\ (10^{-2}-10^{-1})$	$-47\ (10^{-3}-10^{-1})$	$-8\ (10^{-3}-10^{-1})$	$-25\ (10^{-3}-10^{-1})$	$+10\ (10^{-3}-10^{-1})$	$+30\ (10^{-2}-10^{-1})$
	3	$-31\ (10^{-3}-10^{-1})$	$-42\ (10^{-3}-10^{-1})$	$-16\ (10^{-2}-10^{-1})$	$-27\ (10^{-4}-10^{-1})$	$+7\ (10^{-4}-10^{-1})$	$-6\ (10^{-4}-10^{-1})$
	TDDMA		$-86\ (10^{-2}-10^{-1})$		$-25\ (10^{-2}-10^{-1})$		$-10\ (10^{-2}-10^{-1})$
Cl^-	**1**	$-20\ (10^{-4}-10^{-1})$	$-26\ (10^{-4}-10^{-1})$	$-5\ (10^{-3}-10^{-1})$	$-22\ (10^{-4}-10^{-1})$	$+15\ (10^{-3}-10^{-1})$	$-4\ (10^{-4}-10^{-1})$
	2	$-4\ (10^{-4}-10^{-1})$	$-3\ (10^{-4}-10^{-1})$	$+8\ (10^{-4}-10^{-1})$	$-25\ (10^{-4}-10^{-1})$	$+15\ (10^{-2}-10^{-1})$	$0\ (10^{-4}-10^{-2})$
	3	$-13\ (10^{-3}-10^{-1})$	$-36\ (10^{-4}-10^{-1})$	$-6\ (10^{-3}-10^{-1})$	$-44\ (10^{-4}-10^{-1})$	$0\ (10^{-4}-10^{-1})$	$-26\ (10^{-3}-10^{-1})$
	TDDMA		$-46\ (10^{-3}-10^{-1})$		$-37\ (10^{-4}-10^{-1})$		$-21\ (10^{-3}-10^{-1})$
Br^-	**1**	$-25\ (10^{-4}-10^{-1})$	$-38\ (10^{-}-10^{-1})$	$-20\ (10^{-4}-10^{-1})$	$-40\ (10^{-3}-10^{-1})$	$+19\ (10^{-2}-10^{-1})$	$0\ (10^{-4}-10^{-1})$
	2	$-10\ (10^{-3}-10^{-1})$	$-47\ (10^{-4}-10^{-1})$	$+2\ (10^{-4}-10^{-1})$	$-42\ (10^{-3}-10^{-1})$	$-13\ (10^{-2}-10^{-1})$	$+1\ (10^{-2}-10^{-1})$
	3	$-18\ (10^{-3}-10^{-1})$	$-43\ (10^{-4}-10^{-1})$	$-12\ (10^{-3}-10^{-1})$	$-43\ (10^{-4}-10^{-1})$	$-4\ (10^{-2}-10^{-1})$	$-17\ (10^{-3}-10^{-1})$
$H_2PO_4^-$/ HPO_4^{2-}	**1**	$-16\ (10^{-2}-10^{-1})$	$-12\ (10^{-3}-10^{-1})$	$0\ (10^{-4}-10^{-1})$	$-9\ (10^{-3}-10^{-1})$	$0\ (10^{-4}-10^{-1})$	$-2\ (10^{-2}-10^{-1})$
	2	$-14\ (10^{-3}-10^{-1})$	$-35\ (10^{-2}-10^{-1})$	$+13\ (10^{-3}-10^{-1})$	$-14\ (10^{-3}-10^{-1})$	$+19\ (10^{-3}-10^{-1})$	$-14\ (10^{-2}-10^{-1})$
	3	$-18\ (10^{-3}-10^{-1})$	$-32\ (10^{-2}-10^{-1})$	$+16\ (10^{-3}-10^{-1})$	$-28\ (10^{-3}-10^{-1})$	$0\ (10^{-4}-10^{-1})$	$-15\ (10^{-3}-10^{-1})$
	TDDMA		$-21\ (10^{-2}-10^{-1})$		$-30\ (10^{-2}-10^{-1})$		$-13\ (10^{-2}-10^{-1})$

[a] Columns ■ and ● refer to experiments carried out in the absence and presence of TDDMA, respectively, whereas those denoted as just TDDMA (rows) refer to control experiments involving PVC-supported ISEs containing TDDMA as the sole anion recognition species. See text for details.

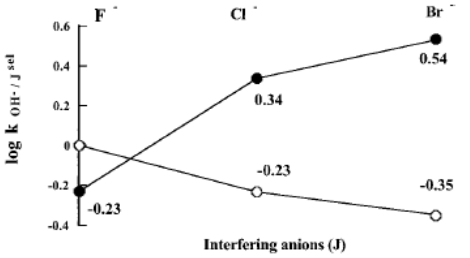

Figure 2. Response selectivities for PVC-membranes based on receptors **1** (open circles) and **2** (closed circles) for OH^- relative to halides (i.e., log $k_{OH^-/J}^{sel}$, where $J = F^-, Cl^-$, and Br^-) at pH 9.0. In these experiments, the selectivity factors (log $k_{I/J}^{sel}$) were determined using the matched potential method[17] as described in the text.

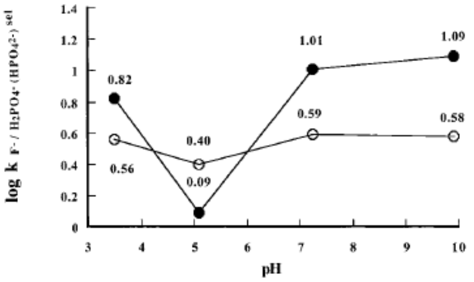

Figure 3. Response selectivities and pH effects for PVC-membranes based on receptors **1** (open circles) and **2** (closed circles) for $H_2PO_4^-$ vs HPO_4^{2-} relative to F^- (i.e., log $k_{F^-/H_2PO_4^-(HPO_4^{2-})}^{sel}$). In these experiments, the selectivity factors (log $k_{I/J}^{sel}$) were determined using the matched potential method[17] as described in the text.

collected in Table 2 (cf. Results and Discussion). In certain instances, especially those wherein a non-Nernstian response was observed, the selectivity factor ($k_{I/J}^{sel}$) was measured using the matched potential method;[17] these results are displayed in Figures 2 and 3.

Results and Discussion

Receptors **1**−**3**, being rather understudied heterocalixarene derivatives, could constitute a homologous series of molecular receptors that might prove useful in the design and assembly of novel electrochemical-based sensors, electrodes, and other supramolecular devices. To test this possibility in a realistic fashion, we elected, for the reasons outlined in the Introduction, to study these materials as potential sensory elements in standard liquid membrane ion-selective electrodes (ISEs). Here, our fundamental goal was to investigate the effects of pH on the magnitude of the ISE response and its contribution, if any, to the potentiometric discrimination that ISEs containing receptors **1**−**3** might display toward the following anions: F^-, Cl^-, Br^-, and ($H_2PO_4^-/HPO_4^{2-}$). A priori, it was expected that, in the case of receptors **1**−**3**, respectively, the selectivity observed toward a given anion would be governed by: (i) what were for the most part specific (i.e., anisotropic) hydrogen-bonding interactions, (ii) a combination of both hydrogen-bonding interactions and generalized Coulombic effects that might be difficult to deconvolute, and (iii) almost purely Coulombic effects.

Given the above, it was expected that the following equilibria, of fundamental molecular recognition interest, would prove critical in terms of mediating the production of a putative potentiometric response:

$$B^+_s + A^-_s + L_m \leftrightarrow B^+_m + L A^-_m \qquad (1)$$

$$H^+_s + L_m \leftrightarrow LH^+_m;\ A^-_s + LH^+_m \leftrightarrow LH^+ A^-_m \qquad (2)$$

$$H^+_s \leftrightarrow H^+_m \qquad (3)$$

$$A^-_s \leftrightarrow A^-_m \qquad (4)$$

$$B^+_s \leftrightarrow B^+_m \qquad (5)$$

Here, L and LH^+ refer to the free and protonated forms, respectively, of a given receptor (i.e., **1**, and **2**, or **3**); B^+ and H^+ represent cationic species such as sodium cation and protons, respectively; A^- represents the anionic analyte species under consideration; and subscripts s and m refer to species either present in the bathing solution or contained in the membrane, respectively.

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[17] Umezawa, Y.; Umezawa, K.; Sato, H. *Pure Appl. Chem.* **1995**, *67*, 507−518.

As can be seen from an inspection of Table 2, ISEs made up from calix[4]pyrrole **1** display both anionic (negative slope) and cationic (positive slopes for Cl^- and Br^-) responses at pH 3.5–5.5 and 9.0, respectively. The anionic response observed at lower pH was as expected, since, under these conditions, **1** should act as a straightforward anion-binding receptor. By contrast the weak cationic response seen at pH 9.0 was not. It is currently rationalized in terms of **1** showing a high selectivity for OH^- and a rather low affinity for Cl^- or Br^-. The ensuing strong interactions between the PVC-supported calix[4]pyrrole and hydroxide anion could lead to the formation of what is effectively a negatively charged complex within membrane phase. This negatively charged complex, in turn, would attract various cations into the polymer membrane with the resulting partitioning leading to the observation of a positive potentiometric response as is indeed seen by experiment (i.e., Donnan failure).[4]

The above proposal, which would also translate into a modest potentiometric anion response, is consistent with potentiometric selectivity studies carried out at pH 9.0 using OH^- as the primary anion. The resulting $\log k_{OH^-/J}^{sel}$ values, where $J = F^-$, Cl^-, and Br^-, are plotted in Figure 2 and serve to demonstrate that ISEs derived from **1** deviate from the so-called Hofmeister selectivity series at pH 9.0. In particular, a selectivity order of $Br^- < Cl^- < OH^- \approx F^- < HPO_4^{2-}$ is observed, whereas one of $HPO_4^{2-} \approx OH^- \approx F^- < Cl^- < Br^-$ would be predicted if pure Hofmeister behavior were observed.[18]

With F^- as the analyte, no special cation-exchange mechanism needs to be invoked at pH 9.0. On the other hand, little sensitivity toward this anion was noted over any portion of the $3.5 < pH < 9.0$ range studied (the so-called S values for F^- range from between −4 and −7 mV/decade in the absence of added TDDMA; cf. Table 2). Such behavior, which is not generally seen in the case of ISEs based on stronger cation-derived anion receptors (cf. control systems based on TDDMA only in Table 2), is thought to reflect the high hydration energy of fluoride anion and kinetic limitations that derive therefrom.[19] In particular, because of its very high free energy of hydration, the process of fluoride anion complexation within the PVC phase would be extremely slow if the transition state associated with anion recognition involved nonsolvated ions.

Not inconsistent with the low sensitivity observed toward F^- is the finding that TDDMA-free ISEs based on **1** are more selective for $H_2PO_4^-/HPO_4^{2-}$ than F^- over a wide range of pH (cf. Table 2 and Figure 3) even though the converse is true in homogeneous dichloromethane solution (for **1**, the relevant K_a values are 17 170 and 97 M^{-1} for F^- and $H_2PO_4^-$, respectively[2]). Apparently, the near-equal experimental and Gibbs hydration enthalpies for these two anions −510 and −522, −465 and −465, for F^- and $H_2PO_4^-$, respectively serves to mask any differences in organic phase binding affinity.[20]

In ISEs derived from receptor **2**, it is expected that both the pyrrolic groups and the pyridine nitrogen atoms will govern the selectivity of the system. For instance, at high pH where the pyridine nitrogens are not expected to be protonated and where internal NH···N (pyrrolic NH···pyridine) hydrogen bonds might pertain, ISEs based on **2** lose functionality. Indeed, the selectivity pattern for such systems at pH 9.0 is fully reversed compared to that for ISEs derived from **1** ($F^- < OH^- < Cl^- < Br^-$; Figure 2) and becomes just what one would expect based on a consideration of the Hofmeister series. This result, which is underscored by the data in Table 2, leads us to conclude that under these more basic conditions the sensing characteristics of ISEs based on **2** are in large measure defined by the substrate lipophilicity rather than some intrinsic property of the receptor. Positive (cationic) deviations are also seen at this pH.

At pH 5.5, it is expected that some fraction of the pyridine centers present in **2** will be protonated (pK_a for pyridinium cation = 5.19[21]). Such protonation processes will serve not only to generate positive sites within the macrocyclic core that can interact with anionic substrates but also to "liberate" the pyrrole NH donor functionality from internal NH···N (pyrrolic NH···pyridine) hydrogen bonds. Thus, an overall improvement in the anion-binding efficiency is expected at this pH. In point of fact, the sensitivity toward F^- (−8 mV/decade) observed for a PVC-membrane ISE based on **2** at this pH is similar to the one observed for ISEs based on **1** at pH 3.5. On the other hand, a rather weak potentiometric response toward chloride, phosphate, and bromide anions is seen for ISEs based on **2** at this pH, with similar observations being made in the case of ISEs based on **3**.

At pH 3.5, the potentiometric response of ISEs based on **2** is greatly increased, as is that of ISEs based on **3**. In both cases, a high level of pyridine protonation and the ready availability of both pyrrole- and pyridine-based NH hydrogen bond donor functionality is expected to improve anion binding. Indeed, both systems show response sensitivities toward fluoride and phosphate anions that are enhanced relative to those seen for ISEs based on **1** (Table 2). With the exception of Cl^- in the case of **2**, this is also true for the halide anions, bromide and chloride. This is consistent with the normal, neutral NH···anion hydrogen-bonding motif of the calixpyrroles being augmented by electrostatic interactions involving the protonated pyridine centers.

The above results can be rationalized in terms of the response function of PVC membranes based on **2** being defined by two underlying and competing mechanisms, namely receptor-derived anion recognition and anion-exchange processes involving the protonated form of the receptor. In fact, ISEs based on **2** are effective carriers for cations (H^+, Na^+) as well as for anions. Indeed, the response of ISEs based on **2**, as well as those based on **3**, may be considered as being that of a pH-sensitive membrane whose basic properties are modulated by an interference-like sensitivity toward anions. For such systems, the extent of anion interference will be a function of (i) the basicity constant (K_b) of the ligand, (ii) the nature and quantity of any ancillary anions present in the sample solution, and (iii) the concentration of the primary cation in the sample, in this case H^+.[22] Thus, at low pH values the membrane-supported ISE based on **2** shows a strong anion response in the presence of F^- (−28 mV/decade), $H_2PO_4^-$ (−14 mV/decade), and a slight, interference-like effect in the presence of either Cl^- (−4 mV/decade) or Br^- (−10 mV/decade). At pH 5.5, the anion interference disappears ($H_2PO_4^-$/ HPO_4^{2-}, Cl^-, Br^-) or becomes increasingly less apparent as is true for F^- (−8 mV/decade) with similar trends being observed for the ISEs based on **3**. At pH 9.0, by contrast, ISE membranes containing receptor **2** display a positive deviation, an effect ascribed to cation interference. While this cation interference clearly serves to modulate the anion binding behavior of ISEs based on receptor **2**, in marked contradistinction to what is true for analoguous systems containing calixpyrrole **1**, the anion selectivities observed at very high pH appear in this instance to reflect not

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[18] Hofmeister, F. *Arch. Exp. Pathol. Pharmakol.* **1888**, *24*, 247–260.

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[22] Buck, R. P.; Cosofret, V. V.; Linder, E. *Anal. Chim. Acta* **1993**, *282*, 273–281.

only the lipophilicity of a given anion but also its ability to complex with the embedded receptor. On the basis of previous solution-phase studies, this latter critical feature is known to correlate quite well with size.[1−3] Thus, the selectivity factor (log $k_{I/J}^{sel}$) observed at pH 9.0 for ISEs based on **2**, namely $F^- < OH^- < Cl^- < Br^- < HPO_4^{2-}$ (cf., Figures 2 and 3), is considered consistent with the idea that both anion lipophilicity and size play competing influential roles. On a more global level, the very fact that the response order for F^-, OH^-, and HPO_4^{2-} is reversed compared to what would be predicted based on a consideration of the Hofmeister series ($HPO_4^{2-} < OH^- < F^-$) serves to establish unequivocally that the response selectivities for ISEs containing **2** are influenced, at least to a considerable degree, by the intrinsic anion-binding properties of the embedded receptor.

One further feature of the ISEs based on **2** that is worth highlighting is that the pH-dependent nature of the binding phenomena makes the fluoride-to-phosphate selectivities of these electrodes quite different from those derived from **1**. Indeed, while these latter systems show pH-independent behavior, ISEs based on **2** display selectivity factors (log $k_{F^-/H_2PO_4^-(HPO_4^{2-})}^{sel}$) that are constant between pH 10 and 7.25, dip down to a minimum at pH 5.1, and then are increased at pH 3.5. Thus, it appears that the special protonation characteristics of mixed calixpyrrole-calixpyridine receptors such as **2** can be potentially exploited to generate ISE systems that are fine-tuned in terms of their anion selectivities simply by choosing appropriately the conditions of the experiment.

