2,7-Diferrocenyl-3,6-diazaocta-2,6-diene: A highly selective dual fluorescent sensor for Zn$^{2+}$ and Ag$^+$ and electrochemical sensor for Zn$^{2+}$

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2,7-Diferrocenyl-3,6-diazaocta-2,6-diene (DFDD), obtained by condensation reaction of acetylferrocene and ethylenediamine, exhibits Zn$^{2+}$ ion-induced enhancement of fluorescence intensity and Ag$^+$ ion-induced quenching of fluorescence intensity. The Zn$^{2+}$ ion shifts the redox potential of DFDD from +0.65 V to +0.615 ± 0.005 V as observed by cyclic voltammetry and square wave voltammetry. Cu$^{2+}$, Mg$^{2+}$, Mn$^{2+}$, Fe$^{2+}$, Co$^{2+}$, Ni$^{2+}$, Cu$^{2+}$, Cd$^{2+}$, Pb$^{2+}$ and Hg$^{2+}$ have very little effect on fluorescence intensity of DFDD. These metal ions together with Ag$^+$ ion have no effect on the electrochemistry of DFDD. Both, fluorescence and UV-visible spectroscopic data show a 1:1 interaction between DFDD and Zn$^{2+}$ with binding constant $K_M = 75 M^{-1}$. The applicability of DFDD as fluorescent sensor for Zn$^{2+}$ ion has been shown in its estimation in commercial tablets.

**Keywords:** Sensors, Electrochemical sensors, Fluorescent sensors, Fluorescence, Cyclic voltammetry, Square wave voltammetry, Zinc, Silver

Design and synthesis of molecular systems which can selectively detect biologically important ions is one of the most challenging areas of research. Zinc is an essential element in all biological systems and is present in more than 70 enzymes. Among its many roles in the human body, zinc regulates gene expression is involved in cellular apoptosis and is a constituent of RNA and DNA polymerases. Deficiency in Zn$^{2+}$ or disorder in Zn metabolism leads to neurological problems and diseases such as Alzheimer’s disease, amyotrophic lateral sclerosis, Guan ALS-Parkinsonism dementia and hypoxia-ischemia and epilepsy. Due to its importance in various cellular functions, its detection is of particular interest.

Fluorescence measurement of specific biological molecules and ions by artificial chemosensors is a versatile technique having high sensitivity, rapid response, and easy performance. Development of new fluorescent sensors for zinc has gained current research interest. A novel pyrazoline derivative synthesized starting from a chalcone and 3-chloro-6-hydratizedilpyridazine is reported to determine Zn$^{2+}$ ion with high selectivity and a low detection limit in CH$_2$CN:EtOH (90/10, v/v)$^{11}$. New bis(pyrrol-2-ylmethylenamine) ligands are also reported to act as selective sensor$^{12}$ for Zn$^{2+}$. [N(NO-di(quinoline-2-methylene)-1,2-phenylenediimine) is reported to exhibit high selectivity toward Zn$^{2+}$ over other metal ions including Cd$^{2+}$ due to the formation of a 1:1 metal:ligand complex$^{13}$. A ratiometric fluorescent sensor for zinc ions based on covalently immobilized derivative of benzoazoxazole is also known$^{14}$. Schiff base derived from benzil and ethylenediamine has been very recently reported as fluorescent Zn$^{2+}$ ion sensor$^{15}$. There are, however, very few reports on electrochemical sensing of Zn$^{2+}$ ion. Reports are available for Zn$^{2+}$ ion determination based on adsorptive stripping voltammetry$^{16,18}$ and cyclic voltammetry$^{19}$ techniques. One single molecule capable of selectively sensing Zn$^{2+}$ ion both by fluorescence and electrochemically is probably not reported.

Although silver is widely used in the electrical industry, photographic and imaging industry, and pharmacy, it has adverse biological effects. Biotoxicity of silver includes inactivation of sulphydryl enzymes, combination with amine, imidazole, and carboxyl groups of various metabolites, causing serious environmental and health problems. A number of reports are available on fluorescence detection of Ag$^+$ ions$^{20,26}$. Reports on dual fluorescent sensors for more than one metal ions are somewhat rare and is known for a few pairs of metal ions$^{27-33}$, e.g., Cu$^{2+}$, Ni$^{2+}$; Zn$^{2+}$, Ni$^{2+}$; Cu$^{2+}$, Zn$^{2+}$ and Pb$^{2+}$, K$^+$. To the best of our knowledge there is no report of fluorescent dual sensor for Zn$^{2+}$ and Ag$^+$. The molecule, 2,7-diferrocenyl-3,6-diazaocta-2,6-diene (DFDD, I) has been reported$^{14}$ for preparing metal complexes and studying their properties. DFDD is the product of condensation of acetylferrocene and ethylenediamine in minimum amount of methanol and exhibited fluorescence of high intensity in the emission range 380 – 680 nm when excited at 360 nm.
Herein, we report DFDD as a selective dual fluorescent sensor for Zn\(^{2+}\) ion (by switch “on” mode) and for Ag\(^{+}\) ion (by switch “off” mode). The redox potential of DFDD undergoes a 0.045 ± 0.005 V negative shift on interaction with Zn\(^{2+}\) ion. Several metal ions like Ca\(^{2+}\), Mg\(^{2+}\), Mn\(^{2+}\), Fe\(^{2+}\), Co\(^{2+}\), Ni\(^{2+}\), Cu\(^{2+}\), Cd\(^{2+}\), Pb\(^{2+}\) and Hg\(^{2+}\), singly or together, do not interfere in the fluorescence property of DFDD while these metal ions along with Ag\(^{+}\) ion do not effect the electrochemical properties of DFDD.

