

# Free-Base Porphyrins as Chemical Probes for Heavy Metal Ions Detection

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**Abstract.** Heavy metals are one of the major contaminants in water. They come from various human activities which include mining, smelting, and industrial discharge to name a few. Heavy metals pose a toxic danger to human beings even at their minute concentration. Current methods of detection suffer from numerous limitations such as complicated sample preparation, high cost of instruments, and the need for professional chemists. These challenges make them unsuitable for in situ and real-time monitoring of samples. Fluorescence spectroscopy has emerged as an alternative for sensing applications. They have high sensitivity, good selectivity, and only require small amounts of fluorescent probes. In this study, free base porphyrins were selected as a fluorescent chemical probe to detect the presence of commonly discharge heavy metals – lead ( $\text{Pb}^{2+}$ ) ions and nickel ( $\text{Ni}^{2+}$ ) ions. Solution-based assay and fluorescent measurements were used throughout the study. From the fluorescent analysis, porphyrins showed more selective and sensitivity towards  $\text{Pb}^{2+}$  ions. The study also investigated the photostability of porphyrins when porphyrins solutions were put under ambient light conditions. This solution-based assay fluorescent measurement shows promising potential application of porphyrins to be used as chemical probes on another sensor medium such as on optical fibre and thin film study.

## 1. Introduction

Heavy metals such as copper, mercury, lead, and nickel have been reported as one of the major contaminations in water as well as inland.[1] The sources of these heavy metals include smelting, mining, and industrial discharge. When exposing to human beings and animals, they could pose a toxic danger even at their minute concentration. Conventional methods for the detection of heavy metals at the lowest concentrations are atomic absorption spectroscopy (AAS), capillary electrophoresis (CE), and inductively coupled plasma emission spectrometry (ICP-AES) to name a few.[2] Despite their high sensitivity and selectivity, these techniques suffer from numerous limitations such as complicated sample preparation, high cost of instruments, and the need for professional chemists.[2] These challenges make them unsuitable for in situ and real-time monitoring of samples, especially for remote applications.

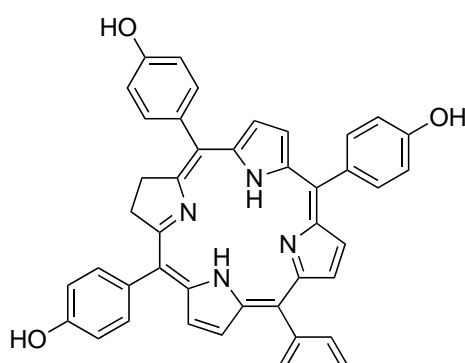
Fluorescent chemical sensors have been developed to detect the presence of a variety of analytes such as  $\text{H}^+$  ions for pH sensors, gaseous ( $\text{O}_2$  and  $\text{CO}_2$ ), and metabolite such as glucose.[3] Among chemical probes that have been reported to detect the presence of heavy metals are fluorescein and porphyrins. Porphyrins are macrocyclic aromatic compounds consisted of four pyrrole rings



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interconnected by the methine bridges. Porphyrins have 26  $\pi$ -electrons in a delocalised system where these electrons are the ones responsible for the optical, electronic, magnetic, redox, and catalytic properties of porphyrins. Porphyrins could exist as either free base porphyrins or metalloporphyrin complexes (with transition metals coordinated at the centre of the ring) [2]. Free-base porphyrins have been reported to be used as chemical probes for heavy metals detection where the absorption principles were used mainly while metalloporphyrins have been widely reported as the best candidate for fluorescent-based oxygen optical sensors [2, 4].

This work is focussed on deploying free-base porphyrins, 5,10,15,20-tetrakis(4-hydroxyphenyl)-21H,23H-porphine, and exploiting the advantages of a fluorescent spectrophotometer to study the sensitivity and selectivity of porphyrins towards two different heavy metals; lead and nickel. This solution-based assay is aimed to study how the presence of different concentrations of heavy metals affect the fluorescence spectra and intensity of porphyrins.



