



Detection of cobalt(II) and copper(II) selectively using versatile fluorochemosensors in parts per trillion level in aqueous solution

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Abstract The tetra terpyridyl appended porphyrin was used for the detection of Co(II) and Cu(II) ions in aqueous media. The porphyrin was synthesized by condensation of a Formyl Phenyl Terpyridine and pyrrole. The fluorescence response and Binding constant value predicated the strong affinity of the fluorophore specifically for Co(II) ion followed by Cu(II) ion. All the metal ion can be detected in 10^{-11} M concentration.

Keywords Metal Cation Sensing, TtpyPP (5,10,15,20-Tetrakis[4'-(terpyridinyl)phenyl]porphyrin), Fluorochemosensing, Porphyrins, Methyl Phenyl Terpyridine (4-methyl-phenyl-2,2':6',2''-terpyridine), Formyl Phenyl Terpyridine(4-formyl-phenyl-2,2':6',2''-terpyridine)".

Introduction

Metal ions are ubiquitous in nature and perform diverse biological functions. It is important to understand the impact of these metal ions in order to elucidate the structure and activity of biological systems [1]. Many metal salts are beneficial but some like Hg (II), Cd(II), Pb(II), etc., are toxic even in trace amounts [2]. Hence detection of these ions in low concentration is an important area which has large ramifications in biological studies as well [3-7]. Large number of work has been carried out to develop suitable fluorophore system for detection of various metal ions. Till date no single system exists which is ideal as the desired sensitivity has not been achieved. Highly sensitive fluorophores can be used in fluorescence sensing which will shed more light on a biological activity [8-13].

Large number of chemo sensors have been developed [14-18] but the complexity of biological systems demands ever increasing improvement in selectivity and sensitivity of the sensors, so that its impact in various biological processes can be targeted, mapped and evaluated. Thus it is important to develop fluorophores of increasing selectivity and sensitivity.

Some of the conjugated fluorophores exhibits an additional shift of λ_{em} along with the traditional enhancement/quenching of fluorescence intensity utilized for detection of metal ions. This feature of shifting of λ_{em} can be used judiciously to enhance the sensitivity as well as the selectivity of the cation detection by the fluorophore. Porphyrin is an extensively studied fluorescent macrocycle to evaluate the various biological activities and has been coupled with terpyridyl units for sensing applications.

The conjugate fluorophore is found to detect Co(II) ion most effectively followed by Cu(II) ion in the range of 10^{-11} M i.e., in 10 ppt level. Other metal ions studied in this work is also detected in ppt level. This is the lowest detection limit reported so far for the metal ions studied to the best of our knowledge.

Materials and Methods

General

The chemicals were obtained from Sigma Aldrich and Merck. Steel bomb for autoclave oxidation was fabricated by the vendor which was of 20 ml and 50 ml capacity. The compounds were characterized using UV-visible Spectrophotometer, ¹H NMR, Mass spectrometry etc.



Synthesis

Formyl Phenyl Terpyridine was synthesized by one pot autoclave oxidation of Methyl Phenyl Terpyridine and was used after chromatographic separation using mixture of CHCl_3 and Hexane as eluent [19]. TtpyPP was prepared by condensation between freshly distilled pyrrole and Formyl Benzyl Terpyridine using Alder Longo method. The free base porphyrin was characterized by $^1\text{HNMR}$ (400 MHz), UV-Visible (Shimadzu 2450), and Mass (MaldiTof) and CHN analysis.

Preparation of Stock Solution

Preparation of Fluorophore Solution

1.54 mg of TtpyPP was added in 10 ml of CHCl_3 to prepare 10^{-4} M TtpyPP solution. 3 μl of stock solution was added to the 3 ml of pure acetonitrile. The Fluorescence spectra of the pure TtpyPP solution in acetonitrile was obtained. The absorption spectra of the free base was obtained whose OD value was less than 0.1, a standard method for studying fluorescence of porphyrins.

Preparation of 10^{-8} M salt Solution

Stock solution of metal acetates were prepared which were diluted to 10^{-8} M solution.

Experimental

3 μl of 10^{-4} M porphyrin solution was added to 3 ml of AR grade acetonitrile taken in the Quartz Cuvette. Absorption spectra was recorded to check the values of O.D such that it did not exceed 0.1. At the wavelength of excitation 423 nm, Fluorescence spectra was recorded. That was followed by the addition of 3 μl of 10^{-8} M salt solution. The solution was allowed to equilibrate for one minute and fluorescence spectra was recorded. After the measurement the solution was discarded and the cuvette was washed several times with distilled water followed by pure acetonitrile. Same process was repeated for all the metal ions studied.

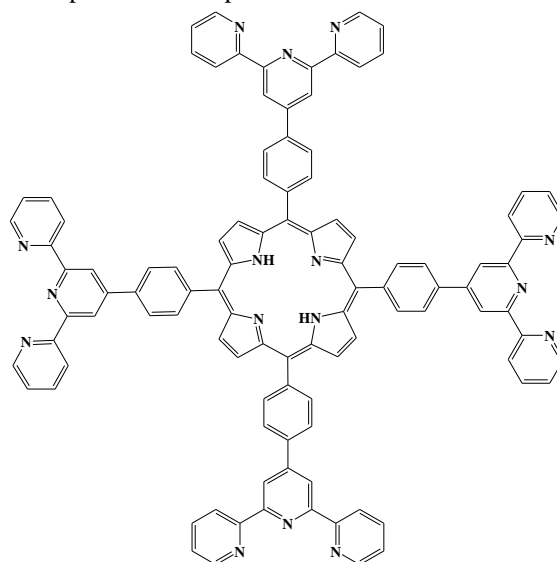


Figure 1: Structure of TtpyPP (5,10,15,20-Tetrakis[4'-(terpyridinyl)phenyl]porphyrin) with four peripheral Terpyridine units

Table 1: Fluorescence Response and Binding Constant values of TtpyPP with respective metal ions.