**Experimental**

All chemicals were from Merck (all metal salts were sulphates except for AgNO\(_3\) and PbNO\(_3\)) and used without any further purification. \(^1\)H-NMR spectra were recorded using a Bruker Ultrasound 300 MHz NMR spectrometer. Chemical shifts are expressed in ppm (in CDCl\(_3\), with TMS as internal standard) and coupling constants (J) in Hz. The UV-visible spectrum was recorded on a Shimadzu UV-vis-1800 spectrophotometer. Fluorescence experiments were performed on a Hitachi F-2500 spectrophotometer at room temperature. FTIR data were collected using KBr pellet, on Perkin Elmer spectrophotometer (RX1).

Electrochemical experiments were carried out at room temperature using CHI 600B electrochemical analyzer (USA) on a conventional three electrode system with Ag/AgCl (3 M KCl) as the reference electrode, a platinum wire as the counter electrode and a glassy carbon disc (GC) as working electrode. Tetrabutylammonium perchlorate (0.1 M) solution was used as supporting electrolyte. Nitrogen gas was purged through the electrolytic solution for at least 5 minutes to remove any dissolved oxygen before every experiment. Nitrogen atmosphere was maintained over the electrolytic solutions during each experiment. Prior to every experiment the GC electrode was cleaned as reported earlier\(^3^5\). For square wave voltammetry experiments, the square wave amplitude was 25 mV, the frequency was 15 Hz and the potential step height for base stair case wave front was 4 mV.

2,7-Diferrocenyl-3,6-diazaocta-2,6-diene (DFDD) was prepared as per reported procedure\(^3^4\) by refluxing a mixture of 1 mmol (0.230 g) acetylferrocene and 0.5 mmol (0.4 mL) ethylenediamine in 10 mL methanol for 2 hours. A brownish red crystalline compound was obtained, which was filtered and recrystallized from methanol. Yield: 68.9 %; m. pt: 90 ºC; soluble in MeOH.

UV-visible spectra of the compound in methanol showed \(\lambda_{\text{max}}\) at 336 nm and 457 nm, originating in the ferrocenes in DFDD. FTIR spectra of the compound recorded in KBr pellet showed the peak for (\(\nu\)C=N) at 1589 cm\(^{-1}\). \(^1\)H NMR of the compound in CDCl\(_3\) was recorded which showed \(\delta\)H values at 1.656 (s) ppm (2H, CH\(_2\)); 2.406 (s) (3H, Me); 4.212 (s) (5H, ferrocene); 4.513, 4.780 (2H, s, ferrocene).

**Results and discussion**

Methanol-water solution, 1:1 (\(v/v\)) of DFDD (10\(^{-4}\) M) shows fluorescence emission in the range 380 - 600 nm with \(\lambda_{\text{max}}\) at 450 nm when excited with 360 nm radiation. The fluorescence intensity remains unaffected for over a period of 1 week and also when \(pH\) was changed from 2 - 10.

The fluorescence titration reaction of 1:1 (\(v/v\)) methanol-water solution of DFDD by a host of M\(^{2+}\) ions was carried out. A steady and smooth enhancement in intensity in the case of only Zn\(^{2+}\) ion was observed which saturates at 1.0 equivalent (Fig. 1).

![Fig. 1 — Changes in fluorescence spectra of DFDD (10\(^{-4}\) M) as a function of added Zn\(^{2+}\) ion concentration in 1:1 (\(v/v\)) CH\(_3\)OH-H\(_2\)O solution. [Inset: Plot of \(I/I_0\) as a function of Zn\(^{2+}\) ion concentration.]]
The overall enhancement was found to be 2.4 fold at saturation. The plot of \( \frac{I}{I_0} \) increases linearly with \( \text{Zn}^{2+} \) ion concentration as shown in inset of Fig. 1 \( (\frac{I}{I_0} = 1.277(\text{Zn}^{2+}) + 0.9599) \). The enhancement in fluorescence intensity may be attributed to the reversal of the photoinduced electron transfer (PET) of the ethylenediamine N lone pairs upon \( \text{Zn}^{2+} \) binding.

