



A simple internal charge transfer probe offering dual optical detection of Co (II) via color and fluorescence modulations

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ABSTRACT

A newly designed internal charge transfer chemosensor, DIPZON exhibits Co²⁺ selective optical responses, which include 112 nm red shift in absorption and a dramatic 37-fold enhancement in the fluorescence output in the buffer CH₃OH/H₂O (1:1 v/v) system. By contrast, the optical responses were not as sensitive with several other biologically relevant metal ions examined with the binding interactions following the sequence Co²⁺ > Cu²⁺ > Zn²⁺ > Cd²⁺ >> Ba²⁺ ≈ Ca²⁺ ≈ Mg²⁺ ≈ K⁺ ≈ Na⁺ ≈ Li⁺.

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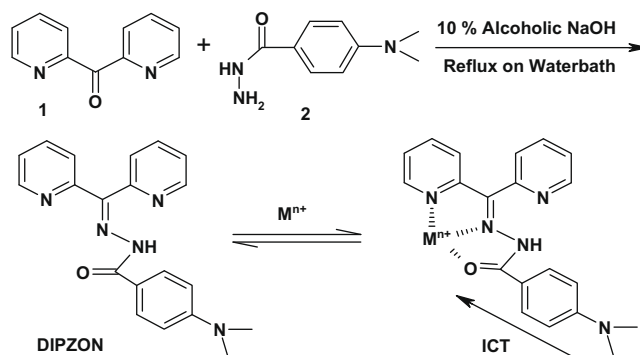
The designing of molecular probes capable of delivering easily recognizable responses upon selective interactions with metal ions of biological and environmental interest is currently an active area of research.¹ Of the many analytical tools currently available for metal ion recognition, the optical spectral technique, particularly the fluorescence spectroscopy is deemed to be of high practical utility due to high sensitivity, non-expensive instrumentation and the possibility of real time measurements.^{2,3} Cobalt is one of the essential trace-elements for all multicellular organisms with high concentrations in liver, bones and kidneys in human body.⁴ Besides being a core component of vitamin B12, cobalt is also involved in DNA synthesis, formation of red blood cells and maintenance of the nervous system.⁵ Cobalt deficiency has been implicated in retarded growth, loss of appetite and anemia.⁶ Furthermore, cobalt is also mildly toxic⁷ and the adverse health effects on workers arising from overexposures to airborne cobalt oxides and salts in cobalt refineries have been documented.⁸

Despite the biological and clinical importance of cobalt, surprisingly little research has been devoted towards developing Co²⁺ selective optical chemosensors. The older literature on cobalt quantification deals mainly with the spectrophotometric method using certain chelating ligands.⁹ However, in most cases the selectivity and the performance accuracies are not satisfactory. In a recent work, the detection of Co²⁺ has been described via fluorescence quenching,¹⁰ a protocol which is prone to error due to photobleaching or extraneous sources of quenching.¹¹ In addition, a few reports exist on cobalt detection using chemodosimeters¹² and chemiluminescent reagents.¹³ Though these approaches offer cobalt sensing via the more reliable fluorescence turn-on signaling, unfortunately the molecular probes are lost due to the chemical modifications. Clearly, there is need to design cobalt selective

chemosensors which could offer sensing, preferably via dual color as well as luminescence modulations.

In continuation of our interest in the internal charge transfer (ICT) concept as a promising metal ion-sensing strategy,¹⁴ we now report an easy to access ICT probe, DIPZON, which offers dual color modulation as well as fluorescence 'off-on' signaling for targeting Co²⁺. Synthesis of DIPZON was readily achieved in 70% yield by condensing dipyrindyl ketone **1** with 4-(*N,N'*-dimethylamino) benzoic hydrazide **2** in 10% alcoholic NaOH under reflux for 4 h. As illustrated in Scheme 1, the metal ion chelation at the three potential binding sites, comprising of the 'pyridyl nitrogen', 'azomethine' and the 'carbonyl' functions could trigger enhanced ICT via the electron donation from the dimethylamino group to the chelating carbonyl ligand. On this premise, we can expect to realize significant photophysical perturbations in the probe in the presence of strongly interacting metal ion(s).

