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Highly Selective Colorimetric Chemosensor for Co^{2+}

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Supporting Information

ABSTRACT: A new highly selective colorimetric chemosensor for Co^{2+} was developed based on coumarin-conjugated thiocarbanohydrazone. The ligand senses Co^{2+} in solution by changing its color from light yellow to deep pink. The sensor has been used in the development of practically viable colorimetric kits and as a staining agent for Co^{2+} in microorganisms.

Cobalt is an essential trace element found in cobalamin and a few other metalloproteins.[1] Cobalamin is necessary for the formation of myelin, an insulating layer found around nerves, in the support of red blood cell production, for the metabolism of fats and carbohydrates, and in the synthesis of proteins.[1] Apart from its biological role, exposure to high levels of cobalt can cause health effects like decreased cardiac output, cardiac and thyroid enlargements, heart disease, elevated red blood cells accompanied by increased cells in the bone marrow, increased blood volume, and vasodilation and flushing.[2] Cobalt is also responsible for allergic contact dermatitis and is possibly carcinogenic to humans.[3] Major sources of cobalt in the atmosphere are soil, dust, seawater, forest fires, and volcanic eruptions. Cobalt is also released to the environment from burning coal and oil, truck and airplane exhausts, and diamond polishing, porcelain, chemical, and hard-metal industries.[4] Therefore, it is crucial to detect trace amounts of cobalt samples in the environment for maintaining good human health. Fluorometric detection is practically difficult because of the fluorescence quenching nature of paramagnetic Co^{2+}.[5] To the best of our knowledge, only a few sensors have been reported for the detection of Co^{2+}.[6] Compared to fluorometric sensors, colorimetric sensors have attracted much attention for allowing "naked-eye" detection in a uncomplicated and inexpensive manner, offering qualitative and quantitative information.[7] Colorimetric chemosensors of cations can be generated based on the right combination of receptor and chromophore. One such combination would be a conjugate of an amine and an aldehyde carrying thioamides formed through Schiff base linkages.[8]

Herein, we report a simple thiocarbonohydrazone system based on a coumarin Schiff base derivative as an efficient colorimetric sensor for Co^{2+} in an aqueous medium. Coumarin and its derivatives have been used as excellent chromogenic and fluorogenic chromophores because of their tunable photophysical properties in the visible region.[9] We have recently reported simple Schiff base conjugates of salicylaldehyde and urea/thiourea as chemosensors for the selective detection of Cu^{2+} based on double deprotonation and extended conjugation.[10] In the present work, we have developed 7-(diethylamino)-2-oxo-2*H*-chromene-3-carbaldehyde-conjugated carbohydrazide/thiocarbohydrazide ligands for selective sensing of Co^{2+}. 7-(Diethylamino)-2-oxo-2*H*-chromene-3-carbaldehyde was treated with carbohydrazide/thiocarbohydrazide in an ethanol/water (1:1) mixture under reflux conditions to form Schiff base derivatives coumarin−carbonohydrazone (**CC**) and coumarin−thiocarbonohydrazone (**CTC**), respectively (Figure 1). These ligands were characterized by NMR, mass spectrometry (MS), and elemental analysis.[11] In ligands **CC** and **CTC**, 7-(diethylamino)coumarin serves as a chromophoric core. The π conjugation and stronger electron-withdrawing ability of the ketone/thioketone group have enhanced charge transfer within the molecule. The imine and keto/thioketo groups can act as chelating sites of the metal cations, in particular, transition- and post-transition-metal cations. The photophysical properties of **CC** and **CTC** were investigated by monitoring the absorption spectral behavior upon the addition of several metal ions such as Li^+, Na^+, K^+, Ba^{2+}, Sr^{2+}, Mg^{2+}, Al^{3+}, Ca^{2+}, Mn^{2+}, Fe^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Ag^+, Cd^{2+}, Hg^{2+}, Pb^{2+}, and Co^{2+} in an aqueous medium [6:4 50 mM HEPES (pH = 7.2)/MeCN]. **CC** shows an absorption band centered around 455 nm and remained unchanged upon the addition of 50.0 equiv of Na^+, K^+, Li^+, Sr^{2+}, Ba^{2+}, Mg^{2+}, Al^{3+}, and Cu^{2+}. The 455 nm band slightly blue-shifted for Hg^{2+} and slightly red-shifted with increased intensity to different extents observed for Ca^{2+}, Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+}, Ag^+, and Pb^{2+}. Therefore, **CC** shows no specific selectivity for any particular metal ion tested (Figure S1 in the Supporting Information, SI). As shown in Figure S2 in the SI, **CTC** exhibits an absorption band centered around 470 nm with an extinction coefficient (ε) of 6.8×10^4 M^{-1} cm^{-1}, which remains unchanged upon the addition of 50.0 equiv of Li^+, Na^+, K^+, Ba^{2+}, Mg^{2+}, Al^{3+}, and Ca^{2+}. Upon the addition of 50.0 equiv of Mn^{2+}, Fe^{2+}, Ni^{2+}, Cu^{2+}, Ag^+, and Hg^{2+}, the absorbance intensity decreases and the band slightly blue-shifted to different extents.