A least squares fitting of the linear plot of log \( \frac{(I-I_0)/(I_{\text{max}}-I)}{I_0} \) versus log \( [\text{Zn}^{2+}] \) yielded the slope as 1.17 ± 0.02, indicating a 1:1 binding between DFDD and \( \text{Zn}^{2+} \). The binding constant was calculated to be 75 M\(^{-1}\).

The effect of various divalent ions on fluorescence intensity of DFDD was studied. Titrations carried out with \( \text{Mg}^{2+}, \text{Mn}^{2+}, \text{Fe}^{2+}, \text{Cd}^{2+}, \text{Hg}^{2+} \) and \( \text{Ni}^{2+} \) ions registered a very small increase in fluorescence intensity. A moderate enhancement in the intensity of DFDD was observed when \( \text{Pb}^{2+} \) and \( \text{Ca}^{2+} \) ions were added. Titrations carried out with metal ions \( \text{Cu}^{2+} \) and \( \text{Co}^{2+} \) exhibited small quenching in fluorescence intensity of DFDD, while titration with \( \text{Ag}^{+} \) ion quenched the fluorescence considerably.

Quenching effect of \( \text{Ag}^{+} \) ion on the fluorescence spectra of DFDD \( (0.5 \times 10^{-4} \text{ M}) \) has been shown in Fig. 2. The plot of \( \frac{I}{I_0} \) versus \( [\text{Ag}^{+}] \) showed a smooth decrease in intensity \( (\frac{I}{I_0} = 0.1126 \text{ [Ag}^{+}] + 0.9963) \) with co-efficient of linearity as 0.9872 (Fig. 2, inset). Addition of NaCl to the already quenched DFDD was found to regenerate the original intensity of DFDD. Hence, fluorescence quenching of DFDD by \( \text{Ag}^{+} \) is reversible with respect to chloride ion. Thus, the steady-state fluorescence data obtained from the titration of DFDD with various \( \text{M}^{2+} \) ions clearly suggests that it can detect \( \text{Zn}^{2+} \) ion by fluorescence enhancement and \( \text{Ag}^{+} \) ion by fluorescence quenching. The lower detection limits were found to be \( 10^{-6} \text{ M} \) for \( \text{Zn}^{2+} \) and \( 0.5 \times 10^{-5} \text{ M} \) for \( \text{Ag}^{+} \) ion.

In acetonitrile, the fluorescence intensity of the sensor molecule is less as compared to that in methanol-water. On addition of \( \text{Zn}^{2+} \), a three-fold enhancement in fluorescence intensity was observed. For the other metal ions, the results were similar to those obtained in methanol-water.

In order to confirm the binding and stoichiometry of \( \text{Zn}^{2+} \) with DFDD, electronic spectra of DFDD was recorded at varying added concentration of \( \text{Zn}^{2+} \) ion. The absorbance at \( \lambda_{\text{max}} \) 336 nm and 457 nm was found to increase with \( \text{Zn}^{2+} \) ion concentration with an isosbestic point at 300 nm, indicating a transition between free species and complexed species. The increase in the 336 nm band is indicative of the interaction of \( \text{Zn}^{2+} \) ion with nitrogen of ethylenediamine.

The absorbance at 457 nm was recorded as a function of [\( \text{Zn}^{2+} \)] ion concentration and the plot of log \( \{A_{\text{aq}}(\text{Ag}^{+})/(A_{\text{aq}}(\text{Ag}^{+}))\} \) versus log \( [\text{Zn}^{2+}] \) was found to be linear. The least squares fitting of data showed that the slope was 1.112 ± 0.002, indicating 1:1 stoichiometric binding between DFDD and \( \text{Zn}^{2+} \) which is in agreement with data obtained from fluorescence. The binding constant 70.795 M\(^{-1}\) was similar to that obtained from fluorescence intensity plot. The \(^{1}H\) NMR of DFDD in presence of 1 equivalent of \( \text{Zn}^{2+} \) ion was recorded and the peaks due to –CH\(_2\) and –CH\(_3\) protons were found to shift downfield while the peaks due to protons in C\(_5\)H\(_5\) rings were unaffected. This result confirms the binding of \( \text{Zn}^{2+} \) to the two imine N of DFDD.

