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Fluorescence-based Probe for Heavy Metal Detection in Water

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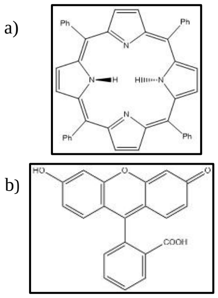
GRAPHICAL ABSTRACT



(a) Porphyrins and (b)Fluorescein

ABSTRACT

Fluorescence is the emission of light by means of a substance that has absorbed light or different electromagnetic radiation. In most cases, the emitted light has a longer wavelength, and consequently decrease energy, than the absorbed radiation. Several fluorescent chemicals can be used as fluorescent probes to detect heavy metals which include fluorescein (FAM) and porphyrins. Fluorescein is a natural compound and dye while porphyrins are a member of heterocyclic macrocycle organic compounds, composed of 4 modified pyrrole subunits interconnected at their α carbon atoms with the aid of methine bridges (=CH−). The objective of this study is to detect the presence of heavy metals ions in water using selected fluorescent chemical probes and to study the sensitivity and selectivity of the selected fluorescent chemical probes towards different concentrations of heavy metals. In general, the study was done by mixing heavy metal solution with the chemical probes to see the changes in fluorescent intensity of the chemical probes in different heavy metal concentration. From the fluorescence analysis result by comparing Ni (II) and Pb (II), it shows that fluorescein was more selective and sensitive towards Ni (II) while porphyrins was more selective and sensitive towards Pb (II). In conclusion, detection of heavy metals using fluorescent chemical probes (FAM and porphyrin) shows promising results with Fluorescein was found to be more selective and sensitive towards Ni (II) while porphyrin was more selective and sensitive towards Pb (II).

Keywords: Fluorescein, porphyrins

1. INTRODUCTION

Heavy metals have been reported as major contamination in water as well as inland. Worldwide, environmental pollution is due to the use of heavy metal, for example, Cu, Hg, and Pb mining, and smelting. These metals pose a toxic danger to human beings and animals even at their low concentration. Conventional techniques 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). Despite high sensitivity and selectivity, these techniques suffer from numerous limitations together with a high cost of instruments, complex sample preparation, the need for a professional chemist, limitation of single composition detection, and preconcentration methods which make them unsuitable for real-time online and continuous monitoring application. In addition, due to the larger size of equipment, they cannot be used for on-site detection [1]. In contrast, the electrochemical method is a powerful sensing device for heavy metal ions detection. This approach presents countless benefits such as accurateness, simplicity, low cost, splendid sensitivity, effectiveness in the multiplexed detection, and on-site detection ability. The sensitivity and selectivity of the electrochemical sensing platform can be similarly improved via chemical modification of the bare electrode with environment-friendly electron mediators.

Several fluorescent chemical probes have been reported to detect heavy metals. These include fluorescein (FAM), porphyrins, quantum dots (QDs), and organic dyes. These fluorescent probes work by their strong coordination ability with metal ions, which can be used as ligands for selective recognition metal ions in coordination chemistry [2]. Fluorescein is a natural compound and dye. It is available as a dark orange/red powder slightly and soluble in water and alcohol. It is broadly used as a fluorescent tracer for many applications. In chemistry the fluorescence of this molecule is very intense; maximum excitation happens at 494 nm and maximum emission at 521 nm. Fluorescein has a pKα of 6.4, and its ionization equilibrium leads to pH-dependent absorption and emission over the range of 5 to 9. Also, the fluorescence lifetimes of the protonated and deprotonated forms of fluorescein are about 3 and 4 ns, Porphyrins are a member of heterocyclic macrocycle organic compounds, composed of 4 modified pyrrole subunits interconnected at their α carbon atoms with the aid of methine bridges (=CH−). Substituted porphyrins are referred to as porphyrins. With a total of 26 π-electrons, of which 18 π-electrons forms a planar, a non-stop cycle, the porphyrin ring shape is frequently described as aromatic [3]. An example of porphyrins that be used as a sensor is cationic porphyrin. It has been used in the UV-vis absorption method for the detection of single or multi heavy metals. When porphyrins bind with metal ion it will form a metal complex which will produce different emission signal.