**Figure 1.** Chemical structure of the free base porphyrins, 5,10,15,20-tetrakis(4-hydroxyphenyl)-21H,23H-porphine.

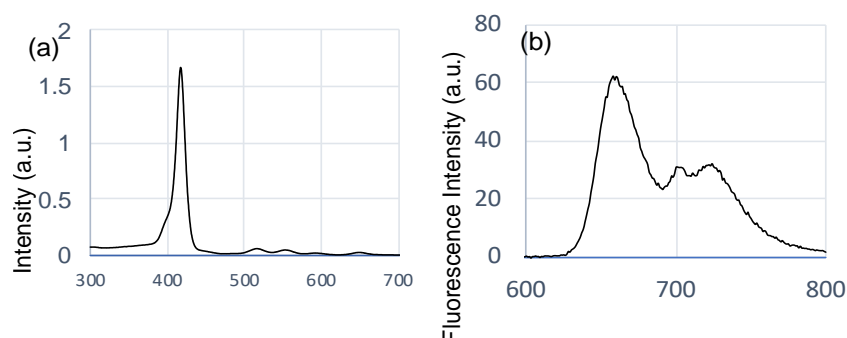
## 2. Experimental

All chemicals were purchased from Merck and were used without further purification unless otherwise stated. The absorption spectra of 5,10,15,20-tetrakis(4-hydroxyphenyl)-21H,23H-porphine were recorded using a UV-Vis spectrophotometer using a 1 cm path length quartz cuvette using ethanol as the solvent. The fluorescence spectra were recorded using a fluorescence spectrophotometer using a 1 cm path length fluorescence clear cuvette. The porphyrins were dissolved in ethanol for the stock solution preparation with a concentration of 1 mg/L. Lead ( $Pb^{2+}$ ) and nickel ( $Ni^{2+}$ ) stock solutions were prepared in distilled water, and the different concentrations of the metals were prepared by serial dilution accordingly. For fluorescence measurement, an equal volume of porphyrins solution and heavy metals solution were mixed in a cuvette. The blank solution consisted of an equal volume of distilled water and ethanol.

## 3. Results and Discussion

### 3.1. Absorption and Fluorescence Spectra

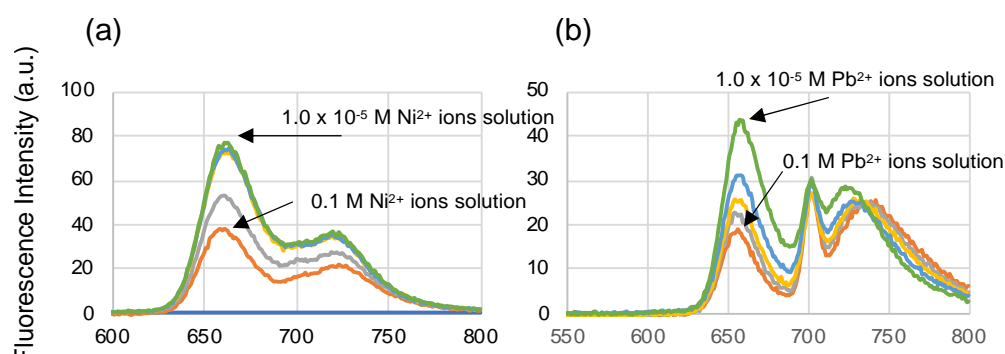
5,10,15,20-tetrakis(4-hydroxyphenyl)-21H,23H-porphine (porphyrins) have highly conjugated pi-electron system thus explaining the colour and the intensity of its absorption and emission spectra. Figure 2(a) show the absorption and fluorescence spectra of 5,10,15,20-tetrakis(4-hydroxyphenyl)-21H,23H-porphine. The maximum absorption peak was observed at 410 nm and smaller peaks between 500 to 600 nm. Fluorescence spectrum of porphyrins is shown in Figure 2(b). When excited at 350 nm, the porphyrins produced an emission at 655 nm and shoulder peaks at around 700 – 720 nm.