The absorption band slightly red-shifted upon the addition of 50.0 equiv of Sr^{2+}, Zn^{2+}, Cd^{2+}, and Pb^{2+}, and no color change in the **CTC** solution was observed. In the case of Co^{2+}, the absorbance band at 470 nm red-shifted to 510 nm ($\Delta\lambda$ = 40 nm) and the color of the solution changed from yellow to deep pink. An absorption study of **CTC** with the sequential addition of increasing concentrations of Co^{2+} (0−3.0 μM) is shown in Figure 2. The absorbance is slowly decreased and the band at 470 nm red-shifted to 490 nm upon the addition of 1.5 μM Co^{2+}. After reaching 2.2 μM, the absorbance started increasing and absorbance maxima overall red-shifted to 510 nm. The absorbance attains saturation with the addition of 3.0 μM Co^{2+} ($\varepsilon = 6 \times 10^4$ M^{-1} cm^{-1}).

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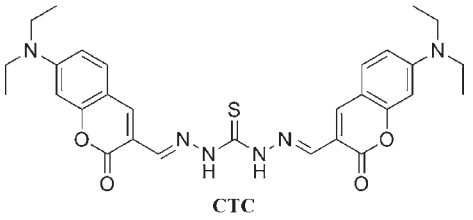

Figure 1. Structure of **CTC**.

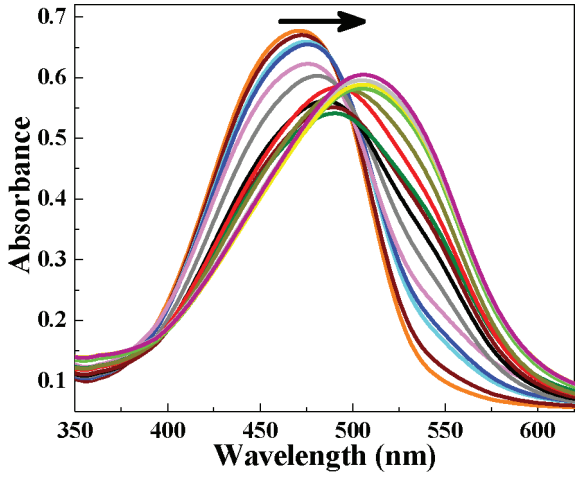

Figure 2. UV/vis absorption spectra of **CTC** (10.0 μM) upon additions of Co^{2+} (0.0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.2, 2.4, 2.6, 2.8, and 3.0 μM) in an aqueous medium.

The preferential selectivity of **CTC** as a colorimetric chemosensor for the detection of Co^{2+} was studied in the presence of various competing metal ions. **CTC** exhibited a distinct color change from yellow to deep pink upon the addition of Co^{2+} (Figure 3a). For competition studies, **CTC** was treated with 1.0 equiv of Co^{2+} in the presence of 5.0 equiv of other metal ions, as indicated in Figure 3b. There was no interference for the detection of Co^{2+} in the presence of Li^+, Na^+, K^+, Ba^{2+}, Sr^{2+}, Ca^{2+}, Mg^{2+}, Mn^{2+}, Fe^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}, and Pb^{2+}, except Ag^+. In the case of Ag^+, a relatively diminished, but detectable, color change was observed for Co^{2+}. Thus, **CTC** can be used as a selective colorimetric sensor for Co^{2+} in the presence of most competing metal ions.

