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Sensing of Lead Ions by a Carboxylate-Substituted PPE: Multivalency Effects

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We report that the structurally simple polymer **4** is a potent sensory platform for lead salts in aqueous solution. Very sensitive fluorescence quenching is observed.

Selective and sensitive determination of heavy metals such as lead and mercury continues to be significant due to their role as environmental pollutants. Lead is found in old water pipes, deteriorating lead-based paints, and occasionally in residential soil from contamination by tetraethyl lead, a now banned antiknock additive to gasoline. If small children are exposed to lead, health effects include anemia and neurological impairment.[1,2] In recent years sophisticated DNA-based approaches for lead sensing have been developed.[3] We have reported that sugar-substituted PPEs are effective in the detection of different metal cations in DMF solution.[4−6] Herein we present PPE **4** as a surprisingly selective and sensitive material to detect lead(II) ions in water.

Schanze reported the synthesis and the amplified fluorescence sensing of a protease by **4** and investigated the photophysics of this fluorescent polyelectrolyte.[7,8] We have independently synthesized **4** via a somewhat different route (Scheme 1): Starting from 2,5-diiodohydroquinone, reaction with 2-bromoethyl acetate in butanone in the presence of potassium carbonate yields the ester **1**. Alkynylation utilizing trimethylsilylacetylene and the $(Ph_3P)_2PdCl_2/CuI$ catalyst system with triethylamine as solvent furnishes **2** after desilylation by tetrabutylammonium fluoride in THF.[4,5] The ester groups are not cleaved under these conditions. Coupling of **1** and **2** utilizing $(Ph_3P)_2PdCl_2/CuI$ in triethylamine at 50 °C for 72 h affords the polymer **3** in 93% yield and with an M_n of 12×10^3 and an M_w/M_n of 4.3 according to gel permeation chromatography (GPC in DMF) with polystyrene standards; **3** is well soluble in polar organic solvents (methanol, DMF, DMSO) and fully characterized. It is insoluble in ethyl ether and hexane, from which it can be precipitated. A solution of **3** in methanol is treated with NaOH to give the deprotected sodium salt **4**. In a similar approach the model compound **5** is synthesized. In Figure 1 the UV−vis and the emission spectrum of **4** is displayed. The polymer shows a strong emission at $\lambda_{max} = 465$ nm, quite typical for a dialkoxy-PPE. The material seems to be unaggregated in solution, probably due to the highly negative charge of **4** which prohibits aggregation in aqueous solution. The quantum yield of **4** in water is 0.08. Addition of transition metal salts to a solution of **4** either in phosphate buffer (pH = 7.2) or in 100 mM $KClO_4$ solution and PIPES−buffer (piperazine-1,4-bis-(2-ethanesulfonic acid); 50 mM; pH = 7.2) led to quenching of the fluorescence. Table 1 shows the obtained Stern−Volmer constants for fluorescence quenching of **4** and its model compound **5**. From Schanze's investigation it is clear that the fluorescence of **4** is generally quenched by a static mechanism.[8] The quencher efficiency, as expressed by the Stern−Volmer constant, is under these conditions equal to the (apparent) complex formation constant of the quencher Q to the polymer **4**.[10] When utilzing no buffer, methyl viologen quenches **4** with a similarly high K_{SV} as reported by Schanze. However, if phosphate or PIPES buffers were utilized to account for constant ion strength and pH, the K_{SV} values decreased significantly due to the diminished electrostatic attraction of **4** and methyl viologen at the higher ion strength. In biological experiments specimens are utilized either in phosphate or in PIPES buffers; however, for the sensing of trace metal ions in cells, PIPES buffer is preferred because phosphate buffers can interfere with metal ions.[8] And indeed, we find in most cases much better binding of metal cations to **4** when performing the quenching experiments in PIPES buffer.[9]

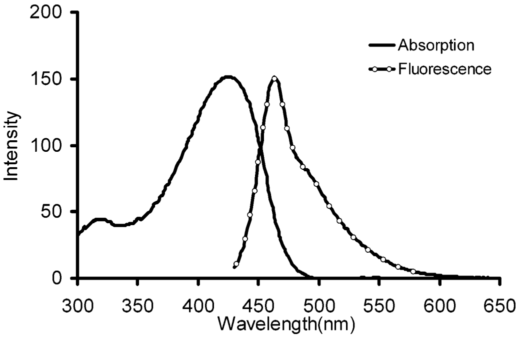

Figure 1. Normalized absorption and emission spectra of **4** in water. $\lambda_{max\ abs} = 430$ nm; $\lambda_{max\ emiss} = 465$ nm.

