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Highly Selective Colorimetric Fluorescent Sensor for Pb^{2+}

Shyamaprosad Goswami*[a] and Rinku Chakrabarty[a]

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Phenanthroline-based colorimetric sensors **1** and **2** have been designed, synthesized, and compared with phenanthrene-based receptor **3** for sensing of Pb^{2+} by color change. Receptor **1** imparts color change (from yellow to red) selectively with Pb^{2+} in acetonitrile/water (9:1) as well as in methanol/water (9:1) when in the presence of other metal ions studied (Li^+, Na^+, K^+, Ca^{2+}, Mg^{2+}, Ba^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}, and Mn^{2+} as their perchlorate salts). Receptor **1** also shows fluorescence enhancement upon addition of lead perchlorate in acetonitrile/water (9:1) solvent possibly due to the chelation enhanced fluorescence (CHEF) effect. However, the binding behavior of **2** with Pb^{2+} is found to be less effective compared to that of receptor **1**.

Introduction

In recent years the design and synthesis of sensors that can selectively bind a particular metal ion has received considerable attention.[1] These sensors have various applications in clinical toxicology, bioremediation, waste management, and so on.[2] Lead is a very useful metal ion because of its widespread use in our daily lives. For example, lead is found in insulation, coating electronics, leaded crystal, and storage batteries. It is also used in coating wicks of some candles and in pool paint, especially those painted by lead-based epoxy paints.[3] We are exposed to trace amounts of lead every day. The main concern is lead exposure with small children, because lead can cause developmental problems. Lead is useful and necessary, but the reality is, any amount of lead is too much. When the body is exposed to lead by inhalation, swallowing, or in a small number of cases, absorption through the skin, it can act as a poison. Although many methods including atomic absorption spectroscopy (AAS) and inductively coupled plasma atomic emission spectroscopy (ICP-AES) have been applied for the quantitative detection of lead ions, all of these processes are highly costly. Therefore, research has been devoted to the detection of lead ions. Among the sensors targeted to determine the lead ion,[4] colorimetric sensors have received considerable attention.[5]

Results and Discussion

We report here phenanthroline-based receptor **1** (Figure 1), which selectively binds Pb^{2+} by changing its color from yellow to red in mixtures of acetonitrile/water (9:1) as well as in methanol/water (9:1). Chromogenic sensor **1** is basically a hydrazone-based receptor, and it displays extreme sensitivity and selectivity for Pb^{2+} even at the micromolar level. Receptor **1** was synthesized by the reaction of phenanthroline dione (**A**) and hydrazide (**C**; Scheme 1) in methanol at room temperature.[6] The structures of receptors **1** and **2** were confirmed by ^1H and ^{13}C NMR spectroscopy and high-resolution mass spectrometry.

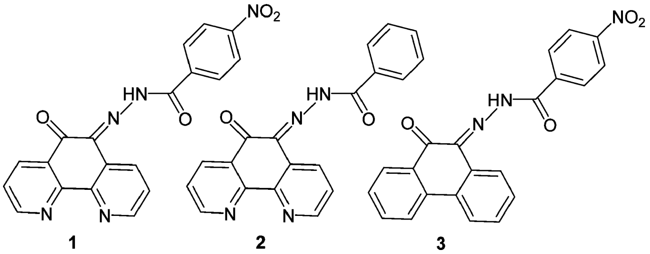

Figure 1. Structures of receptors **1**–**3**.

Synthesis

The NH proton of receptor **1** appears considerably downfield (δ = 15.52 ppm) as a broad singlet because this compound adopts a six-membered ring conformation in which a strong hydrogen bond between the NH proton and the carbonyl group of the phenanthroline moiety exists (Figure S4, Supporting Information).[6] Again, strong electron-withdrawing resonance of such conjugated carbonyl and adjacent amide carbonyl moieties are also responsible.