2. EXPERIMENTAL

The experiment was divided into three main stages. The first stage focused on the absorbance measurement of porphyrins by using a UV-VIS spectroscopy using ethanol as a solvent while Fluorescein was conducted using distilled water as a solvent. Fluorescent chemical probes (1 mg) was dissolved in 1 L to make the concentration of 1mg/L. The second stage was fluorescent measurement by using a fluorescence spectrometer. Stock solutions of 1.0 M for Ni and Pb heavy metal was prepared in distilled water. Then, a series of heavy metal solutions of different concentrations were prepared using serial dilution. Fluorescein was prepared in distilled water and porphyrins solutions were prepared in ethanol and were diluted where appropriate. Each measurement was conducted in ethanol or distilled water as a solvent which was based on the probe used using a glass cuvette (path length of 1 cm). All measurements used equal concentration of probe and heavy metal solution (of different concentrations). The last stage was analysis and evaluation of results where calibration curves for individual ions Ni (II) and Pb (II) were constructed to confirm the linear dependence of measured signals and content of elements. This was done by calculating the correlation coefficients. For fluorescent probe sensitivity, very low concentrations of heavy metals solutions were prepared and used for fluorescent analysis.

3. RESULTS AND DISCUSSION

3.1 UV-VIS Study of Fluorescence Compounds

3.1.1 Fluorescein

Fluorescein in aqueous solutions with different pH values can exist in four different forms: cation, neutral, monoanionic, and dianion, leading to different excitation and emission wavelengths of fluorescence [4]. The absorption of fluorescein in deionized water was initially explored by UV-Vis spectroscopy to get certain excitation and emission wavelengths for the fluorescein. By this, it shows fluorescein is primarily present in its form by the UV-Vis absorption spectrum of fluorescein in water. The wavelength of maximum absorbance (λmax) was observed at 490 nm (shown in Figure 1). This shows that once excited by light at a wavelength of 490 nm, fluorescein in the water medium can emit fluorescence light at a wavelength of 520 nm by reference [5]. Therefore, the excitation and emission wavelengths of 490nm and 520 nm, respectively, were selected as operating wavelengths for the fluorescence emission of Fluorescein.

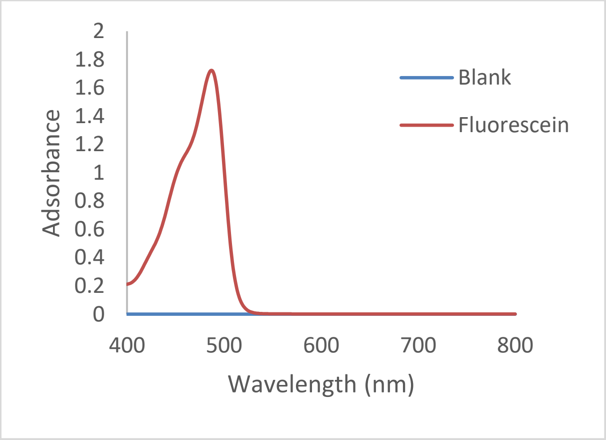


Figure 1. UV-Vis spectrum of fluorescein in water where the concentration was 1mg/L

3.1.2 Porphyrin

It was recognized early that the intensity and colour of porphyrins are derived from the highly conjugated p-electron systems and the most fascinating feature of porphyrins is their characteristic UV-visible spectra that consist of two distinct region regions in the near-ultraviolet and in the visible region. The absorption spectrum of porphyrins has long been understood in terms of the highly successful four-orbital (two highest occupied p orbitals and two lowest unoccupied p* orbitals) which show the importance of charge localization on electronic spectroscopic properties. The absorption bands in porphyrin systems arise from transitions between two HOMOs and two LUMOs, and it is the characteristic of the metal centre and the substituents on the ring that affect the relative energies of these transitions [6][7]. By using 1 mg/L in ethanol the range of absorption is between 380-440 nm (shown in Figure 2) favourable spectroscopic features of porphyrins. Porphyrins absorb light energy intensively in the UV region which favourable operating for the fluorescence emission. Therefore, the excitation and emission wavelengths of 380nm and 440 nm, respectively. From the UV-vis spectrum, it is also good to note that there are smaller peaks between 500 to 600 nm showing possible excitation wavelengths at those wavelengths.

