



EVALUATION OF A ZINC(II) PHENANTHROLINE COMPLEX AS A POTENTIAL INORGANIC PHOSPHATE SENSOR

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INTRODUCTION

Phosphorus is an essential nutrient for life, most commonly in the form of phosphates. The excess of inorganic phosphate ions is linked to eutrophication, which causes aquatic dead zones. The development of biocompatible receptors with high sensitivity and selectivity for inorganic phosphates is vital for the purpose of qualitative identification, quantification and the removal of phosphates present in water bodies. Most importantly, high selectivity over other anions must be a key feature of such receptors as water bodies are saturated with a wide variety of anions. For example, Toth and co-workers reported that a picolinate based Gd complex has high affinity for phosphate ions, but the assay result is interfered by bicarbonate and citrate ions (Palinkas et al., 2009). When it comes to metal based phosphate probes, indicator displacement assay is one of the most acceptable strategies with high selectivity, sensitivity, convenience and efficiency (Raju et al., 2020). In indicator displacement assay, the indicator, a weak ligand, binds reversibly with metal receptors and upon the presence of phosphate ions, the indicator displaces and gives out colorimetric or some other form of readout. For this strategy to be successful, the binding affinity of the metal receptor for phosphate ions must be greater than the affinity for the indicator. In this study, we modified a Zn(II) phenanthroline complex using Eriochrome Black-T (EBT) to probe inorganic phosphate via indicator displacement assay.

Zn(II) metal ion was selected due to its interesting properties such as biocompatibility, flexible coordination preferability (Kumar et al., 2021), fast interconversion among different coordination states and high kinetic lability compared to other similar metal complexes (quick ligand exchange in its coordination sphere). Further, the Zn(II) ion has fully filled d^{10} electronic configuration liable for its redox inertness and very low affinity to generate reactive oxygen species. The ligands in a metal complex play a key role in the overall reactivity of the complex and in the nature of interactions involved in the recognition and sensing of target sites (Alemu, 2017). The selected ligand here, 1,10-phenanthroline, is a commonly available heterocyclic bidentate N-donor ligand. A pH sensitive dye, Eriochrome Black-T (EBT), is chosen as the indicator due to its excellent complexing ability with zinc ions (Vega et al., 2011) and the distinguishable visual change upon displacement. Blue colour EBT solution at pH 10 turned to red upon the addition of $[Zn(phen)_2]^{2+}_{(aq)}$ complex which enabled us to confirm the complex formations and displacements visually. To our knowledge, the proposed $[Zn(phen)_2(EBT)_2]^{2+}_{(aq)}$ complex or its applicability as an inorganic phosphate probe is not reported.

METHODOLOGY

Materials: Zinc nitrate hexahydrate, 1,10-phenanthroline monohydrate and Eriochrome Black-T were used as received. All other reagents and solutions were prepared with standard procedures and as required.

Synthesis and characterization of $Zn(phen)_2(NO_3)_2 \cdot 2H_2O$ complex: The Zinc complex, $Zn(phen)_2(NO_3)_2 \cdot 2H_2O$, was prepared by a method reported in literature (Alemu, 2017; Matos et al., 2019). The resultant white solid was recrystallized via slow evaporation and dried over silica gel under vacuum. The purity and in-solution stability were confirmed by FTIR, 1H NMR and UV/Vis characterization.

[Zn(phen)₂](EBT)₂]²⁺_(aq) complex formation: A series of solutions containing 0.3 mM EBT and 0.1 mM of [Zn(phen)₂]²⁺_(aq) were prepared in double distilled water with varying pH from 1 to 12, in order to determine optimal pH for the [Zn(phen)₂(EBT)₂]²⁺_(aq) complex formation. The stoichiometric ratio between [Zn(phen)₂]²⁺_(aq) and EBT was determined using Job's plot of continuous variation ($\lambda_{\max} = 546 \text{ nm}$). The Benesi-Hildebrand method was used to determine the association constant of [Zn(phen)₂(EBT)₂]²⁺_(aq).

Phosphate ion probing analysis: A 2 ml aliquot of [Zn(phen)₂(EBT)₂]²⁺_(aq) solution (50 μM , pH 10) was taken in a cell and the UV/Vis absorbance spectra were recorded before and after the addition of different amounts of phosphate ions (final total volume was 4 ml). The association constant and limit of detection of [Zn(phen)₂(HPO₄)_n]^x_(aq) were determined using Benesi-Hildebrand method and OriginPro software, respectively.