To understand better the mechanistic origins of the above results, the potentiometric characteristics of PVC-membranes based on **1**−**3** with added TDDMA were surveyed. It was observed that the cationic responses seen in many cases disappeared upon the addition TDDMA (Table 2). These results support the idea that the observed positive response is the result of cation exchange within the membrane phase. Other, more generalized background effects are also seen upon the addition of TDDMA. For instance, the pH-dependence of the potentiometric response for ligands **2** and **3** is, in large measure, seen to disappear. Likewise, the higher relative sensitivity toward F^- seen for ISEs based on **2**, as compared to **1**, is found to be diminished in the presence of TDDMA. In fact, in a more generalized sense, the anion selectivity patterns observed for all three receptors **1**−**3** is found to be reduced in the presence of TDDMA. However, the absolute response of the ISEs containing TDDMA toward various anions is seen to be increased relative to that of the original receptor-only derived systems. This is consistent with the TDDMA-containg ISEs acting as cation-exchange mediators and interacting with anions via straight electrostatic interactions (i.e., Coulombic attractions).

Conclusions

In summary, the potentiometric selectivity for membrane ISEs (PVC-membranes) based on dichlorocalix[2]pyrrole[2]pyridine (**2**) and tetrachlorocalix[4]pyridine (**3**) toward a range of investigated anions, namely F^-, Cl^-, Br^-, $H_2PO_4^-/HPO_4^{2-}$, was found to be pH dependent. In the specific case of the hybrid system **2**, the potentiometric selectivity reflects the presence of both pyridinium-derived Coulombic attractions and "pure" calixpyrrole-like pyrrole NH···anion hydrogen-bond donation interactions, as is particularly apparent in analyzing the ISE response toward hydrophilic anions such as fluoride and phosphate. Also potentially important in the case of this receptor are proton-induced receptor deformations and adjustments to the binding cavity size, effects that could help rationalize the lower selectivities seen for the larger halides Cl^- and Br^-. Such protonation effects are not believed to be operative in the case of the ISEs based on **1**. Here, pure pyrrole NH···anionic substrate hydrogen-bonding interactions are thought to mediate anion recognition. As a consequence, the potentiometric selectivity of ISEs based on **1** is for the most part pH independent. However, some small changes in potentiometric response for Cl^- and Br^- are observed that most likely reflect the greater lipophilicity of these anions.

On a more forward-looking level, the present results lead us to suggest that the calixpyrroles and their derivatives could have an important role to play in the generation of PVC-based ISEs. The fact that anti-Hofmeister anion recognition behavior is seen for receptors **1** and **2** under experimental conditions whose defining attributes include a well-defined aqueous sample/membrane interface leads us to propose further that, using these systems or close analogues, key insights into the mode and mechanism of binding could be obtained for a range of other anionic analytes, including ones such as nucleotides, salycilates, and barbituates, that are of obvious biological importance.

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Functionalization of PVC membrane with ss oligonucleotides for a potentiometric biosensor

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Abstract

A novel application of a single stranded (ss) oligonucleotide as an active component of polymeric membrane in an ion-selective electrode (ISE) is described. The original oligonucleotides, oligo(dA)$_{15}$, modified by cholesterol, triphenylmethyl and hexadecyl derivatives, were immobilized into poly(vinyl chloride) (PVC) membrane using extraction protocol. In parallel, the adsorption protocol was used to immobilize unmodified oligo(dA)$_{15}$ on the PVC membrane based on tridodecylmethyammonium chloride (TDDMA$^+$Cl$^-$). Immobilization of ss oligonucleotide probe through spacer was more effective for the potentiometric detection of the hybridization between complementary oligonucleotides. It was found that cholesterol-oligo(dA)$_{15}$ modified membranes were sensitive toward complementary oligo(dT)$_{15}$ in the concentration range 2–80 nM at pH 7. An explanation for the detection mechanism is proposed.

Keywords: Ion-selective electrode; Oligonucleotide; Immobilization; Hybridization

1. Introduction

There is a growing interest in the development of analytical techniques suitable for DNA sequence recognition. Fluorescence (Epstein et al., 2002; Park et al., 2006; Ranasinghe and Brown, 2005; Szunerits et al., 2005; Tawa et al., 2005), surface plasmon resonance (Wu et al., 2001; Fortin et al., 2005; Szunerits et al., 2005; Zhang et al., 2005; Tawa et al., 2005), acoustic transduction (Cavic and Thompson, 2002) and various electrochemical methods (Wang, 2002) have been used for that purpose.

Due to a great potential of miniaturisation and direct measurement of electrochemical signal, the popularity of the latter methods has been increasing continuously during the last few years. Within electrochemical devices designed for detection of DNA hybridization, direct and indirect methods have been developed. Indirect methods are based on the detection of electroactive indicators that intercalate or otherwise associate with double-stranded DNA (Steel et al., 1998; Wang et al., 1997, 2001; Sistare et al., 2000; Takenaka et al., 2000; Maruyama et al., 2001; Li et al., 2004; Teh et al., 2005; Flechsig et al., 2005). On the other hand, direct methods mostly rely on the intrinsic electroactivity of the nucleobases (Napier et al., 1997; Wang et al., 1998, 1999). There are several attempts to detect DNA by its intrinsic molecular charge using field-effect devices (Souteyrand et al., 1997; Fritz et al., 2002; Uslu et al., 2004). A practical realization of field-effect devices for the pure electrostatic detection of charge associated with the probe or target molecules in relatively high ionic-strength solution (e.g. in physiological solutions) is problematic (Poghossian et al., 2005). As an alternative, it is proposed a new method for the label-free DNA detection using an ion-sensitive field-effect devices that sense the changes in the ionic strength of the electrolyte. The top of such ion-sensitive field-effect device is modified with immobilized ssDNA probe molecules arranged with a center-to-center average interprobe distance. The remaining surface of the ion-sensitive layer between the immobilized molecules is in contact with the electrolyte solution and detect a change in ion concentration within the intermolecular spaces before and after hybridization.

Herein, the potentiometric detection of the hybridization event is demonstrated applying oligonucleotide modified poly(vinyl chloride) (PVC) membrane electrode. A new protocol of the preparation of a PVC membrane with anchored ss

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oligonucleotides as probe sequence was developed. In general, hydrophilic compounds cannot be directly used as active components of membrane electrodes. This is because the lack of lipophilicity leads to leaching of the active component from the membrane to solution disturbing the potentiometric response. To overcome this obstacle, chemically modified ss oligonucleotide probes comprising both lipophilic and hydrophilic constituents in one molecule were synthesized. The lipophilic part of the probe should fix the molecule into PVC matrix, while the hydrophilic part, represented by ss oligonucleotide, is expected to protrude into aqueous being ready to recognize selectively the complementary ss oligonucleotide sequence present in solution.

This report summarizes preliminary results for application of a ss olidonucleotide as an active component of polymeric membrane in an ion-selective electrode (ISE). Potentiometric data are discussed and an explanation for the detection mechanism is proposed.

2. Experimental

2.1. Reagents

Poly(vinyl chloride) (PVC, high molecular weight type), tridodecylmethylammonium chloride (TDDMACl), tetrahydrofuran (THF) were of "Selectophore" grade from Fluka. Dioctylphthalate (DOP) was purchased from Aldrich. The oligonucleotides used for immobilization and hybridization experiments were synthesized in Rosenberg's group (Institute of Organic Chemistry and Biochemistry, Academy of Science, Czech Republic) according to a methodology described earlier (Beaucage and Froehler, 1993; Agrawal, 1994; Hianik et al., 2001). The probe oligonucleotides (oligo(dA)$_{15}$) were modified by cholesterol (ligand A), triphenylmethyl (ligand B) and hexadecyl (ligand C) derivatives, as the target and noncomplementary oligonucleotides were used oligo(dT)$_{15}$ and oligo(dG-dC)$_{10}$ (Generi Biotech, Hradec Kralove, Czech Republic) (Fig. 1).

Oligonucleotide stock solutions were kept frozen at −20 °C. For hybridization experiments, the following chemicals were used: $MgCl_2 \cdot 6H_2O$, Tris(hydroxymethyl) aminomethane hydrochloride (TRIS), ethylenediaminetetraacetic acid (EDTA) all "Cell culture" grade (Sigma) and benzene (Lachema, Brno, Czech Republic).

2.2. Immobilization of oligo(dA)$_{15}$ probes into PVC membrane

2.2.1. Adsorption protocol

Polymeric membranes derived from TDDMACl were cast using the conventional method for ISE membrane preparation (Král et al., 1999). In the present study, 0.7 ml THF was used to dissolve approximately 100 mg of a mixture composed of TDDMACl, PVC and plasticizer (DOP) in a weight ratio 1:33:66. Then THF was left to evaporate. The resulting small disk (Ø5 mm) of the polymeric membranes was incorporated into electrode body (Crytur, Czech Republic) and dipped in 2 ml of aqueous solution (0.05 M Tris buffer (pH 7) + 0.01 M $MgCl_2$ + 0.001 M EDTA) containing 200 nmol unmodified oligo(dA)$_{15}$ for 60 min and then hybridization procedure was followed straightway.

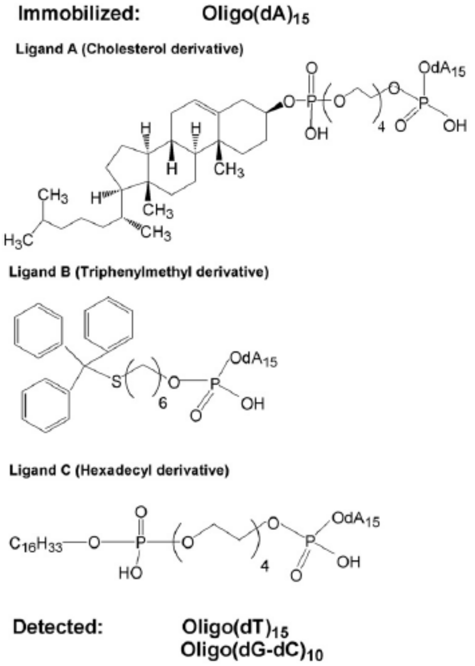


Fig. 1. Investigated probe and target oligonucleotides.

2.2.2. Extraction protocol

A 16.5 mg DOP and 33 mg PVC were dissolved in 400 µl of a mixture benzene + THF, 1:3 (v/v). Then 500 µl of aqueous solution of modified oligo(dA)$_{15}$ at final concentration 1 µM was added and resulting mixture was vigorously shaken for 10 min in Eppendorf tube. Separation of aqueous from organic phase was supported by centrifugation for 5 min. In order to attain a slow evaporation of the mixture of the organic solvents (THF + benzene), the assembly was stored at 4 °C. Within about 1 week a thin polymeric film (membrane) was formed on the top of the aqueous layer. Prior to the hybridization measurements, the bottom of Eppendorf tube with the aqueous solution was cut off and the obtained membrane was washed with a mixture consisting 0.05 M Tris buffer (pH 7) + 0.01 M $MgCl_2$ + 0.001 M EDTA.

2.3. Hybridization experiments with oligo(dA)$_{15}$ probe-immobilized PVC membrane

The prepared PVC membrane electrodes were inserted in the measuring chamber of the cell (Fig. 2) and washed with the supporting electrolyte until their potential became constant.

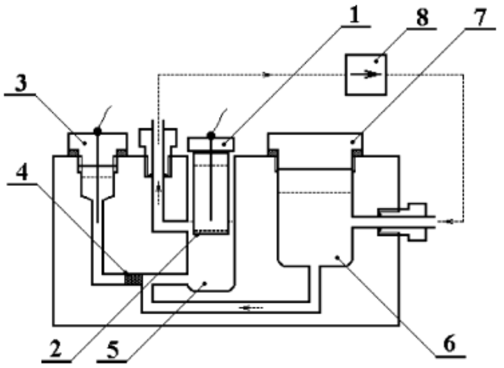


Fig. 2. Schematic representation of the potentiometric cell. (1) Internal reference electrode (Ag/AgCl electrode (0.05 M Tris buffer (pH 7) + 0.01 M $MgCl_2$ + 0.001 M EDTA)); (2) probe-immobilized PVC membrane; (3) external reference electrode (Ag/AgCl electrode (0.003 M KCl)); (4) diaphragm (porous ceramic roller); (5) measuring chamber; (6) dosing chamber; (7) screw cap; (8) peristaltic pump.

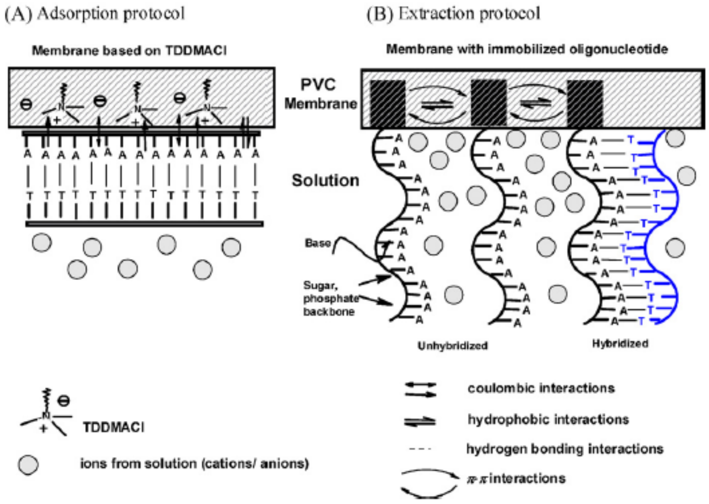
To generate various oligo$(dT)_{15}$ concentrations, aliquots of the concentrated oligo$(dT)_{15}$ stock solution were added to the background electrolyte (0.05 M Tris buffer (pH 7) + 0.01 M $MgCl_2$ + 0.001 M EDTA). All solutions were stored in a refrigerator.

The reproducibility of the potentiometric measurements was investigated by comparing the hybridization signal obtained between the PVC membrane with fixed modified oligo$(dA)_{15}$ and the same concentrations of complementary target oligonucleotide $(dT)_{15}$. For each experiment, at least three replicate membranes were prepared and measured.

After hybridization, regeneration was performed by immersing the membrane in 0.01 M NaOH for 15 min. Then the tested membranes were thoroughly rinsed under stirring (15 min) in an excess of the background electrolyte (0.05 M Tris buffer (pH 7) + 0.01 M $MgCl_2$ + 0.001 M EDTA). The membranes not in use were stored at −20 °C in refrigerator.

2.4. *Potentiometric measurements*

The potentiometric measurements were carried out using the custom-made plexi-glass cell (V_{whole} = 1.5 ml) at 25 ± 1 °C (Fig. 2). The cell consisted of a measuring chamber, a dosing chambers and a chamber containing in-house prepared Ag/AgCl reference electrode (Ag wire with Ø0.5 mm, length 10 mm; inner solution 0.003 M KCl). The measuring and the dosing chambers were connected by the PVC tube (Ø1.5 mm; length 5.0 cm) and were protected from an evaporation of sample solution with a screw cap. The experimental electrodes (Ø6 mm) were placed into the measuring chamber. In order to mix and homogenize sample solutions, an externally joined peristaltic pump (Nisa type 597, Czech Republic; maximal pumping rate 1 ml/min) cycled the sample solution through the measuring and the dosing chamber and *vice versa*. The conductive contact between the reference electrode and the measuring/dosing chambers was accomplished *via* the porous ceramic roller (Ø2 mm, length 5 mm). The data were measured using voltammeter with high input impedance (PHI 04 MG, Labio, Czech Republic) controlled by custom made dataloging software programmed in LabView 6.1 (National Instruments, USA).



Scheme 1. Potentiometric sensor for detection of hybridization between complementary ss oligonucleotides on the phase boundary PVC membrane/solution. The redistribution of the ion concentration within intermolecular regions induced by hybridization process close to the PVC membrane should be detected by the potentiometric sensor. The ss oligonucleotide probe may be arranged either flat (A) or normal (B) to the membrane surface during immobilization protocol. After hybridization, an arrangement of the immobilized ss oligonucleotide probe molecules can prevent (A) and open (B) the membrane surface toward ion-exchanger.

3. Results and discussion

3.1. Concept for formation of the potentiometric response

Two different approaches of the oligonucleotide probe immobilization into PVC membrane, the adsorption and the extraction procedure, are compared (Scheme 1).

The signal of the potentiometric sensor is result of ion-exchanger processes occurring onto the phase boundary between the membrane and solution. It was supposed that the redistribution of the ion concentration within intermolecular regions induced by hybridization process (Poghossian et al., 2005) close to the PVC membrane should be detected by the potentiometric sensor. With this point of view, the PVC membrane surface with either adsorbed or anchoring oligonucleotide should have the "useful" area, available for the ion exchanger.

The dependence ΔE versus $\log c_{\text{target}}$ should be linear in certain concentration range and it is reasonable to expect an existence of some "breaking point" where the saturation of the electrode surface is reached and the electrical potential becomes independent on the concentration of the complementary oligonucleotide. Thus, the linear dependence ΔE on $\log c_{\text{target}}$ and the location of "the breaking point" may be used for the evaluation of the surface coverage density of the membrane electrode modified with oligo(dA)$_{15}$.

