

Bathophenanthroline as Turn-off Fluorescence Sensors for Selective and Sensitive Detection of Fe(II)

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Abstract

Bathophenanthroline (BPhen) is a versatile bidentate ligand for transition metals, also frequently used as a universal colorimetric probe for Fe(II). The effect of pH on the fluorescence intensity of BPhen and quenching constant of Fe(II) were studied at different pH values using working solutions of 50% ethanol buffered with acetate buffer. Fluorescence intensity of a 3.0 μM Bphen solution in 50% ethanol buffered at pH 6.6 with acetate buffer was selectively quenched by Fe(II) at 25 °C. The LOD of 19 nM at 3.3 σ and the linear range of 63 nM – 224 nM with $R^2 = 0.9919$ revealed that this method is more sensitive than the colorimetric method (detection range = 1.8 μM - 18 μM). Quenching of the BPhen-Fe(II) complex is temperature-dependent, which may be due to the increased stability of the formed complex with temperature, supporting a static quenching mechanism. Interference from foreign ions on the fluorescence of the BPhen-iron(II) complex was also studied. A tolerance limit exceeding 5% was observed and recorded. The interference from cations, Ni(II), Co(II), and Cu(II), was relatively higher, while the most common anions showed only a little to no interference. In response time analysis, the fluorescence intensity, which remains constant after 10 minutes, reveals that the system approached equilibrium. This method would be quicker and more accurate than a colorimetric method due to the absence of a solvent extraction step.

1. Introduction

Several methods, such as atomic absorption spectroscopy (AAS) [1], inductively coupled plasma mass spectrometry (ICP-MS) [2], and inductively coupled plasma atomic emission spectroscopy (ICP-AES) [3] are available to detect metal ions in trace amounts. Although these techniques provide sensitive and accurate results, significant drawbacks like non-portability, time-consuming sample processing, high cost, and sophisticated operation [4] have led to researchers' search for alternative methods.

The development of fluorescence sensors is an increasing research interest among researchers as these sensors have unique features such as high sensitivity and simplicity. Any process that results in a change in fluorescence intensity, wavelength, anisotropy, or lifetime can be used for sensing studies. A typical fluorescence probe has two moieties; a receptor (the recognition site) and a signaling unit (fluorophore) capable of absorbing and emitting light. Thus, the development of cost-effective, susceptible fluorescent sensors with low detection limits has gained much attention in recent years. [5]

A decrease in fluorescence intensity is called fluorescence quenching, and it can be static or dynamic. Dynamic quenching occurs due to collisions of the quencher with the fluorophore. Static quenching is caused by the interactions of the ground state of the fluorophore with a quencher. Static quenching, quencher concentration, and formation constants are essential factors, while excited state lifetime is critical for collisional quenching. [6–7] Fluorescence quenching is temperature dependant in accordance with the mechanism of quenching. [8–11]

Iron is a crucial trace metal in living systems. It participates in various metabolic processes, including oxygen transportation, DNA synthesis, chlorophyll synthesis, electron transportation, and photosynthesis. [12–13] Iron exists as the Fe(II) or Fe(III) state, and depending on the oxidation state, it shows a unique reactivity. Disorders in iron metabolism cause a broad spectrum of diseases in humans, ranging from anemia to Parkinson's disease. Labile iron, which is not bound to proteins, produces reactive oxygen species, causing potential cell damage and various iron-related disorders. The form of endogenous labile iron is Fe(II) rather than Fe(III). [14] When considering the oxidative degradation of cellulose by Fe(II), monitoring Fe(II) in trace amounts has a significant role in biomedical studies, water quality assessments, and several other aspects like paper conservation and the textile industry. Furthermore, Fe(II) impurities are common in reagents, and determining these levels is necessary for analytical applications. [15–17]

1,10-Phenanthroline and its derivatives are chemically versatile ligands due to their promising structural and chemical properties. Phenanthroline plays an important role in coordination chemistry due to its unique features like planarity, rigidity, hydrophobicity, aromaticity, and chelating capability [18]. 1,10-Phenanthroline ($\lambda_{\text{ex}} = 266 \text{ nm}$, $\lambda_{\text{em}} = 366$), is a fluorescent molecule. A non-fluorescent complex with Fe(II) formation makes 1,10-Phenanthroline a sensitive fluorescence probe for the iron with a LOD of 24 nM at 3σ . (Linear range 0.5 μM - 20 μM). [19]

The compound 4,7-diphenyl-1,10-phenanthroline, known as Bathophenanthroline (BPhen), [A], is one of the most common phenanthroline derivatives. BPhen can form a stable 1:3 red coloured complex (B) selectively with Fe(II) but not with Fe(III), known as the ferroin reaction. [20, 21]. This reaction can be used to detect extremely low concentrations of Fe(II) ions in aqueous solutions. The use of BPhen as a sensitive chromophore to detect iron was initially described by the Smith method. After this, various modifications have been made to increase the effectiveness of the Fe(III) to Fe(II) conversion in the original method described by Smith *et al.* [22]. These colorimetric-based procedures to detect Fe(II) with BPhen include an organic extraction step, as Fe(II) is generally in an aqueous medium. The use of BPhen (molar absorptivity = 22,400) which gives a detection range of 1.8–18 μM , is a significant improvement over 1, 10-phenanthroline (molar absorptivity = 11,100) for the colorimetric determination of Fe(II) [15], [23–24].

In this study, a novel turn-off fluorescence probe based on BPhen (A) was developed successfully to quantify Fe(II) in a 50% ethanol solvent system. Compared to 1,10-phenanthroline, improved reproducibility solubility and selectivity were observed with the novel probe. The improved detection limit of this probe is 19 nM.

2. Experimental

2.1 Apparatus

All the fluorescence studies were carried out by using a Hitachi F7000 fluorescence spectrophotometer equipped with a 150 W xenon lamp using a $1 \times 1 \text{ cm}^2$ quartz fluorescence cuvette with a 1.0 cm quartz cell holder and a thermostat bath. The excitation and the emission slit widths were set to 5 nm. The absorbance studies were done on a Labomed Inc. (USA) double beam UV-Visible spectrophotometer. Homogenization of solutions was done using a sonicator. Precisa XB 120 analytical balance was utilized for weighing purposes, and the pH was determined using a pHS-2C pH-meter (Shanghai DaPu Instruments Co., Ltd, Shanghai, China) at ambient temperature.

