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# A Highly Selective and Sensitive Inorganic/Organic Hybrid Polymer Fluorescence “Turn-on” Chemosensory System for Iron Cations

Li-Juan Fan and Wayne E. Jones, Jr.*

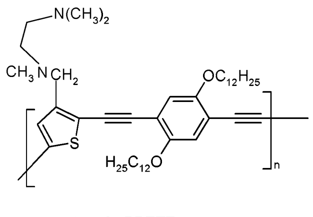
*Department of Chemistry, State University of New York at Binghamton, Binghamton, New York 13902-6016*

Received February 22, 2006; E-mail: wjones@binghamton.edu

The detection of metal ions in environmental or biological systems by fluorometric methods has gained tremendous attention in recent years.[1] Fluorescence methods have several advantages over other techniques, including ease of detection, sensitivity, and tunability. Numerous literature reports have appeared that explore fluorescence sensing of main-group and transition-metal ions, such as $K^+$, $Hg^{2+}$, $Cu^{2+}$, $Cd^{2+}$, $Zn^{2+}$, and $Ag^+$.[2] Interestingly, relatively few examples have been reported as sensors for $Fe^{2+}$ and $Fe^{3+}$ cations. [3,4] Both $Fe^{2+}$ and $Fe^{3+}$ play vital roles in many biological processes, and deficiencies or excesses of these ions are toxic or can lead to a variety of diseases.[3c,5] Though the human body can regulate iron to some extent, detection and analysis of bioactive iron represents an important healthcare challenge.

Most literature reports use fluorescence quenching as the readout mechanism for the sensor response.[3] Very few involve a fluorescence “turn-on” response.[4] Birck et. al. recently published an elegant example involving small molecule sensors that selectively identified iron cations by amplified fluorescence.[4a] The greatest advantage of fluorescence “turn-on” sensors related to “turn-off” sensors is the ease of measuring low-concentration contrast relative to a “dark” background. This reduces the likelihood of false positive signals and increases the sensitivity, as demonstrated by numerous studies.[6]

In our previous work, poly[*p*-(phenyleneethynylene)-*alt*-(thienyleneethynylene)] (PPETE) with a *N*,*N*,*N′*-trimethylethylenediamino receptor loaded on the thienylene ring (tmeda-PPETE) was synthesized on the basis of a strategy we advanced for a series of fluorescence “turn-on” chemosensors.[7] The fully characterized polymer was found to have a number-average molecular weight of $3.67 \times 10^4$ g/mol with a polydispersity of 2.73. It showed varying fluorescence “turn-on” behavior in the presence of cations including $Li^+$, $Na^+$, $K^+$, $Mg^{2+}$, $Ca^{2+}$, $H^+$, $Mn^{2+}$, $Fe^{2+}$, $Co^{2+}$, $Ni^{2+}$, $Zn^{2+}$, $Cd^{2+}$, and $Hg^{2+}$. The chelation-enhanced fluorescence (CHEF) mechanism has been described as involving coordination of the cation to a redox active amino receptor and subsequent termination of the photoinduced electron transfer (PET) quenching process with the fluorophore.[1a,b] Although this mechanism was effective in our system, the overall sensitivity was limited by a relatively high background fluorescence.



**tmeda-PPETE**

Interestingly, $Cu^{2+}$ has been found to exhibit a noteworthy exception to the fluorescence enhancement behavior in this system. The fluorescence is significantly quenched with $Cu^{2+}$ in solution, as observed in typical PPETE chemosensors.[8] On the basis of $Cu^{2+}$ quenching, we hypothesized that an inorganic/organic hybrid system may provide for both enhanced sensitivity and selectivity by preloading $Cu^{2+}$ onto the amino receptor to totally quench the initial background fluorescence. Here, we report the design and evaluation of a highly selective and sensitive fluorescence “turn-on” chemosensory system for iron cations on the basis of a tmeda-PPETE/ $Cu^{2+}$ hybrid system.

The tmeda-PPETE/$Cu^{2+}$ hybrid system was prepared from THF solutions of tmeda-PPETE with a repeat unit concentration of 5 $\mu$M. $CuCl_2$ was added from aqueous stock solution to reach a final concentration of 5 $\mu$M and to achieve a 1:1 ratio of $Cu^{2+}$ to receptor. Consistent with previous studies,[7,8] absorption peaks for the solution were observed around 440 nm and can be assigned to $\pi-\pi^*$ transitions from the conjugated polymer backbone. Thus, the solution was found to have no shift in the UV−vis absorption. The emission intensity was quenched by more than 98% at $\lambda_{max} = 494$ nm relative to the initial tmeda-PPETE polymer (see Supporting Information). This result is consistent with previous $Cu^{2+}$ quenching results in the literature.[9]

The titration of $Fe^{2+}$ was carried out by adding small aliquots of $FeCl_2$ aqueous stock solution into the THF solutions containing the tmeda-PPETE/$Cu^{2+}$ hybrid system. We observed a greater than 100-fold enhancement in the fluorescence intensity upon titration of 10 $\mu$M aqueous ferrous chloride (Figure 1). During the titration, the emission maximum did not shift and UV−vis spectra showed negligible changes. This suggests no significant change in the overall electronic structure of the polymer upon addition of $Fe^{2+}$. The final fluorescence intensity of the titrated solution was almost the same as for the titration of the pure polymer in the absence of $Cu^{2+}$. On the basis of these results, we assume that the $Fe^{2+}$ has displaced the $Cu^{2+}$ from the receptor. Attempts to directly observe the binding event by NMR and EPR have, to date, proved unsuccessful.