3.2. Adsorption procedure

Original oligonucleotides consist of monomeric units having both hydrophobic and hydrophilic moieties, moreover, some groups are ionizable. At neutral pH, the overall charge of the oligonucleotides is negative due to deprotonized phosphogroups. Theoretically, positively charged species localized on the membrane surface should assist the adsorption of oligonucleotide by Coulombic interaction. We supposed that the PVC membrane based on TDDMACl (anionic exchanger) might serve that purpose (Scheme 1A). The overall adsorption process thus might be based on a combination of hydrophobic and Coulombic interaction. For hydrophobic interaction it can be written:

$$(\text{oligo(dA)}_{15})^{z-}_{\text{aq}} \Leftrightarrow (\text{oligo(dA)}_{15})^{z-}_{\text{org}} \qquad (1)$$

and for Coulombic interaction:

$$z\text{TDDMA}^{+}\text{Cl}^{-}_{\text{org}} + (\text{oligo(dA)}_{15})^{z-}_{\text{aq}} \Leftrightarrow (z\text{TDDMA}^{+}(\text{oligo(dA)}_{15})^{z-})_{\text{org}} + z\text{Cl}^{-}_{\text{aq}} \qquad (2)$$

Obtained experimental data showed that the potentiometric response of PVC membrane based on TDDMACl was anionic, but very weak and the electrode cannot be used as a sensing probe for the complementary oligonucleotide. It seems that the adsorption procedure leads to unsuitable geometry of the oligo(dA)$_{15}$ on the membrane surface for the formation of the potentiometric signal at hybridization. Probably the negative charge localized on the oligonucleotide interact strongly with a positively charged molecules of TDDMA^{+} in the membrane in accordance with (Eq. (2)). Immobilized oligo(dA)$_{15}$ might be tightly hold on the membrane in more or less parallel arrangement and prevent ion-exchanger. This explains the observed low sensitivity of the electrode to oligo(dT)$_{15}$.

3.3. Extraction procedure

To immobilize the hydrophilic oligonucleotide molecules into PVC membrane, the original probe oligonucleotide, oligo(dA)$_{15}$, was modified by cholesterol (membrane A), triphenylmethyl (membrane B) and hexadecyl (membrane C) derivatives, respectively. The idea behind this concept stemmed from the presumption that the hydrophobic part of the synthesized molecule should fix it within PVC matrix while the hydrophilic oligo(dA)$_{15}$ remained exposed to the solution being available for interaction with a complementary oligonucleotide strand (Scheme 1B). Accordingly to common practice, the membrane electrodes based on PVC are prepared from the membrane cocktail consisting of PVC, plasticizer and lipophilic ligand in weight ration 33:66:1. A ligand have to be hydrophobic to facilitate its solubility in organic phase and to insure its stability within the resulting membrane. Lipophilisation of ligand is usually achieved by attaching of lipophilic groups to a original molecule. In the classical procedure, a ligand is incorporated into the membrane by dissolution of the membrane cocktail in a small amount of organic solvent with its subsequent evaporation. However, in our procedure, a ligand is incorporated into the matrix within the extraction process being underway between an organic and an aqueous phase. This should lead to a significant improvement in control of the immobilization procedure and it created a favourable conditions for a suitable orientation of the long pendant chain of oligonucleotide into aqueous phase.

Cholesterol-based spacer is the best among the tested spacers for oligonucleotide anchoring into PVC/DOP membrane. As can be seen from Fig. 3A, the magnitude of the potentiometric response increased with increasing amount of the hybridized sample for membranes A in the intervals of 2–80 nM:

Added oligo(dT)$_{15}$ (nM)	Potential change (mV)
2	−21 ± 4
20	−80 ± 8
60	−103 ± 14
80	−133 ± 8

The fact that the potentiometric response (−140 mV) remained without remarkable changes at 800 nM of the oligo(dT)$_{15}$ might be explained by a saturation effect on the modified surface. The potentiometric signal of these membranes to noncomplementary oligo(dG-dC)$_{10}$ is significantly reduced (Fig. 3B), in contrast to complementary oligo(dT)$_{15}$ (Fig. 3A). With 20 nM of oligo(dG-dC)$_{10}$, the potentiometric signal is rarely reversed, namely, from negative on positive (+4 ± 1 mV). The actual reason for such occurrence is not clarified. Possibly, this phenomenon may be result of the new ion redistribution within the intermolecular spaces near the membrane surface. The modified membranes B–C react only on addition of 2 nM of complementary oligo(dT)$_{15}$ with potential −17 to −29 mV, the further increasing of concentration does not lead

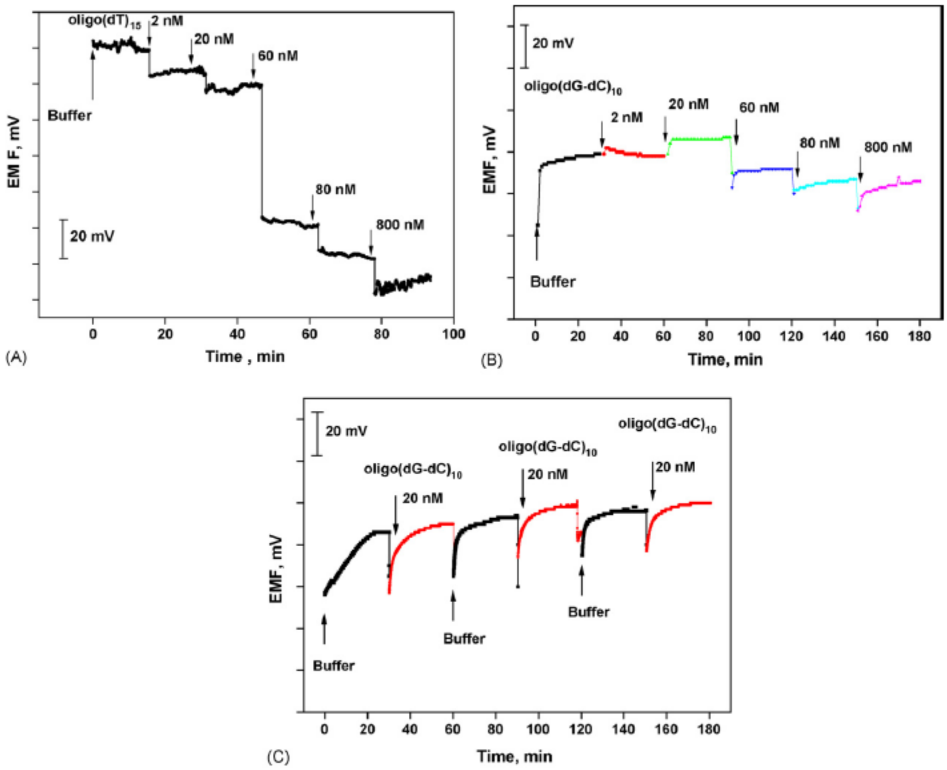


Fig. 3. Application of PVC membrane functionalized with cholesterol-oligo(dA)$_{15}$ for potentiometric detection of the hybridization with complementary oligo(dT)$_{15}$ (A) and unspecific interactions with noncomplementary oligo(dC-dG)$_{10}$ (B) oligonucleotides. (C) Reproducibility of the potentiometric signals of the PVC membrane functionalized with cholesterol-oligo(dA)$_{15}$ after regeneration in 0.01 M NaOH for 15 min. (Downward arrows indicate injections of the corresponding oligonucleotides and upward arrows indicate injection of buffer into the potentiometric cell.)

to changes of the potentiometric signal. Seemingly, it could be surprising that only cholesterol derivative of oligo(dA)$_{15}$ showed a good response in reasonable concentration range to the complementary oligonucleotide. However, the explanation might be relatively straightforward. It is well known that cholesterole molecules can organize themselves in dimers and other highly structured assemblies in solution (Farooqui et al., 1991; Salunkhe et al., 1992; Letsinger et al., 1993; Zhang et al., 1996). This can aid and positively influence also an arrangement of spacer molecules (e.g. cholesterol) into the PVC membrane during the extraction procedure. Suitable distances among the pendant oligonucleotide strands and their appropriate orientation within the membrane can be therefore achieved as a cooperative effect of the extraction preparation procedure and an intrinsic physico-chemical properties of the competently chosen liphophilic oligo(dA)$_{15}$ modificator.

Repeated usability is a desired feature for biosensors in practical applications. In present study, chemical regeneration with 0.01 M NaOH solution was applied. The electrode response returned to its original signal, indicating that the double-stranded hybrid was dissociated and that the signal of the immobilized probe oligonucleotide was not destroyed. The regenerated sensor produced a similar changes of the potentiometric signal for both the complementary oligo(dT)$_{15}$ (see table above) and noncomplementary oligo(dG-dC)$_{10}$ (Fig. 3C).

4. Conclusion

Results presented here clearly show that functioning ISE based on immobilized oligonucleotides can be prepared. The extraction preparation procedure described here proved as a very promising approach. It was demonstrated that the appropriate combination of the synthetic procedure with proper chemistry can lead to the creation of the working sensing device for oligonucleotides. It seems that the outlined concept is viable and could be a new step for the development of the potentiometric sensors.

Acknowledgements

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Potentiometric response and mechanism of anionic recognition of heterocalixarene-based ion selective electrodes

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Abstract

The ion selective electrode (ISE)-based potentiometric approach is shown to be an effective means of characterizing the anion recognition sites in the molecular receptor calix[2]pyridino[2]pyrrole (CPP). In particular, potentiometric pH-measurements involving the use of experimental PVC-membranes based on CPP revealed the existence of both mono- and diprotonated forms of the receptor under readily accessible conditions. Based on these analyses, apparent surface protonation constants for this heterocalixarene were found to lie between 8.5–8.9 (pK_{B1}) and 3.3–3.8 (pK_{B2}). CPP was found to interact with targeted anionic analytes based on both coulombic and hydrogen bond interactions, as inferred from varying the kinds of ionic sites present within the membrane phase. Potentiometric selectivity studies revealed that CPP preferred "Y-shaped" anions (*e.g.* acetate, lactate, benzoate) over spherical anions (*e.g.* fluoride and chloride), fluoride over chloride within the set of spherical anions, and the *ortho*-isomer over the corresponding *meta*- and *para*-isomers in the case of hydroxybenzoate (salicylate and congeners). In the context of this study, the advantages of potentiometric determinations of acetylsalicylic acid using optimized PVC-membranes based on CPP relative to more conventional PVC-membrane ISEs based on traditional anion exchanger were also demonstrated.

Keywords: Potentiometry; Ion-selective electrodes; Hydroxybenzoate; Heterocalixarene; Anion recognition

1. Introduction

The challenge of constructing analyte-specific ion selective electrodes (ISEs) is increasingly attracting the attention of supramolecular chemists. This is because such systems could provide a convenient means of characterizing, under interfacial organic–aqueous conditions, the substrate binding characteristics of receptors that might not otherwise be amenable to study in the presence of water due to, *e.g.*, poor aqueous solubility and/or overly weak binding affinities. ISEs are also of obvious interest because they can help translate the chemistry of new substrate binding systems into tools that can be used to recognize selectively various targeted species in the presence of potentially interfering analytes. In the specific case of anion recognition, this approach has been explored extensively by Umezawa, Meyerhoff, Simon, Reinhoudt, Schmidtchen and others using a range of receptors including bis-guanidinium [1,2], porphyrins [3], protonated polyamines [4–7], and protonated sapphyrins [8–10], as well as a variety of Lewis acidic systems such as calixarenes [11–15], uranyl salophenes [16,17], metalloporphyrins [18–22], metallocenes [23], other organometallic derivatives [24–26], and fluorinated compounds [27].

The *meso*-octamethyldichlorocalix[2]pyridino[2]pyrroles (CPPs; Scheme 1) represent a class of macrocycle that should act as anion binding agents. Unlike the better studied calix[4]pyrroles, the CPPs contain both pyrrolic and pyridine sites within their macrocyclic cores. Thus, the putative anion-receptor interactions of CPPs are expected to reflect contributions from both electrostatic and hydrogen bonding interactions in varying degrees depending on the conditions (*e.g.*, degree of protonation, nature of solvent, *etc.*). In other words, it could be anticipated that under certain conditions the CPPs would act like pyridinium-type receptors and under others, pyrrole-based receptors. Because of this potential duality, it was proposed that studies of appropriately chosen analytes, capable of multiple types of supramolecular interactions, would provide invaluable insights into the fundamental molecular recognition properties of this class of receptor.

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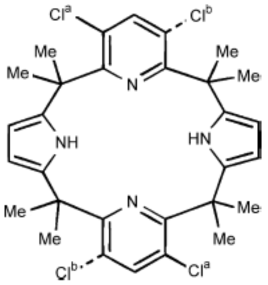


Scheme 1. *meso*-Octamethyldichlorocalix[2]pyridino[2]pyrrole. Each of the individual pyridine subunits bears a single chlorine substituents at either the 3 or 5 positions (Cl^a or Cl^b), but not both.

With such considerations in mind, the salicylate anion and its congeners were selected as test analytes of choice. The carboxylate group and aromatic core present in the salicylate anion makes it potentially capable of entering into ion-pairing, hydrogen bonding, and π-stacking interactions with a putative receptor. Indeed, the salicylate anion has previously been the subject of analysis-based recognition efforts, including ones focused on the selective coordination to tetraphenylporphyrin [20,28–31], phthalocyanine [32–34], and salophenes [35] metal centers. The supramolecular interaction properties of salicylate have been exploited in the case of anion-exchange- [36–38], calix[4]phyrin- [39] and guanidinium derivative-based [1] polymeric membranes. In this paper, the interactions of CPP with salicylate anion have been studied using a potentiometric approach. On the basis of these studies the primary recognition sites within the receptor framework, namely the neutral pyrrole NH protons and the protonated pyridinium centers, could be identified and the role of these disparate sites in achieving anion recognition in large measure "deconvoluted" and assessed.

2. Experimental

2.1. Reagents

High molecular weight poly(vinyl chloride) (PVC), 2-nitrophenyl octyl ether (NPOE), tridodecylmethylammonium chloride (TDDMACl), sodium tetraphenylborate (NaTPB), tetrahydrofuran (THF) (stored over molecular sieves) and 2[*N*-morpholino]ethanesulfonic acid (MES) were purchased from Fluka (Switzerland) and Sigma–Aldrich (Germany). Acids, sodium hydroxide, and various inorganic salts (analytical grade) were obtained from Lachema (Czech Republic). The synthesis of CPP has been reported previously [40]. The analgesics used in this study, Aspirin-C (Bayer AG, Leverkusen, Germany), Acylcoffin and Acylpyrin (Slovakofarma, Hlohovec, Slovakia), were purchased in the form of commercial tablets from a local drugstore in Prague, Czech Republic.

2.2. Membranes

Polymeric membranes derived from either TDDMACl or NaTPB and CPP were cast using a conventional method for ISE membrane preparation [41]. In the present study, 0.7 mL of THF was used to dissolve 100 mg of a mixture composed of the active component, CPP, (3 wt.%), of PVC and plasticizer in a 1:2 (w/w) ratio. When indicated, quantities of TDDMACl or NaTPB (specified as mol% relative to CPP) were added (Table 1). The resultant mixtures were poured into a metallic ring of 16 mm internal diameter resting on a glass plate, and the THF was allowed to evaporate at room temperature.

2.3. Electrodes and EMF measurements

A disk of 10-mm diameter was punched from the ion-selective membrane prepared as described above, glued onto a polymeric ring of 8 mm internal diameter with a PVC/THF paste, and mounted in the electrode body for EMF measurements. An aqueous solution of 10^{-3} M sodium salicylate was used as the inner filling of the electrode. MES buffer was used to obtain the desired experimental pH of 5.5 and 9. Prior to and after the potentiometric experiment, the electrodes were soaked and regenerated in a solution of 10^{-3} M sodium salicylate. All measurements were carried out at 25 °C with cells of the following type: Ag; AgCl; 3 M KCl|sample solution|membrane|inner filling solution; AgCl; Ag. The EMF values were measured using a custom made five-channel electrode monitor [42]. The pH response of the experimental electrode was obtained by titrating 0.05 M HCl with NaOH in the absence and presence of sodium salicylate. The pH was monitored by using a glass electrode PHR 01 and a PHI 04 MG ion- and pH-meter (Labio, Czech Republic).