2.2 Reagents

Analytical grade purity solvents and reagents were used without any further purification. A Sigma Aldrich (USA) 99% Bathophenanthroline was used to prepare the BPhen stock solutions. Fe(II) solutions were made by using an analytical grade $(\text{NH}_4)_2\text{Fe}(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$ from Merck Specialities Private Limited (India).

Analytical grade CH_3COONa (Daejung chemicals and metals, South Korea) and Sigma Aldrich 99.8% acetic acid were used to prepare the buffer solution. AR grade absolute ethanol (VWR Chemicals, France) was used to make ethanol solutions, and doubly distilled deionized water was used wherever dilution was needed. All glassware was washed with diluted sulphuric acid along with deionized water and oven-dried before each experiment.

2.3 Preparation of stock solutions

2.3.1 BPhen stock solution (3.0 mM)

A mass of 10.0 mg of BPhen was dissolved in 100% ethanol in a 10 ml volumetric flask using a sonicator and made up to the mark. The working solution of BPhen was freshly prepared by diluting the appropriate volume of the stock solution using the solvent system.

2.3.2 Preparation of Fe(II) stock solution (5.0 mM) in water

A mass of 20.0 mg of analytical grade $(\text{NH}_4)_2\text{Fe}(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$ was dissolved in double-distilled deionized water in a 10 ml volumetric flask using a sonicator and made up to the mark. Fe(II)'s working solution was freshly prepared by diluting the appropriate volume from the stock solution using the solvent system.

2.3.3 Preparation of working solutions of pH 4.5, pH 5.5, and pH 6.6 using acetate buffer

Acetate buffers are used to prepare the working solutions at pH 4.5, pH 5.5, and pH 6.6.

2.3.4 BPhen in 50% ethanol (3.0 μM)

A volume of 10 μl of the $1.5 \times 10^{-3} \text{ M}$ BPhen in 50% ethanol was added to a 5 ml volumetric flask and made up to the mark with 50% ethanol, without buffer. Another set of working solutions of pH 4.5, pH 5.5, and pH 6.6 (made using the acetate buffer) were prepared separately with 3.0 μM BPhen in 50% ethanol.

Sodium Acetate Anhydrous extra pure AR (99%), $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, MgSO_4 , CaCO_3 all were purchased from Techno Pharmachem, India, AR grade $\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$, NaBr , LiCl , $\text{Al}_2(\text{SO}_4)_3 \cdot 18\text{H}_2\text{O}$, $\text{Pb}(\text{NO}_3)_2$ were purchased from Fluka Chemical Co., USA, $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, FeCl_3 , $\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$ and NaCO_3 (SRL Chemicals, India) were used in this study.

3. Results And Discussion

3.1 Excitation and emission spectra of BPhen

In UV-Visible absorption studies, the fluorophore's stock solution was diluted enough to avoid the molecules' aggregation. In the absorption and the emission spectra of BPhen in 50% ethanol (Fig. 1), two distinct peaks were observed at 224 nm and 272 nm. They were assumed to be due to $\pi \rightarrow \pi^*$ transitions of phenyl and phenanthroline groups of BPhen, respectively. Computational studies revealed no $n \rightarrow \pi^*$ transition is possible in the absorption spectrum of the phenanthroline derivative. [25, 26]. The emission spectrum of BPhen ($\lambda_{\text{ex}} = 272 \text{ nm}$) is a broad single peak with λ_{max} at 385 nm.

3.2 Effect of the concentration of BPhen on fluorescence

The fluorescence intensity, as well as the degree of fluorescence quenching, depends on the fluorophore concentration. Two different solvent systems, 50% ethanol and 100% ethanol, were used to optimize the appropriate solvent system for further studies.

The fluorescence intensity of BPhen was higher in 50% ethanol than in absolute ethanol. In 50% ethanol, the maximum intensity was observed at the concentration of 4.5 μM of BPhen. After this point, the intensity gradually decreases due to self-quenching. The BPhen concentration of 3.0 μM in a 50% ethanol solvent system was selected to avoid any possibility of self-quenching in further studies.

3.3 pH effect on BPhen fluorescence intensity

The medium's pH intensely affects the fluorescence intensity of BPhen and quenching constants of $\text{Fe}(\text{II})$. The emission spectra of a series of 3.0 μM BPhen solutions containing 0.10 μM of $\text{Fe}(\text{II})$ were recorded at different pH values. The pH of the solutions was adjusted using 98% conc. H_2SO_4 and a saturated NaOH solution. When the emission maximum at 385 nm (I_{385}) was recorded against the solution pH, a reasonably linear range was obtained between pH 3.5 and 11, as illustrated in Fig. 3. At higher pH, the decrease in free $\text{Fe}(\text{II})$ in the system due to hydroxide formation lowers the freely available $\text{Fe}(\text{II})$ ions, decreasing the quenching response. When a 3.0 μM BPhen solution was titrated with H^+ , a gradual decrease in pH resulted, and the intensity of the peak at 385 nm was also reduced. Another peak was observed at 444 nm, as shown in Fig. 4, indicating the formation of a fluorescent protonated BPhen complex.

3.4 Quenching effect of Fe(II) on BPhen fluorescence

3.4.1 When the system is not buffered (50% ethanol without buffer)

The fluorescence intensity of a 3.0 μM BPhen solution in 50% ethanol with increasing Fe(II) concentration was studied. Fluorescence intensity at 385 nm reduces, and no other fluorescence signals were observed upon the addition of Fe(II). The intensity of the BPhen fluorescence peak at 385 nm was plotted against the Fe(II) concentration and is shown in Fig. 5. This observation suggests that Fe(II) effectively quenches the fluorescence of BPhen and forms a non-fluorescent complex with BPhen.

The Stern-Volmer equations;

For collisional / Dynamic quenching; $F_0/F = K_D [Q] + 1$ (1)

For static quenching; $F_0/F = K_S [Q] + 1$ (2)

According to the equation, since the gradient is equal to the quenching constant K in Fig. 5 (b), the quenching constant is 0.0072 nM^{-1} .

3.4.2. *When the system is buffered (50% ethanol with the acetate buffer)*

Although the calibration curve plotted without a buffer in the system gave good linearity, a working solution buffered with acetate buffer was used in this study. Working solutions buffered with acetate buffer at three different pH values were used to study the difference in quenching responses to select an appropriate buffer system for the probe. Three working solutions were prepared, and in all working solutions, the concentration of BPhen was maintained as 3.0 μM , whereas the concentration of Fe(II) was increased from 0 nM to 40 nM.