**Figure 2.** (a) The absorption spectrum of 5,10,15,20-tetrakis(4-hydroxyphenyl)-21H,23H-porphine in ethanol and (b) the fluorescence spectrum of 5,10,15,20-tetrakis(4-hydroxyphenyl)-21H,23H-porphine in ethanol

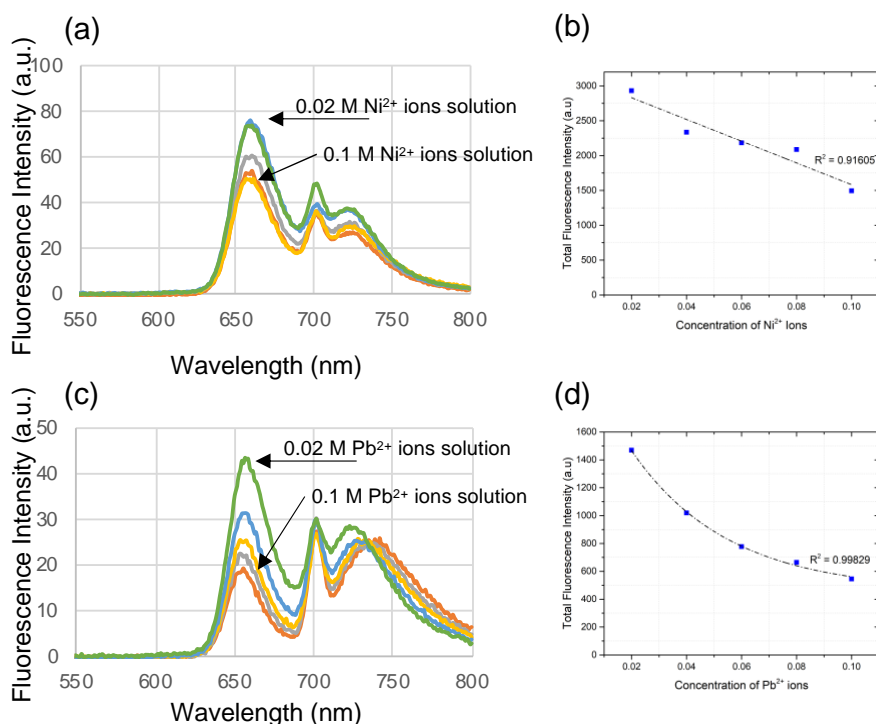
### 3.2. Fluorescent Studies of Porphyrins on $Pb^{2+}$ and $Ni^{2+}$ ions

Porphyrins are water-insoluble, which limits their application in fluorescence analysis and detection. Therefore, to improve the solubility of porphyrins, porphyrins solutions were prepared in ethanol. In this study, a qualitative on-off experiment using a fluorescence spectrophotometer was conducted to confirm the fluorescence quenching by Ni(II) and Pb(II). Simply, 0.1 M to  $1.0 \times 10^{-5}$  M range solution of Ni(II) and Pb(II) was added into porphyrin suspension containing 1 mg/L of porphyrin of ethanol. All the sample solutions were then mixed thoroughly. Figure 3 shows the fluorescence spectra of porphyrins when Ni(II) ions and Pb(II) ions were added into the porphyrins solutions, respectively. The results show clearly that both Pb(II) and Ni(II) ions quench the fluorescence emitted by porphyrin. From this large range concentration fluorescence analysis, the range between 0.02 M to 0.1 M was selected for linear calibration because of the larger difference in spectra intensity between lower and higher Ni(II) and Pb(II) concentrations.



**Figure 3.** (a) Fluorescence spectra of porphyrin (0.5 mg/L) at 0.1 M, 0.05 M, 0.001 M, 0.0005 and 0.00001 M of Ni (II) solutions in distilled water/ethanol, and (b) fluorescence spectra of porphyrin (0.5 mg/L) at 0.1 M, 0.05 M, 0.001 M, 0.0005 and 0.00001 M of Pb (II) solutions in distilled water/ethanol.

In this part, the fluorescence quenching of porphyrin by Ni(II) and Pb(II) was examined quantitatively by fluorescence spectroscopy at 0.02 M, 0.04 M, 0.06 M, 0.08 M, and 1.0 M of Ni(II) and Pb(II) solutions. Figure 4 shows the fluorescence spectra and the integrated fluorescence spectra of porphyrins when 0.02 M, 0.04 M, 0.06 M, 0.08 M, and 1.0 M of Ni(II) and Pb(II) solutions were used. From the spectra, the fluorescence intensity of porphyrin decreased with the increasing amount of Ni(II) and Pb(II) concentration, while there was no change in emission spectra during the quenching process, suggesting no observable photochemical reaction between porphyrin and Ni(II) and Pd(II) [12]. By comparing the fluorescence spectra of porphyrin between Ni(II) and Pb(II), porphyrin is more sensitive towards Pb(II) than Ni(II). This is because Pb(II) ions at different concentrations could quench the emission of porphyrin more than Ni(II) ions with distinct intensity at each concentration.