Table 1
Composition of membranes based on CPP in *o*-NPOE/PVC (2:1) and their potentiometric properties towards salicylate anions

Membrane	Composition, wt.% (mol% relative to receptor)			Sensitivity, mV decade^{-1}	Linear range, M
	CPP	NaTPB	TDDMACl		
2A	3.0	0.52 (26.4)		+53.0 ± 2	10^{-2} to 10^{-1}
2B	3.0	0.26 (13.8)		−34.6 ± 2	10^{-5} to 10^{-1}
2C	3.0			−35.3 ± 2	10^{-3} to 10^{-1}
2D	3.0		0.29 (8.8)	−53.2 ± 0.6	10^{-3} to 10^{-1}
2E	3.0		0.57 (17.4)	−54.2 ± 0.5	10^{-5} to 10^{-1}
2F	3.0		1.63 (49.4)	−58.3 ± 0.5	10^{-5} to 10^{-1}
2G	3.0		4.94 (150)	−53.3 ± 0.5	10^{-5} to 10^{-1}
TDDMACl			3.0	−53.3 ± 0.7	10^{-5} to 10^{-1}

In studies of the potentiometric response and anion selectivity, working solutions of the analytes in question were prepared by diluting concentrated stock solutions. Calibration curves were constructed by plotting the potential *versus* the logarithm of the concentration of the anion present in the tested solution. Potentiometric selectivity coefficients (log $K_{I/J}^{\mathrm{Pot.}}$) were then determined by the separate solution method (SSM) [43], with the primary (I) and interfering (J) ion concentrations being 10^{-2} M. In those membranes where no theoretical potentiometric response was observed, the potentiometric selectivity was estimated using the matched potential method (MPM) [43]. In the MPM, a 10^{-4} M concentration of sodium salicylate was used as the background. The values were calculated from the concentration of the interfering ion, which induced the same amount of potential change as that induced by increasing the concentration of sodium salicylate to 5.0×10^{-4} M.

2.4. Sample analysis

The content of acetylsalicylic acid in commercial analgesic products (Acylpyrin, Acylcoffin, Aspirin-C; please, see Section 2.1) was assayed using both volumetric and potentiometric methods.

The volumetric analysis was based on the following procedure: briefly, five tablets containing acetylsalicylic acid were ground and 0.4 g of the resulting powder was dissolved in 10 mL ethyl alcohol. The solution was then titrated quantitatively with a solution of 0.1 M sodium hydroxide using the color change of phenolphthalein as the end point; the resulting solution was mixed with 30 mL of 0.1 M sodium hydroxide and was heated for 10 min. After cooling at the ambient temperature, the excess sodium hydroxide was neutralized with an appropriate quantity of dilute aqueous sulfuric acid (0.05 M). A blank experiment was carried out in the same manner.

The potentiometric analysis of analgesics was carried out without subjecting acetylsalicylic acid to hydrolysis and using the method of standard addition at pH 5.5 [44].

3. Results and discussion

In the case of CPP, the expected binding sites are the pyrrole and pyridine subunits. Each one is capable of promoting anion recognition, albeit in a somewhat different fashion. For instance, the pyrrole subunits provide NH hydrogen bond donors that are inherently neutral, although nonetheless subject to potential protonation, as has been demonstrated in the case of ISEs based on calix[4]pyrroles [45]. Thus, at least under many conditions, these subunits might be expected to support anion recognition through rather straightforward $\mathrm{NH}\cdots\mathrm{X}^-$ hydrogen bonding interactions. In contrast, the acid–base properties of the pyridine nitrogen should allow it to bind anionic species through either $\mathrm{N:}\cdots\mathrm{HO}$ hydrogen bonds at higher pH or coulombic $\mathrm{NH}^+\cdots\mathrm{X}^-$ interactions at low pH that could operate independently or in concert with hydrogen bonding interactions. Thus, it was expected that, depending on the specific conditions of use, CPP might act as mixed anion carrier, *i.e.*, either a charged or neutral carrier.

The main goal of the present study was thus to use a potentiometric approach to obtain insights into the nature of the interaction modes potentially operative in CPP anion substrate recognition. Towards this end, salicylic acid was chosen as the test substrate, because (1) it is potentially capable of interacting with the CPP core through a variety of multi-point interactions, (2) earlier studies with other related systems had established that this is a species which gives a good response in receptor-functionalized ISEs [1,28–35,39], and (3) its derivative, acetylsalicylic acid, represents important pharmaceuticals.

3.1. pH effects

Given the potential pH sensitivity of the CPP receptor (as the presumed result of pyridine N: protonation), our initial interest was to define roughly the pH ranges where the mono- and diprotonated forms prevail. The potential of CPP-based membranes were measured in aqueous media as a function of solution pH in the presence and absence of salicylate anion (Fig. 1A). In the absence of salicylate anion, there were pH ranges where a weak (−31 mV/pH unit: pH 2–5) and sub-Nernstian (−47 mV/pH unit: pH 8–9) responses were seen, as well as ones where pH independent behavior was obtained (below pH 2 and at 6–8). The addition of salicylate anion (sodium salt) led to a negative deviation in two pH ranges, namely, 3.5–6.0 (−11 mV/pH unit) and 8–9 (−25 mV/pH unit). This negative deviation is ascribed to the favorable charge–charge interactions between the protonated CPP pyridinium subunits and the salicylate anion at

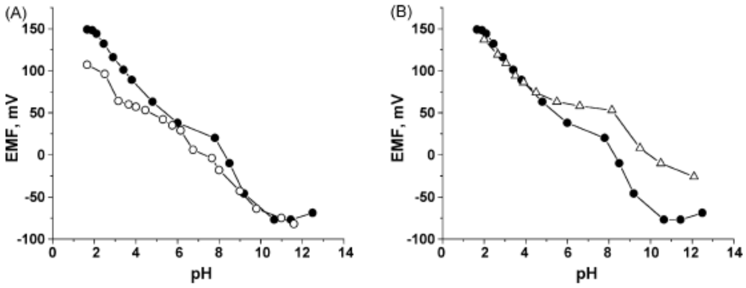
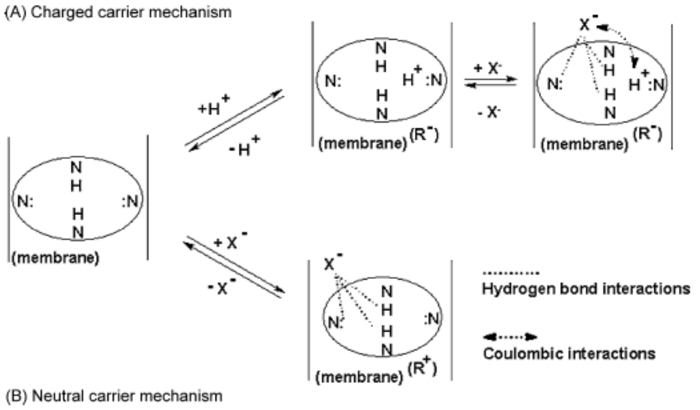


Fig. 1. pH dependence of the potentiometric response for (A) CPP-based membrane in the absence (●) and presence (○) of 10^{-3} M sodium salicylate, (B) CPP-based membrane in the absence (●) and presence (Δ) of anionic sites (13.8 mol% NaTPB relative to receptor).



Scheme 2. Possible mechanism of the potentiometric response seen for CPP-based membranes, showing how this receptor system can act as a either a singly charged (A) or neutral (B) carrier at pH 5.5 (X^-, analyte anion; R^- and R^+ are added lipophilic anionic and cationic sites, respectively).

the membrane/sample solution interface. The stepwise decrease in sensitivity observed in passing from the 9–8 to the 6–3 pH regimes is, accordingly, most easily rationalized in terms of the predominant existence of the mono- and diprotonated forms of CPP, respectively.

Recently, Umezawa et al. demonstrated that anionic sites (including the sodium tetraphenylborate, NaTPB, employed in the present study) within the membrane phase can play an important role in modulating receptor protonation and thus their interaction with targeted anionic analytes [46]. These effects mostly take place at the membrane/water interface, since anionic sites help attract protons to the interfacial region. As such, they serve not only to enhance receptor protonation, they also help suppress unfavorable charge–charge repulsions between the resulting protonated receptor molecules and protons present at the interface. Since the anionic site used in the present study, NaTPB, is a weak Brönsted base, it itself is not subject to extensive protonation at the interface. Thus, the pH dependences seen in the case of the CPP-containing membranes can be attributed to changes in receptor protonation, presumably at the membrane surface. Consistent with this proposal is the fact that parallel pH dependent profiles were seen for the CPP electrodes in the presence and absence of NaTPB (Fig. 1B).

The above findings lead us to propose that the CPP receptor is protonated within the membrane over the pH range 2–9. Within the context of this global assesment, we propose that specific surface protonation effects might intervene between 8.5–8.9 (pK_{B1}) and 3.3–3.8 (pK_{B2}). Support for this latter hypothesis comes from proton binding studies of a pyridine-strapped 5,12-dioxocyclam-based macrocycle [47]. Meyer et al. showed that this elaborated pyridine-containing receptor behaves as a diprotic base with $pK_{B1} = 8.94(1)$ and $pK_{B2} = 2.32(9)$. However, in this latter instance the diprotonated species was thought to be stabilized by additional intramolecular hydrogen bonding within the cavity. For the CPP receptor of the present study, such internal hydrogen bonding interactions are not possible in the diprotonated form (all the core nitrogens are protonated). Accordingly, the relevant pK_{B2} for CPP is expected to fall in the 3.3–3.8 range.

3.2. *Effect of ionic additives on the behavior of CPP-based membranes*

Taking into account its susceptibility to protonation, we believe the CPP core should be considered as a mixed anionic carrier, namely a positively charged or neutral carrier (Scheme 2) depending on the effective pH. To the extent such a suposition is true, the observed potentiometric selectivity should depend on both the amount and nature of the ionic sites incorporated within the membrane [48–50].

Because of this potential ambiguity, most potentiometric measurements were carried out at pH 5.5, where the receptor and salicylate anion exist predominantly in the form of the respective monocations and monoanions. The potentiometric results obtained under these well-defined conditions are presented in Table 1.

In the PVC membrane, the CPP receptor, neutral in itself, is protonated at the membrane surface and, consequently, recognizes analyte anions in the sample solution (Scheme 2A). The pyridine nitrogen basicity helps establish limits on what are expected to be optimal concentrations of added anionic sites. An excess of added anionic sites (*i.e.*, sodium tetraphenylborate) could compete with the protonated receptor and actually reverse the potentiometric response of a membrane such that it displays a cationic response rather than the expected anionic response. By deacreasing the concentration of anionic sites in the membrane phase, the anionic potentiometric response resulting from the receptor protonation is expected to become stronger [50]. Nonetheless, membranes 2C and 2B, made up without and with ~13.8 mol% NaTPB, respectively, both showed sub-Nernstian anionic responses of −35.3 to −34.4 mV decade^{-1} from

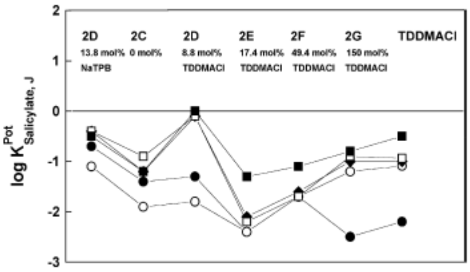


Fig. 2. Plot showing the potentiometric anion selectivity, log $K^{\text{Pot}}_{\text{Salicylate,J}}$[a], of the CPP-based membranes containing various concentrations of anionic and cationic sites at pH 5.5. (F^- (●), Cl^- (○), benzoate (■), lactate (□), acetate (◆)). [a]All values of the potentiometric selectivity coefficients were obtained using MPM, as detailed in Section 2.3.

10^{-5} M.[1] However, the addition of ~26.2 mol% NaTPB led to a loss in the anionic response in favor of a cationic response.

The addition of cationic sites into the CPP-containing membrane phase should support salicylate binding *via* a neutral mechanism as outlined in Scheme 2B. In this case, the both pyrrolic and pyridine groups should be involved in the formation of hydrogen bonds. However, the nature of the interactions is different in the case of these two subunits. This is because the anionic (carboxylate) group present on the salicylate anion can interact with the pyrrole NH proton acting as a hydrogen bond donor, whereas it is expected that the pyridine nitrogen atom will act as a hydrogen bond acceptor binding the OH group present in the analyte. In addition to these first order effects, the formation of stacking interactions between the aromatic subunits within the receptor and those present in the analyte might enhance the overall process of receptor-analyte recognition. Consistently with such hypotheses, it was found that the addition of ~17.4 and 49.4 mol% TDDMACl, a known cationic site additive, to CPP-containing membranes at pH 5.5 served to increase the potentiometric sensitivity relative to what was seen in its absence (membranes 2E and 2F, respectively). In fact, at these concentrations of TDDMACl, near-Nernstian response characteristics were seen at analyte concentrations $\geq 10^{-5}$ M.

The potentiometric selectivity, which was seen to be dependent on the kind of lipophilic additive employed, was thought to reflect interference caused by the carboxylate anions. It leads to the presumption that electrode membranes containing CPP would display selectivity for "Y-shaped" anions (*e.g.*, acetate, lactate, benzoate) over spherical ones (*e.g.*, fluoride and chloride). As a test of this hypothesis, a range of species was tested as reflected in Fig. 2. As can be inferred from an inspection of this figure, a selectivity sequence was seen that was different from that predicted based on the Hofmeister series (membrane containing only TDDMACl), a finding that is completely consistent with the involvment of specific receptor–analyte interactions in the anion recognition and sensing process. Moreover, the fact that the addition of either anionic or cationic sites had a different effect on the carboxylate/halide discrimination ratio is consistent with the pyrrolic and pyridine subunits providing a different, and hence non-Hofmeister contribution to the recognition process. In particular, the presence of anionic sites was expected to enhance the effect of both coulombic and hydrogen bond interactions and thus lead to the greatest level of generalized anionic interference. In fact, the greatest such interference was observed for membranes containing ~13.8 mol% NaTPB (membrane 2B). In contrast to what is seen with anionic sites, the presence of cationic sites was expected to enhance the discrimination between anionic interferents in accord with the number of receptor–analyte hydrogen bonds that could be stabilized. To a first approximation, this too was seen. However, it should be noted that the interference from fluoride anion was relatively greater than that produced by chloride. Such a difference argues in favor of a cooperative effect, wherein both coulombic and hydrogen bonding interactions contribute to the recognition of such spherical halide anions. Finally, as noted above, once a large excess of cationic sites is added into the membrane phase (membrane 2G), the selectivity pattern begins to resemble that of membranes based only on anion exchangers (cf. membrane containing only TDDMACl; Table 1).

More recently, a new series of calixphyrin oligopyrrolic macrocycles has been proposed for oxoanions recognition [39]. Their structure lies between that of porphyrins and calixpyrroles and, like CPP, consists of two pyrrolic units and two basic nitrogens. As part of our study, we were interested to compare the affinity of these analogues as receptors for oxoanionic species in ISEs. Unfortunately, the potentiometric results reflecting the presumed binding properties of a prototypical monoarylcalix[4]phyrin revealed a pattern of selectivity only at pH 9 (the potenciometric selectivity coefficients were measured by SSM). At this pH, the monoarylcalix[4]phyrin thus appears to behave as a neutral carrier. In contrast, CPP (membrane 2F) displays interference from acetate, lactate, and benzoate, a finding we interpret as meaning that CPP is more sensitive towards carboxy groups and their presence in analytes under these same experimental conditions.

Table 2
The potentiometric selectivity coefficients, log $K^{\text{Pot}}_{o\text{-Hydroxybenzoate,J}}$[a], of salicylate electrodes prepared with different membrane active components

Interfering anion	Aliquat 336S[b]	Sn[TPP]Cl_2[c]	CPP[d]
Acetate	<−2.70	−3.9	−2.10
Lactate	−2.2	−4.0	−2.2
Benzoate	−1.15	−1.4	−1.3
meta-Hydroxybenzoate	−1.34	−1.10	−2.51
para-Hydroxybenzoate	−1.30	−1.00	−3.32

[a] All values of the potentiometric selectivity cofficients were obtained using the SSM, with the primary (I) and interfering (J) ion concentrations being 10^{-2} M in 0.05 M MES, pH 5.5.

[b] Aliquat 336S membranes were prepared with 63 wt.% *n*-dibutyl phthalate, 30 wt.% PVC and 7 wt.% Aliquat, as detailed in ref. [28].

[c] Sn[TPP]Cl_2-based membranes were prepared with 66 wt.% DBS, 33 wt.% PVC, and 1 wt.% carrier, as detailed in ref. [28].

[d] Membrane composition corresponded to that of membrane 2F (Table 1).

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[1] Anionic impurities are always present in plasticized PVC membranes. Therefore, membrane 2C likely contains a small amount of anionic impurities incorporated within it even though it is nominally free of NaTPB.

Table 3
Results of the determination of acetylsalicylic acid in commercial analgesic products

Tablet sample (label value, mg tablet^{-1})	Acetylsalicylic acid (% of the nominal value)		
	Potentiometric analysis		Volumetric analysis
	CPP-membrane	TDDMACl-membrane	
Acylpyrin (500)	99.6 ± 4.4	105.4 ± 4.4	95 ± 4.4
Acylcoffin (450)	102 ± 4.2	106 ± 6.2	102 ± 5.8
Aspirin-C (400)	99.5 ± 4.8	107 ± 5.5	–

3.3. Effect of positional isomers on potentiometric selectivity

To follow up on the above and to obtain greater insights into the presumed binding modes, isomers of salicylate anion (*ortho*-hydroxybenzoate), namely, *meta*- and *para*-hydroxybenzoate, were studied, at pH 5.5. This series of potentiometric measurements was carried out so as to determine the effect, if any, of the position of the hydroxyl group on the observed potentiometric response. Therefore, for the sake of comparison, selectivity data for a previously reported salicylate electrode based on Aliquat 336S [28] and Sn[TPP]Cl$_2$ [28] is also included.