Figure 6 illustrates the fluorescence emission spectra, calibration plot, and Stern Volmer plot obtained when titrating 3.0 μM BPhen in pH 6.6 working solution with increasing Fe(II) concentration. Table 1 summarises the analytical data obtained for pH = 4.5 and pH = 5.5 working solutions buffered with acetate buffer.

The LOD, LOQ standard deviation of the intercept and the slope obtained for probe BPhen are summarized in Table 1. LOD and LOQ values were calculated using the following equations [27], $\text{LOD} = 3.3 \sigma/S$, $\text{LOQ} = 10 \sigma/S$, where σ is the standard deviation of the regression intercept line.

Table 1
The analytical data of the calibration plots and Stern Volmer plots obtained for the working solutions of pH 4.5, pH 5.5, and pH 6.6.

Parameter		Calibration plot			Stern Volmer Plot		
pH of the solution		4.5	5.5	6.6	4.5	5.5	6.6
LOD	nM	2.20	3.42	1.58	1.165	4.30	2.72
	ppb	0.12	0.19	0.09	0.07	0.24	0.15
LOQ	nM	6.67	10.36	4.79	3.53	13.03	8.24
	ppb	0.37	0.58	0.27	0.20	0.73	0.46
Slope (nM^{-1})		-0.54	-0.54	-0.69	0.017	0.023	0.023
Linear Range (nM)		10–35	10–35	0–40	0–40	20–40	19–40
R^2		0.97	0.93	0.98	0.99	0.97	0.98

Since the gradient of the Stern - Volmer plot is equal to the quenching constant K , the highest quenching constant was observed in pH 5.5 and pH 6.6. The steepest slope in the calibration plot was obtained for the pH 6.6 system, indicating the highest sensitivity. Its R^2 value implied the highest correlation between the fluorescence intensity and Fe(II) concentration. Based on the LOD and LOQ values and the linear range, it was concluded that the pH 6.6 working solution was the most appropriate of these three buffered solutions.

3.4.3 The blank study that corresponds to the addition of Fe(II) in pH 6.6 working solution

This study was carried out to clarify if quenching is due to the Fe(II) solution or a pH change in the system. In Sect. 3.4.2, the quenching of 3.0 μM BPhen in pH 6.6 working solution with increasing Fe(II) concentration was studied by spiking 25 μM Fe(II) in 50% ethanol with 0.2 μl increments as in Fig. 6 (b). A blank study was performed by spiking a 50% ethanol solution to a 3.0 μM BPhen in pH 6.6 working solution.

A plot of fluorescence intensity vs the volume of added 50% ethanol is shown in Fig. 7(a). Similarly, 25 μM Fe(II) in 50% ethanol was spiked with 0.2 μl increments to a solution of 3.0 μM BPhen in pH 6.6 working solution and plotted in Fig. 7(b).

It was observed that the change of fluorescence intensity due to the addition of 50% ethanol up to the volume of 8 μl (0.2–40 μl) was insignificant. However, when Fe(II) was added, the intensity decreased without any significant pH change. Therefore, a pH 6.6 working solution was selected for further studies.

The LOQ value reported for the fluorescence probe based on **(A)** was 7.5×10^{-10} M (4.2×10^{-5} ppm). A selective fluorescence sensor for Fe(II) determination, based on pristine 1,10-phenanthroline, reported a LOD of 24 nM at 3.3σ in a previous study [28]. The calculated LOD value for **(A)** through this study was lower than that, indicating a higher sensitivity that has not been reported so far.

3.5. Temperature studies

3.5.1 The temperature effect on the quenching of BPhen by Fe(II).

When a particular concentration of Fe(II) ions is added to the solution at once, the quenching is lower than when it is added gradually to the solution. Therefore it is essential to clarify whether this phenomenon can be affected by temperature. The effect of temperature on the quenching process is also a key parameter for determining the quenching mechanism [29]. Thus, a temperature study was carried out to determine whether there is a temperature effect for the quenching by Fe(II) ions. The temperature of 3.0 μ M BPhen in pH 6.6 working solution was increased from room temperature 25 $^{\circ}$ C to 60 $^{\circ}$ C in the presence of 20 nM Fe(II) solution. Quenching of the BPhen-Fe(II) complex was increased with temperature. Figure 8 depicts that the fluorescence decreases with increasing temperature in the presence of 20 nM Fe(II).

Even though the temperature studies suggest that the quenching mechanism may be dynamic, previously reported studies to indicate that the phenanthroline-Fe(II) complex formation is static. Further confirm the effect of temperature on the quenching of Fe(II) ions, the ratio between fluorescence intensity at room temperature (I_0) and fluorescence intensity at a particular temperature (I) were plotted against temperature as in Fig. 9. The plot I_0/I vs. $[Q]$ also resulted in a straight line, which can be taken as evidence for static quenching [30].

After adding BPhen, the solutions were kept for one hour to ensure complete solubility of BPhen, and then Fe(II) was added. According to these studies, the quenching of BPhen by Fe(II) ions was increased with increasing temperature due to the increased stability constant of the formed complex with temperature, which supported the static quenching mechanism. However, these studies were done at an average room temperature of 25 $^{\circ}$ C.

3.6 Response time with a higher Fe(II) concentration

The fluorescence intensity of a 3.0 μ M BPhen solution buffered at pH = 6.6 with and without 0.10 μ M of Fe(II) reduced with time, as shown in Fig. 10. However, after 10 minutes, the intensity remained constant, indicating that an equilibrium [31] was reached. Hence in further studies, emission intensity was measured after 10 minutes.

3.7 Calibration curve

Based on the optimum conditions obtained, using a 3.0 μM BPhen solution in pH 6.6 working solution and measuring the fluorescence intensity 10 minutes after Fe(II) was spiked gave the calibration curve given in Fig. 11(a).

The calibration curve obtained using the conditions optimized was linear in the Fe(II) concentration range 63.86 nM – 224.4 nM ($R^2 = 0.9919$). Accordingly, LOD for the method was 19.16 nM at 3.3 σ , and LOQ was 63.86 nM at 10 σ at 25°C.