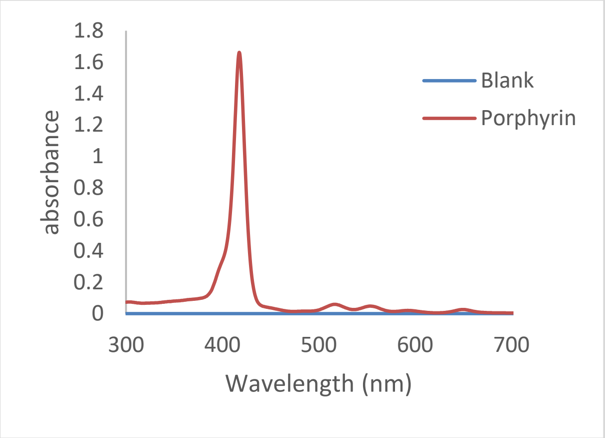


Figure 2. UV-Vis spectrum of porphyrins in ethanol where the concentration was 1mg/L

3.2. Selectivity and Sensitivity of Fluorescein and Porphyrins

3.2.1. Nickel and Lead in Fluorescein

It has been previously reported that Fe(III) at a concentration of 5 x 10-4 M (and above) could completely quench the fluorescence of 20 ppm fluorescein in water, and the quenching effects of Fe(III) on fluorescein fluorescence [8]. The results demonstrate that low concentrations of Fe (III) quench fluorescence to such an extent that visual acuity would perceive fluorescence as being markedly reduced. In this study, a qualitative on-off experiment confirming the fluorescence quenching of Fluorescein by either Ni (II) and Pb (II) was first investigated by fluorescence microscopy. In general, a range of different concentrations (0.1 M – 1 x 10 -5 M) solution of Ni (II) and Pb (II) was added into Fluorescein suspension containing 1 mg/L of Fluorescein of water. All the sample solutions were then mixed thoroughly. Results clearly show that both Pb (II) and Fe (III) Ni (II) and Pb (II) could quench the fluorescence of Fluorescein. The peak intensity of fluorescence spectra decreased as the concentration of metal ions increased. Blanks confirmed that the fluorescence emission was not observed for either of Ni (II) and Pb (II) solutions. The area under the graph analysis between 500 and 700 nm shows good linearity between the total fluorescence intensity and the concentration of Ni (II) and Pb (II). From this area under the graph 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.