It was found that at pH 5.5, ISEs derived from CPP-based membrane displayed a preference for the *ortho*-isomer of hydroxybenzoate (salicylate) relative to the *meta*- and *para*-isomers. For these latter two species, studied in competition with salicylate, the interference gradually decreased with a distance of the hydroxyl group from carboxyl group (Table 2). In other words, the effect of the *para*-isomer was less pronounced than the *meta*-isomer. While this proved specifically true just for the ISEs made up using CPP, there is simply not enough data to tell whether this generazition will hold in the case of ISEs made up from Aliquat 336S and Sn[TPP]Cl$_2$.

The differences in selectivity seen for the CPP-based electrodes relative to the other ISE receptors included in Table 2 are believed to reflect differences in the intrinsic recognition modes for the receptors in question. In the case of the Aliquat 336S-based membrane system, recognition is based on nonselective coulombic interactions with the quarternary ammonium salt. Likewise, for the Sn[TPP]Cl$_2$-based membrane hydroxybenzoate (salicylate) recognition is the result of its selective coordination to the metal center as an axial ligand. In contrast, CPP is capable of recognizing hydroxybenzoates *via* hydrogen bond interactions (*e.g.*, pyrrolic NH···carboxylic, pyrrolic NH···OH, pyridine···HO), as well as coulombic effects, especially pyridinium $\mathrm{NH^+\cdots{}^-O_2C}$ salt bridges. When these effects can be made to be synergetic, as in the case of salicylate binding, the selectivity for one particular regioisomer would be expected to be particularly high, a finding that is very much in accord with the experimental findings.

3.4. Analytical application

Acetylsalicylic acid is one of the best known and most widely used salicylate derivatives. Based on its structure, and its ability to interact with CPP through more than one recognition mode, it was anticipated that ISEs containing this latter receptor would prove particularly sensitive. As a corollary of this hypothesis, it was anticipated that the ISEs of this study would allow for the detection of acetylsalicylic acid present in various commercial analgesic products. Towards this end, three commercially available analgesic products containing acetylsalicylic acid were tested. The results obtained by potentiometric analysis using the CPP-based electrodes of this study proved to be in good agreement with those recorded using standard volumetric procedures (Table 3). In accord with the mechanistic rationale given above, the CPP-based PVC-membranes proved more accurate than those derived from just TDDMACl (cf. last entry in Table 1).

4. Conclusions

The potentiometric measurements provided insights into the fundametal recognition processes that underlie the observed potentiometric response and the basic chemical properties of CPP. For instance, pH-dependence experiments served to support the notion that the apparent protonation constants of this particular heterocalixarene might lie between 8.5–8.9 (pK_{B1}) and 3.3–3.8 (pK_{B2}). Parallel studies, that involved varying the kinds of ionic sites present within the membrane, served to demonstrate that both the charged and neutral forms of this particular heterocalixarene display an inherent affinity for "Y-shaped" anions (*e.g.* acetate, lactate, benzoate) over spherical anions (*e.g.* fluoride and chloride). The fact that the level of this discrimination could be made to vary depending on whether anionic or cationic sites were added to the membrane, lends credence to the conclusion that both hydrogen bonding and coulombic interactions (involving the pyrrolic and pyridine units in CPP) contribute to the recognition process and that the relative importance of these disparate effects varies with the experimental conditions. In contrast to several other ISEs based on other kinds of synthetic receptors, the membranes containing CPP proved capable of discriminating between the three positional isomers of salicylate (*i.e.*, *ortho*-, *meta*-, and *para*-hydroxybenzoate). Given this selectivity and the fact that acetylsalicylic acid could be detected in commercial pharmaceutical products, leads us to suggest that CPP and ISEs derived from it could have a role to play in pharmaceutical analysis, including in high-throughput or sensor array systems, such as those embodied in so-called "electronic tongues".

Acknowledgements

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Full Paper

A Novel Way to Improve Sulfate Recognition

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Abstract
We report a novel way to improve the recognition of highly hydrated sulfate anions. It is shown that polyaniline (PANI) layer deposited on the poly(vinyl chloride) (PVC) membrane favors transport of highly hydrated anions due to stronger PANI hydration in their presence.

Keywords: PVC membrane, Polyaniline hydration, Recognition of sulfate, Membranes, Hydrates

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1. Introduction

Traditionally, ion-selective electrodes (ISE) are composed of a polymeric matrix, usually poly(vinyl chloride) (PVC), plasticizer, ionophore and lipophilic ionic additives [1]. The inert polymeric matrix provides the necessary mechanical stability. Despite that there have been attempts to replace PVC with other polymers, such as silicone rubber [2–4], polyurethane [5–7], Teflon [8], PVC still remains the most commonly used membrane matrix. The quantity of plasticizer present in the membrane affects the physical properties of the polymeric membrane and ensures mobility of its constituents [9]. The lipophilic ionophore (neutral or charged) governs the selectivity of the potentiometric sensor. The lipophilic additives (e.g., alkylammonium salts for anion sensing or tetraphenylborates for cation sensing) lower the plasticized PVC membrane bulk resistance, ensure permselectivity, and optimize the selectivity of the membrane.

It is especially challenging to achieve selectivity for the strongly hydrophilic sulfate and phosphate anions [10–13]. The interface transfer of small ions and multiply charged ions is less favorable than transfer of larger and singly charged ions [10]. Our previous study showed an enhancement of the potentiometric response of the PVC membrane based on tridodecylmethylammonium chloride (TDDMACl) to strongly hydrated anions after coating with polyaniline (PANI) [14–15].

In present study we explain the anion transport through the PANI on its hydration which is influenced by the structure of the anions present in the surrounding aqueous medium. The better hydrated anions possessing stronger water-structure-perturbing capacity can provide more efficient hydration of the amine nitrogens of the PANI chains and, as consequence, can improve the water permeability through the PANI layer.

2. Experimental

2.1. Materials

Poly(vinyl chloride) (PVC) of high molecular weight, 2-nitrophenyl octyl ether (NPOE), TDDMACl, tetrahydrofuran (THF; stored over a molecular sieve) were purchased from Fluka (Switzerland). Aniline (>99%) and 2-[4-(2-hydroxyethyl)-1-piperazino]-ethanesulfonic acid (HEPES) were from Sigma-Aldrich (Germany). Acids, sodium hydroxide, and various inorganic salts were of analytical grade from Lachema (Czech Republic).

2.2. Preparation of Membranes

1 mg of TDDMACl, 33 mg of NPOE and 66 mg of PVC were dissolved in 1.0 mL THF at ambient temperature. To prepare 1 mm thick membranes, 1.0 mL of the solution was deposited onto a metallic ring of 16 mm internal diameter resting on a glass plate, and dried in air.

2.3. Preparation of PANI Coated Membranes

The oxidation polymerization of aniline was carried out with 10 mL of aqueous solution of aniline (8×10^{-2} M) and $(NH_4)_2S_2O_8$ (8×10^{-2} M) in 1.5 M HCl at 0°C in the presence of PVC membrane. After polymerization, the PANI-coated membrane was removed, rinsed with 1.5 M HCl aqueous solution, treated ultrasonically for 10 min in 1.5 M HCl aqueous solution and dried at ambient temperature. The PANI films were very stable and they were not removed neither during immersion in aqueous solutions for at least several months nor by rinsing with a jet water. A light absorption at characteristic wavelength ($\lambda = 800$ nm at pH < 4, $\lambda = 680$ nm at pH 7, and $\lambda = 580$ nm at pH > 7) measured for PANI-PVC treated membranes was used for the determination of the PANI quantity adsorbed at PVC surface. Before the potentiometric measurements, the PANI-coated membranes were immersed in 2×10^{-2} M NaOH for 10 min and then washed with distilled water.

2.4. Characterization of Membranes

X-ray diffraction experiments of the PANI layers were carried out at ambient temperature using a Philips X'Pert diffractometer in reflection and transmission modes (CuK$_\alpha$ radiation, $\lambda = 1.542$ Å).

Scanning electron microscopy (SEM) images of the PANI layers were obtained using a FEI Quanta 400 microscope.

2.5. Potentiometric Study of the Membranes

A disk of 10 mm diameter was punched from the prepared membrane, glued to a polymeric ring-support of 8 mm internal diameter with a PVC/THF mixture, and mounted in an electrode body for potentiometric measurements. An aqueous solution of 0.1 M Na_2SO_4 and 1×10^{-6} M NaCl was used as the inner filling of the electrode. All measurements were carried out at ambient temperature with cells of the following type: Ag; AgCl; 3 M KCl || sample solution | membrane | inner filling solution; AgCl; Ag. The electromotive force (ΔE) was measured with digital ion-meter PHI 04MG (Labio, Czech Republic). The electrodes were immersed in 50 mL of distilled water and calibrated by the subsequent additions of 2 µL, 10 µL, 100 µL, 1 mL and 10 mL of 0.1 M solution of sodium sulfate. Potentiometric selectivity coefficients $\left(k^{\mathrm{MPM}}_{\mathrm{SO_4^{2-}},J}\right)$ were determined by the matched potential method in solutions buffered with 0.1 M HEPES [13]. A 1×10^{-3} M solution of the primary ion (SO_4^{2-}) was used as a background.

2.6. Study of Anionic Transport through Membranes

The tested membrane was fixed into a cylindrical Teflon tube. The individual tested membrane separated acceptor (2 mL) and donor (2 mL) aqueous phases. The donor solution consisted of 0.1 M sodium salts of chloride, sulfate and nitrate. Distilled water served as the acceptor solution. The acceptor phase was vigorously stirred with a magnetic stirrer. The amount of Cl^-, SO_4^{2-}, NO_3^- anions diffused through the membrane to the acceptor solution was determined with capillary electrophoresis (CE) (Beckman Coulter, UK). The samples were introduced into the fused silica capillary (the internal diameter 75 µm, the total length of 37 cm and an effective length of 30 cm; Composite Metal Services, USA) by hydrodynamic injection for 30 s. The run voltage for all experiments was kept constant at 10 kV and the temperature was set at 25 °C. The background electrolyte consisted of a mixture of 5×10^{-3} M K_2CrO_4 and 5×10^{-4} M tetradecyltrimethylammonium bromide. The anions were monitored with a diode array detector by indirect technique at 254 nm. The preconditioning of a new capillary involved rinsing in 5 min periods with the following sequence of solutions: 0.1 M HCl, deionized water, 0.1 M NaOH, deionized water. Rinsing of the capillary with the background electrolyte for 2 min preceded each measurement.

3. Results and Discussion

According to SEM analysis, the PANI layer coating the PVC membrane consists of particles with diameters of about 30–40 nm (Fig. 1A). EDS analysis of the PANI coated PVC membranes revealed 12–14 wt % of nitrogen in the overlayer. A semi-crystalline structure of the particles was

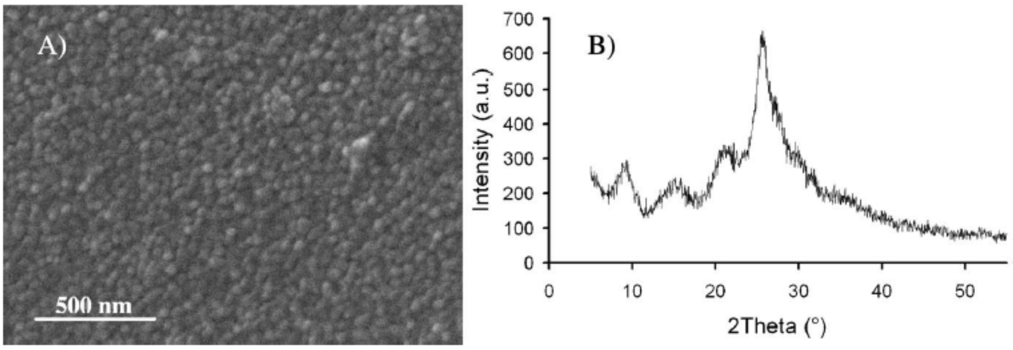

Fig. 1. A) SEM image and B) X-ray diffraction pattern of PANI layer deposited on the surface of the PVC membrane.

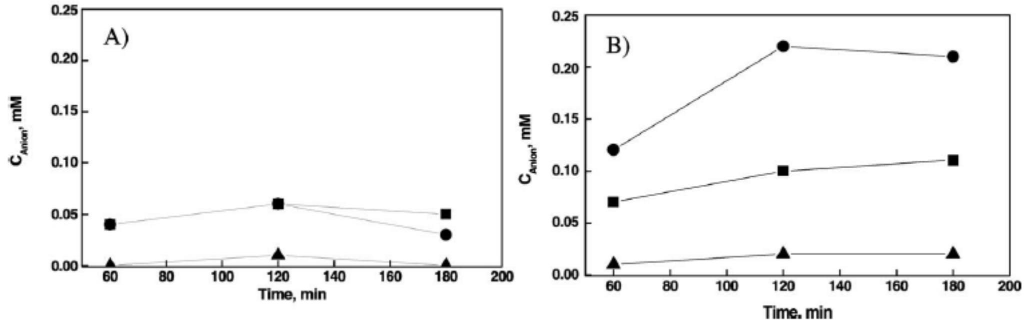

Fig. 2. Time dependence of concentration in the acceptor solution for SO_4^{2-} (full circles), Cl^- (full squares), NO_3^- (full triangle) diffused through uncoated (A) and the PANI-coated (B) membranes.

Table 1. Comparison of the potentiometric selectivity coefficients $\left(k^{MPM}_{SO_4^{2-},J}\right)$ obtained in the present study and known from literature [13].

Interfering anion, J	Experimental membranes [a]		Literature [13]	
	Uncoated	PANI coated	TDDMACl [b]	Sulfate ISE [c]
SCN^-	4.1 ± 0.1	2.3 ± 0.4	5.0	2.9
NO_3^-	3.3 ± 0.2	2.3 ± 0.2	2.9	1.6
Br^-	2.1 ± 0.2	1.5 ± 0.3	2.2	1.1
Cl^-	0.9 ± 0.1	0.3	0.8	−0.1
CH_3COO^-	1.0 ± 0.2	−1.4 ± 0.3	−0.8	−1.5

[a] Measurements were performed with three membranes.
[b] Membrane contained 31.4 wt% PVC, 62.6 wt% NPOE and 6 wt% TDDMACl (without ionophore).
[c] Membrane contained 33 wt% PVC, 66 wt% NPOE, 1 wt% ionophore and 50 mol% TDDMACl relative to bis-thiourea ionophore.

monitored by X-ray analysis (Fig. 1B). The XRD analysis of uncoated plasticized PVC membrane revealed its amorphous structure (not shown here). Thus, we can describe the PANI layer as a densly packed film built up by solid particles. It is reasonable to suppose that the transport of low-molecular species in such solid-like structure can take place only through inter-particle spaces.

The PANI coated membranes showed a stable and reproducible near-Nernstian response −22.4 ± 1.6 mV decade^{-1} to sulfate in a broad concentration range from 4×10^{-6} up to 2×10^{-2} M for 35 days. Importantly, the observed potentiometric selectivity of the PANI-coated membrane was found to be comparable with the sulfate selective electrode reported by Umezawa et al. [13] (Table 1). The high selectivity to sulfate was confirmed by the results from anion diffusion through membranes. In contrast to uncoated membrane, the PANI-coated membrane transfers the hydrophilic anion, SO_4^{2-}, more easily than lipophilic Cl^- and NO_3^- (Fig. 2).

Both potentiometric and transport selectivity cannot be explained by any ion exchange property of PANI. High diffusion of the hydrophilic anions compared to lipophilic ones through the PANI layer we interpret by the strong dependence of the PANI hydration on the nature of the anions present in surrounding aqueous solution. This phenomenon was recently discovered by N. Gospodinova et al. [16].

It is well known that the introduction of different anions and cations in solution can lead to appreciable variation in the water solubility of neutral species [17 – 19]. Though the exact mechanism of this process still remains unclear, the capability of the ions to perturb the intrinsic water structure seems to be responsible for the alteration of hydration even of neutral species. This capability is certainly related to the ions hydration characteristics. For this reason, we assume that better hydrated anions such as sulfate ions should perturb the water structure more efficiently than chloride anions. The weakening of the intermolecular interactions between the water molecules in the presence of highly hydrated anions can provide an efficient hydration of the amine nitrogens on the surface of the PANI particles. Stronger water-PANI interactions seems to be favorable for the transport of hydrophilic solutes through the PANI layer.

4. Conclusions

Favored transport of highly hydrated sulfate ions through the PANI-coated PVC membranes is the intrinsic property of the PANI layer. The increased hydration of PANI in the presence of highly hydrated anions is responsible for their more efficient diffusion. The discovered property of PANI offers a novel way to recognize another hydrophilic species.