3.8 Interference study

3.8.1 The study of tolerance limits of interfering ions.

The interference effect, which might be caused by typical fluorescence quenchers such as Co(II), Ni(II), Cu(II), and Fe(III), was studied. Fluorescence quenching by Co(II), Ni(II), Cu(II), and Fe(III) may be due to the paramagnetic nature of these metal ions. The unpaired d-electrons can effectively quench the fluorophore's singlet excited state and promote the excited state's energy dissipation mainly through non-radiative emission [32]. Moreover, the interference of metal ions commonly present in actual analytical samples such as Na(I), Li(I), Ca(II), Mg(II), Zn(II), Pb(II), Mn(II), and Al(III) was also investigated. In this study, a 3.0 μM solution of BPhen containing 0.10 μM concentration of Fe(II) was titrated with different interfering metal ions. The tolerance limits [19] for the interfering ions were calculated and are given in Table 2. The tolerance limit is the maximum concentration in which there is less than a 5% effect on fluorescence intensity. The higher the molar ratio, the less the ion's interference in the determination of Fe(II) [11], [33].

Table 2
Tolerance limits of interfering species for Fe(II)
determination at pH 6.6, when BPhen
concentration is at 3.0 μM and Fe(II) at 0.10 μM .

Interfering species	Mole ratio
Al ³⁺ , Ca ²⁺ , Na ⁺ , Li ⁺ Cl ⁻ , CO ₃ ²⁻ , Br ⁻	1000
Mn ²⁺	400
Fe ³⁺ , Mg ²⁺ , Pb ²⁺	200
Zn ²⁺	100
Cu ²⁺ , Ni ²⁺	3
Co ²⁺	2

4. Conclusion

In this study, a turn-off fluorescence sensor was successfully developed based on BPhen for Fe(II) 's selective quantification at room temperature. The method excludes time-consuming extraction procedures. Fe(II) colorimetric determination with BPhen, which contains an organic solvent extraction step, gave a detection range of 0.1 – 1 ppm (1.8 – 18 μ M). Although using Bathophenanthroline Sulphonate (BPS), the water-soluble analogous of BPhen can also be used for colorimetric determination of Fe(II) without an extraction step; both analogs' sensitivity is similar [1]. This method proposed that aqueous Fe(II) solutions in 50% ethanol can be detected directly using only a 3.0 μ M solution of BPhen in 50% ethanol buffered at pH 6.6. LOD of 19 and the linear range of 63 nM – 224 nM of the new method reveals the capability of BPhen to detect trace Fe(II) amounts using fluorescence more than colorimetry.

Therefore, BPhen can be used as a turn-off fluorescence sensor to determine nano-level Fe(II) concentrations with minimum interference from other common foreign ions. Co(II), Ni(II), and Cu(II) were identified as the foreign ions, which bring a significant interference for the selective determination of Fe(II). However, it is generally observed when using phenanthroline-derived compounds as fluorescence sensors. Fe(II) quenching was increased with increasing temperature, possibly due to the formed complex's increased stability constant. According to the interference study, the blank study, and the temperature studies, the quenching process of BPhen involved a combination of both static and dynamic quenching.

5. Declarations

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Conflicts of interest/Competing interests

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Ethics approval/declarations

Not applicable

Consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material/ Data availability

Data available on request from the authors

Code availability

Not applicable

Authors' contributions

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Figures

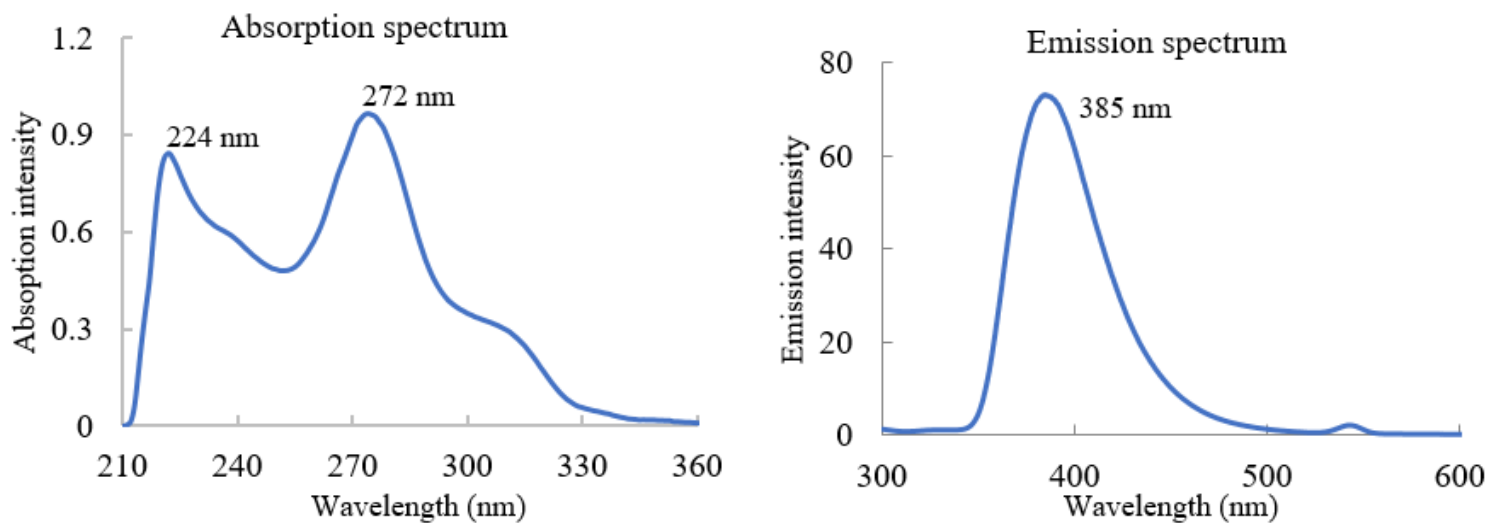


Figure 1

(a) UV Visible absorption spectrum of 9.0 μM BPhen in 50% ethanol; (b) Fluorescence emission spectrum of 3.0 μM BPhen in 50% ethanol.