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[a] Department of Chemistry, Bengal Engineering and Science University,
Shibpur, Howrah 711103, India
Fax: +91-33-2668-2916
E-mail: spgoswamical@yahoo.com

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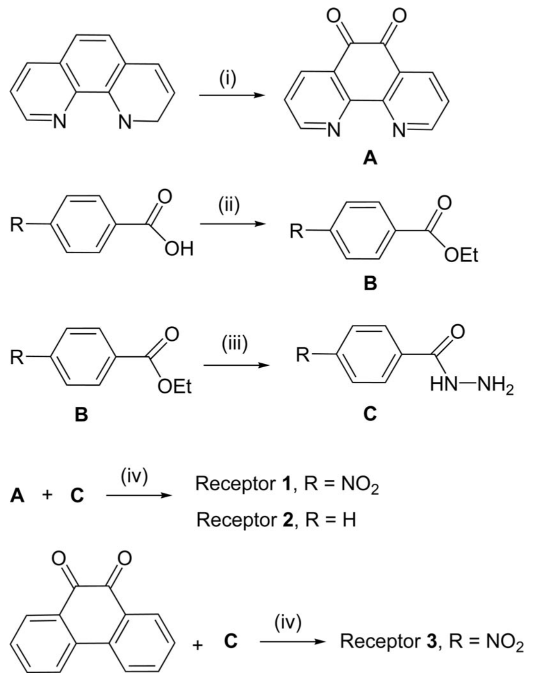

Scheme 1. Reagents and conditions: (i) H_2SO_4, HNO_3, 80 °C; (ii) dry ethanol, reflux, 12 h; (iii) hydrazine hydrate, ethanol, reflux, 2 h; (iv) dry methanol, room temp.

Naked-Eye Detection

Naked-eye detection experiments were carried out initially in acetonitrile/water (9:1) by addition of the corresponding cation solutions in an acetonitrile/water mixture as their perchlorate salts. The addition of the solution of lead perchlorate into the solution of receptor **1** resulted in a color change from yellow to red (Figure 2) as a result of the appearance of a band at 483 nm. Thus, the resulting species is stabilized through aromaticity. The azo[7] form of the receptor generated from the hydrazo moiety is probably responsible for the formation of the color (Figure S5, Supporting Information).[6] To realize the necessity of the 4-nitrophenyl group in receptor **1**, we synthesized receptor **2** lacking a nitro substituent. Receptor **2** also displays a red color with Pb^{2+}, but at a much higher concentration compared to that of **1**. The detection limit[5c] of Pb^{II} to the naked eye is 1.6×10^{-7} M with receptor **1**.

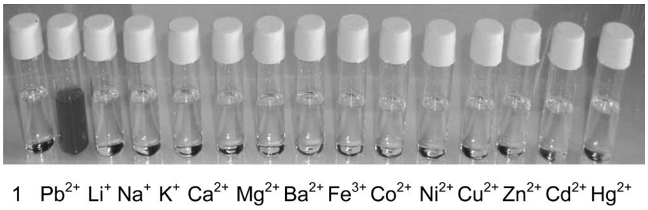

Figure 2. Change in the color of receptor **1** (1.1×10^{-5} M) after addition of different metal ions [from left: receptor **1**, **1** + Pb^{2+} (red solution), **1** + Li^+, **1** + Na^+, **1** + K^+, **1** + Ca^{2+}, **1** + Mg^{2+}, **1** + Ba^{2+}, **1** + Fe^{3+}, **1** + Co^{2+}, **1** + Ni^{2+}, **1** + Cu^{2+}, **1** + Zn^{2+}, **1** + Cd^{2+}, **1** + Hg^{2+}] as their perchlorate salts (49 µM, 1 equiv.) in CH_3CN/H_2O (9:1).

Binding Studies

Binding Studies by UV/Vis Method

The binding behavior of receptor **1** with metal ions as their perchlorate salts was studied by UV/Vis. The UV/Vis titration of receptor **1** was carried out in an aqueous acetonitrile medium in the presence of HEPES buffer solution (pH 7.4, 10 mM). The UV/Vis spectra of receptor **1** reveal an absorption maximum at 352 nm. Addition of Pb^{2+} ions very strongly influences the absorption maximum. It is worth noting that a new peak appears at 483 nm ($\Delta\lambda$ = 131 nm), which increases with an increase in the concentration of Pb^{2+}. The isosbestic point at 420 nm indicates the formation of a new complex between **1** and Pb^{2+}. The corresponding spectrum is depicted in Figure 3b. The binding constant (K_a)[6] values for receptor **1** were calculated by using the UV/Vis titration experiment. The K_a value determined by this method, that is, the K_a value of receptor **1** with Pb^{2+}, is 4.56×10^5 M^{-1}. The binding constant values of receptor **1** with alkali and alkaline earth metal ions are lower compared to those of other transition metal ions, for example, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, and so on (Figure 3c).[6]