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Important aspects influencing stability of the electrochemical potential of conductive polymer-based electrodes

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Abstract In this article, important aspects influencing the signal stability of the potentiometric sensors based on various conductive polymers on platinum support are discussed. The initial step is the choice of a suitable cleaning method (mechanical stripping, burning out) of the platinum support. The second point consists in the selection of an appropriate procedure employed to mature the freshly prepared film on the metallic support (soaking, potential treatment, drying). The third crucial stage involves the application of a proper regeneration procedure (pH value of a buffer, anionic surfactant). The mentioned aspects have been tested using polythiophene, polyaniline, polypyrrole, poly(neutral red), poly(5,15-bis(2-aminophenyl)-2,8,12,18-tetraethyl-3,7,13,17-tetramethyl-porphyrine), poly(5,15,25,35-tetrakis(4-hydroxyphenyl)porphyrine) or poly(2,3-bis-(1H-pyrrol-2-yl)-quinoxalin-5-ol) as sensitive layers. The combination of the prepared electrodes into an electrode array (electronic tongue) and the development of a suitable protocol of the measurement allowed us to distinguish the individual brands of the Czech beers, specifically, Bernard 10°, dark Kozel 10°, Kozel 10°, Bráník 10°, Radegast, and Staropramen. Besides the platinum support, the conductive polymers were also deposited on a nonconductive support (membrane). The latter experiments demonstrated that the introduction of a conductive polymer as the outer layer onto poly(vinyl chloride) membranes led to the significant stabilization of their potentiometric response.

Introduction

A very important general aspect of the electrochemical sensors is their long-term signal stability. Our attention was directed specifically to the stability of potentiometric sensors based on conductive polymers (CPs). The essential step in the development of potentiometric sensors consists in the preparation of a selective and stable electropolymerized film on a suitable support. Most studies have been devoted to the preparation of the CPs and their application as potentiometric sensors [1]. The chemical composition and the surface and bulk properties of the polymer represent the most important factors determining the sensor characteristics. While the selectivity of CPs may be improved by introducing selective receptors into polymer backbone or combining single electrodes in an array, there is no single recipe on how to improve the stability of CPs [1].

The CPs can be prepared either chemically (with the participation of an oxidant) or electrochemically under potentiostatic [2, 3], galvanostatic [4], or cyclic voltammetry conditions [5, 6]. In the course of the polymerization, the film is held in one specific redox state (for potentiostatic polymerization [3]); its redox state is continuously changed according to the potential applied by the galvanostat to maintain a constant current (galvanostatic polymerization [4]); or the redox state is switched from one to the other form by cyclic voltammetry [5, 6]. For all of these three approaches it is common that the redox state is determined by external factors. After the disconnection of the electrode from the electrical circuit, the film begins to drift toward a state that is in equilibrium with its open-circuit potential. During the redox process, the film changes its volume and absorbs or expels small molecules from its bulk. Most CP films have a porous structure [7–9] and contain counter ions [10], molecules of solvents and

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monomers [11], specific molecules [12], and short chains (oligomers) [13]. These can be exchanged by other ions or molecules from the solution, a monomer, or an oligomer unit can attach to the film, etc. Final rearrangement of the polymer structure takes some time, the length of which depends on the kind of a polymer, polymerization conditions, and other factors like pH, solution redox activity, and temperature. Therefore, it is always very useful to know the relaxation period required for the CP reorganization and the factors affecting this process.

Polyaniline (PANI), poly(pyrrole) (PPy), poly(thiophene) (PT), and their derivatives are the most frequently used polymers in the development of potentiometric sensors based on CPs [14]. In these sensors, the CPs are either selective membranes or ion-to-electron transducers placed between a solid-state electrode support and a typical ion-selective membrane. Stability of CPs has been mostly discussed in the literature for the potentiometric sensors applying CPs in the form of ion–electron transducer. Bobacka et al. [15] have found that a sufficiently high bulk (redox) capacitance of CPs (e.g., poly(3,4-ethylenedioxythiophene, PEDOT) used as the solid contact is a prerequisite for obtaining a stable electrode potential of the all-solid-state ion-selective electrodes (ISEs). In these cases, the redox reaction occurring in CP layer facilitates the transduction of an ionic signal into an electronic one. Moreover, the stability of the PANI solid contact in K^+ –ISEs based on plasticized PVC depends on partial conversion of conducting emeraldine salt form to nonconducting emeraldine base form [16]. UV–visible spectroscopic studies show that this conversion takes place during long-term (1–3 months) but not short-term measurements (4 days).

Lewenstam's group has presented results on the stability of the potentiometric signal of electrodes based on the PPy and PEDOT doped with adenosine triphosphate (ATP) and heparin [17, 18]. There has been a correlation between the film topology and the potentiometric response of PEDOT doped with ATP, which was sensitive to Ca^{2+} and Mg^{2+} [17]. In general, smoother films have shown a more stable potentiometric response than the rougher ones. The response remained unchanged even after one year of soaking indicating the exceptionally high stability of this type of electrodes [18]. The influence of various chemical processes on properties, structure, composition, and possible degradation of PPy (often used as potentiometric sensors) has been discussed in detail in an excellent article of Maksymiuk [19]. Such chemical processes can be based on participation of typical components of surrounding media (i.e., oxygen, acids, bases, redox reactants, water, and organic vapors) or on the incorporation of nonreactive ions and surfactants from solutions. The benefits and disadvantages of such processes for analytic characteristic of PPy-based electrochemical sensors have been evaluated.

Various analytic applications of CPs have been developed since the 1980s, and they have been discussed in several reviews over the past few years [20–22]. These include the application of CPs in biomedical engineering [21], in chemical sensors, and arrays [14, 20, 22], and they reflect the wide interest in this subject within the scientific community.

The aim of this study is to summarize the experience acquired in an effort to suppress the signal drift of potentiometric sensors based on CPs prepared in our laboratory. The results refer to the stand-alone coated-wire electrodes, their arrays, and the conventional PVC electrodes coated with a conductive polymer. We believe that the approaches discussed in this study can contribute to the widespread application of the potentiometric sensors in the analysis of real samples.

Materials and methods

Reagents

3-Amino-7-dimethylamino-2-methylphenazine (neutral red, NR), sodium dodecylsulfate (SDS), all inorganic salts, and acids were obtained from Lachema, Penta, and Lach-Ner (Czech Republic). 2-(N-Morpholino)ethanesulfonic acid hydrate (MES), tris(hydroxymethyl)aminomethane (TRIS), acetonitrile, tetrabutylammonium perchlorate (TBAP), aniline.H_2SO_4, pyrrole, and thiophene were obtained from Sigma–Aldrich (Germany). 5,15-bis(2-aminophenyl)-2,8,12,18-tetraethyl-3,7,13,17-tetramethyl-porphyrine (TTP), 5,15,25,35-tetrakis(4-hydroxyphenyl)porphyrine (THP), and (2,3-bis-(1H-pyrrol-2-yl)-quinoxalin-5-ol (BPQ) were prepared according to the general procedure described elsewhere [23, 24]. Poly(vinyl chloride) high molecular weight (PVC), 2-nitrophenyl octyl ether (NPOE), tridodecylmethylammonium chloride (TDDMACl), tetrahydrofuran (THF) (stored over a molecular sieve), persulfate ammonium, and aniline hydrochloride were purchased from Fluka (Switzerland).

Working solutions for potentiometric measurements were prepared using redistilled water. The Czech beers (Bernard 10°, dark Kozel 10°, Kozel 10°, Bráník 10°, Radegast, Staropramen) originated from local stores, all samples being taken from the same manufacturing batch.

Preparation and stability evaluation of conductive polymers on various supports

Metallic support

Platinum electrodes, used as metallic support, were designed either as wires (0.4 mm diameter, 8 mm length) or a set of rings (1 mm diameter). The electrodes were polished with alumina (0.3 µm) or burned out in an oxidative or reductive part of the flame. The stability of the potential was verified by measuring the potential in 0.05 M MES solution with pH 5.5. The potentiometric signal of the bare platinum surface was examined by measuring the pH and chloride dependences for the individual platinum electrodes (number of electrodes $n = 4$).

Polymer-coated platinum electrodes were prepared by polymerization of the corresponding monomers onto the platinum electrode using cyclic voltametry. BPQ was specifically reduced before electropolymerization to the corresponding 5-aminoquinoxaline derivative [25]. Before polymerization, the working electrodes were polished with alumina powder slurry and then rinsed with distilled water. The polymerization conditions used are summarized in Table 1. The prepared electrodes were stored in distilled water. The signal stability was tested both independently with single electrodes and simultaneously within a group of electrodes combined into an electrode array.

Postpolymerization treatment of the polymer film based on poly(neutral red) (PNR) was carried out one day after deposition. According to the postpolymerization treatment, these electrodes were divided into three groups. The first group of electrodes was held at constant potential of 150 mV vs. Ag/AgCl reference electrode for 2 h in the stirred background electrolyte (0.02 M TRIS + 0.1 M KNO_3 + 0.001 M sodium citrate). The second group of electrodes was dried for 2 h at 60°C. The third group of electrodes was left without any treatment with the goal to compare the effect of the individual postpolymerization treatment procedures. Then, the stability of their potentiometric signal was measured in background electrolyte.

The group of ring electrodes ($n = 16$) was combined into an electrode array and then used to test the signal stability of the CPs during the measurement of real samples (various beer samples).

PVC support

PVC/NPOE (2:1 mass ratio) membranes with 3 wt. % TDDMACl were cast using a conventional method for the ion-selective electrode membrane preparation [26]. The experimental membranes were immersed in a freshly prepared solution consisting of aniline hydrochloride (0.08 M) and persulfate ammonium (0.08 M) in 1.5 M HCl at 0°C [27]. After polymerization, the polymer coated membranes ($m = 0.1$ g, diameter 1.28 cm, thickness 0.11 mm) were removed, rinsed with 1.5 M HCl aqueous solution, treated in an ultrasonic bath for 10 min in 1.5 M HCl aqueous solution, and dried at ambient temperature.

Apparatus and measurement conditions

Single electrodes based on conductive polymers

Cyclic voltammetry was carried out using the PAR263 potentiostat/galvanostat (Princeton Applied Research, USA) controlled with GPIB commands using LabView. An analog PA2 polarographic analyzer (Laboratory Devices Prague, Czech Republic) was used for constant potential treatment. An Ag/AgCl (3 M KCl) and platinum plate electrode (area ca 0.25 cm^2) were used as a reference and auxiliary electrode, respectively. The pH was monitored using a type SEUJ 212 glass electrode (Monokrystaly Turnov, Czech Republic) with a PHI 04 MG pH-Meter (Labio, Czech Republic). The potential dependence of the platinum electrodes ($n = 4$) on pH was tested in Britton-Robinson buffer over a pH range from 2 to 12.

All potentiometric measurements were carried out at 25°C with the cells of the following type:

Table 1 Conditions of polymer preparation on metallic support

Composition of polymerization bath				Polymerization parameters		Application
Monomer	Monomer concentration (M)	Solvent	Supporting electrolyte	Potential limits (V) vs Ag/AgCl	Time (min)	
Aniline H_2SO_4	0.05	1 M H_2SO_4	1 M H_2SO_4, 60 µM PVP	0.00–1.20	15	s, a
Thiophene	0.4	Acetonitrile	50 mM TBAP	−0.20–1.90	4	a
Pyrrole	0.2	Acetonitrile	50 mM TBAP	−0.30–1.25	5	s, a
Neutral red [21]	0.005	Acetonitrile	50 mM TBAP	−0.20–1.80	20	s, a
Neutral red [21]	0.005	3 M H_2SO_4	3 M H_2SO_4	−0.05–1.25	20	s
THP	0.0025	Acetonitrile	50 mM TBAP	−0.20–1.30	15	a
TTP	0.005	3 M H_2SO_4	3 M H_2SO_4	−0.05–1.50	30	a
BPQ [19]	0.005	Glacial acetic acid	1 M H_2SO_4	−0.30–1.25	30	a

Electrodes used as a part of an electrode array (*a*) or as a single electrode (*s*)

Ag|AgCl|3MKCl|sample solution|membrane|inner filling solution|AgCl|Ag

and

Ag|AgCl|3M KCl|sample solution|conductive polymer|Pt

The electrochemical potentials were measured using a custom-made five- and seven-channel ISE tester for membrane and coated wire electrodes (Academy of Sciences in cooperation with the Institute of Chemical Technology, Czech Republic) [28].

Electrode array based on conductive polymers

The electrode array was composed of 16 ring platinum electrodes (diameter of 1 mm). Two electrodes for each type of polymer, PANI, PPy, PT, PNR, poly(BPQ), poly(THP) and poly(TTP), were prepared (Table 1), and two electrodes were left without a polymer (bare platinum electrodes). All polymerizations were carried out by cyclic voltammetry with a scan rate of 100 mV s^{-1}.

The electrochemical potential of the electrodes intended for the measurement of different beer samples was determined by an Electronic Tongue instrument [29] and evaluated by principal component analysis (PCA; Camo Unscrambler 7.8 and MathWorks Matlab 6.0). The beer samples were percolated with nitrogen for 10 min before the analysis. The testing sequence consisted of alternating sequences of two washes with the same washing solution (0.1 M acetic buffer at pH 5.5 or 0.001 M SDS in 0.1 M acetic buffer at pH 5.5) followed by two measurements of the same beer sample, and this was repeated five times overall. Electrochemical potential was measured at a fixed time 4 min and 40 s from the beginning of the measurement of each sample. The dead time of the cell was 1 min and 30 s.

Leaching studies

Quantitative leaching studies were carried out using membranes based on TDDMACl and coated with the PANI film. The membranes uncoated with the PANI film were used for the comparison purposes. The membranes were exposed to 10 mL of 0.01 M NaCl (soaking solution), and they were vigorously shaken in this solution for several hours. A 20 μl aliquot of soaking solution with the leached TDDMACl was withdrawn and then added to 1 mL of methanol. The obtained methanolic solutions were analyzed by a mass spectrometer (Q-Tof Micro™, Waters, USA). Absolute magnitudes of leached TDDMACl in water phase (ng mL^{-1}) were determined using a calibration curve obtained for a series of TDDMACl standard solutions of different concentrations in methanol.

Results and discussion

Effect of platinum support on stability of potentiometric signal

The stability of the potentiometric signal of the electrodes based on CPs depends on the treatment of the support material as well as the surface properties of the deposited polymer. First, bare platinum electrodes were employed to evaluate the effect of the pretreatment process and the effect of the platinum oxide layer on the stability of the potentiometric signal. Second, the stability of the potentiometric signal of the CP-coated platinum electrodes was evaluated. Two kinds of polymers, the frequently used PPy and PNR, recently introduced by us [30], were used in these experiments.

Platinum used as the electrode material supports the electron transfer across the platinum/solution phase boundary and accelerates polymerization because of the catalytic effect of the platinum oxide layer. The thickness and structure of this oxide layer depends on the surface treatment conditions [31, 32]. The first factor affecting the stability of the signal is the cleanliness of the surface of the

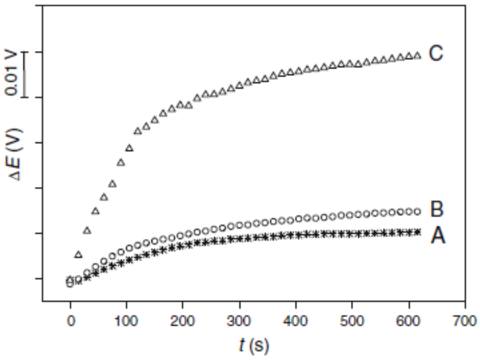

Fig. 1 Stability of the potential of bare platinum electrode measured in 0.05 M MES solution at pH 5.5 after polishing with alumina (*A*), burning out in oxidative (*B*) and reductive (*C*) part of the flame. For more facile comparison, the curves are adjusted to the same initial point

electrode support. The recycling and stripping off the old polymeric film constitute the key procedure. Mechanical abrasion by alumina and burning the polymers from the metal surfaces in a flame belong to the well-established techniques. Cleaning by abrasion using alumina is the most convenient method because the resulting electrodes produce the lowest signal drift (Fig. 1—curve A). The surface layer of the platinum oxides [32] is removed by polishing and then the signal drifts until a new layer is established. The stability of the signal is significantly different for burning in the oxidative (Fig. 1—curve B) or reductive (Fig. 1—curve C) part of the flame. The platinum should not be exposed to the reductive flame because this leads to the formation of non-stoichiometric platinum carbides [33] and, as a consequence, to a high drift of the electrode potential.