The optical spectral sensitivity of the probe towards selected metal ions of biological relevance, namely Li⁺, Na⁺, K⁺, Ba²⁺, Ca²⁺, Mg²⁺, Cd²⁺, Zn²⁺, Co²⁺, and Cu²⁺ was investigated by adding metal



Scheme 1. Synthesis and the proposed interaction of DIPZON with metal ions, Mⁿ⁺.

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perchlorates in $\text{CH}_3\text{OH}/\text{H}_2\text{O}$ (1:1 v/v) buffered by 10 mM Tris–HCl to maintain a pH of 7.4 ± 0.1 . The absorption spectrum of the probe displayed absorption maxima at 267 and 368 nm with molar extinction coefficients of $1.76 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ and $2.01 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$, respectively. The lower energy maximum can be assigned to the ICT type transition of the dimethylaminobenzhydrazone chromophore on the basis of the positive solvatochromic behavior of the probe, while, the higher energy band can be attributed to the local excitation (LE) originating from the dipyriddy moiety.¹⁵

As shown in Figure 1, except for a slight decrease in the absorption maxima, the absorption profile of the probe remained largely invariant to the added Li^+ , Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Ba^{2+} , and Cd^{2+} up to 100 equiv, implying very poor affinities of these metal ions towards the probe. The most remarkable spectral modifications were observed upon adding 1 equiv of Co^{2+} , which resulted in the displacement of the λ_{ICT} from 368 to 480 nm, amounting to a red shift of 112 nm. Additionally, Co^{2+} also caused ca. 25% enhancement in the absorbance of the LE maximum. On the other hand, Cu^{2+} and Zn^{2+} at their limiting concentrations exerted less pronounced perturbations in the absorption behavior of the probe. Addition of Cu^{2+} caused the λ_{ICT} to be shifted to the red by a lower margin of 74 nm, while Zn^{2+} induced just a weak shoulder in 410–435 nm region and largely retained the original ICT absorption at 368 nm. Furthermore, in contrast to Co^{2+} , no detectable effects on the LE transition of the probe could be seen with either Cu^{2+} or Zn^{2+} .

The spectrophotometric titration of DIPZON ($2.83 \times 10^{-5} \text{ M}$) with Co^{2+} is depicted in Figure 2 and the inset shows the plots of changes in 368 and 480 nm maxima as a function of increasing concentrations of Co^{2+} . At 1:1 ligand/ Co^{2+} mole ratio, the original ICT band of the probe was fully replaced by the relatively more intense 480 nm absorption band. The observation of isosbestic points at 334 and 404 nm suggests the formation of a well-defined DIPZON- Co^{2+} complex, for which 1:1 complexation stoichiometry was established from the Job plot analysis (see Supplementary data).

The pronounced spectral variations, in particular the red shift of the ICT absorption by Co^{2+} (and to lesser extents by Cu^{2+} and Zn^{2+}) are consistent with the anticipated increase in the ICT interaction mediated by the electron donation from the donor dimethylamino group all the way to the chelating amide carbonyl, acting as an acceptor. This proposition is confirmed by the shift of the carbonyl stretching frequency in the IR from 1665 cm^{-1} in the unbound probe to 1603 cm^{-1} in the probe- Co^{2+} complex.

As expected, addition of the perchlorate salts of the weakly interacting metal ions, Li^+ , Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Ba^{2+} , and Cd^{2+} into

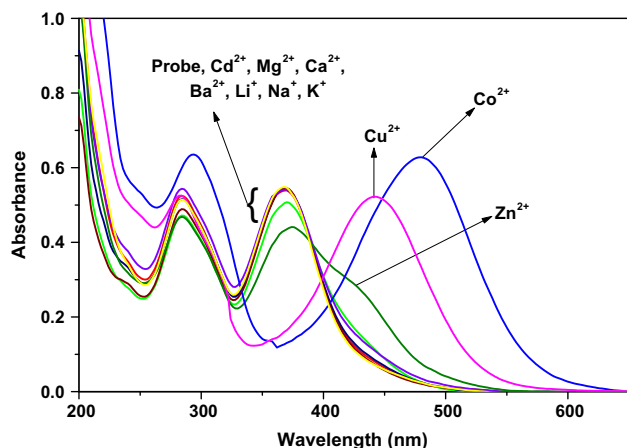


Figure 1. Absorption spectra of DIPZON ($2.83 \times 10^{-5} \text{ M}$) without and with the added Li^+ , Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Ba^{2+} and Cd^{2+} ($2.83 \times 10^{-3} \text{ M}$ each), Co^{2+} ($2.83 \times 10^{-5} \text{ M}$), Cu^{2+} ($5.83 \times 10^{-5} \text{ M}$) and Zn^{2+} ($7.47 \times 10^{-5} \text{ M}$) in $\text{CH}_3\text{OH}/\text{H}_2\text{O}$ (1:1 v/v) at pH 7.4 ± 0.1 .