Interference study: The UV/Vis absorbance of [Zn(phen)₂(EBT)₂]²⁺_(aq) complex (50 μM , pH 10) was recorded before and after the addition of anions; SO₄²⁻, HPO₄²⁻, Br⁻, Cl⁻, HCO₃⁻, F⁻, NO₃⁻ and CH₃COO⁻ by keeping the ratio of [Zn(phen)₂]²⁺_(aq): anion as 1:10. The spectral changes and visual colour changes were recorded.

RESULTS AND DISCUSSION

The Zn(phen)₂(NO₃)₂·2H₂O complex (Figure 1(a)) is in octahedral geometry with two bidentate phenanthroline ligands (Matos et al., 2019). These two phenanthroline ligands are the strong ligands which ensures that Zn(II) complex is always complexed in solution. The purity and complex formation were confirmed by comparing obtained UV/Vis (Figure 1(b)), FTIR and ¹H NMR spectroscopic data with literature (Matos et al., 2019).

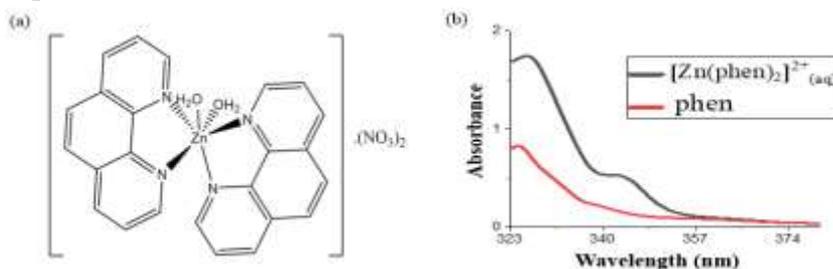


Figure 1: (a) The structure of Zn(phen)₂(NO₃)₂·2H₂O complex
(b) UV/Vis absorbance of phen ligand (1 mM) and [Zn(phen)₂]²⁺_(aq) complex (1 mM) in DMSO at room temperature

The stability of the Zn(phen)₂(NO₃)₂·2H₂O complex in working solutions was evaluated by monitoring the UV/Vis absorbance spectra: at neutral (pH 7) and basic (pH 10) conditions, spectra did not exhibit any changes with time. However, in acidic medium (pH 2), the spectral shape was distorted perhaps due to stacking of the heterocyclic rings of phenanthroline.

[Zn(phen)₂(EBT)₂]²⁺_(aq) Complex Formation

The optimal pH for the [Zn(phen)₂(EBT)₂]²⁺_(aq) complex formation was found to be in the range of 9 to 10.5 and therefore this pH was maintained throughout the experiment. Experimentally obtained Job's plot of continuous variation (Figure 2(a)) indicated the stoichiometric ratio between [Zn(phen)₂]²⁺_(aq) and EBT as 1:2. Thus, for the probing analysis, this ratio was used to prepare the [Zn(phen)₂(EBT)₂]²⁺_(aq) probe solutions.

Phosphate Sensing Analysis

Upon the addition of [Zn(phen)₂]²⁺_(aq) to EBT solution (1:2), approximately 19 nm bathochromic shift was observed at 565 nm and the colour of the solution turned from blue to red. Simultaneously, one new band appeared at 600 nm and these two bands correspond to the binding of [Zn(phen)₂]²⁺_(aq) to the EBT. As expected, the new bands at 565 nm and 600 nm in [Zn(phen)₂(EBT)₂]²⁺_(aq) gradually disappeared upon the consecutive addition of phosphate ions as shown in Figure 2(b) and also red colour turned to blue indicating the release of free EBT upon

phosphate binding.

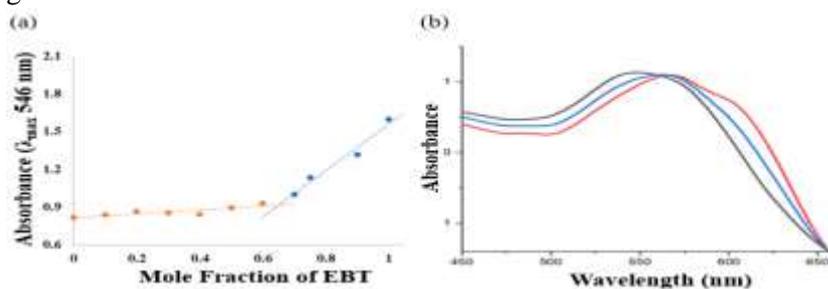


Figure 2: (a) Absorbance was recorded at 546 nm to determine $[\text{Zn}(\text{phen})_2]^{2+}_{(\text{aq})}$: EBT stoichiometric ratio via Job's plot of continuous variation (b) UV/Vis absorbance of EBT (black), $[\text{Zn}(\text{phen})_2(\text{EBT})_2]^{2+}_{(\text{aq})}$ (red) and $[\text{Zn}(\text{phen})_2]^{2+}_{(\text{aq})}$ with excess phosphate ions (blue)