We find that the model compound **5** is only moderately quenched by metal salts; Stern−Volmer constants of 10−700 result. When the polymer **4** is examined under the same condtions, much higher K_{SV} values, up to 8.8×10^5, for lead(II) salts result. Swager[10a] has attributed the enhanced quenching of conjugated polymers when compared to that of monomeric model compounds to a molecular wire effect: An exciton is delocalized over many chromophores in the polymeric receptor, and if only one of the receptors binds to the analyte, the fluorescence of the whole chain is quenched. An enhanced signal is observed. Typically enhancement is 40−70-fold, and for zinc, copper, and manganese as well as for methyl viologen we find enhancements of that magnitude. In the case of lead the enhancement is signifcantly larger, up to 1.5×10^3. The dramatic enhancement must be attributed to an additional effect. The crystal structure of lead(II) acetate is instructive: "...The lead atom is coordinated to eight oxygen atoms of which two belong to water molecules...".[11] Consequently, lead(II) coordinates three acetate molecules. If we assume that lead ions will coordinate up to three carboxylate groups in solution, then the model compound will not bind particularly strongly because the second carboxylate arm seems to be too far away to bind to the metal center.

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Scheme 1. Synthesis of the Polymer 4 and the Model Compound 5

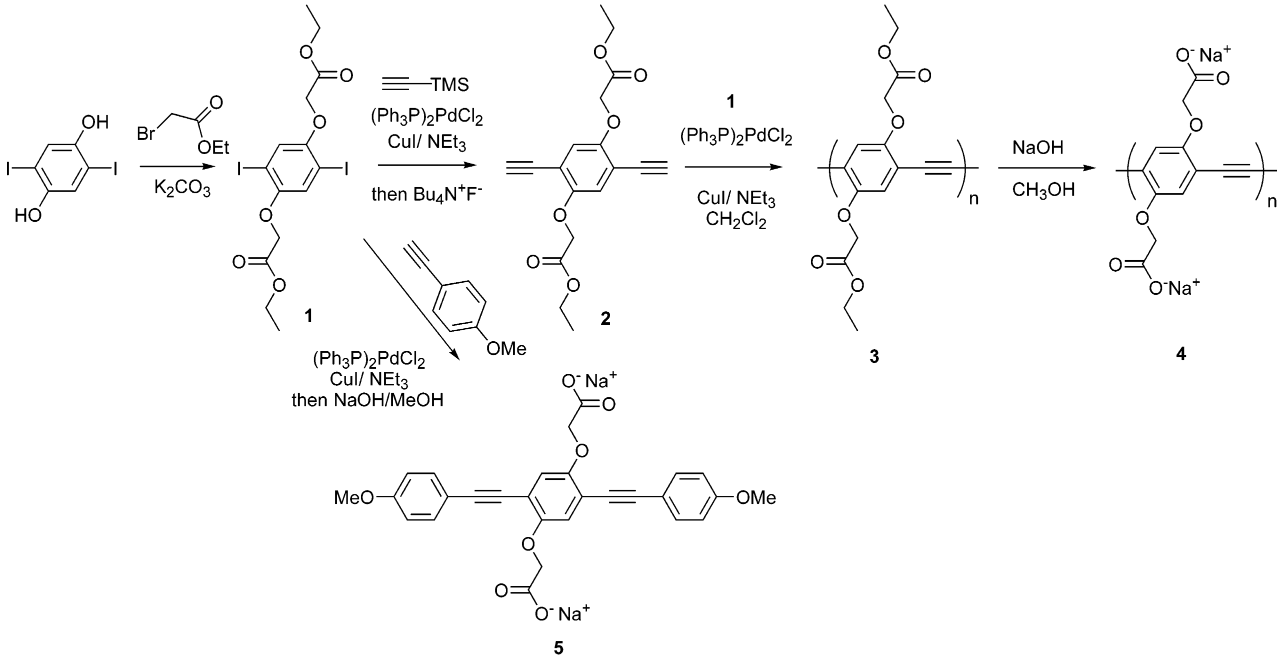

Table 1. Quenching of 4 and 5 by Metal Salts; Shown Are the Stern−Volmer Constants (K_{SV})