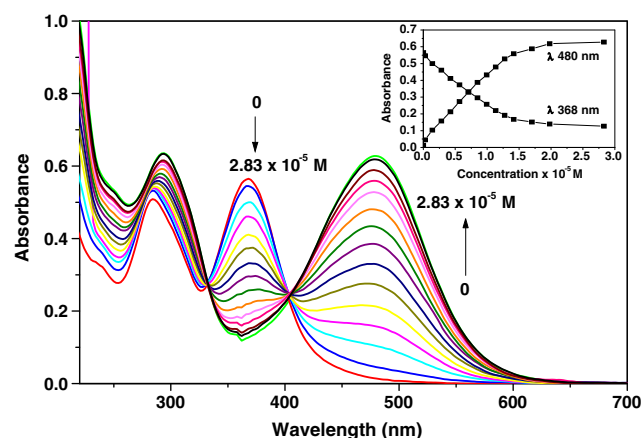


Figure 2. Spectrophotometric titration of DIPZON ($2.83 \times 10^{-5} \text{ M}$) with Co^{2+} (0– $2.83 \times 10^{-5} \text{ M}$). Inset: absorbance plot of DIPZON against increasing of Co^{2+} at λ_{max} 368 and 480 nm.

the solution of the probe did not cause noticeable color change, whereas the weakly interacting Zn^{2+} and Cu^{2+} turned the pale yellow solution of the probe into deeper shades of yellow. On the other hand, a distinctive color change from pale yellow to the red was observed upon adding Co^{2+} , indicating the potential of the probe to function as a ‘naked eye’ chemosensor for this ion.

Fluorescence behavior of the probe was also monitored to evaluate the sensitivity of DIPZON towards Co^{2+} . The free probe, when excited at 404 nm revealed a weak, structureless emission band at 428 nm with a quantum yield, $\phi_f = 0.007$ calculated with reference to coumarin151 ($\phi_f = 0.7$).¹⁶ This weakly emissive band probably has its origin in the ICT type excited state, which in many other cases is known to exhibit predominantly non-emissive character.¹⁷ As shown in Figure 3, the fluorimetric titration of the probe in the buffered $\text{CH}_3\text{OH}/\text{H}_2\text{O}$ (1:1 v/v) system with Co^{2+} resulted in a linear increase in the emission intensity of the 428 nm maximum. Interestingly, an overlapping but slightly blue shifted emission band also developed simultaneously at 400 nm. Though the nature of the excited states involved in this phenomenon is presently undefined, it is clear that two distinct emissive channels are involved within the metal-bound chromophore.¹⁸

At a limiting concentration of $3.5 \times 10^{-4} \text{ M}$ of Co^{2+} , the intensity of both the emission maxima peaked leading to ca. 37-fold emission enhancement with respect to that of the free probe at 428 nm. The ϕ_f for DIPZON- Co^{2+} complex at the saturating Co^{2+}

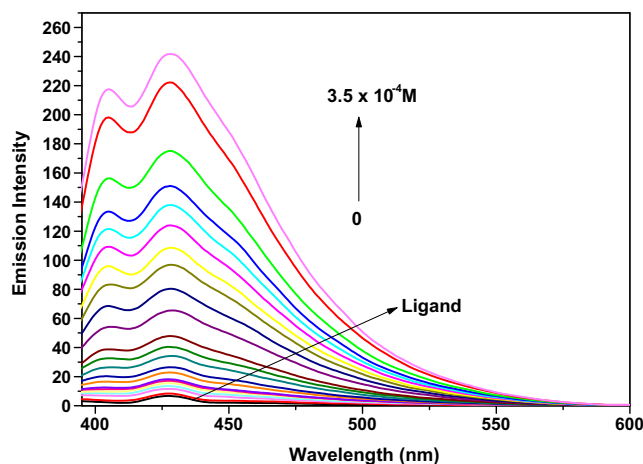


Figure 3. Fluorimetric titration ($\lambda_{\text{ex}} = 404 \text{ nm}$) of DIPZON ($1.67 \times 10^{-6} \text{ M}$) with Co^{2+} (0– $3.5 \times 10^{-4} \text{ M}$) in $\text{CH}_3\text{OH}/\text{H}_2\text{O}$ (1:1 v/v) at pH 7.4.