Metal Ion	Normalized I/I^0 (Fluorescence Response)	K_s (Binding Constant) $\times 10^8$
Hg(II)	0.827988	1.09124
Zn(II)	0.412495	3.19768
Co(II)	0.081508	20.2435
Cu(II)	0.161778	9.70306
Mn(II)	0.607711	1.84925
Ni(II)	0.57486	2.01208
Cd(II)	0.632903	1.73584
Pb(II)	1	0.73152
Na(I)	0.954308	0.81443



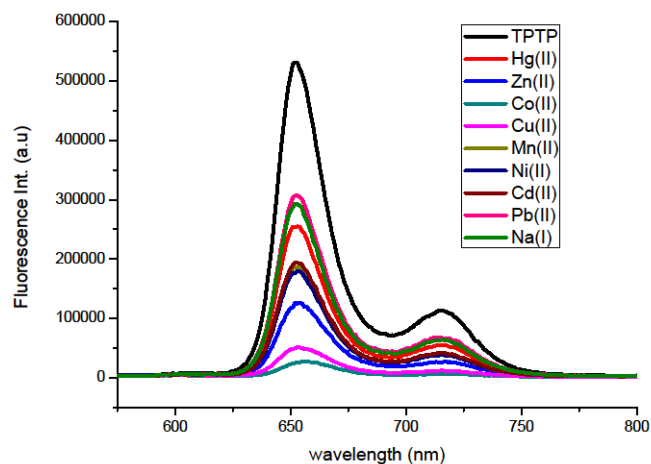


Figure 2: Fluorescence Quenching of different metal ions when added to the $10^{-7}M$ TPTP solution.

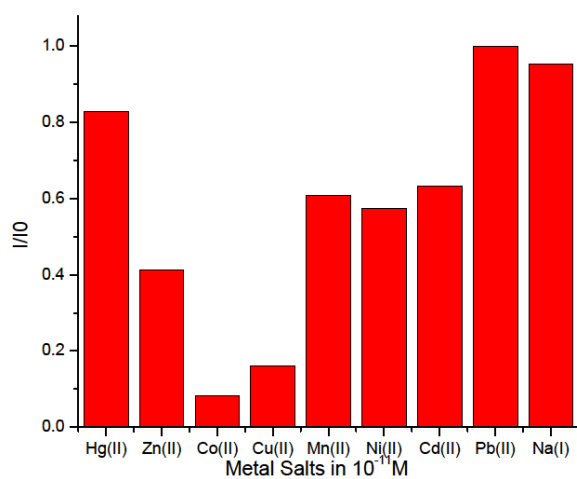


Figure 3: Bar diagram representing Fluorescence Response of TptyPP in presence of different metal ions

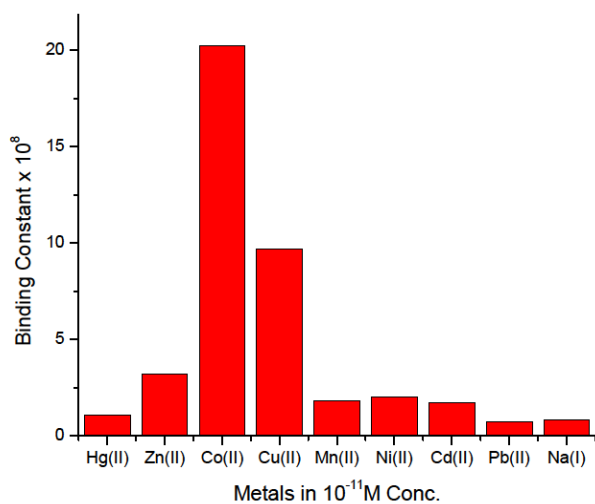


Figure 4: Bar Diagram representing binding constant of different metal ions with Free base TptyPP



Result and Discussion

As shown in the Figure 1, the four terpyridyl units appended to porphyrin core via phenyl rings are suitably placed at right angle to each other to interact with the metal ions in the vicinity. Any interactions with the terpyridyl unit is expected to cause a change in photophysical properties of porphyrin if they are strongly coupled with each other.

It is observed in the present study that all the metal ions studied in this work results in quenching of fluorescence emission of the porphyrin when excited at 423 nm.

One can anticipate that at the concentration of metal ions used and the ambient temperature, the porphyrin core is not getting metallated by metal ions such as zinc. The fluorescence emission does not indicated metalation of porphyrin in detectable amount. Any metalation would have reflected in the blue shift of the emission band.

From figure 3, it is clear that Co(II) ion shows maximum fluorescence response followed by Cu(II). Least response is exhibited by Pb(II) followed by Na(I). Fig.4 clearly shows the strong binding of the Co(II) to Terpyridine units. Table 1 shows the numerical value for normalized fluorescence response and binding constant of Fluorophore to the diff metal ions. The present study indicates that the sensor molecule exhibits moderate selectivity and high sensitivity for Co(II) ions. All the metals individually can be detected at a concentration of 10^{-11} M.

Conclusion

Tetraterpyridyl appended porphyrin is excellent sensor for the detection of especially Co (II) and Cu(II) ions present in aqueous solution. Further work is under progress to evaluate the sensitivity and selectivity of sensor towards other metal ions especially those that are toxic to the living beings.

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