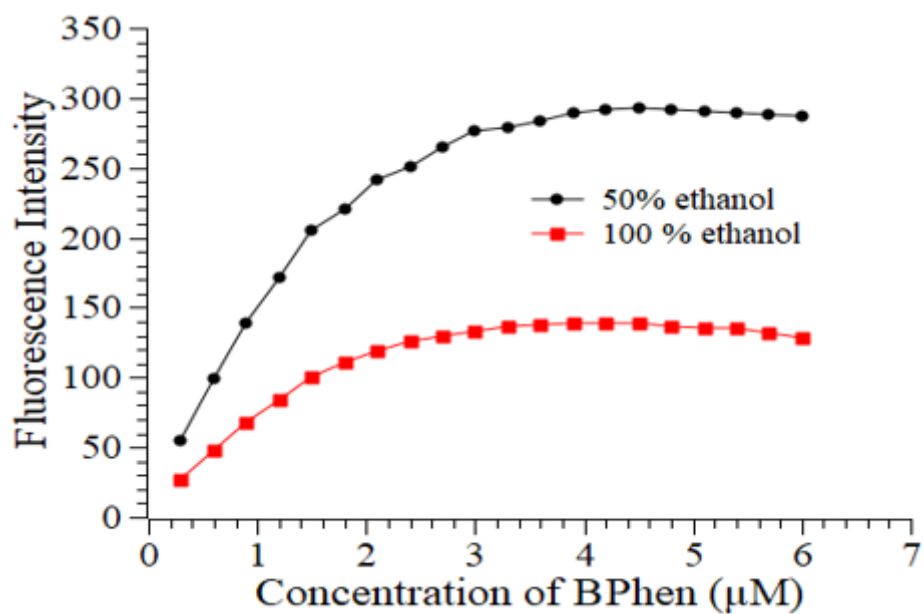


Figure 2

Fluorescence intensities of fluorescence peak at 385 nm with increasing concentration of BPhen from 0 μM to 6 μM in 50% ethanol and 100% ethanol.

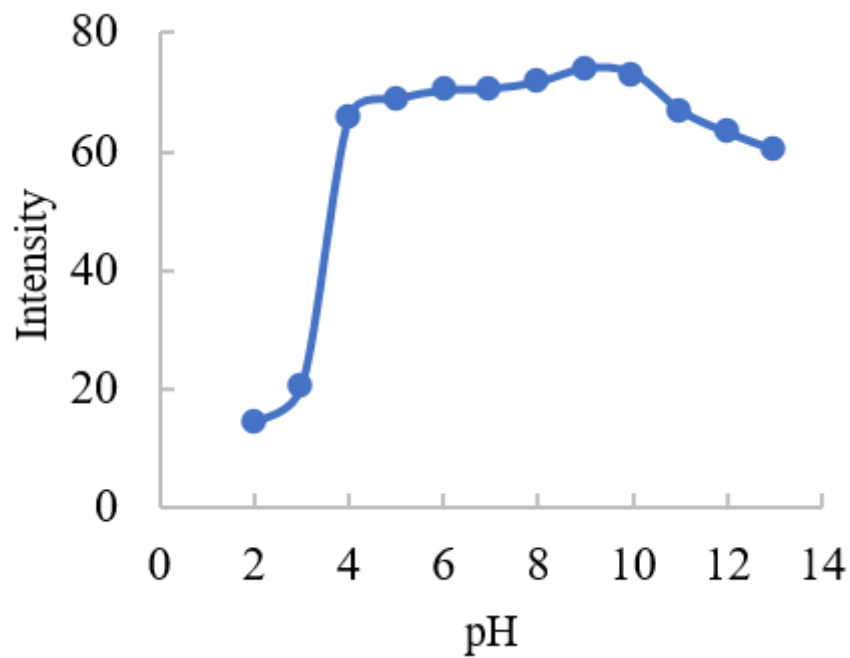


Figure 3

The emission intensity of 3.0 μM BPhen solutions containing 0.10 μM Fe(II) at different pH values

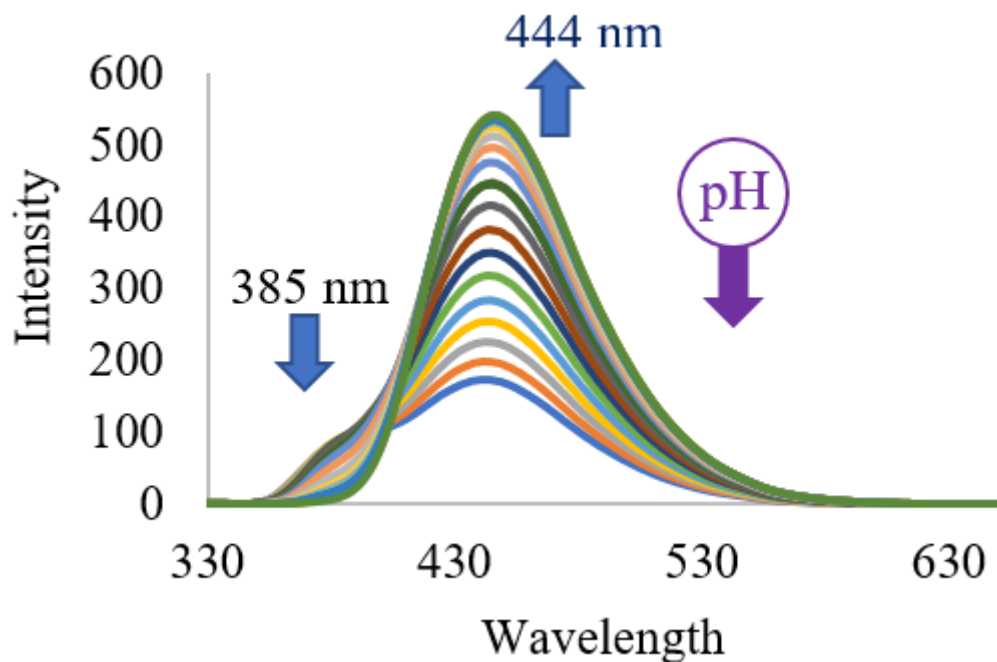
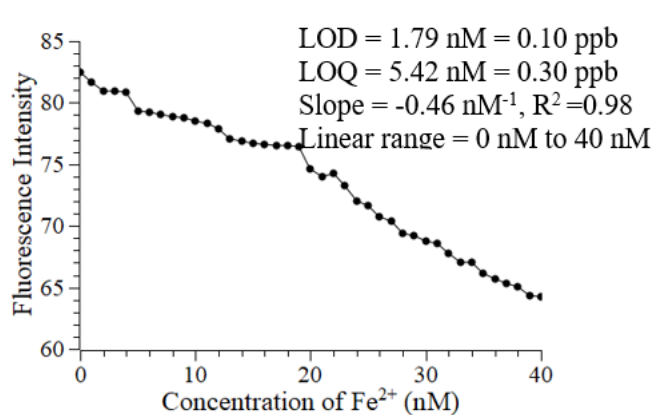
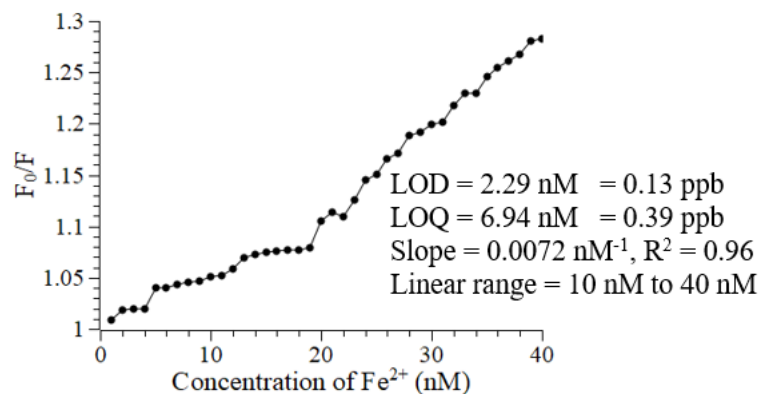