For comparison purposes, the influence of some other common divalent metal chlorides was investigated, and the data is collected on the basis of the relative change in fluorescence, $I/I_0$, in Figure 2. In each case, a total amount of 10 $\mu$M of cations was added into the tmeda-PPETE/$Cu^{2+}$ solution. $Ca^{2+}$ was selected as a representative alkali earth cation and $Hg^{2+}$, $Zn^{2+}$, $Ni^{2+}$, $Co^{2+}$, and $Mn^{2+}$ as divalent transition-metal cations. To investigate the influence of solution acidity, the addition of HCl aqueous solution into the sensory system was also carried out.

As shown in Figure 2, the system is highly selective for $Fe^{2+}$ fluorescence enhancements of 150-fold. No other metal cations showed significant response, with the exception of $Hg^{2+}$ with a 10-fold enhancement. The addition of all the cations into pure tmeda-PPETE THF solution is also shown for comparison. Results showed there was no significant difference in fluorescence enhancement among any of these cations for the pure chemosensor polymer. In all cases, the fluorescence enhancement was significantly smaller

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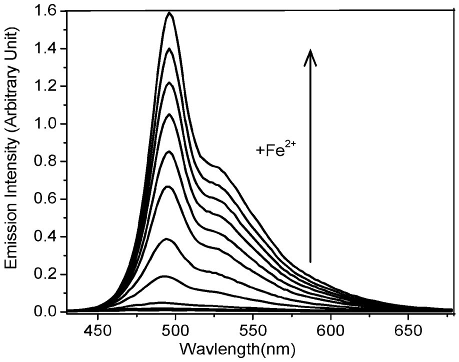

***Figure 1.*** Fluorescence response following excitation at 408 nm from tmeda-PPETE/Cu$^{2+}$ solutions upon addition of Fe$^{2+}$ aqueous solution. The concentrations of the Fe$^{2+}$ for the spectra (from bottom to top) were 0, 1, 2, 3, 4, 5, 6, 7, 8, 10 $\mu$M; The concentrations of tmeda-PPETE (with respect to the repeat unit) and Cu$^{2+}$ were fixed at 5 $\mu$M.

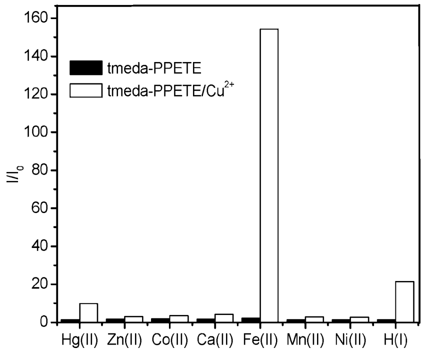

***Figure 2.*** Fluorescence response of tmeda-PPETE/Cu$^{2+}$ (white) or tmeda-PPETE (black) to various 10 $\mu$M cations in room-temperature solution; The concentrations of tmeda-PPETE (with respect to the repeat unit) and Cu$^{2+}$ were fixed at 5 $\mu$M.

for the model compared to the tmeda-PPETE/Cu$^{2+}$ system. Thus, the high sensitivity and selectivity required the polymer/Cu$^{2+}$ hybrid.

Although there was a 21-fold fluorescence enhancement in the presence of H$^+$ and iron or other aqueous cations can generate protons by hydrolysis,[10] the change in fluorescence intensity for the metal cations is not a function of acidity alone. The hydrolysis p$K_a$'s provide insight into the relative acidities of the cation solutions[10,11]( see also Supporting Information). The acidity of Fe$^{2+}$ in aqueous solution is very similar to Zn$^{2+}$, and the $K_{SP}$ of their hydroxides is also very close. In addition, HCl and HgCl$_2$ are much more acidic than the FeCl$_2$ solution. However, Zn$^{2+}$, H$^+$, and Hg$^{2+}$ showed limited fluorescence enhancement in the system studied. Therefore, we conclude that the metal interaction with the polymer is required mechanistically.

The tmeda-PPETE/Cu$^{2+}$ system was highly selective toward Fe$^{2+}$ cations in solution. Because this selectivity is not present for the parent tmeda-PPETE solutions, it must not be based simply on the association constant between the receptor ligand and the cation. It is likely that the selectivity is related to the relative ability of the cations to replace the Cu$^{2+}$ already in coordination with the receptor. Competitive binding has previously been observed in small molecule sensors.[13] The relative coordination of metal cations can often be related to their Lewis acid−base properties. However, Fe$^{2+}$ and other cations do not follow a consistent trend, so more work is necessary to specifically identify the mode of action for the selectivity.

In summary, a highly selective and sensitive sensory system toward iron cations in solution was achieved by preparing a transition metal-derivatized conjugated polymer. By preloading Cu$^{2+}$onto the polymer receptor, fluorescence enhancements of over 2 orders of magnitude were achieved. This unique hybrid inorganic/organic polymer method may be useful in the detection of iron cations or modified for other analytes.

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**Supporting Information Available:** Experimental details, UV−vis spectra and emission spectra, quenching data, and cation physical constants and pH calculations are available free of charge via the Internet at http://pubs.acs.org.

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