A second key factor, which affects the potentiometric response of CP-coated-wire ISEs, is the compactness of the coverage of the platinum support by the polymeric film. In the case of compact polymeric coating, the response of the electrode should be determined by the polymeric film itself, and the influence of the platinum support should be marginal [34]. The platinum itself is sensitive to pH [31, 32], the presence of redox compounds [35], and many various ions, especially halogenides [36]. Therefore, the platinum support often significantly affects the potentiometric response obtained with electrodes based on CPs in cases where the coating is insufficiently compact. On the other hand, the compact PPy coating remarkably improves the reproducibility of the potentiometric signal toward pH (Fig. 2a) and chlorides (Fig. 2b). In the case of the bare Pt electrodes, a significant variability of the individual potentiometric calibration points demonstrates the incomplete coverage of the platinum with an oxide layer (with an ill-defined composition [31, 32]). The potential variability was about 80 mV for pH (Fig. 2a) and 40 mV for the chloride response (Fig. 2b). The variance of the potential responses of bare platinum electrodes toward pH was nearly four times higher than that for PPy electrodes. PPy electrodes show a half of the variance of bare platinum electrodes for chlorides. The presumption that a suitable coverage is vital for the correct and stabile electrode function was independently verified using electrodes based on PNR. The polymerization of PNR runs better in organic media leading to the formation of highly compact films [30]. On the other hand, the polymerization of the same compound from aqueous solution resulted in an incompact hole-containing film. Electrodes made in organic media provide a lower variability for all calibration points (20 mV) in comparison to the electrodes made in aqueous media (about 40 mV) (Fig. 2c). Thus, the complete coverage of the support metal improves significantly the reproducibility of the obtained potentiometric signal. From the mentioned experiments, it is evident that the negative effects of the support material may be avoided or at least significantly restricted forming the more compact and adherent polymeric film on the surface of the support.

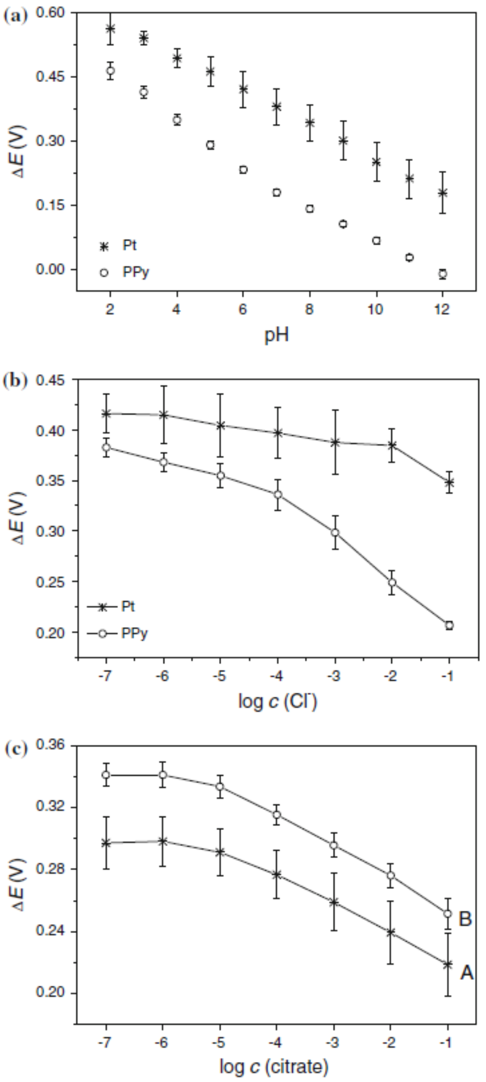

Fig. 2 Dependence of potentiometric responses of bare (Pt) and polypyrrole (PPy)-coated platinum electrodes on pH (**a**) and chloride concentration (**b**). Dependence of potentiometric responses of poly(neutral red) electrode with inhomogeneous (*curve A*) and homogeneous polymer film (*curve B*) on citrate concentration (**c**); standard deviation expressed as error bars, $n = 4$

Stabilization of potentiometric signal of single electrodes

In order to stabilize the potentiometric signal and to reduce the signal drift, the electrodes based on CPs traditionally undergo the postpolymerization treatment before their subsequent application [4]. According to the applied postpolymerization treatment, the experimental electrodes based on PNR were assigned into three groups. The first group of freshly prepared electrodes was left in distilled water, and its potential was recorded for about 50 days in 0.001 M citrate solution with TRIS and KNO_3 at pH 8.5 [37]. The soaking of the coated-wire electrodes in a suitable background electrolyte is one of the frequent procedures of the electrode treatment. For example, electrodes based on the PPy [2] and poly(1-aminoanthracene) [38] films were conditioned in phosphate buffer, and PT electrodes [39] were macerated in 0.1 M KCl. For PNR based electrodes, the initial change in potential was in the range of tens of milivolts (Fig. 3a—curve A). The drift of the signal significantly decreased after eight days, but the potential was not perfectly constant, and the remaining drift must be taken into account. This remaining minor drift could be eliminated by a frequent calibration preferably immediately before the measurement.

The second group of PNR electrodes was treated by maturation at constant potential at background electrolyte (see "Materials and Method" section) (Fig. 3a—curve B). In contrary to the treatment based on sole soaking of the electrodes, the initial change in the potential of the matured film was in the range of few milivolts and, in addition to that, it remained nearly constant throughout the long-term period. The conditioning time was a half of that required for the treatment in the absence of the applied potential.

The third group of the tested electrodes was measured immediately after their drying. A lower drift of the potential was achieved at the beginning of the measurement (during 1–3 days; Fig. 3a—curve C). However, the film gradually absorbed water and swelled. Swelling changed the properties of the polymeric layer, and this was reflected in the potential change and, also in the decrease of the adhesion of the film to the metal surface.

It is important to note that the postpolymerization treatment applied for the PNR electrodes had a similar effect on their potentiometric behavior as it was observed for the classical PANI electrodes (Fig. 3b). The best signal stability was obtained for the electrodes treated with a constant potential, using the same protocol as for PNR. Their potential was stable for about six weeks since their preparation. This demonstrates that the treatment of the polymeric films at a constant potential can be successfully applied also for other polymeric films to stabilize the potentiometric signal of the prepared electrodes.

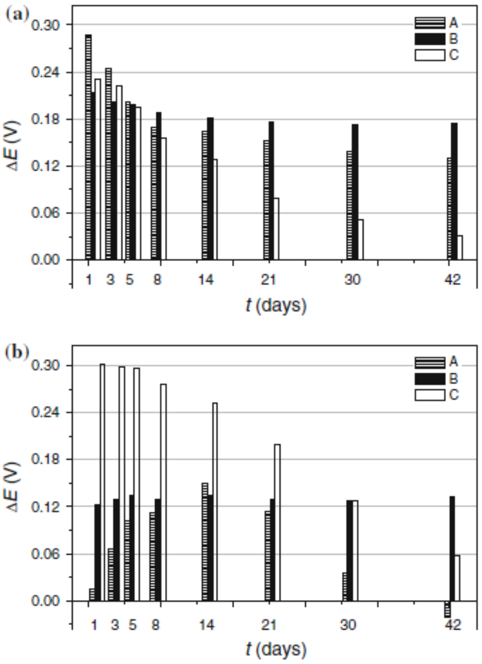

Fig. 3 Influence of postpolymerization treatment of electrodes based on poly(neutral red) (PNR) (**a**) and polyaniline (PANI) (**b**) on their long-term stability in 0.001 M sodium citrate dissolved in 0.02 M TRIS with 0.1 M KNO_3 at pH 8.5: *(A)* no treatment, *(B)* at constant potential vs Ag/AgCl, *(C)* dried at 60°C for 2 h ($n = 3$)

Stabilization of potentiometric signal of electrode array

The development of an electrode array—electronic tongue—[29] is of special interest in many prosperous and practically oriented fields of industry including, for instance, the brewing industry as the well-designed electrode array can provide quick and reliable data on the quality and authenticity of beer samples. As it can be seen in Fig. 4, the assembled electrode array is capable to distinguish the individual brands of the Czech beers.

The recognition pattern obtained with the electrode array was shifted slightly after three days (data not shown). This finding demonstrates the need for the regular recalibration of the array. Taking into account the chemical composition of beer, it is clear that it represents a very complex sample for the analysis. It consists of many various low- and high-molecular weight compounds (e.g., minerals, proteins, sugars) [40, 41]. These compounds gradually contaminate/block the CP surface of the electrodes. Therefore, a suitable washing procedure constitutes a substantial part of the measuring cycle of the beer

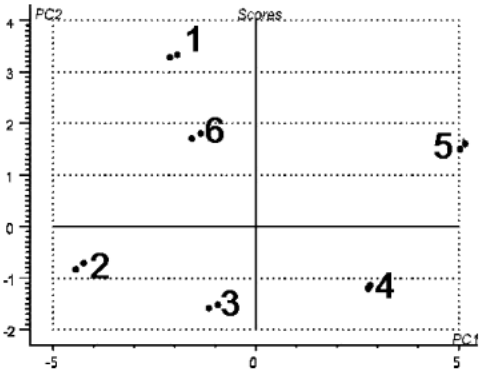

Fig. 4 PCA score plot showing the ability of the electrode array to distinguish the Czech beers of different brands. The recognition pattern was measured with the following beer samples: *1* Bernard 10°, *2* dark Kozel 10°, *3* Kozel 10°, *4* Bráník 10°, *5* Radegast, *6* Staropramen ($n = 5$). These measurements were carried out in thermostated cell at 25°C. The tested electrodes were washed with distilled water between measurements

samples. To eliminate the undesirable effect of the dissolved gases, especially carbon dioxide and oxygen, the beer samples are bubbled with nitrogen before each individual measurement. In addition to the possible poisoning of the electrodes by the components of the complex matrix of the sample, a variability of pH values of the measured samples might adversely influence the response of the electrodes and thus, negatively affect reliability of the measurement. Thus, we proposed two potential washing/regenerating solutions, (i) a plain acetate buffer, pH 5.5, and (ii) the same buffer with the addition of an anionic surfactant, SDS.

In the first step the sole acetate buffer with pH 5.5 as washing solution was tested. In this case, the stabilization of the potential of the electrode array was relatively fast (~20 min) in the plain buffer solution. However, in real beer samples the stabilization took about 2 h. Thus, our results showed that a simple pH control of the washing solution is insufficiently effective.

In the second step with SDS added into the acetate buffer, the stabilization time decreased very significantly to about 20 min even in the real beer samples. It is important that the addition of the anionic surfactant to the acetate buffer did not change the recognition pattern obtained by the electrode array for beer samples. We assume that the presence of SDS in the washing solution effectively suppresses the sorption of polysaccharides and other potential contaminants onto the electrodes' surface. Thus, the simultaneous presence of the acetate buffer (pH 5.5) and the anionic surfactant in the washing solution stabilized the electrode array calibration and extended its applicability from three days to two weeks.

Stability of potentiometric signal of PANI-coated membrane ion-selective electrodes

The use of CPs as the active/contact layer of coated-wire ion selective electrodes is not the only application in potentiometric sensors [1]. CPs, such as PANI, can be deposited also on the surface of a PVC membrane by chemical polymerization. In this case, the PANI layer influences the ion exchange processes occurring at the membrane–solution phase boundary [27, 42, 43]. The polycationic layer of the PANI partially plays the role of a lipophilic additive in the membrane based on a neutral carrier (calix [4]pyrrole). Such phenomenon might result from the synergistic effects of both the polycationic character of the PANI layer on the

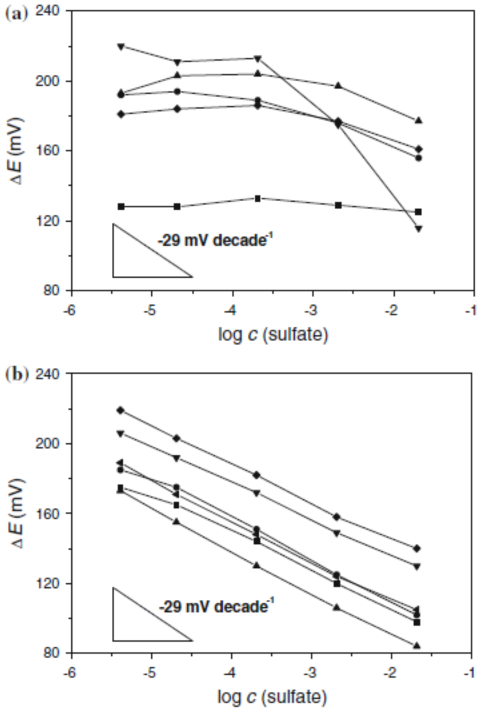

Fig. 5 Potentiometric responses of TDDMACl-based membrane (PVC/NPOE mass ratio 2:1 and 3 wt. % of TDDMACl) uncoated (**a**) and coated (**b**) with the PANI film toward sodium sulfate in time: *filled square* fresh membrane; after *filled circle* 1 day, *filled triangle* 7 days, *filled left pointing triangle* 10 day, *filled inverted triangle* 18 days, *filled diamond* 35 days. Membranes were conditioned in 0.1 M Na_2SO_4 before these measurements were carried out

Table 2 Absolute amount of TDDMACl leached from NPOE/PVC (1:2 m/m) membrane uncoated and coated with the PANI film ($n = 3$) into water phase

Contact time (h)	Amount of TDDMACl leached from membrane (ng mL^{-1})	
	Uncoated membrane	PANI coated membrane
2.5	461 ± 23	89 ± 5
8	563 ± 43	103 ± 6
32	461 ± 27	105 ± 15
96	489 ± 35	96 ± 6
168	372 ± 6	113 ± 16

membrane surface and the bulky cation of the lipophilic additive in the membrane phase [42].

The NPOE/PVC (1:2 m/m) membrane based on TDDMACl shows a fast and near-Nernstian potentiometric response toward anions after being coated with the PANI film [27, 43]. The long-term stability of PANI-coated membranes based on TDDMACl toward hydrophilic sulfate anions was proved and successfully verified by the repeated calibration measurements (Fig. 5). As it can be seen from the comparison of Fig. 5a, b, the membranes coated with the PANI layer provided a stable and reproducible potentiometric response toward sulfate for 35 days. In addition, the PANI layer onto the TDDMACl-based membrane is capable to prevent the leaching of the active anion-exchanger from the membrane (Table 2). Thus, a suitable coating procedure represents one of the possible methods to stabilize the potentiometric signal of the ion-selective electrodes. In addition, this approach does not require the covalent attachment of an active component to the membrane matrix.

Conclusions

We discussed and proposed several procedures enabling to reach a substantial suppression of signal drift in potentiometric sensors with conductive polymers. Our experimental findings indicate that a suitable pretreatment of the support material followed by a formation of the compact polymeric film possess prerequisites necessary to obtain electrodes with the stable electrochemical potential. Moreover, we also demonstrated that postpolymerization treatment of the prepared electrodes can further significantly improve the stability of the prepared electrodes. The discussed factors are of crucial significance, and they must be always considered when CP-based ion-selective electrodes are designed and prepared.

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ORIGINAL PAPER

Terahertz split-ring metamaterials as transducers for chemical sensors based on conducting polymers: a feasibility study with sensing of acidic and basic gases using polyaniline chemosensitive layer

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Abstract We report on the first application of terahertz metamaterials acting as transducers for chemical sensors based on conducting polymers. In our feasibility study aimed at sensing of gaseous hydrochloric and ammonia, a two-dimensional sensor metamaterial consisting of an array of split-ring resonators on the surface of undoped silicon wafer was prepared. The surface of the resonator was coated with a 150-µm layer of polyaniline. Binding of hydrogen chloride to polyaniline leads to distinct changes in the resonance frequency of the metamaterial. Measurements can be performed both in the reflection and transmission mode. A numerical simulation of the response revealed an increase of both the real and the imaginary components of the dielectric function of the polyaniline film. These changes are attributed to the transition from emaraldine base to emeraldine salt. The results demonstrate a new approach for formation of highly sensitive transducers for chemical sensors.

Keywords Metamaterial · Split-ring resonator · Chemical sensor · Transducer · Conducting polymer · Dielectric permittivity

Introduction

During the last 20 years conducting polymers were established as a convenient and versatile material for sensing of various analytes like pH, HCl, NO_2, O_3, SO_2, inorganic ions or volatile organic compounds, for reviews see e.g. [1–6]. Such polymers have some intrinsic receptor properties toward different analytes, like for example redox active or acidic/basic compounds. Oxidation/reduction and for some polymers also protonation/deprotonation remove charge carriers in the conjugated backbone, whereas conformational changes alter planarity of the conjugated backbone leading to some restriction in the mobility of charge carriers and corresponding decrease of conductivity of this material. Chemical selectivity of conducting polymers can be modified by introducing additional receptor groups, by using molecularly imprinted polymerization of this material [2] or by formation of composites of conducting polymers with different types of nanomaterials [7–10].

A detection of analyte binding to conducting polymers can be performed by different techniques, encompassing various transducing mechanisms and frequency ranges. They comprise quartz microbalance [11], fluorescence measurements [12], UV–vis—spectroscopy [13], surface plasmon resonance [14, 15] and potentiometry [16]. Recently also radio-frequency identification devices (RFID) were successfully applied for this purpose [17]. Obviously, the mostly applied transducing mechanism is based on the measurements of the polymer resistance. A number of different measurement configurations of conductometric chemosensors with conducting polymers were suggested [18]. In the simplest sensors, two interdigitated electrodes are used. A number of more sophisticated measurement configurations, such as four-electrode chemoresistors and electrochemical chemotransistors with 3- or 6-electrode configuration were reported [18, 19].