Figure 4

The emission spectra of a 3.0 μM BPhen solution in 50% ethanol spiking with an 18.0 μM H^+ solution



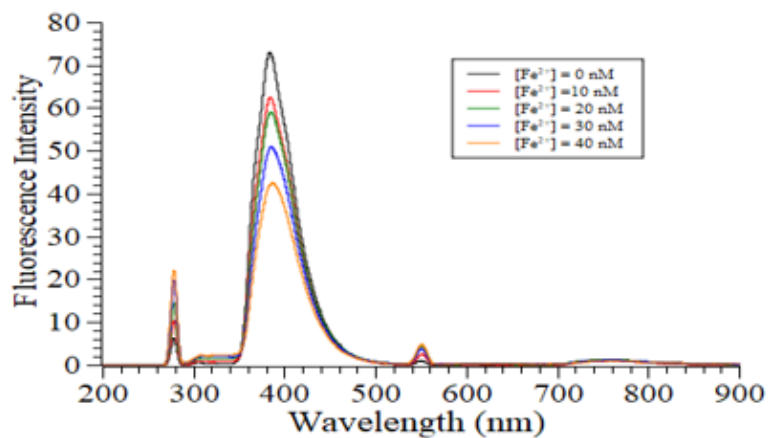
(a)



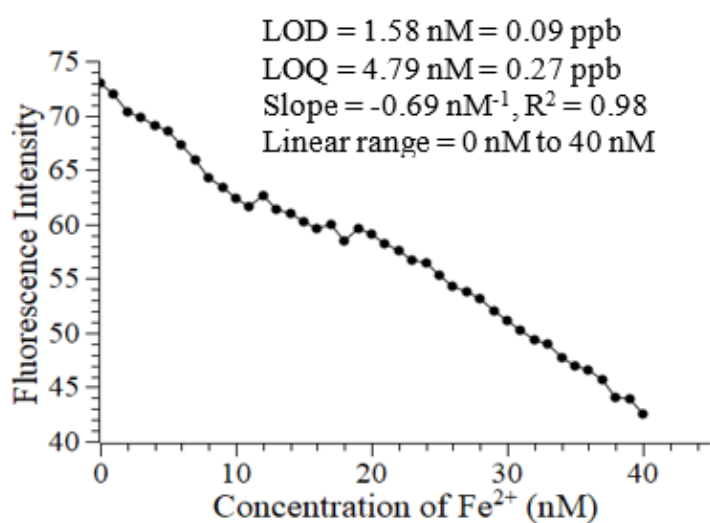
(b)

Figure 5

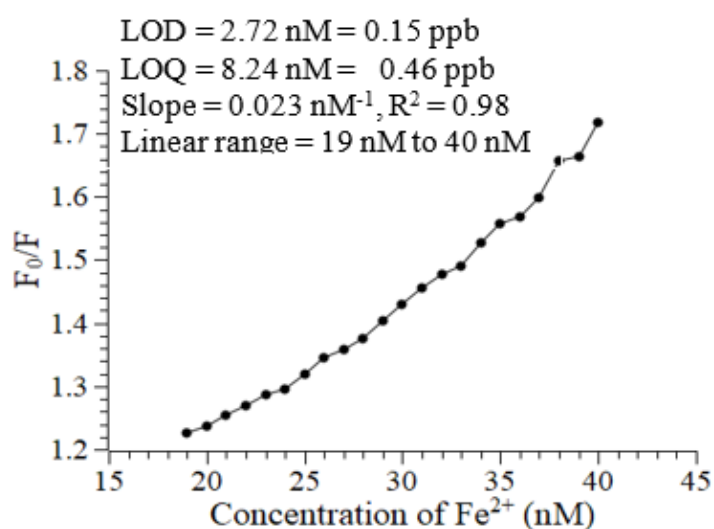
(a) Change in fluorescence intensity of peak at 385 nm (b) The Stern Volmer plot of the 3.0 μM Bphen in 50% ethanol (without acetate buffer), with increasing $\text{Fe}(\text{II})$ concentration from 0 nM to 40 nM



(a)



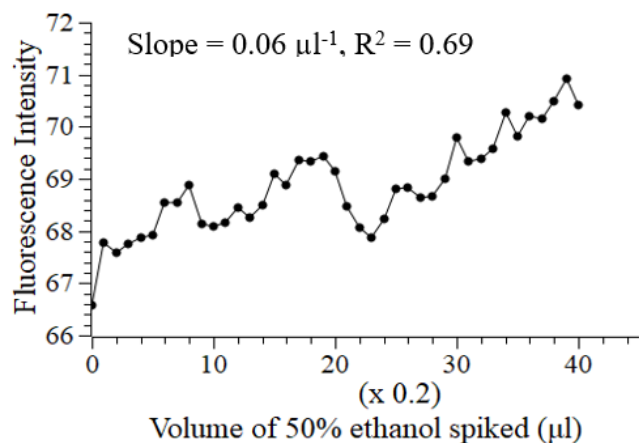
(b)



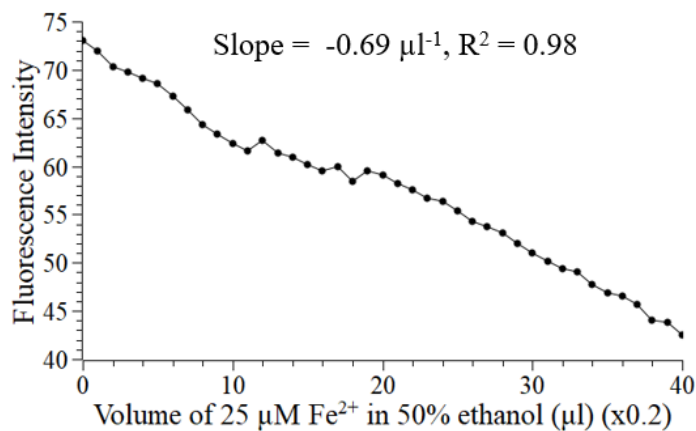
(c)

Figure 6

(a) Fluorescence emission spectra, (b) Change in intensity of the fluorescence peak, and (c) Stern Volmer plot for the quenching of 3.0 μM BPhen in pH 6.6 working solution with increasing $\text{Fe}(\text{II})$ concentration.



