Sensing of Aqueous Phosphates by Polymers with Dual Modes of Signal Transduction

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Accurate determination of levels of phosphate-related anions such as pyrophosphate and nucleotide-phosphates is an important factor in monitoring numerous metabolic processes.[1] Very few sensors exist that display high selectivity and reliability, and that function in aqueous media without interference from endogenous substrates such as chloride anion. That is because selective and reliable sensing of anions is generally difficult to accomplish. As compared to isoelectric cations, anions often display a high energy of hydration, display tautomerism, and possess low surface-charge density, features that make the binding of anions less effective.[2]

The increase in receptor−anion affinity may be achieved by utilizing positively charged moieties as a part of the receptor. Unfortunately, electrostatic interactions are nondirectional, and, as a result, all anions present in the medium are attracted to such receptor. It is, therefore, desirable to also include weak directional interactions such as hydrogen bonding to improve the selectivity in receptor−anion association. Recently, new receptors that comprise both positive charges and hydrogen bond donors acting in a synergistic manner were prepared.[3] Additionally, conductive polymers were shown to yield sensor materials for cations, biomacromolecules, and neutral analytes.[4] Only a few such materials were used for anion sensing.[5]

We reasoned that a conductive polymer with incorporated receptors capable of hydrogen bonding while being able to undergo an adjustable degree of p-doping could provide an inexpensive alternative to the synthesis of multifeature sensors (Figure 1). We decided to utilize the propensity of polythiophenes[6] to undergo p-doping. The positive charge in the polymer can be adjusted by an externally applied potential. We also used receptors capable of change in color in addition to polymer conductivity, to obtain two independent modes of signal transduction in the sensor material, thus increasing the overall reliability of the sensing process.
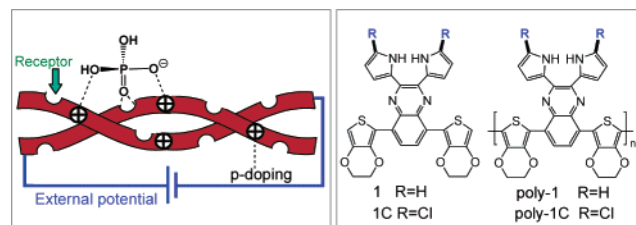

Figure 1. Left: A schematic drawing of synergy in a p-doped conductive polymer with integrated hydrogen-bonding receptors. Right: Structures of monomers **1** and **1C**, and sensor materials poly-**1** and poly-**1C**.

In the design of a sensor material, we used a polythiophene conductive polymer with integrated quinoxaline moieties modified with two pyrrole units. Dipyrrolylquinoxaline (DPQ) is capable of binding anions via hydrogen bonding,[7a] while more or less a pronounced color change occurs.[7a,b] Because DPQ alone does not yield stable conductive polymers, we modified the quinoxaline ring with two polymerizable ethylenedioxythiophene (EDOT)[8] units to obtain monomer **1** (Figure 1, right). To avoid potential polymeri-

zation through α-pyrrole positions, we also synthesized monomer **1C** with chloro-substituents blocking the α-positions.

Careful evaluation of titrations of monomers **1** and **1C** in DMSO with various aqueous anions showed that **1** and even more so **1C** show changes in color upon binding of pyrophosphate^{2-} (PP^{2-}), fluoride, and, to a lesser extent, phosphate (H$_2$P$^-$) and cyanide anions.[9] Analysis of anion-induced changes in the absorption spectra of **1** and **1C** (Figure 2) allowed for determination of the respective binding constants (Table 1).
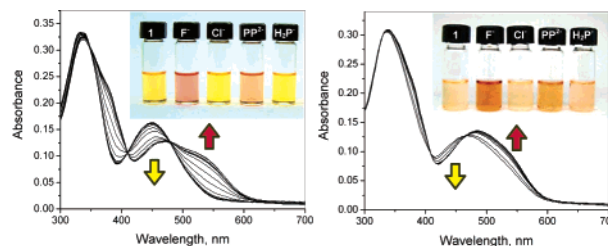

Figure 2. Left: Titration of **1** with pyrophosphate^{2-} (inset: photograph of 50 μM DMSO solutions of **1** treated with anions (50 equiv)). Right: Titration of **1C** with pyrophosphate^{2-} (inset: photograph of 50 μM DMSO solutions of **1C** treated with anions (50 equiv)).