Job's plot obtained from absorbance spectral studies showed 2:1 stoichiometric complexation between **CTC** and Co^{2+} (Figure S3 in the SI). These data were further supported by MS analysis of **CTC**−Co^{2+} complex formation.[11] Matrix-assisted laser desorption ionization time-of-flight MS shows the formation of a complex between two molecules of deprotonated **CTC** and a cobalt ion [m/z 1177.38 (2**CTC** + Co^{2+} − $2H^+$); calcd for $C_{58}H_{64}CoN_{12}O_8S_2$ m/z 1179.37; Figure S4 in the SI]. The response parameter α, which is defined as the ratio of the free ligand concentration to the initial concentration of the ligand, was plotted as a function of the Co^{2+} concentration. This plot can serve as the calibration curve for the detection of Co^{2+} (Figure S5 in the SI). The association constant (log K_a) for the complexation of **CTC** and Co^{2+} was found to be 7.95 M^{-2} as determined from Li's equations.[11] We have recorded the absorbance of the [2**CTC**:Co^{2+}] complex as a function of the pH. The absorbance intensity at 510 nm was found to increase gradually from pH 2.0 to 12.5 (Figure S6 in the SI). Co^{2+} can be clearly detected by the naked eye or UV/vis absorption measurements using **CTC** over a wide pH range of 2.0−12.5.

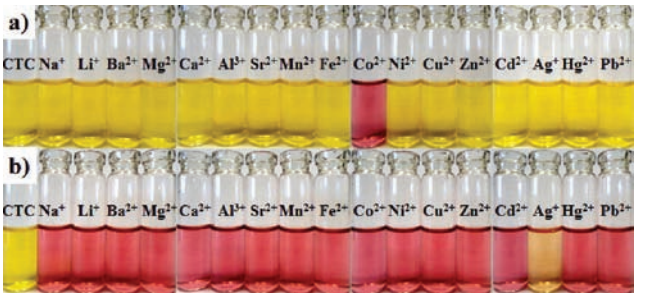

Figure 3. (a) Observed color changes of a **CTC** solution (10.0 μM) upon the addition of 5.0 equiv of different metal ions in aqueous media. (b) Color changes of a **CTC** solution (10.0 μM) containing Co^{2+} (1.0 equiv) in the presence of different metal ions (5.0 equiv) in aqueous media.

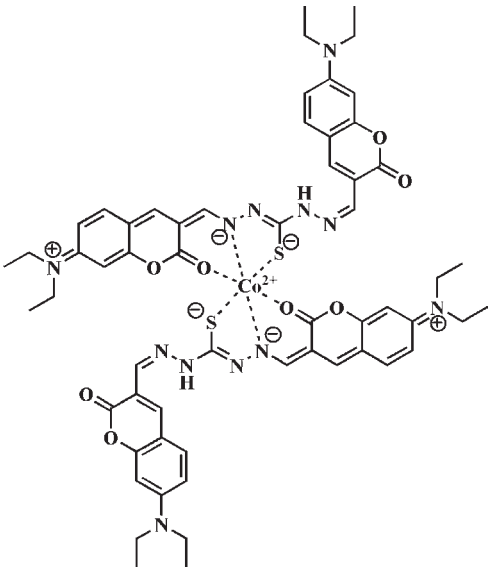

Figure 4. Proposed structure of a 2**CTC**:Co^{2+} complex.

In colorimetric sensor **CTC**, binding and signaling subunits are electronically conjugated to each other to effectively induce color changes. Sulfur analogues (C═S) are less stable because of the poor overlap between the $2sp^2$ orbital on carbon and the $3sp^2$ orbital on sulfur. In **CTC**, thioamide undergoes conjugation with nitrogen to stabilize the weak C═S bond.[10] Tautomerized thioamide coordinates to Co^{2+} after deprotonation, which leads to a red shift in the absorbance band in the visible region. The proposed mode of 2:1 stoichiometric complexation of **CTC** and Co^{2+} is shown in Figure 4.

For practical applications, we have developed a colorimetric test kit. The test kit uses ligand-coated filter paper strips, which were prepared by immersing them into a **CTC** solution (2.0 mM). The aqueous solutions of different metal ions were sprayed onto these strips, and a yellow-colored strip changed its color to deep pink exclusively when a Co^{2+} solution was sprayed (Figure 5). In addition, the intensity of the colored strips can be used to estimate the different concentration ranges of Co^{2+}. This experiment exhibits steady colorimetric changes for increasing concentrations of Co^{2+} with at least down to 1.0 μM detection limit.