(a)



(b)

Figure 7

Change in fluorescence intensity of the fluorescence peak of $3.0 \mu\text{M}$ BPhen in pH 6.6 working solution upon (a) spiking of 50 % ethanol; (b) spiking of $25 \mu\text{M}$ Fe(II) in 50% ethanol.

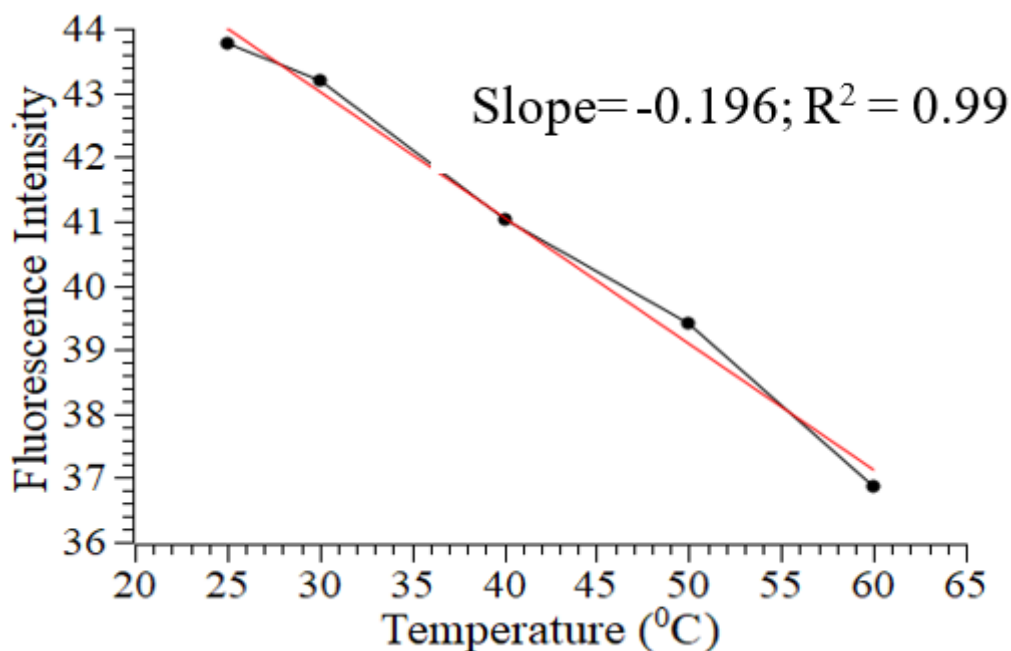


Figure 8

Change in fluorescence intensity in pH 6.6 working solution of $3.0 \mu\text{M}$ BPhen with increasing temperature from 25°C to 60°C on the 20 nM Fe(II) ions.

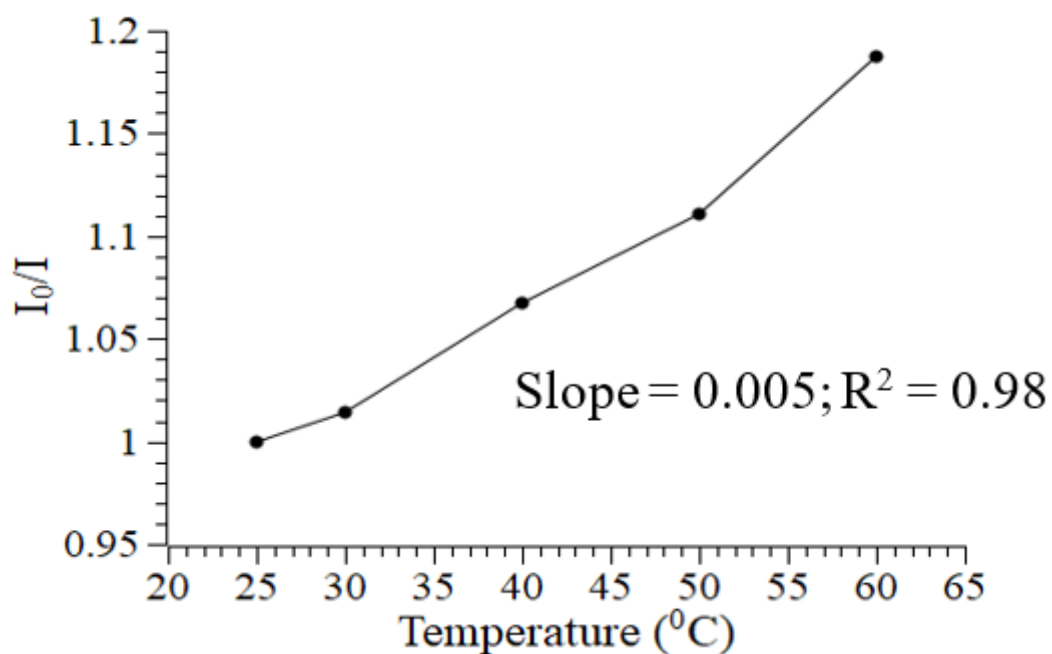


Figure 9

The ratio between fluorescence intensity at room temperature (I_0) and fluorescence intensity at the particular temperature (I), with increasing temperature.