	4 PIPES	**5** PIPES	enhancement	**4** phosphate	**5** phosphate	enhancement
$CaCl_2$	260	50	5.2	40	500	0.08
$Ca(NO_3)_2$	700	500	1.4	3.7×10^3	600	6
$Ca(OTf)_2$	900	500	1.8	1.0×10^3	1.1×10^3	0.9
$Zn(OTf)_2$	2.7×10^3	200	14	500	600	0.8
$ZnCl_2$	3.4×10^4	340	10^2	1.2×10^4	600	20
ZnI_2	3.2×10^3	40	80	1.0×10^3	0	$>10^3$
$Hg(OAc)_2$	0	100	0	2.5×10^3	100	25
$Hg(OTFAc)_2$	700	600	1.2	1.8×10^3	500	4
$HgCl_2$	800	60	13	2.6×10^3	90	29
$Hg(NO_3)_2$	0	300	0	0	400	0
$Mg(NO_3)_2$	600	600	1	2.7×10^3	1.0×10^3	2.7
$Mg(ClO_4)_2$	600	700	0.86	400	700	0.6
$Mg(OTf)_2$	500	500	1	600	700	0.9
$MgSO_4$	900	70	13	1.2×10^3	70	1.8×10^2
$Pb(NO_3)_2$	8.8×10^5	600	1.5×10^3	$2. x 10^3$	200	10
$Pb(OAc)_2$	6.9×10^5	500	1.4×10^3	1.8×10^3	0	$>10^3$
$CuBr_2$	4.2×10^4	600	70	1.4×10^3	50	28
MV^{2+}	2.3×10^4	400	58	7×10^4	7.0×10^3	10
$MnCl_2$	810	10	81	0	0	0

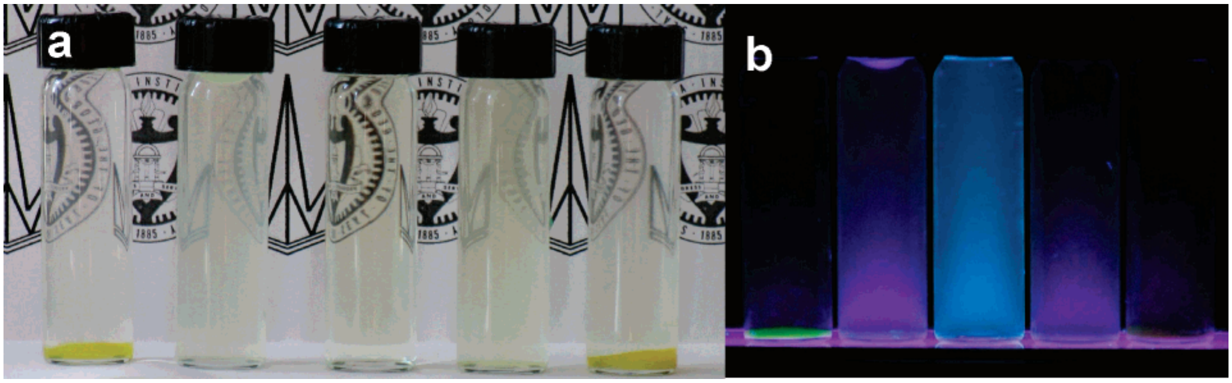

Figure 2. (a) Polymer **4** upon exposure to lead salt. Outer left/right: concentrated solution of **4** exposed to an excess of $Pb(OAc)_2$ or $Pb(NO_3)_2$. Inner left/right: dilute solution of **4** exposed to an excess of $Pb(OAc)_2$ or $Pb(NO_3)_2$. Middle: dilute blank solution of **4**. Visible is the preciptiation of **4** by lead ions. (b) Same arrangement of **4** and lead ions under blacklight illumination. Visible is the fluorescence of **4** (blank) in the middle.

However, lead can probably bind easily to a total of two carboxylate units of two adjacent phenyleneethynylene monomer units, i.e., partake in a multivalent binding event with the third coordination site available for binding to a second chain of **4**.[12] In the samples that were used for the quenching experiments, the concentration of **4** was so low that multichain processes can be excluded because clouding was not observed. Removal of the lead from the solution of **4** by precipitation cannot be the reason for the observed enhanced quenching. Multivalent binding plays a role in host−pathogen interactions and is well established in the interaction of sugar-substituted PPEs with lectins[13] but seems to be as well at work in the lead sensing of **4**.

Lead acetate is very soluble in water; its reaction with a moderately dilute solution of **4** leads to immediate clouding and formation of a thick and barely fluorescent yellow precipitate (Figure 2), supporting our hypothesis of multivalent binding which is followed by precipitation. We assume that in concentrated regimes not only intrachain but also interchain complexation of lead acetate will occur.

In conclusion, we have shown that (a) the fluorescence of an aqueous PIPES-buffered solution of **4** is efficiently quenched by lead ions with a $K_{SV} \approx 10^6$ and (b) that **4** is by a factor of 1.5×10^3 more sensitive toward quenching than its model compound **5**. (c) Multivalent binding is an important factor in the observed sensitivity of this assay. (d) As a cautionary note, we wish to reiterate that the use of the correct buffer (or its omission) will dramatically influence the binding constants of conjugated polymers to metal ions in aqueous solution. That is a particular caveat for experiments performed with conjugated polymers on biological samples.[14,15]

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Supporting Information Available: Details of the synthesis and spectroscopic characterization of **4** and **5**. This material is available free of charge via the Internet at http://pubs.acs.org.

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