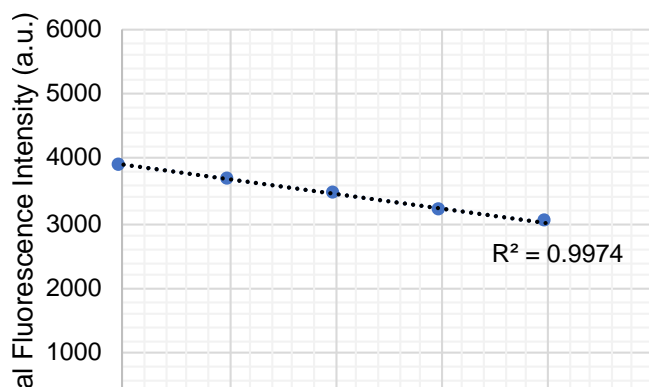


**Figure 4.** (a) Fluorescence spectra of porphyrin (0.5 mg/L) at 0.2 M to 1.0 M of Ni (II) solutions in distilled water/ethanol and (b) integrated fluorescence spectra of porphyrins at different concentrations of Ni(II) solutions. (c) Fluorescence spectra of porphyrin (0.5 mg/L) at 0.2 M to 1.0 M of Pb (II) solutions in distilled water/ethanol and (d) integrated fluorescence spectra of porphyrins at different concentrations of Ni(II) solutions.

There are few possible quenching mechanisms to explain why the emission intensity of porphyrins decreases upon the addition of a higher concentration of Pb(II) and Ni(II) ions. Firstly, the quenching mechanism is the dynamic collision between fluorophores (at their excited state) and the metal ions (the quencher) [5]. This mechanism could be the best to describe the mechanism in this study. The next mechanism is static quenching wherein the mechanism involves the formation of a non-fluorescent ground-state complex between the ground-state fluorophore and quencher [5,6]. Another mechanism is the external heavy metal effect where the heavy metal elements enhancing the rate of intersystem crossing in the fluorophore thus quenching the fluorescence emission [7].

### 3.3. Photostability of Porphyrins

One of the problems when dealing with fluorescein and porphyrin dye is the efficiency of their characteristic towards absorbing light. Because of their sensitivity toward the light, photobleaching may happen before qualitative analysis was done. Therefore, in this part, the photostability of both fluorescein and porphyrins was conducted. Porphyrins (in ethanol) was exposed to light, and their fluorescence spectra were recorded at 0 minutes (before exposure), 10 minutes, 20 minutes, 30 minutes, and 40 minutes. Figure 5 shows the integrated fluorescence spectra of porphyrins over time shows that total intensity decreased over a long exposure to the light source. Although the fluorescence intensity of porphyrins decreased when exposed to the light source, the intensity drop between each exposure was very small and not that significant which could be corrected by introducing an internal reference probe. From this observation, it shows that photobleaching could occur towards any chemical probe in the presence of light. This photobleaching problem could be minimized by conducting the experiments in a dark room where random sources of light from others could be minimized.



**Figure 5.** The integrated spectra of porphyrins emission spectra upon photostability studies. The total fluorescence intensity decreased upon longer irradiation time.

#### 4. Conclusion

Detection of heavy metals using free base porphyrins as a fluorescent chemical probe was investigated in this study. The fluorescence of the free base porphyrins was found to be quenched in the presence of Ni(II) and Pb(II) ions. Porphyrins probe was found to be more sensitive toward Pb(II) ions compared to Ni(II) ions. This is because Pb(II) ions at different concentrations could quench the emission of porphyrin more than Ni(II) ions with distinct intensity at each concentration. In terms of photostability, the fluorescence intensity of porphyrins decreased upon longer exposure time to light source. Future works include solving the solubility problem of porphyrins by modification of the chemical structure of porphyrins and also to immobilised the porphyrins onto any sensor medium for the fabrication of robust chemical sensors.

#### Acknowledgement

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