Oxidative electropolymerization of monomers **1** and **1C** yielded conductive materials poly-**1** and poly-**1C**. The band gaps were estimated[6] as 1.39 eV for poly-**1** and 1.36 eV for poly-**1C**.[10]

To test the anion sensing ability of the polymers, the poly-**1** and poly-**1C** films were titrated at a constant potential of 0.0 V by anions added as tetrabutylammonium (TBA) salts (5.0 mM, pH ≈ 6.5) in water while vis−NIR spectra were recorded (Figure 3). As expected, the addition of fluoride, pyrophosphate, and in the case of poly-**1C** also phosphate anions into the cell containing the sensor films resulted in gradual changes in the absorption spectra. The respective binding isotherms that show well-defined saturation were obtained, and apparent affinity constants were calculated (Table 1).[11]

Additionally, we used the electrochemical quartz crystal microbalance method (EQCM)[12] to confirm the anion binding in poly-**1** and poly-**1C**. After the deposition of poly-**1** on the EQCM probe, aqueous solutions of the anions were added. The addition of anions (PP^{2-}, F$^-$, H$_2$P$^-$ but not Cl$^-$) caused a rapid increase of the mass
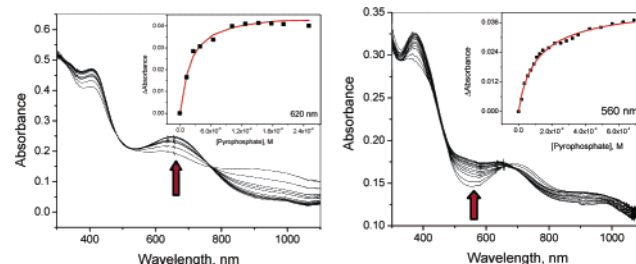

Figure 3. Poly-**1** (left) and poly-**1C** (right) titrated with aqueous pyrophosphate (5 mM). Insets show the respective binding isotherms. Supporting electrolyte: 0.1 M TBAClO$_4$ in DMSO.

Table 1. Affinity Constants[a] for **1** and **1C**, and Apparent Affinity Constants[b] for Poly-**1** and Poly-**1C** (M^{-1}) in DMSO (0.1% H_2O) Derived from Titrations with Aqueous Anions

	sensor			
anion	**1**[a]	poly-**1**[b]	**1C**[a]	poly-**1C**[b]
F^-	$>10^6$	48 000	$>10^6$	24 000
Cl^-	<100	<100	2000	<100
PP^{2-}	$>10^{6c}$	61 100	$>10^6$	11 000
H_2P^-	<100	3800	$>10^6$	90 000

[a] In units of $dm^3\ mol^{-1}$. Fits were performed using 1:1 stoichiometry as determined from Job plots. [b] Apparent affinity constants[11] were calculated with unknown concentration of the receptor in the material surface using the equation for 1:1 stoichiometry. [c] Binding isotherm displays a biphasic (sigmoidal) behavior.

of the deposited polymer. EQCM titration experiments resulted in a typical saturation profile, thus confirming that the changes in vis−NIR spectra are, indeed, a result of anion binding.

To test both our hypotheses that p-doping of the conductive polymers may be used to tune anion-sensor affinity, and that conductive polymers may be used to obtain two independent modes of signal transduction useful in the sensing process, we have constructed sensing devices schematically shown in Figure 4 using an interdigitated ITO electrode (IME).[13] The interdigitation was biased in such a way that the polymer grew from digit A until it reached bare digit B, causing closing of the circuit as observed by the dramatic increase in conductivity. The film thickness estimated by the surface profiler was typically 50−100 nm. The conductivity (poly-**1**, σ_{max}= 54 S/cm; poly-**1C**, σ_{max}= 23 S/cm) plot suggested that the potential for conductivity measurements should be ≥0.70 V (Figure 4).
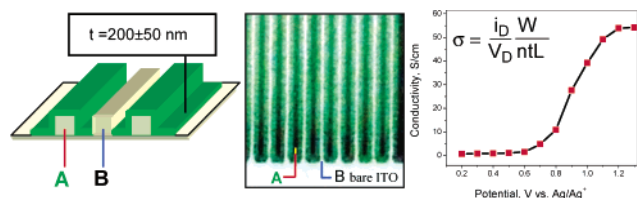

Figure 4. Left: A schematic representation of polymer deposition. Center: Optical micrograph of the interdigitation with poly-**1**. Right: Change in conductivity of the poly-**1** film as a function of a gate potential.