Titrations were also carried out with other metal ions used as their perchlorate salts like Li^+, Na^+, K^+, Ca^{2+}, Mg^{2+}, Ba^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}, and Mn^{2+}. For the cases of other metal ions stated earlier, the changes in the spectra of receptor **1** are almost negligible (Figure 3a). The binding constant for receptor **2** and Pb^{2+} is found to be 1.22×10^3 M^{-1}, which is less than receptor **1** (for spectra, see Figure S8, Supporting Information).[6]

The stoichiometry of complexation was determined from the Job plot (Figure S2, Supporting Information)[6] by UV/Vis experiment between receptor **1** and Pb^{2+}. A continuous variation method was applied to determine the stoichiometry in the Job plot. The break at 0.5 indicates the 1:1 stoichiometry of the receptor with Pb^{2+}.

The nitrogen atoms of the phenanthroline core probably remain innocent towards the binding process. The explanation may be found from the azo form of the Pb^{2+}-bound protonated intermediate[8] (Figure S6, Supporting Information). As a result of the protonation of the phenanthroline ring nitrogen atoms, the azo form with the H atom attached is stabilized, and therefore, these ring nitrogen atoms do not take part in the binding process. Therefore, the nitrogen atoms of the phenanthroline core play an important role in the binding process. For comparison, we also synthesized receptor **3** (Scheme 1), which shows red coloration on prolonged exposure to Pb^{2+}. Thus, even the phenanthroline system is a more effective sensor for Pb^{2+}, receptor **3** may also be used for this sensing purpose.

From the experimental read out, it can be concluded that receptor **1** possesses high selectivity and sensitivity towards Pb^{2+} in acetonitrile/water (Figure 3c). The other metal perchlorates, for example, Li^+, Na^+, K^+, Ca^{2+}, Mg^{2+}, Ba^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}, and Mn^{2+}, had no significant influence. Thus, receptor **1** is selective for the recognition of Pb^{2+}.