As previously discussed, the range of 0.1 M to 0.02 M of metal solutions was selected. In this part, the fluorescence quenching of fluorescein by Ni(II) and Pd(II) was examined quantitatively by fluorescence spectroscopy at 0.02 M, 0.04 M, 0.06 M, 0.08 M, and 0.1 M of Ni(II) and Pb(II) solutions. Figure 3(a) and (b) display the fluorescence emission spectra of Fluorescein (1 mg/L in water) with varying concentrations of Ni (II) and Pd (II) from 0.1 M to 0.02 M also prepared in water. It can be seen from the fluorescence intensity of fluorescein decreased with the increasing of Ni(II) and Pd(II) concentration, while there was no change in emission spectra during the quenching process, suggesting no observable photochemical reaction between fluorescein and Ni(II) and Pd(II) [50]. By comparing the fluorescence spectra of fluorescein between Ni (II) and Pb (II), fluorescein is more sensitive towards Ni (II) than Pb (II). This is because Ni (II) ions at different concentrations could quench the emission of fluorescein more than Pb (II) ions. As reported in the literature [9], this quenching mechanism is related to the dynamic collision between fluorescein and the metal ions. The dynamic quenching mechanism results from diffusive collisions between the fluorophore (fluorescein) and the quencher (metal ions) during the lifetime of the excited state, whereas the static quenching occurs as a result of the formation of a nonfluorescent ground-state complex between the ground-state fluorophore and quencher [10]. Moreover, heavy metal elements can also quench fluorescence by strongly enhancing the rate of intersystem crossing (the change from triplet to singlet states or vice versa) and this relaxation mechanism is therefore known as the *External Heavy Atom Effect* [11]. The nearby heavy atom only favours the spin-flip in the originally excited molecule but is not excited itself. The nearby heavy atom only favors the spin flip in the originally excited molecule but is not excited itself. Also, contact quenching (static quenching) may cause by relies on the possible complex formation between the fluorescein and quencher heavy metal ions which results in the formation of non-fluorescent complex before the excitation of fluorescein. Complex formation between the fluorescein and heavy metal ions can result either by the transfer of electron from fluorescein to metal cations or from metal ions to the fluorescence probe but, the case might be, quenching results [16].

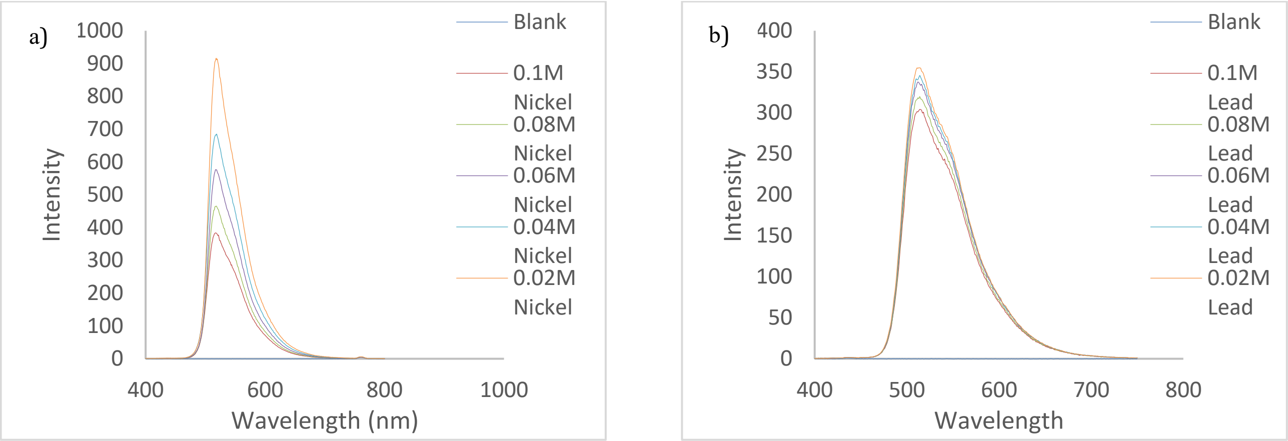


Figure 3. Fluorescence spectra of 1mg/L fluorescein at 0.02 M ,0.04 M, 0.06 M, 0.08 and 0.1 M (a) Ni (II) solutions in distilled water and (b) Pb (II) solutions in distilled water

The fluorescence spectra of fluorescein were then integrated and the area under the graph was then calculated. Figure 4(a) and (b) show the area under the graph analysis for fluorescein in Ni (II) and Pb (II) solutions. From the analysis, both calibration plots in Figure 4(a) and (b) show good linearity with $R^2 > 0.99$ whereas the total fluorescence intensity decreased as the concentration of metals increased. By comparing the two plots, calibration plot for Ni (II) is steeper than the one in Pb (II) – showing that fluorescein is more selectivity and sensitive towards Ni (II).