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Simultaneous application of 2- and 4-electrode configurations provides an internal sensor integrity control [19, 20] while a 6-electrode configuration allows one to get a fast sensor recovery [19]. Changes of physical properties of conducting polymers caused by their oxidation/reduction or protonation/deprotonation can be detected in a wide frequency range, starting from zero frequency (measurements of conductivity changes in DC mode), through Hz-kHz (electrochemical impedance spectroscopy) and MHz- (RFID) ranges up to the near infrared (FTIR or Raman spectroscopy [21]), and visible regime (colorimetric technique [13, 22] or surface plasmon resonance [14, 15]). Terahertz frequency range has not yet been exploited for such applications so far. However, this frequency range provides a unique advantage: it allows using metamaterials working as microscopic antenna for detection of local changes of physical properties of closely placed chemosensitive materials. Large chemosensitivity of polymers at THz/infrared frequencies and availability of straightforward and scalable fabrication methods for metamaterials, make this approach highly attractive.

We report here a new type of transducer for chemical sensors which is based on planar arrays of split-ring resonators (SRRs) embedded in conducting polymers. SRRs belong to the mostly studied types of terahertz metamaterials [23, 24] and are usually investigated in vacuum or in air. We show that due to the strong spatial confinement of the resonator mode, small changes of electrical or optical properties of the material in the gap region (conductivity, dielectric permittivity) lead to large changes of the resonance frequency. These changes of physical properties are caused by adsorption of some compounds. Deposition of receptor layers which provide selective binding of defined analytes makes this effect chemically selective. In the current work polyaniline (PANI) synthesized directly on the surface of SRRs was used as a pH-sensitive layer. The measurements were performed in THz range of frequencies corresponding to the wavelength range in vacuum 100–250 μm. Numerical simulation of the sensor response revealed an increase of both real and imaginary components of the dielectric function of the polyaniline film. The approach can be used in remote sensors based on conducting polymers.

Materials and methods

The SRR array with dimensions indicated in Fig. 1(a) was formed from gold by photolithography on undoped 0.6 mm thick silicon (Si) -wafer. The wafer was coated by a 10 nm titan (Ti) sublayer followed by a 100 nm thick gold (Au) layer. Photolithography was performed to fabricate the SRRs. The shape and dimensions of the resonators are shown in Fig. 1(a). Each resonator consists of an inductive loop and a capacitive gap, which define the *LC* resonance characteristics for light polarized along the *x*-axis [25]. In this case, the incident electric field $\boldsymbol{E}_{\text{in}} \parallel x$ drives a current through the structure which, on resonance, leads to charge accumulation at the capacitive gap and hence strong local near fields, and reduced far-field transmission. For $\boldsymbol{E}_{\text{in}} \parallel y$, the capacity of the gap does not play a role due to symmetry, resulting in a significantly higher resonance frequency since the structure responds mostly like a *dipolar* antenna [26]. The spacing between two resonators was chosen to be 3 μm in order to avoid cross-talk. The macroscopic size of the array of SRR is 10×10 mm^2. The wafer with the resonators was treated by oxygen plasma and coated by PANI. The coating conditions were selected according to the data reviewed in [27]. By that 250 μL of 0.3 M solution of aniline in 0.5 M sulfuric acid and 250 μL of 0.27 M aqueous solution of ammonium peroxydisulfate were cooled to 0 °C, mixed and deposited onto the surface of the cooled wafer. The reaction was performed for 2 h at 0 °C. After polymerization, the formed PANI film was washed with distilled water, treated with 25 % ammonium hydroxide and dried. This leads to the conversion of the PANI film into the low conducting form of emeraldine base. The thickness of the polymer film was measured using a confocal optical profiler Pluneox from Sensofar and a value of 307±33 nm was obtained. All chemicals were obtained from Merck (www.merck.com).

Transmission experiments in the THz range were performed using a standard Fourier Transform Infrared (FTIR) spectrometer Vertex-80 (Bruker, www.bruker.com), covering the frequency range from around 1 to 8 THz. The orientation of the applied electric field in the *x*-*y* plane was manipulated using broadband wire grid polarizers [28, 29]. A vacuum sealed aluminum flow cell with quartz/TPX (4-methyl-penten) windows [30] at both ends was used for transmission experiments. The illuminated area of SRRs was about 1 mm in diameter, therefore the number of excited resonators is estimated to be of the order of 10^5.

Results and discussion

The transmission through the SRR array was measured for both principle polarizations (cf. Fig. 1(b)). For $\boldsymbol{E}_{\text{in}} \parallel x$, the black line in Fig. 1(c) clearly shows a pronounced, narrow transmission minimum at 2.03 THz, which corresponds to the fundamental *LC* resonance. At around 6 THz, a more broadband feature which originates from a higher mode is observed. For $\boldsymbol{E}_{\text{in}} \parallel y$, the *dipolar* resonance leads to a minimum at around 5 THz. In the following, we will focus our discussion on the fundamental *LC* resonance at 2.03 THz which is most suitable to our purpose due to its narrow width and large local field enhancement, as detailed later. The measured spectra are superimposed by high-frequency oscillations caused by multiple interferences in the substrate. The periodicity of these oscillations fits to the thickness of the Si- substrate. The curves

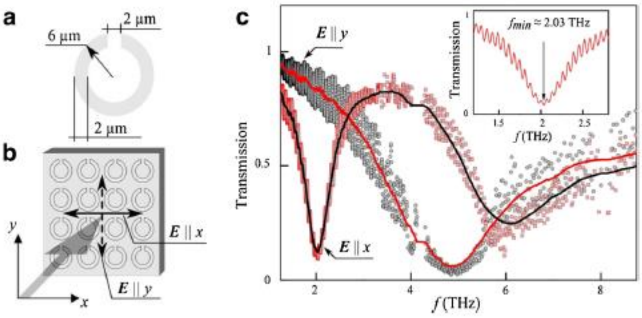

Fig. 1 **a** Design and dimensions of the split-ring resonators. **b** Experimental geometry: SRRs are oriented in the *x*-*y*-plane. The spacing between the centers of two neighboured rings in both *x*- and *y*-direction is 15 µm. *Dashed* and *full lined arrows* indicate orientations of the radiation's polarization in parallel to *x*- and *y*-direction. Transmission was measured for the resonators array illuminated at normal incidence, i.e. for the radiation electric field oriented in plane of the split-ring resonators. **c** Transmission spectra for two perpendicular polarizations: dots and squares are measured results, full lines are smoothed curves; Inset shows *LC* resonance at 2.03 THz

were smoothed by averaging over 100 neighbored points, i.e. over the spectral bandwith of 0.1 THz.

Depending on its oxidation and protonation, polyaniline can have different chemical states [31]. Polyaniline deposited by chemical polymerization has the low-conducting form of emeraldine base [27]. A deposition of this layer on the SRR array does not lead to any visible changes of the transmission spectrum of this array. However, an incubation of the sample with gaseous hydrogen chloride results in well distinguished red shift of the resonance, see Fig. 2(a). While the center frequency of the resonance shifts to around 1.8 THz, the total transmission increases by about 50 %. Subsequent treatment of these probes with ammonia (vapor over a 25 % aqueous solution) results to the blue shift of the adsorption band. The extent of the sensor regeneration depends on the incubation time (left panel in Fig. 3). The same effect is observed after heating of these probes till 150 °C for 15–30 min, see right panel in Fig. 3. An incubation of the sensor in aqueous solutions of different pH (a droplet of unbuffered HCl solutions was deposited on the sensor) leads to the pH-dependent shift of the adsorption spectrum (Fig. 4). This demonstrates that the value of the resonance shift can be used for quantitative measurements. However, a downscaling of the SRR geometry, which shifts the absorption band into near infrared range, would be very favorable for in-line applications of this sensor in aqueous environment.

We calculate the transmission characteristics of our resonators using a finite-difference frequency-domain (FDFD)

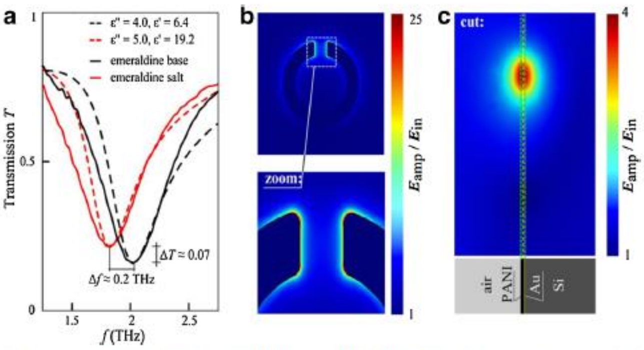

Fig. 2 **a** Black curves—transmission spectra of an array of split-ring resonators coated by polyaniline in the form of *low conducting* emeraldine base. Red curves—transmission spectra measured in the presence of HCl. The spectrum is red-shifted due to the interaction with gaseous hydrogen chloride resulting in conversion of emeraldine base into the *highly conducting* emeraldine salt. Solid lines shows measured spectra, dashed lines correspond to the results of computer simulation. The fitting parameters are ε=6.4+2.0i for the black curve and ε= 19.2+ 5.0i for the red curve showing polymerized structure in contact with HCl. **b**–**c** Color maps of the electric field distribution under resonance condition. Plotted is the ratio between the amplified electric field, $\boldsymbol{E}_{\mathrm{amp}}$, divided by the incoming field, $\boldsymbol{E}_{\mathrm{in}}$. **b** Zoom into the gap region of the SRRs. **c** Cut through the layer profile

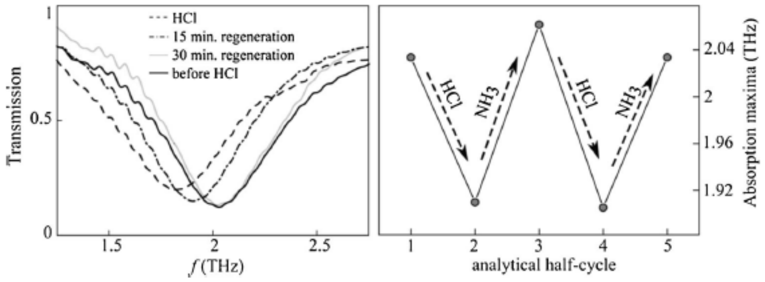

Fig. 3 Sensor regeneration by temperature and by chemical treatment. Left panel shows time-dependent recovery of the samples due to heating: The resonance shifts almost back to its starting position shown by the dashed line. Right panel depicts recovery of the transmission spectrum of the sensor contacted with 240 ppm gaseous hydrogen chloride by treatment with ammonia: The resonance shows well reproducible behavior for several analytical half-cycles

approach. The calculation yields the full near-field distribution of the resonators as a function of their dielectric environment. We use $\varepsilon_{Si}=12.6$ for the silicon substrate. For gold, a range of values for the dielectric permittivity are documented in literature, often found to depend on the crystalline state and the deposition method. However, since $\varepsilon_{Au}>>\varepsilon_{Si}$ at THz frequencies [32], the results depend only very weakly on the precise value of ε_{Au}. Hence, we approximate $\varepsilon_{Au}=\varepsilon'_{Au}+i\,\varepsilon''_{Au}\approx-10^5+10^5i$ [32]. With the given geometry of our structures, we vary the dielectric permittivity of the PANI layer, ε_{PANI}, to match the experimentally obtained transmission spectra, as shown in Fig. 2(a). Note that for the values which the complex dielectric permittivity $\varepsilon_{PANI}=\varepsilon_{PANI}'+i\varepsilon_{PANI}''$ assumes for our experiment, the resonance frequency of the structure is predominantly dependent on ε_{PANI}', while the width of the transmission feature depends mostly on ε_{PANI}''. We obtain $\varepsilon_{PANI,base}=6.4+4i$ for emeraldine base and $\varepsilon_{PANI,salt}=19+5i$ for emeraldine salt. The observed increase of ε_{PANI}', which is caused by the conversion from emeraldine base into emeraldine salt correlates with an increase of the polymer molecular weight and polymer density [27].

In Fig. 2(b, c), the near-field distribution of the THz mode at 2.03 THz is plotted along with the geometry of our structure. Figure 2c shows the large enhancement factors $\boldsymbol{E}_{amp}/\boldsymbol{E}_{in}$ of the near field of the resonator $\boldsymbol{E}_{amp}$ relative to the incident field $\boldsymbol{E}_{amp}$. These strong near fields which prevail in the gap region of the structure demonstrate the high spatial confinement of the resonator mode, yielding a mode volume of only a few μm^3. Note that it is this volume in which the resonance frequency of the structure is most sensitive to changes of the dielectric permittivity. Although the PANI layer thickness of our structure, 300 nm, is only approximately a tenth of the near-field extension in the direction perpendicular to the surface, the well-pronounced shift of the resonance as discussed above was observed. Calculations for a 3 μm thick PANI layer with larger spatial overlap with the resonator mode (not shown) lead us to expect a 5 times larger spectral shift for the same change in dielectric properties. This aspect suggests a straight-forward pathway towards maximizing the signal-to-noise ratio and downscaling the required volume of analyte via tailoring the PANI volume to fit the mode volume of the resonator. Ultimately, this may lead to structures with μm^3-sized microreactor cells residing in the gap of a single resonator, requiring only nanoliters of analyte volume. As an important result, the simulation verifies that the fundamental mode of the SRR is highly spatially confined to the gap region, i.e., to a volume on a scale of only 10^{-3} of the corresponding vacuum wavelength, which leads to the large sensitivity of the resonance frequency of the structure to changes of the dielectric function in its gap region.

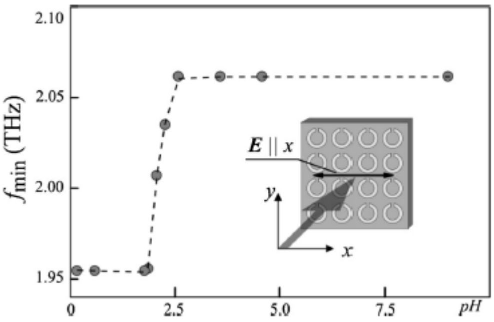

Fig. 4 pH-dependence of the red-shift of the transmission minimum

The obtained values of dielectric permittivity and their changes due to interaction with acidic compounds are very close to that reported for interaction of gaseous hydrogen chloride with poly-N-methyl-aniline at 650 nm: $\varepsilon_{base}=4.34+1.09i$ and $\varepsilon_{salt}=10.1+2.05i$ [14]. A decrease of frequency should lead to some higher values. The value of real part of dielectric permittivity at the used measurement frequency (2 THz) for a composite of polyurethane and undoped polyaniline is ~2.2; a 5 % acidic doping increases this value till ~6 [33]. It is logical to expect that pure polyaniline has a higher value of dielectric permittivity which is further increased with increasing of doping extent. Therefore, the value of dielectric permittivity obtained from computer simulation of experimental data and its change due to interaction with acidic compounds, are close to the available literature data. The strong effect of this change on the resonance frequency of the array of split ring resonators provides a possibility to use this type of metamaterials as a new transducer to detect such interaction.

Chemosensitive properties of polyaniline which we used in this work as chemosensitive material are well known. Its selectivity towards particular analytes can be optimized or adjusted using chemical modification [1–3, 13, 34–38]. Instead of polyaniline other types of chemosensitive materials can be used [1–6].

In this work we have performed spectral measurements which allowed us to make a complete characterization of the transducer and recalculation of changes of optical parameters of chemosensitive layer. For particular applications in chemical sensing, measurements at single wavelength using a laser or other monochromatic light source can be performed. Scaling of metamaterial provides a technological possibility to adjust the adsorption spectrum of the sensor to the irradiation wavelength of suitable lasers. The value of the intensity change in the current work was about 50 % at 2.03 THz for 240 ppm of HCl. Assuming typical values of dynamic range for the measurements of light intensity as ~10^4–10^5, we can estimate the instrumental value of the limit of detection of detection of this sensor as tens of ppb which is about the same as for conductometric sensors. Further improvement of sensitivity can be reached by optimization of the design of metamaterials with the goal to get a narrow resonance peak [39], such works are now in progress.

Conclusion

The lack of simple and inexpensive instrumentation for terahertz range resulted to the ignoring of possibilities of this range in the development of technologies of chemical sensing. The situation was changed during last years—an intensive development of irradiation sources and measurement techniques made this range also perspective for different applications. In the case of analytical applications, this provided a possibility to exploit a novel class of transducers for chemical sensors—metamaterials. Here we have demonstrated a new transducing mechanism based on planar array of SRRs. One can expect that a further implementation of this allows one to combine advantages of optical measurement techniques, such as remote sensing and parallelized reading of sensor arrays, with high sensitivity and dynamic range of electrical measurement techniques.

To summarize, we have demonstrated that split-ring metamaterials based on conducting polymers can be applied as transducer elements in chemical sensing. Since the fundamental mode of the SRR is highly spatially confined to the micron-sized gap region enables local or even spatially resolved studies of chemicals, or, more courageously, downscaling devices to single-resonator structures with fluidic microchannels which may ultimately enable processing nanoliter-scale analyte volumes. While our measurements are carried out on simple spit-ring resonators excited at terahertz frequencies a large variety of easily scalable designs of state-of-the-art metamaterials with lower line widths and mode volumes would allow developing sensors with substantially higher physical sensitivity and a wide range of frequencies.

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