VIP Potentiometric Sensing

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Potentiometric Sensing of Neutral Species Based on a Uniform-Sized Molecularly Imprinted Polymer as a Receptor**

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Polymeric membrane ion-selective electrodes (ISEs) have been routinely used for determination of ionic species in clinical applications and for the determination of water quality owing to their attractive features including excellent selectivity, low cost, ease of use, and high reliability.[1–3] This well-established analytical technology has undergone a quiet revolution over the last few years. The detection limits of ISEs have been lowered from the micromolar to the subnanomolar range, and the discrimination of interfering ions has been improved by many orders of magnitude.[4] Currently, the application of ISEs has evolved to provide a promising measurement technique for environmental trace analysis and potentiometric biosensing. However, it has remained an open challenge for analytical chemists to develop potentiometric sensors for uncharged molecules, since the prerequisite for the general mechanism of potentiometric response is the occurrence of a charge on an analyte. Very few ISEs have been reported for which the membrane potentials are affected by neutral molecules. Electrodes formulated with plasticized poly(vinyl chloride) (PVC) membranes containing tetraphenylborate salts of barium complexes with given polyethoxylates[5a–c] or hydrogen-ion carriers[5d] show cationic responses to acyclic polyether-based nonionic surfactants, which are attributed to the partitioning of the surfactant/metal cation complexes coextracted into the membranes. Anionic responses are induced by undissociated neutral phenols using PVC matrix liquid membranes containing lipophilic nitrogen-containing compounds[6a–b] or metal porphyrins[6c] as sensory elements. The net movement of protons from the membrane interface to the aqueous phase stimulated by uncharged phenols is responsible for the anionic response. Although these approaches have made great contributions toward the potentiometric detection of neutral species, the sensors developed show rather poor selectivities because the potential response is governed mainly by the lipophilicity of the neutral molecules, rather than specific molecular recognition. So far, a potentiometric sensor with a synthetic carrier that selectively binds neutral species is still apparently unknown.[2b]

As highly suitable receptors, molecularly imprinted polymers (MIPs) have emerged as attractive, simple, and seemingly general materials for the selective binding of a wide range of analytes with affinities and selectivities similar to those of antibodies, enzyme, and hormone receptors.[7–10] Compared to their biological counterparts, MIPs are more stable, less costly, and easier to produce. Such materials are synthesized in the presence of functional monomers, template molecules, and a cross-linking agent by covalent,[11] noncovalent,[12] and sacrificial spacer methods.[13] Binding sites with molecular recognition properties are formed after template molecules have been removed from the polymerized material, leaving behind cavities for the subsequent rebinding process that are complementary in size and shape to the template. MIPs have gained wide acceptance as new molecular recognition materials in chemical sensors. Although MIP-based ISEs have been developed for numerous ionic species,[14] none has been reported for uncharged molecules. Herein, we describe a novel strategy for the selective and sensitive detection of neutral species using a polymeric membrane ISE, which is based on a uniform-sized MIP as the sensing element for molecular recognition and a charged compound with a structure similar to that of the analyte as an indicator ion for the transduction of potential signal. It is anticipated that this strategy will lay a foundation for the development of potentiometric sensors for measuring neutral species at trace levels.

Chlorpyrifos (CPF), a representative organophosphorus pesticide, was chosen as the model neutral species; it has been linked to the potential risk of behavioral deficits both in animals and children.[15] According to our strategy, CPF-imprinted polymer beads of regular size and shape were synthesized by precipitation polymerization[16] in the presence of template (CPF), functional monomer methacrylic acid (MAA), and two different cross-linkers, trimethylolpropane trimethacrylate (TRIM) and divinylbenzene (DVB), by radical initiation (Scheme 1a). The nonimprinted polymer (NIP) was synthesized by a similar procedure in the absence of template molecules. These polymers were embedded into the PVC membrane to function as the conventional ionophores of the ISEs. The membranes contained MIP or NIP (7.6 wt%), the anion-exchanger tridodecylmethylammonium