Table 1

Apparent stability constants, $\log K_s$ of DIPZON in the presence of metal ions in $\text{CH}_3\text{OH}/\text{H}_2\text{O}$ (1:1 v/v)

Ligand (L) + metal ions	$\log K_s$ (absorption)	$\log K_s$ (fluorescence)
L \subset Li ⁺	<0.1	<0.1
L \subset Na ⁺	<0.1	<0.1
L \subset K ⁺	<0.1	<0.1
L \subset Mg ²⁺	<0.1	<0.1
L \subset Ca ²⁺	<0.1	<0.1
L \subset Ba ²⁺	<0.1	<0.1
L \subset Cd ²⁺	0.91	1.04
L \subset Zn ²⁺	1.39	1.47
L \subset Cu ²⁺	2.72	2.55
L \subset Co ²⁺	3.97	3.84

was determined to be 0.24 with reference to coumarin151.¹⁶ In contrast to Co²⁺, the weakly coordinating Cu²⁺ and Zn²⁺ induced significantly less emission enhancements by ca. 9- and 6-fold, respectively. Consistent with the spectrophotometric results discussed above, the emission behavior of the probe was also not significantly modified upon adding Li⁺, Na⁺, K⁺, Mg²⁺, Ca²⁺, Ba²⁺, and Cd²⁺ up to 5×10^{-3} M (\leq 3-fold emission enhancements), implying poor binding interactions in the excited states as well (see Supplementary data). The chelation-induced fluorescence quenching is commonly induced by redox active Co²⁺ and Cu²⁺ ions via either ligand-to-metal electron or energy transfer mechanisms.^{10,19} Though not unprecedented,²⁰ the observation of fluorescence enhancements by these ions, including Zn²⁺ in the present circumstances may be ascribed to the conformational rigidification imposed on the probe upon metal ion complexation.^{14e,21} Hence, the non-radiative pathways prevailing in the free probe might be suppressed, leading to fluorescence switch-on in the presence of complexing metal ions.

The apparent stability constants ($\log K_s$) of complexations were determined by both spectrophotometric and fluorimetric titrations using the nonlinear curve fitting method. As shown in Table 1, the binding interactions follow the sequence Co²⁺ > Cu²⁺ > Zn²⁺ > Cd²⁺ >> Ba²⁺ \approx Ca²⁺ \approx Mg²⁺ \approx K⁺ \approx Na⁺ \approx Li⁺ and the $\log K_s$ determined by these two methods are of similar magnitudes. This observation suggests that the binding interactions in both the ground and excited states are of comparable strength. The detection limits of Co²⁺ calculated from the absorbance and fluorescence data were found to be 1.4×10^{-7} and 7.07×10^{-7} M, respectively (see Supplementary data). These detection limits are comparable and correspond well with the $\log K_s$ derived from the spectrophotometric and fluorimetric titrations, respectively. In comparison to several metal ions investigated including Cu²⁺ and Zn²⁺, at least over an order higher $\log K$ for cobalt signifies its relatively superior binding interaction.

In conclusion, a simple chemosensor, DIPZON has been developed along the lines of the internal charge transfer concept. The probe can selectively sense biologically significant Co²⁺ under the buffer conditions via the 'naked eye' detection and an efficient fluorescence 'off-on' signaling response. By contrast, biologically coexisting Na⁺, K⁺, Mg²⁺, Ca²⁺, Cu²⁺, and Zn²⁺ as well as Li⁺, Ba²⁺, and Cd²⁺ of some biological import, display negligible or relatively weak impact on the photophysical properties of the probe. Work to examine the nature of excited states involved in the complexations and the potential of the probe towards in vivo imaging applications are in progress.

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Supplementary data

Supplementary data (synthesis and characterization of DIPZON, spectral data, solvatochromic study, quantum yield determination, Job's plot, relative fluorescence enhancements, color variations in the presence of different metal ions and the plots of the detection limits) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.12.132.

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