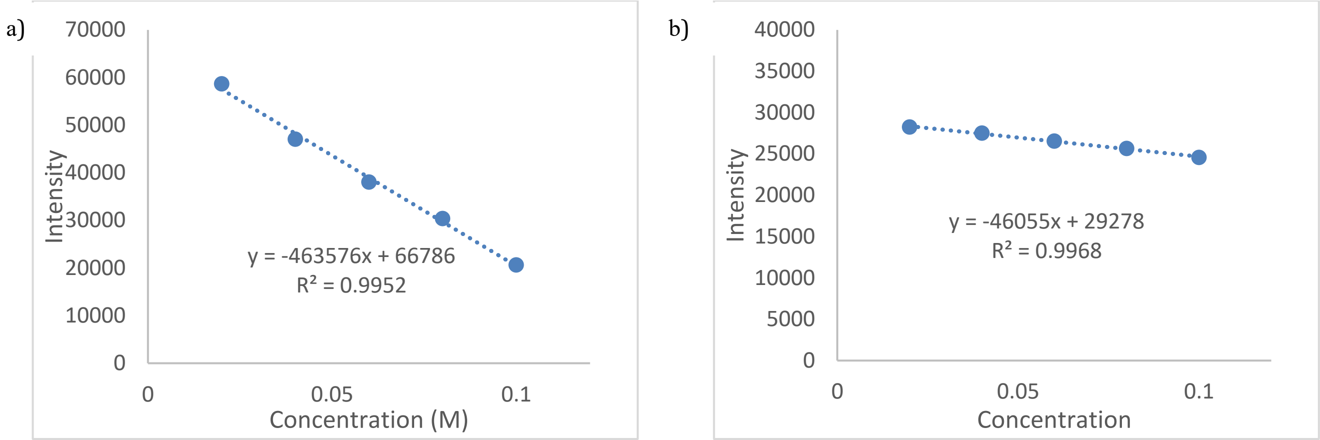


Figure 4. Area under the graph analysis of spectra from Figure 3(a) of fluorescence spectra of fluorescein for Ni(II) area between 500 nm and 700 nm was used and integrated for the analysis (b) Area under the graph analysis of spectra from Figure 3(b) of fluorescence spectra of fluorescein for Pb(II) area between 500 nm and 700 nm was used and integrated for the analysis.

3.2.2. Nickel and Lead in Porphyrin

Porphyrin has a large planar conjugated system, which gives it good optical properties. Thus, the porphyrin molecule is a good class of metal ions probes. However, porphyrins are water-insoluble, which limits their application in metal ions analysis and detection. Therefore, to improve the solubility porphyrin, porphyrins were prepared in ethanol as a fluorescent sensor for metal ion detection. In this study, a qualitative on-off experiment confirming the fluorescence quenching of porphyrin by either Ni (II) and Pb (II) was first investigated by fluorescence microscopy. Simply, 0.1 M to 1 x 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. From results show clearly that both Pb (II) and Ni (II) could quench the fluorescence that emits by porphyrin. The ethanol peak that gives at blanks at fluorescence emission was eliminated for not disturb the observed area for Ni (II) and Pb (II) solutions. The area under the graph analysis between 650 and 800 nm shows good linearity between the total fluorescence intensity and the concentration of Ni (II) and Pb (II). From this area under the graph 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.

As previously discussed, the range of 0.1 M to 0.02 M of metal solutions was selected. 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 5 (a) and (b) 4.16 display the fluorescence emission spectra of porphyrin (1 mg/L in), ethanol with varying concentrations of Ni (II), and Pb (II) from 0.1M to 0.02M prepared in distilled water. It can be seen from the fluorescence intensity of porphyrin decreased with the increasing 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.