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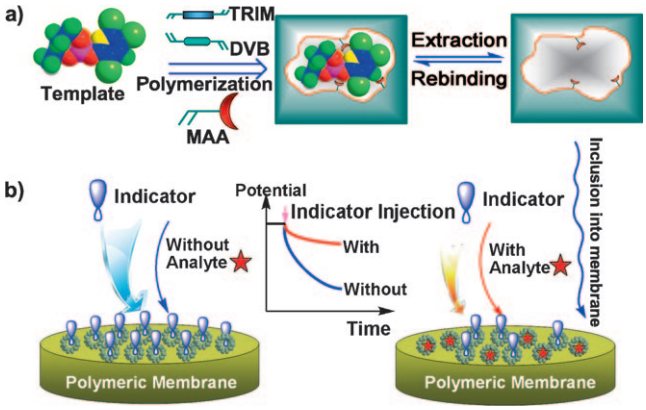
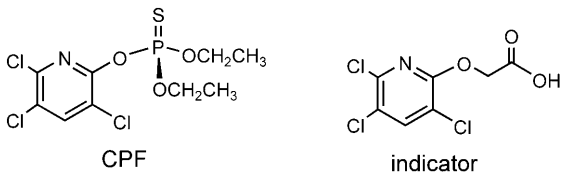


Scheme 1. Representations of a) the synthesis of an MIP and b) the potentiometric detection of neutral species.

chloride (1.5 wt%), *o*-nitrophenyl octyl ether (60.6 wt%), and PVC (30.3 wt%). The membrane ISE was electrically contacted to a potentiometer for electromotive force (EMF) measurements. The internal filling and conditioning mediums of the ISE were 0.05 mol L^{-1} phosphate buffer solution (PBS) of pH 7.4. Further information about the materials and methods is available in the Supporting Information.

The proposed potentiometric detection of neutral species involves two steps: the first is the molecular recognition and simultaneous accumulation of the neutral analyte in the polymeric membrane phase through the selective interaction between the analyte in the sample solution and the MIP in the membrane; the second is the potential measurement of the indicator ion by using the ISE membrane with the accumulated analyte, in which case the membrane is removed from the sample solution and placed in a detection solution containing a fixed amount of indicator ion. When the electrode is in contact with the sample solution, the MIP, which has binding cavities as receptors in the polymeric membrane, selectively extracts the target molecules that are identical to the original template into the organic membrane phase by means of hydrogen bonding and hydrophobic interactions. This extraction process can reduce the number of the available binding sites in the membrane, thus decreasing the subsequent potential response to the indicator ions (Scheme 1b). After preliminary binding assays (Figure S1 in the Supporting Information), 3,5,6-trichloro-2-pyridyloxyacetic acid with a binding capability similar to that of CPF was chosen as the indicator, which has a pK_a of 3.97 and exists as an anion in the detection solution of PBS of pH 7.4. The structures of CPF and its indicator are shown in Scheme 2.



Scheme 2. Chemical structures of a) chlorpyrifos (CPF) and b) its analogue 3,5,6-trichloro-2-pyridyloxyacetic acid (indicator).

For most of the MIP-based chemical sensors, the traditional bulk polymerization method with ethylene glycol dimethacrylate (EGDMA) as the cross-linker has been commonly used.[17] However, this method suffers from problems of lengthy procedures for particle grinding and sieving, low production yields, and irregularity in terms of particle size and shape. In the present work, we employed the synthesis of uniform-sized MIPs which is based on precipitation polymerization[16] using TRIM/DVB and a near-θ solvent mixture (a θ solvent is one that does not affect the polymer conformation) of acetonitrile and toluene as the cross-linkers and porogenic solvent, respectively. Using the proposed method, uniform imprinted beads can be readily obtained after the polymerization, and there is no need for particle grinding.

To evaluate the selective recognition and special binding properties of the MIP obtained by precipitation polymerization, the CPF recognition abilities of the MIP and control polymer were investigated by the classical steady-state binding method (see the Supporting Information). As shown in Figure 1, the imprinted polymer exhibits a much higher

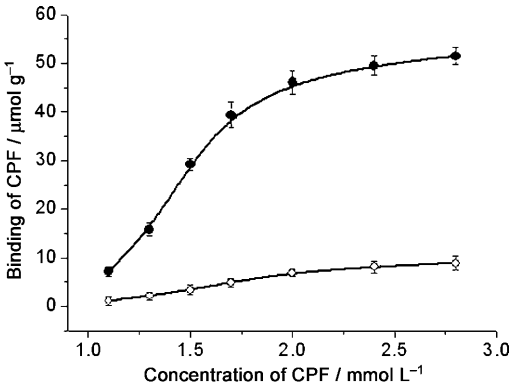


Figure 1. Equilibrium binding isotherm for the uptake of CPF by MIP (●) and NIP (○) in toluene/acetonitrile (1:1 v/v). Each data point represents a mean value ± standard deviation for three measurements.