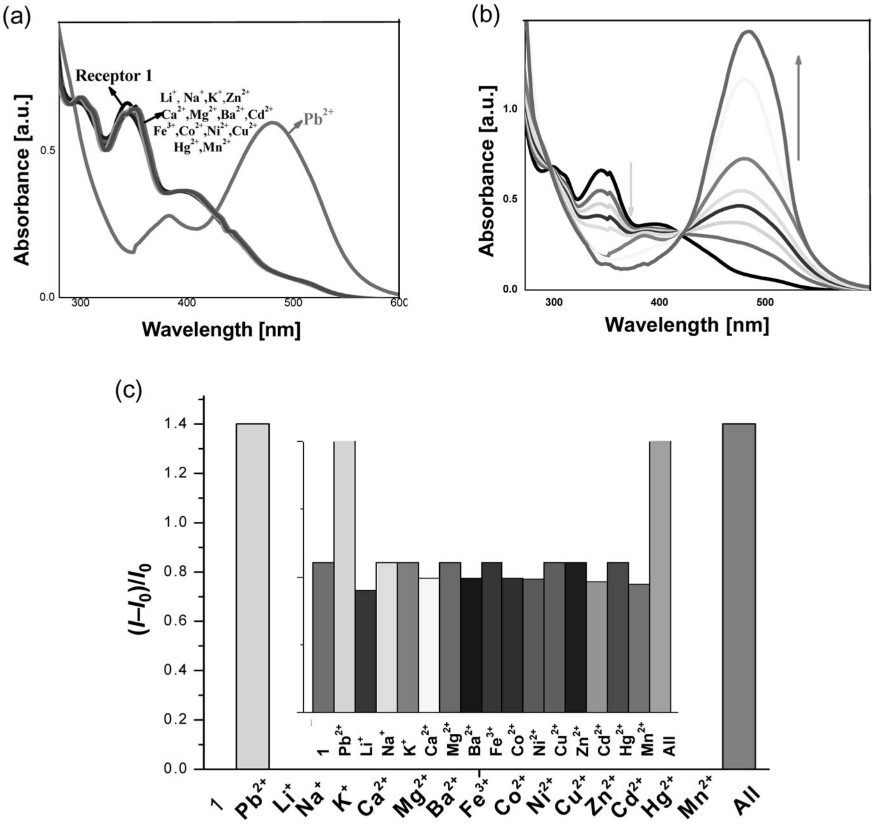

Figure 3. (a) UV/Vis spectra of receptor **1** with the addition of different metal ions; (b) change in the UV/Vis spectra of receptor **1** ($c \approx 1 \times 10^{-5}$ M) after addition of increasing amounts of Pb^{2+} ($c \approx 1 \times 10^{-4}$ M) in acetonitrile/water (9:1) [0, 0.5, 1, 1.5, 2, 3, 5, and 7 equiv. of Pb^{2+}]; (c) the response of receptor **1** (at 483 nm) in UV/Vis towards metal ions after the addition of 1 equiv. of metal ions, which shows the response toward the Pb^{2+} of 10^{-4} M plus coexisting metal ions at 10^{-4} M. "All" means all the tested interfering metal ions are present but at a concentration of 10^{-5} M. [Receptor **1**] = 10^{-5} M.

Binding Studies by Fluorescence Method

The binding behavior of receptor **1** with different metal perchlorates (Li^+, Na^+, K^+, Ca^{2+}, Mg^{2+}, Pb^{2+}, Ba^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}, and Mn^{2+}) was also studied by fluorescence spectroscopy. All the titrations were carried out by using a 1×10^{-5} M concentration of **1**. By using the metal ions mentioned earlier, **1** possibly showed the CHEF (chelation-enhanced fluorescence)[4a,5a,5b] effect only with Pb^{2+} [other metal ions show negligible change after addition of a large excess of the metal ions (100 equiv.)], resulting in an increase in intensity in the observed fluorescence spectra (Figure 4).

Receptor **1** showed emission maximum at 395 nm at an excitation wavelength of 352 nm. As a result of the addition of Pb^{2+} ions, the emission maximum is shifted towards slightly longer wavelengths and appears at 401 nm. The value of K_a calculated by the fluorescence method was found to be 4.67×10^5 M^{-1}. The stoichiometry for the formation of the complex of receptor **1** was again determined by a Job plot in the fluorescence method (Figure S3, Supporting Information).[6]

The quantum yield (Φ_F) of the system (receptor **1**) is 0.0039 in acetonitrile/water at 298 K with the use of tryptophan as a standard. After the addition of Pb^{2+} the value of Φ_F increases.

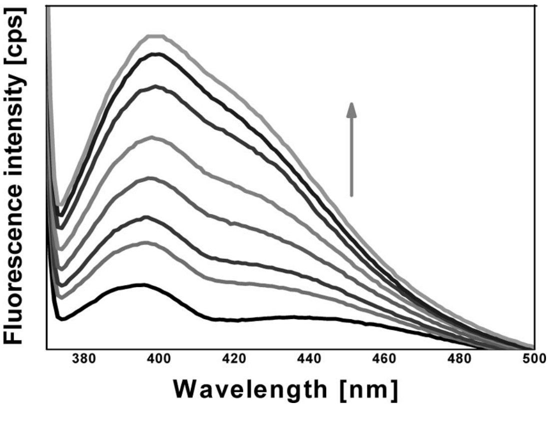

Figure 4. Fluorescence spectra of receptor **1** ($c \approx 1 \times 10^{-5}$ M) with the addition of increasing amounts of Pb^{2+} perchlorate ($c \approx 1 \times 10^{-4}$ M) [0, 0.5, 1, 1.5, 2, 3, 5, and 7 equiv.].

NMR Studies

^1H NMR experiments (Figure S7, Supporting Information)[6] were performed in CD_3CN to understand the coordination mechanism of receptor **1** and Pb^{2+} (Figure 5). As a result of the complexation process, the NH proton of hydrazone undergoes an upfield shift from δ = 15.55 to 15.38 ppm. Again noticeable upfield chemical shifts have been induced for the protons of the benzene ring as well as those of the phenanthroline rings. This indicates that the azo form, which was the minor form in solution before the addition of Pb^{2+}, probably predominates[7a] in the presence of Pb^{2+} in the case of receptor **1**.