As reported in the literature [13], this quenching mechanism is related to the dynamic collision between porphyrin and the metal ions. The dynamic quenching mechanism results from diffusive collisions between the fluorophore (porphyrin) and the quencher (metal ions) during the lifetime of the excited state, whereas the static quenching occurs as a result of the formation of a nonfluorescent ground-state complex between the ground-state fluorophore and quencher[13][14]. Moreover, heavy metal elements can also quench fluorescence by strongly enhancing the rate of intersystem crossing (the change from triplet to singlet states or vice versa) and this relaxation mechanism is therefore known as the *External Heavy Atom Effect* [15]. The nearby heavy atom only favours the spin-flip in the originally excited molecule but is not excited itself. The nearby heavy atom only favours the spin flip in the originally excited molecule but is not excited itself. It is well-established that, for reasons of symmetry, spin-orbital coupling is exceptionally weak in planar n-electron systems built-up exclusively from $2p_z$, atomic orbitals [17]. However, upon insertion of a transition-metal ion into the centre of the porphyrin ring a new spin-orbital coupling pathway is opened. This mechanism derives from the conjugation of the metal d_{xz}, d_{yz} and T-type atomic orbitals with the first pair of antibonding p^* orbitals on the porphyrin nucleus to which the electron is promoted upon excitation. For heavy metal porphyrins, this mechanism should be efficient, and the spin-orbital coupling should be dominated by the one-centre contribution that comes from the central metal ion [17].

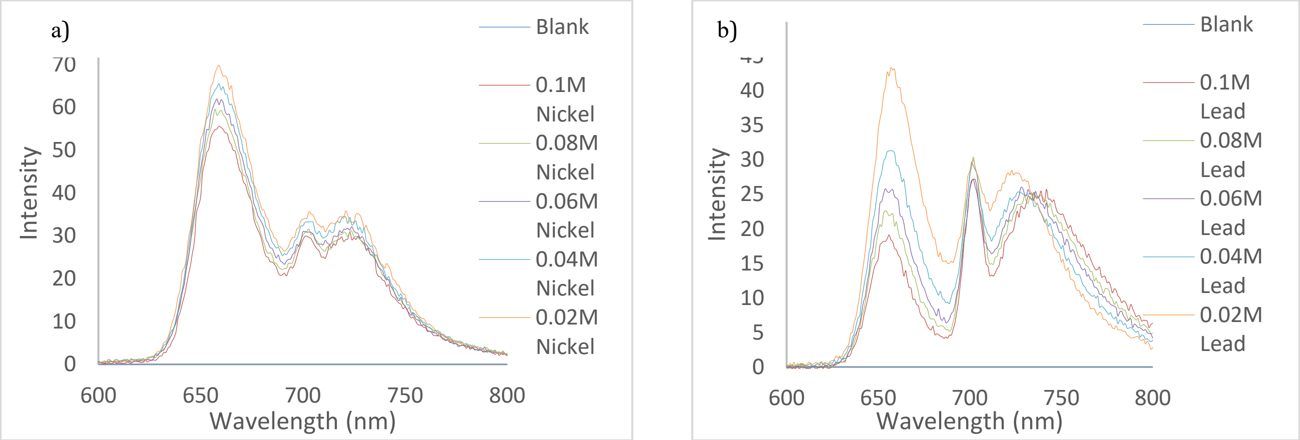


Figure 5. Fluorescence spectra of 1mg/L porphyrin at 0.02 M ,0.04 M, 0.06 M, 0.08 and 0.1 M of (a) Ni (II) solutions in distilled waterand (b) Pb(II) solutions in distilled water.

The fluorescence spectra of porphyrin were then integrated and the area under the graph was then calculated. Figure 6 (a) and (b) show the area under the graph analysis for porphyrin in Ni (II) and Pb (II) solutions. From the analysis, both calibration plots in Figure 6 (a) and (b) show good linearity with $R^2 > 0.99$ whereas the total fluorescence intensity decreased as the concentration of metals increased. By comparing the two plots, calibration plot for Pb (II) is steeper than the one in Ni (II) – showing that porphyrin is more selective and sensitive towards Pb (II).