DFDD showed quasi reversible cyclic voltammetric response in 1:1 CH\(_3\)OH:H\(_2\)O on GC electrode; both the cathodic and anodic currents were found to increase with scan rate. The redox potential was found to be 0.660 ± 0.005 V (\( \Delta E = 0.182 \text{ V} \)) at scan rate 0.050 V s\(^{-1}\) versus Ag-AgCl as reference. The plot of cathodic and anodic current against square root of scan rate was found to be linear, indicating a reversible redox process. As ferrocene is a well known electrochemically reversible redox molecule, the observed cyclic voltammogram is due to ferrocene/ferroceenium couple presents in DFDD.

![Fig. 2 — Changes in fluorescence spectra of DFDD (0.5 \times 10^{-4} \text{ M}) as a function of added \text{Ag}^{+} \text{ ion concentration in 1:1 (v/v) CH\(_3\)OH-H\(_2\)O solution. [Inset: Plot of } \frac{I}{I_0} \text{ as a function of } \text{Ag}^{+} \text{ ion concentration]}


Figure 3 shows the effect of Zn\(^{2+}\) ion concentration on the cyclic voltammogram of DFDD. Addition of aqueous solution of Zn\(^{2+}\) ion into the electrolytic medium shifted the cyclic voltammogram of DFDD in negative direction and the redox potential became 0.615 ± 0.005 V (i.e., 0.045 V shift was observed) at Zn\(^{2+}\) ion concentration 1.23 mM. The plot of redox potential versus concentration of Zn\(^{2+}\) ion shown in Fig. 3 (inset) clearly reveals that both the cathodic and anodic currents increased due to the interaction with Zn\(^{2+}\) ion. The enhancement in cathodic and anodic currents of DFDD was linear with Zn\(^{2+}\) ion concentration and maximum enhancement was approximately 1.20-fold at 1 equivalent Zn\(^{2+}\) ion concentration.

The square wave voltammogram of DFDD at different added concentration of Zn\(^{2+}\) ion is given in Fig. 4. For a given addition of Zn\(^{2+}\) ion, the shift in redox potential values obtained were in good agreement with that obtained by cyclic voltammetry. The plot of current versus Zn\(^{2+}\) ion concentration was linear as shown in Fig. 4 (inset).

Cyclic voltammogram and square wave voltammogram of DFDD was also recorded in presence of Ca\(^{2+}\), Mg\(^{2+}\), Mn\(^{2+}\), Fe\(^{2+}\), Co\(^{2+}\), Ni\(^{2+}\), Cu\(^{2+}\), Cd\(^{2+}\), Pb\(^{2+}\) and Hg\(^{2+}\) ions. No significant effects in the cyclic and square wave voltammograms were observed when these ions were added into the electrolytic medium singly or together. This indicates the selectivity of the sensor towards Zn\(^{2+}\) over the above mentioned metal ions. The electrochemical behavior of DFDD remained the same in acetonitrile as in methanol-water medium.

Applicability of the sensor DFDD was tested in the estimation of concentration of Zn\(^{2+}\) in multimineral tablet (Supra Cal, MMC Health Care, H.P, India). The test solution was prepared by dissolving one tablet in 50 mL phosphate buffer solution (PBS) (pH 7.0) and the standard solution of Zn\(^{2+}\) ion was prepared by dissolving 4 mg in 50 mL PBS. The fluorescent intensity of 0.1 mM solutions of DFDD was measured at varying concentrations of Zn\(^{2+}\) (0.025 mM, 0.05 mM, 0.075 mM, 0.1 mM, 0.125 mM, 0.150 mM) by adding appropriate volume of the test solution. Another set of experiments was performed by adding appropriate volume of the standard Zn\(^{2+}\) solution to 0.1 mM DFDD so that Zn\(^{2+}\) ion concentration is the same as in the test solution. The relative increase in intensity for these two sets of measurements was found to be comparable (Table 1), showing the applicability of DFDD as a sensor for Zn\(^{2+}\) ion.
In summary, the present study shows that 2,7-diferrocenyl-3,6-diazaocta-2,6-diene acts as a fluorescent sensor for Zn$^{2+}$ ion by “switch on” mode while it acts as fluorescent sensor for Ag$^+$ by “switch off” mode. The fluorescence quenching of DFDD by Ag$^+$ ion is reversible with respect to chloride ion. DFDD can also recognize Zn$^{2+}$ ion electrochemically.

Several metal ions, viz., Fe$^{2+}$, Cu$^{2+}$, Cd$^{2+}$, Co$^{2+}$, Pb$^{2+}$, Hg$^{2+}$, Ca$^{2+}$, Mn$^{2+}$, Ni$^{2+}$ and Mg$^{2+}$ do not interfere with the fluorescent determination of Zn$^{2+}$ and Ag$^+$ ion. This group of metal ions and Ag$^+$ also do not interfere in the electrochemical determination of Zn$^{2+}$.

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