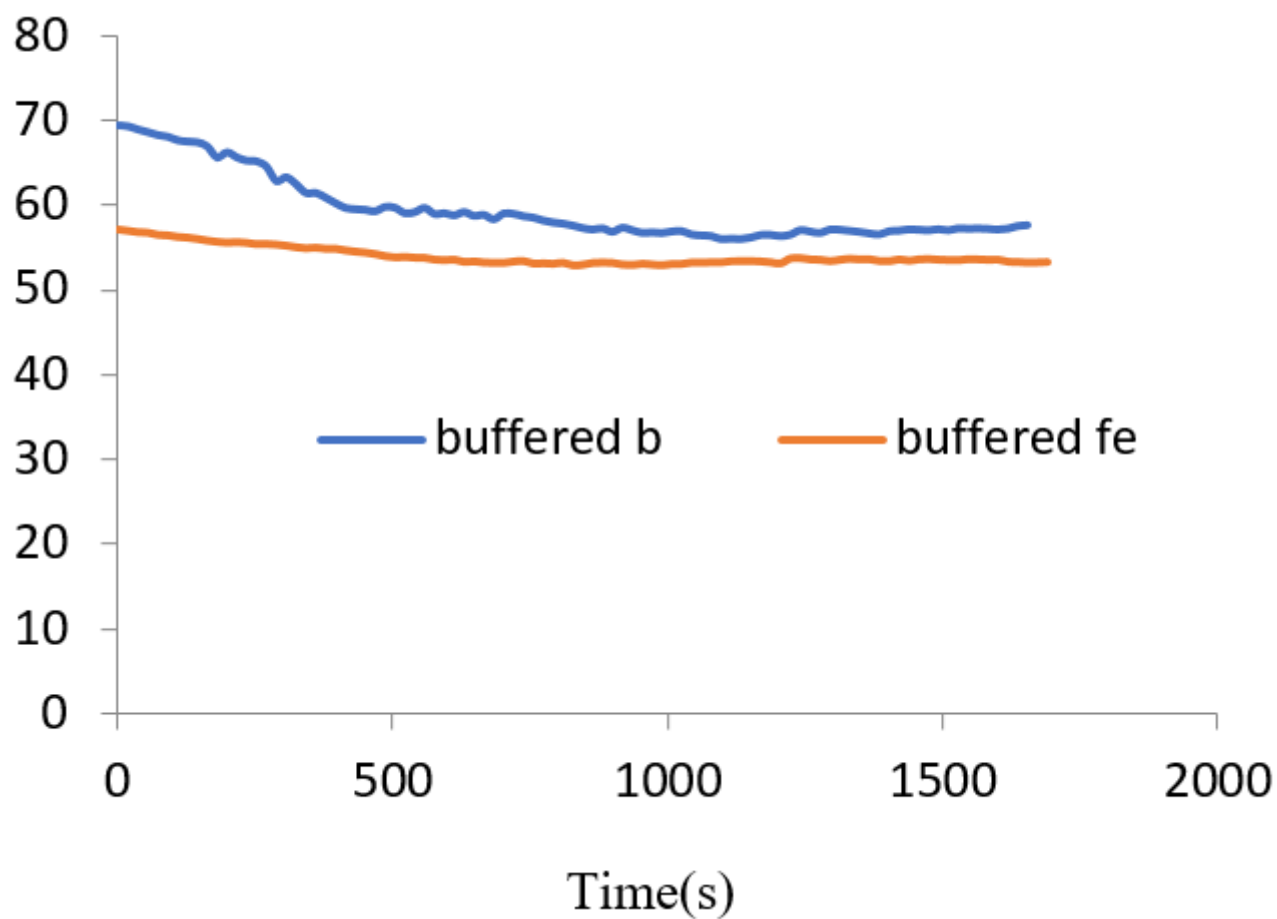


Figure 10

I₃₈₅ of 3.0 μ M BPhen solutions over time with and without the addition of Fe(II) (concentration is 100 nM).

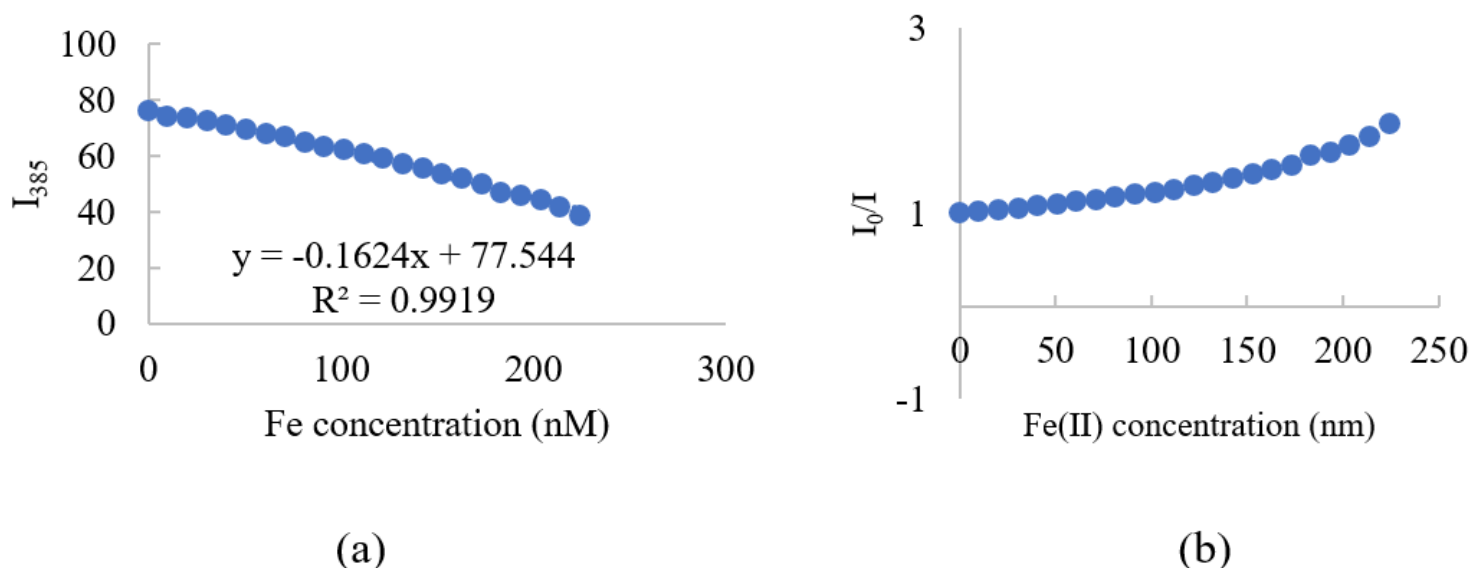


Figure 11

(a) Change in emission intensity of a 3.0 μ M BPhen solution in 50% ethanol at 385 nm against the concentration of Fe(II); (b) Stern-Volmer plot obtained by spiking a 2.5 μ M Fe(II) solution into a 3.0 μ M BPhen solution at pH 6.6.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [structure1.png](#)