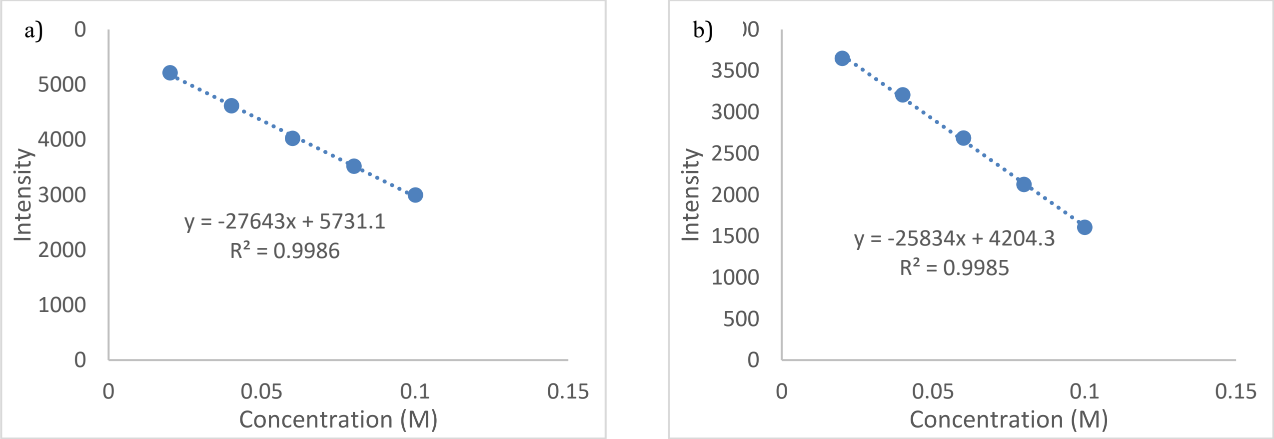


Figure 6. Area under the graph analysis of spectra from Figure 5(a) of fluorescence spectra of porphyrin for Ni(II) area between 650 nm and 800 nm was used and integrated for the analysis and (b)Area under the graph analysis of spectra from Figure 5(b) of fluorescence spectra of porphyrin for Pb(II) area between 650 nm and 800 nm was used and integrated for the analysis.

3.3 Photo-stability and Photobleaching of Fluorescein and Porphyrin

The fluorescent dye is good to make qualitative analysis for heavy metals by analysing the different changes in the intensity of its emission. The sensitivity and selectivity of this fluorescent dye towards different heavy metals make it a good candidate to be used as a chemical probe. 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. Fluorescein (prepared in water) and porphyrins (in ethanol) were exposed to light, and their fluorescence spectra were recorded at 0 minutes (before exposure), 10 minutes, 20 minutes, 30 minutes, and 40 minutes. Figure 7(a) shows the fluorescence spectra of fluorescein at the different duration of light exposure. The area under the graph analysis as shown in Figure 7(b) shows that total intensity decreased over a long exposure to the light source. A similar observation was also recorded in porphyrins as shown in Figure 8(a) and (b). Although the fluorescence intensity of both fluorescein and 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.