The association constant was calculated for $[\text{Zn}(\text{phen})_2(\text{EBT})_2]^{2+}_{(\text{aq})}$ complex formation and $[\text{Zn}(\text{phen})_2(\text{HPO}_4)_n]^{x+}_{(\text{aq})}$ complex formation from Benesi-Hildebrand plot and their affinity were found to be $2.7 \times 10^3 \text{ mM}^{-1}$ and $1.6 \times 10^4 \text{ mM}^{-1}$ respectively. The affinity of $[\text{Zn}(\text{phen})_2(\text{EBT})_2]^{2+}_{(\text{aq})}$ was one order of magnitude lower than the affinity of $[\text{Zn}(\text{phen})_2(\text{HPO}_4)_n]^{x+}_{(\text{aq})}$ complex, which corroborate the fact that EBT ligands can be readily displaced by the inorganic phosphate ions. The limit of detection for phosphate ions was found to be 0.087 mM (Figure 3).

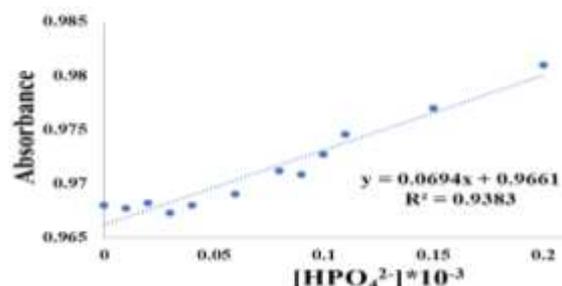


Figure 3: Absorbance at 546 nm was recorded for $[\text{Zn}(\text{phen})_2(\text{EBT})_2]^{2+}_{(\text{aq})}$ (50 μM) with varying concentration of phosphate ions to obtain limit of detection

An interference study was carried out in order to confirm that none of the anions except phosphate ions alter assay results. An observable colour change of red to blue was observed only in the sample with phosphate ions (Figure 4(b)). Moreover, the spectral change in Figure 4(a) clearly illustrates the displacement of EBT due to phosphate ions, but no such change was observed with the presence of other anions. It confirmed that the new probe exhibits excellent selectivity towards phosphate ions over the other anions. This sensor is of particular interest due to its convenient ability to detect phosphates by the naked eye, without spectrophotometer.

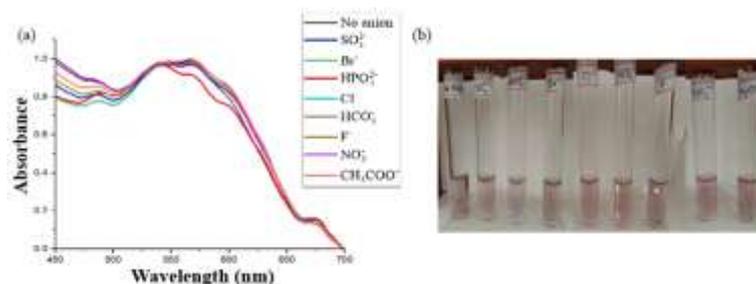


Figure 4: (a) Spectral changes of $[\text{Zn}(\text{phen})_2(\text{EBT})_2]^{2+}_{(\text{aq})}$ (50 μM) in basic medium (pH 10) was observed in the presence of different anions (5 mM) (b) Change in colour of $[\text{Zn}(\text{phen})_2(\text{EBT})_2]^{2+}_{(\text{aq})}$ (50 μM) in basic medium (pH 10) was observed in the presence of different anions (5 mM)

CONCLUSIONS/RECOMMENDATIONS

In summary, we have assembled a novel colorimetric sensor, $[\text{Zn}(\text{phen})_2(\text{EBT})_2]^{2+}_{(\text{aq})}$, which can detect inorganic phosphate ions spectrophotometrically and visually. This sensor has high selectivity towards phosphate anions over a variety of other anions. The limit of detection was found to be 0.087 mM. With further investigations, the proposed sensor could be developed to use in quantitative analysis of inorganic phosphates in water. Further, as Zinc(II) phenanthroline complexes reported to be fluorescent, $[\text{Zn}(\text{phen})_2(\text{EBT})_2]^{2+}_{(\text{aq})}$ might have the potency to produce fluorometric responses with the presence of inorganic phosphates, which could only be confirmed with further experiments.

ACKNOWLEDGMENTS

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