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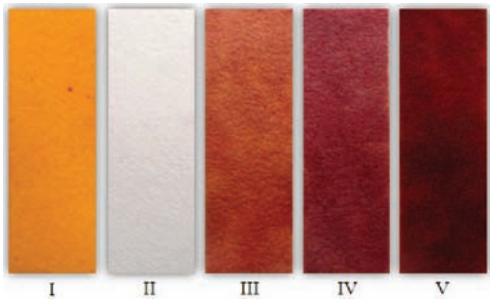

Figure 5. Colorimetric test kit. Photographs of the filter paper coated with **CTC** (10.0 μM) used for the detection of different concentrations of Co^{2+} in aqueous solutions: I, **CTC** (control); II, Co^{2+} (500 μM, control); III–V, **CTC** filter paper sprayed with 1.0, 2.0, and 3.0 μM Co^{2+}, respectively.

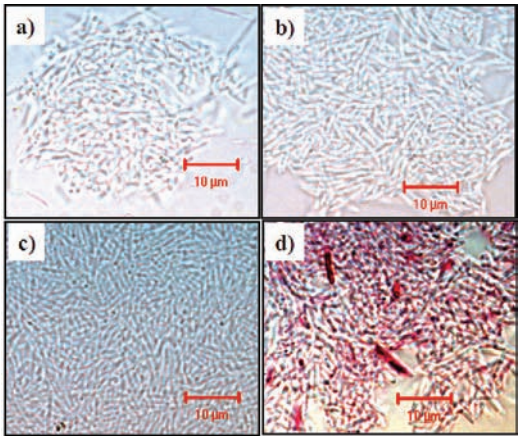

Figure 6. Light microscopic images of (a) control cells of *E. coli*, (b) cells exposed to only Co^{2+} (5.0 μM), (c) cells exposed to only **CTC** (10.0 μM), and (d) cells exposed to Co^{2+} (5.0 μM) and **CTC** (10.0 μM).

We have further demonstrated the use of **CTC** as a colorimetric reagent for the detection of Co^{2+} in microbes. The *E. coli* strain DH5α was grown overnight in LB media (HIMEDIA) at 37 °C incubation. The cells were harvested and vortexed to make the homogeneous suspension in sterile distilled water. The cultured cells were first exposed to Co^{2+} (5.0 μM) in a 50 mM HEPES/CH_3CN buffer (3:2, v/v; pH 7.2) for 30 min at 25 °C. The excess Co^{2+} present in the cultured media was removed through centrifugation. This process was repeated after the addition of ~5 mL of a 50 mM HEPES/CH_3CN buffer to remove traces of Co^{2+} that may be present on the microorganism surfaces in order to avoid the background color during recording of the optical microscope images. The centrifuged bacterial cells were finally exposed to **CTC** (10.0 μM) in a 50 mM HEPES/CH_3CN buffer. The treated bacterial cells were examined at 100× magnification on a confocal laser scanning microscope (LSM 510 META, Carl Zeiss) and captured using a LSM5 Image Examiner (Figure 6). The microscopic images of bacterial cells in the absence and presence of Co^{2+} were observed for comparison. *E. coli* exposed to Co^{2+} followed by **CTC** developed a deep-pink color, as shown in the light microscopic image (Figure 6d). This clearly revealed that detection and staining of Co^{2+} in the cell is possible using colorimetric reagent **CTC**.

In summary, a new colorimetric chemosensor **CTC** was designed and synthesized. The sensitivity and selectivity of **CTC** to Co^{2+} over other metal ions in aqueous media were demonstrated by its optical response, ascribed to the formation of a push–pull Co^{2+} Schiff base complex. We have developed a **CTC**-based colorimetric kit for Co^{2+} detection and as a staining agent for Co^{2+} in cellular microorganisms. Therefore, molecular sensor **CTC** with optical responses in the visible region can be used as a practically viable probe for the environmental and biological sensing of cobalt ions.

■ ASSOCIATED CONTENT

Supporting Information. Experimental procedure, UV/vis, mass, and NMR spectra, and Job plots. This material is available free of charge via the Internet at http://pubs.acs.org.

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