The poly-**1C** IME devices were then tested for anion sensing in custom-made spectroelectrochemical cells connected to a fiber optic spectrometer and bipotentiostat. The bipotentiostat was used to apply voltage on the ITO-interdigitation (gate potential 0.70 V, drain potential V_D = 40 mV) and record the drain current (i_D) passing through polymer-modified interdigitation while the vis−NIR spectra were recorded.[14] As expected, the addition of anions resulted in dramatic changes in both vis−NIR spectra as well as drain current (conductivity) (Figure 5). The low level p-doping (at 0.70 V) and a corresponding positive charge in the polymer resulted in a dramatic increase of the anion affinity. For example, the apparent affinity constant ($dm^3\ mol^{-1}$) for poly-**1**/pyrophosphate recorded at 0.70 V was calculated as $K_{PP}(0.7\ V)$ = 260 000, while the constant recorded at 0.00 V was $K_{PP}(0.0\ V)$ = 61 100. Similarly, for poly-**1C**, the values are $K_{PP}(0.7\ V) > 10^6$ and $K_{PP}(0.0\ V)$ = 11 000.[15] The conductivity measurements[16] allowed for simultaneous observation of changes in drain current as a function of anion concentration, thus providing a second independent signal usable for sensing. Figure 5 shows examples of vis−NIR and conductivity data acquired simultaneously. It is also important that the sensing process is reversible and the deposited materials are reusable.

In summary, we have demonstrated a new approach to sensing of aqueous phosphate-related anions based on chromogenic conduc-
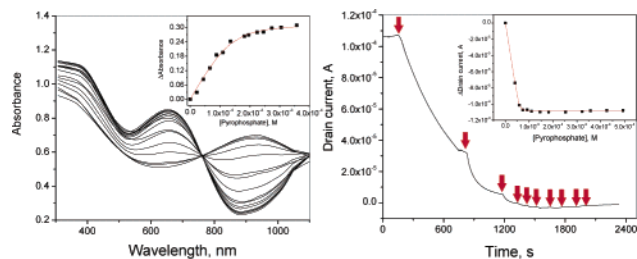

Figure 5. Left: Titration of poly-**1** modified IME with aqueous pyrophosphate at 0.70 V. Right: Simultaneous decrease in conductivity of poly-**1** as a result of pyrophosphate binding. Insets show the respective binding isotherms. Supporting electrolyte: 0.1 M TBAClO$_4$ in DMSO.

tive polymers. This method utilizes synergy between low-level p-doping in a polythiophene polymer and hydrogen bonding to increase anion-sensor affinity. These chromogenic conductive polymers show reversible anion-specific changes both in color and in conductivity upon increasing concentration of anions, thus providing two independent modes of signal transduction. We believe that this concept will yield more reliable anion sensors soon.

Supporting Information Available: Analytical data for **1**, **1C**, poly-**1**, and poly-**1C**, and experimental details for material deposition including anion titrations (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(10) The polymers are green (pale blue) in the reduced (oxidized) form.
(11) Apparent affinity constants were calculated using the equation for 1:1 stoichiometry. The equation uses the concentration of added anion to be a sum of a free anion (unbound) and anion bound by the receptor. The latter is considered equal to the receptor concentration. Because the receptor concentration on the surface of the material is unknown, the affinity constant is designated as "apparent", and the respective values are relative. In fact, judging from the dynamic range of the sensors, the affinity of poly-**1** and poly-**1C** for anions is equal to or higher than the affinity of the monomers **1** and **1C**.
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(13) Abtech Scientific, Inc.; IME characteristics: digit length, 5 mm; number of digit pairs, 50; digit width, 10 μm; interdigit space, 10 μm.
(14) For more details on experimental conditions, see Supporting Information.
(15) Chloride anion, which is not bound by the monomers, is also not bound by poly-**1** and poly-**1C** regardless of the doping level. Both poly-**1** and poly-**1C** display $K_{Cl}(0.7\ V)$ = 1200 $dm^3\ mol^{-1}$ or lower.
(16) PEDOT used as reference material. See also: Kingsborough, R. P.; Swager, T. M. *J. Am. Chem. Soc.* **1999**, *121*, 8825−8834.

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