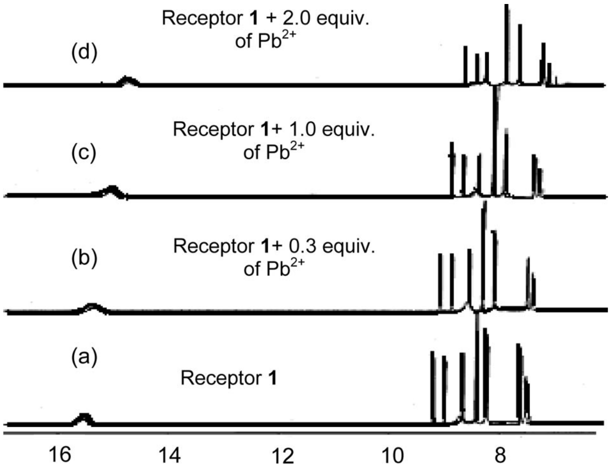

Figure 5. Evolution of the ^1H NMR spectra of **1** in CD_3CN upon the addition of (a) 0, (b) 0.3, (c) 1.0, and (d) 2.0 equiv. of Pb^{2+}.

We acquired the ^1H NMR spectra of receptor **1** and Pb^{2+} in the presence of 1 equiv. of Pb^{2+} and either Zn^{2+} or Na^{2+}. In both cases, the ^1H NMR spectra of the mixture of Pb^{2+} and Zn^{2+} or Pb^{2+} and Na^{2+} were the same. Therefore, we can conclude that the interferences of other metal cations to the binding process of receptor **1** and Pb^{2+} in CH_3CN is negligible.

The ^{13}C NMR spectrum (Figure 6) also reveals a strong interaction between receptor **1** and Pb^{2+}. Complexation induces downfield shifts of the carbonyl carbon atom ($\Delta\delta$ = 0.31 ppm) as well as the benzene and phenanthroline ring carbon atoms.[6] The reversibility of the binding process can be proved by the fact that after the addition of an excess amount of ethylenediamine[4b] to the red-colored solution of receptor **1** and Pb^{2+}, the solution became yellow (color of **1** itself).

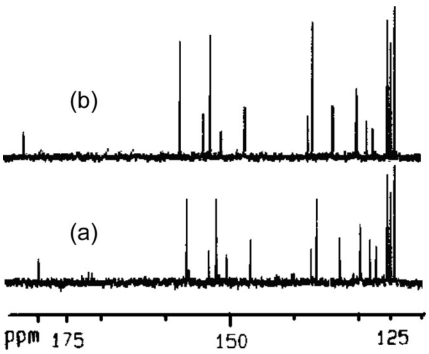

Figure 6. ^{13}C NMR spectra of **1** in CD_3CN upon addition of (a) 0.0 and (b) 0.5 equiv. of Pb^{2+}.

We also performed another experiment to understand the real-life application of sensor **1**. To an aqueous solution of Pb^{2+} was added a solution of sensor **1** in chloroform, and the mixture was shaken well. The water/chloroform interface became red, and the yellow-colored chloroform layer also became reddish (due to the lower solubility of the receptor **1**–Pb^{2+} complex in chloroform). Thus, receptor **1** shows potential practical value in the extraction of Pb^{2+} from water. Further improvements and modification to the receptor to increase the solubility of the receptor–Pb^{2+} complex in chloroform is in progress in our laboratory. The IR spectra of receptor **1** and different metal ions in acetonitrile also support the strong binding of **1** and Pb^{2+} (Figure S1, Supporting Information).[6]

Conclusion

In conclusion, designed receptor **1** was demonstrated to be a selective colorimetric as well as a fluorescent sensor for the recognition of Pb^{2+}. Receptor **1** shows high selectivity and sensitivity towards Pb^{2+} by its color change from yellow to red in acetonitrile/water (9:1). Receptor **1** also shows fluorescence enhancement due to the addition of lead perchlorate because of the chelation enhanced fluorescence enhancement (CHEF) effect.

Supporting Information (see footnote on the first page of this article): ^1H and ^{13}C NMR and mass spectra (HRMS) of receptors **1**–**3**; general procedure for their synthesis; table of binding constants.

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