capacity than the control polymer for CPF. When the CPF concentration is 1.5 mmol L^{-1}, the amount bound by the MIP beads is 29.2 μmol g^{-1} while that bound by the control polymer beads is 3.4 μmol g^{-1}. These results indicate that the functionalities on the CPF molecules are responsible for the imprinting effect of MIP.

The effect of particle regularity on the sensor response was investigated by using the irregular imprinted polymer particles and well-defined polymer beads synthesized with the traditional bulk polymerization and with the precipitation polymerization methods, respectively. As illustrated in Figure 2, the membrane ISE prepared with the uniform MIP beads shows a much shorter response time (ca. 2 min), defined as the time required to achieve 95% of the stable signal,[18] than that obtained from irregular particles (ca. 6 min). Moreover, much lower noise levels were observed for the potential response with uniform polymer beads. These observations might be attributed to the fact that the uniform beads with a diameter of roughly 1 μm (see the image in

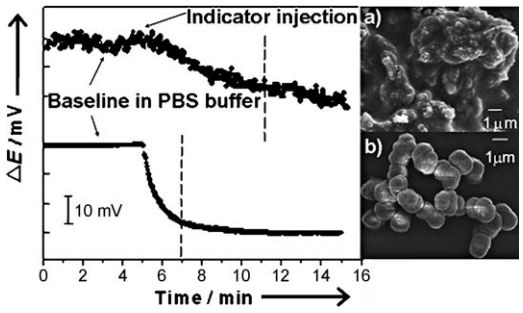


Figure 2. Left: Potential responses to 1.0×10^{-5} mol L^{-1} indicator in 0.05 M phosphate buffer (pH 7.4) using ion-selective electrodes based on a) MIP particles prepared by conventional bulk polymerization and b) uniform-sized MIP beads prepared by precipitation polymerization; the dashed lines indicate the response time. Right: SEM images of both MIPs.

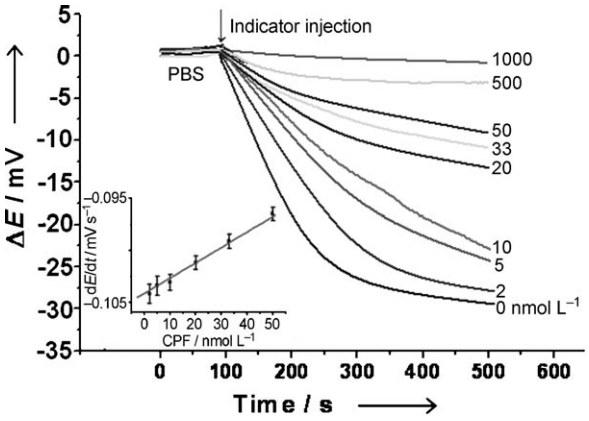


Figure 3. Potentiometric responses to 1.0×10^{-5} mol L^{-1} indicator in 0.05 M phosphate buffer (pH 7.4) after incubation of the proposed sensor with increasing concentrations of CPF from 2–1000 nmol L^{-1}. Inset shows the initial slopes of the EMF changes over the concentration range 2–50 nmol L^{-1}. Data represent an average ± standard deviation for three measurements.

Figure 2) can be well dissolved in the polymeric ISE membrane, thus leading to more available binding sites in the membrane and lower membrane impedance than in the membrane prepared with irregular particles having a broad size distribution of 1–100 μm (see the image in Figure 2).