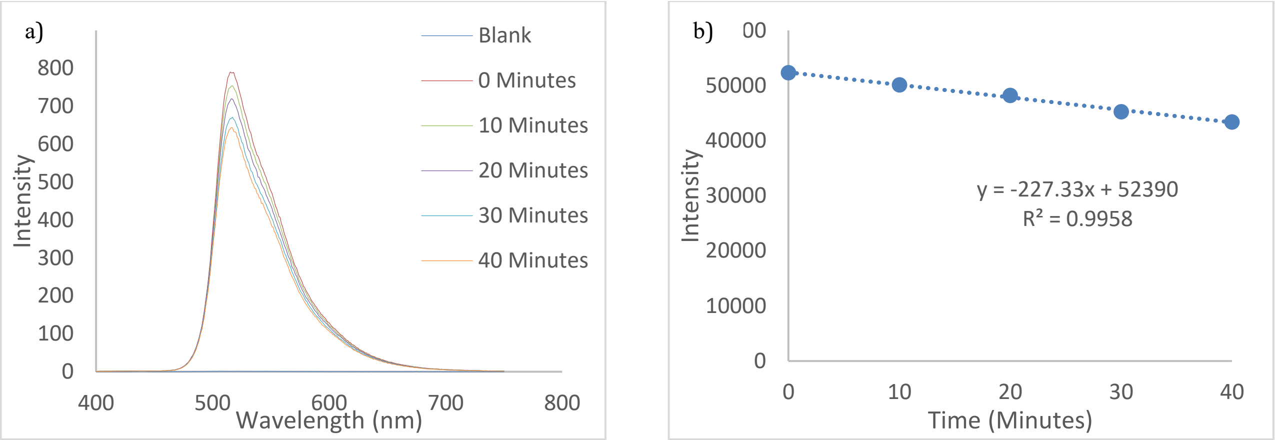


Figure 7. (a) Fluorescence spectra of fluorescein 1mg/L at different duration of light exposure in distilled water and (b) Area under the graph analysis of spectra from Figure 7(a) of fluorescence spectra of fluorescein. The area between 500 nm and 700 nm was used and integrated for the analysis.

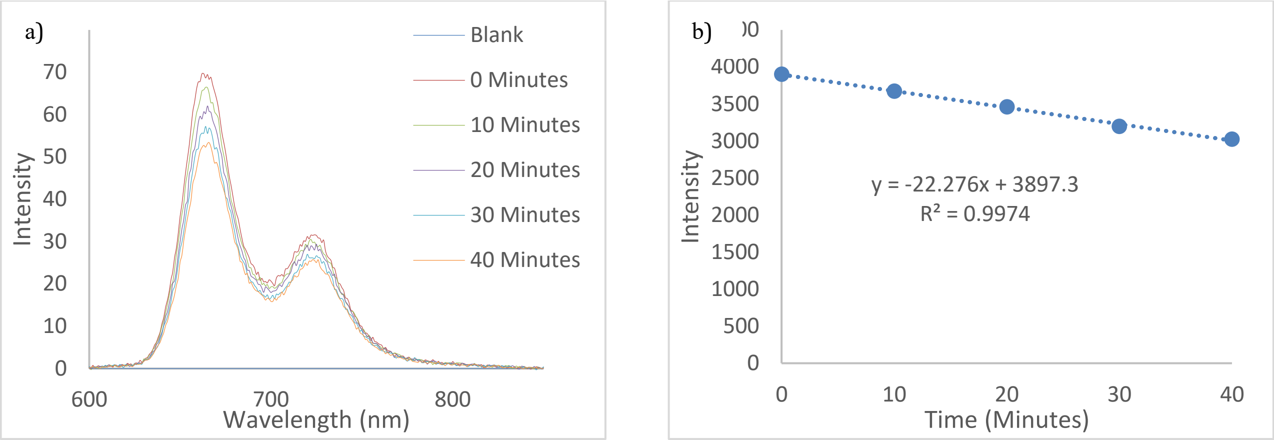


Figure 8. (a) Fluorescence spectra of porphyrins 1mg/L at different duration of light exposure in ethanol and (b) Area under the graph analysis of spectra from Figure 8(a) of fluorescence spectra of porphyrins. The area between 650 nm and 800 nm was used and integrated

4. CONCLUSION

Detection of heavy metals using fluorescein (FAM) and porphyrins as fluorescent chemical probers was investigated in this study. Both chemical probes were quenched in the present of Ni(II) and Pb(II) ions. Sensitivity and selectivity study of fluorescein and porphyrins at different concentration of heavy metals show that fluorescein was more sensitive and selective towards nickel, Ni (II) ions, while porphyrin was more sensitive and selective toward lead, Pb (II) ions. This result has been confirmed by the steepness of the calibration curve graphs of fluorescence intensity of fluorescein and porphyrin at different concentrations of heavy metals. In term of photostability, the intensity of both fluorescein and porphyrins decreased upon longer exposure time to light source. Future works include to solve the solubility problem of Porphyrin by modified. Solution chemical probe can be future study to make as film which can been reusable in fluorescence spectroscopy.

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