The molecular recognition and simultaneous preconcentration of CPF can be readily performed by immersing the uniform-sized MIP-based polymeric membrane ISE in the sample solution. However, preliminary experiments showed that the incubation process would take a rather long time (ca. 60 min) especially for the detection of lower amounts of CPF (<0.1 μmol L^{-1}). Since the rotating configuration has been proved to be an effective way to decrease the diffusion layer thickness of the aqueous phase and enhance mass transfer of the analyte to the membrane/sample interface,[19] we attached the ISE membrane to a rotor to improve the detection limit and shorten the incubation time. After incubation in the CPF aqueous solution for 10 minutes and rotation at 3000 rpm, the MIP membrane was washed and transferred to a separate electrochemical cell containing 0.05 M PBS (pH 7.4) for subsequent potentiometric detection. Notably, experiments showed that there was no loss of CPF (i.e. no release from the ISE membrane into solution) during the washing and transferring processes probably because of the high-affinity binding of the imprinted polymer to the CPF molecules. Figure 3 shows the ISE potential responses to 1.0×10^{-5} M indicator after incubation with CPF at different concentrations in the sample solution. As can be seen, the measured potential of the ISE membrane decreases rapidly with the injection of indicator; this is a result of the favorable extraction of the indicator anion through the effective interaction between the indicator and the MIP in the membrane phase.

The selectivity of this MIP-based ISE was characterized by using Bakker's method to evaluate the influence of the discriminated ions[20] (Figure S2 in the Supporting Information). The logarithmic Nikolskii coefficients for the indicator anion ($K^{pot}_{IndicatorJ}$) over Cl^- and HPO_4^{2-} are -3.76 (±0.04) and -5.51 (±0.06), respectively. Indeed, a much larger response to the indicator anion was obtained when phosphate buffer was used as the detection solution rather than Tris-HCl buffer because chloride is less discriminated and causes a higher background. As expected, the potential response to the indicator anion can be largely inhibited by incubation of the MIP-based ISE membrane with CPF in the sample solution, which causes less binding sites to be available in the membrane phase. Detailed analysis of the experimental results reveals that there is a linear dependence of the initial slope of the EMF change, which was evaluated by a numeric fit of initial part of the EMF change (<5 mV) to a first-order polynomial,[21] on the concentration of CPF in the range of 2.0–50 nmol L^{-1} ($\gamma=0.998$) with a detection limit of 0.96 nmol L^{-1} (3σ) (Figure 3). The detection limit is two orders of magnitude lower than those reported by other researchers.[22] Although some lipophilic anions such as thiocyanide could show high response on the ISE membrane, experiments indicate that these anions are not sensitive to the changes in the binding sites of MIPs in the ISE membrane induced by the molecular recognition of analyte, which suggests the specific interaction between the proposed indicator anion and the MIP in the membrane. The influence of different structures in the indicator is shown in Figure S3 (see the Supporting Information). The proposed potentiometric sensor shows an excellent selectivity over other related organophosphate pesticides such as parathion, parathion-methyl, and phoxime (see Scheme S1 in the Supporting Information). Compared with the response to CPF, neglectable changes in the initial rates of potential decrease can be observed for these three pesticides after incubation (Figure 4), which suggests the specific recognition of the target analyte by using the MIP as the receptor. Moreover, control experiments with NIP confirm that the measured potential changes are caused exclusively by the high-affinity binding of the imprinted polymer to the CPF molecules (Figure S4 in the Supporting Information).

In conclusion, we have demonstrated a novel strategy for the potentiometric detection of neutral species using a uniform-sized MIP as a sensing element for molecular recognition and a charged compound with a structure similar to that of the analyte as an indicator ion for transduction of

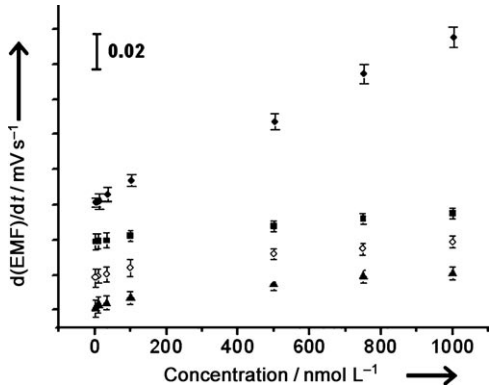


Figure 4. Initial slopes of the EMF changes with respect to the increasing concentrations of CPF (●), parathion (■), parathion-methyl (○), and phoxime (▲) at 2, 10, 33, 100, 500, 750, and 1000 nmol L^{-1}. Each data point represents an average ± standard deviation for three measurements.

potential signal. The proposed polymeric membrane ISE is highly selective and sensitive, and exhibits a lower detection limit of 0.96 nmol L^{-1} for chlorpyrifos. This methodology may pave the way to using ISEs for measuring non-ionic species at trace levels.

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