A Novel Copper(II) Sensor Based on a Unique Water-soluble Porphyrin

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A unique water-soluble porphyrin [5,10,15,20-tetra(3-ethoxy-4-hydroxy-5-sulfonate)phenyl porphyrin, H₂TEHPPS] was designed and synthesized, which could be used as a potential sensor for the determination of Cu²⁺. Studies were performed in the solution. The concentration of H₂TEHPPS was 5 μmol L⁻¹. The optical properties of H₂TEHPPS were investigated based on the absorption spectra. The results show that the absorbance of H₂TEHPPS at 417 nm is directly proportional to the concentration of Cu²⁺ in the range of 0 - 2.5 μmol L⁻¹. H₂TEHPPS can thus be used as a novel class of Cu²⁺ sensors.

Keywords Water-soluble porphyrin, absorption spectrum, copper, sensor

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Introduction

Copper is an essential trace element that is vital to the health of all living things. In humans, copper is essential for the proper functioning of organs and metabolic processes, such as bone formation, hematopoietic function, cellular respiration, the prevention of cardiovascular diseases and connective tissue development. Copper is absorbed, transported, distributed, stored, and excreted in the body according to complex homeostatic processes that ensure a constant and sufficient supply of the micronutrient while simultaneously avoiding excess levels. If an insufficient amount of copper, or too much copper, is ingested this can lead to tissue injury and disease. In addition to being an essential nutrient for humans, copper is vital for the health of animals and plants, and plays important roles in agriculture. Thus, the detection and quantification of copper ions have become an important issue in chemical analysis as well as bioanalytical and environmental protection.

So far, many methods have been described for the detection of Cu²⁺, including atomic absorption spectrometry, inductively coupled plasma-mass spectrometry (ICP-MS), inductively coupled plasma-atomic emission spectrometry (ICP-AES), and capillary electrophoresis. Although each method has its own advantages, these methods are limited in their use for in situ and on-line monitoring because of their size, expensive instruments or tedious separation. In recent years, UV-vis spectrophotometry has indeed received increasing interest, due to the relatively simple and inexpensive instrumentation involved.

Porphyrins are a class of naturally macrocyclic compounds that play a very crucial role in the metabolism of living organisms. Porphyrins have unique biological activity and excellent physicochemical property as a result of their characteristic ring structure of conjugated double bonds, and have been widely used in many fields, such as sensors, solar cells, photodynamic therapy, and so forth. The spectrophotometric characteristics of porphyrin could be altered by bonding or interactions with other molecules and ions. The greater is the association energy and association constant, the greater is the spectra change that is essential for forming the basis of optical sensor elements. Recently, a considerable stack of reports have been published concerning the use of porphyrins and its derivatives in sensors for detecting toxic gases, metal ions, herbicides, and so forth.

Experimental

Reagents and chemicals
3-Ethoxy-4-hydroxy-benzaldehyde, propionic acid, chlorosulfonic acid, CHCl₃ and pyrrole were purchased from Shanghai Chemical Reagents (Shanghai, China), and used as received. N,N-Dimethylformamide (DMF) was freshly distilled from P₂O₅. Silica gel (mash 300 - 400) was purchased from Beijing.
Chemical Factory (Beijing, China). Acetic acid and sodium acetate were purchased from Beijing Chemical Reagent Corporation (Beijing, China). Solutions of metal ions were prepared from their salts, which were purchased from Beijing Chemical Reagent Corporation (Beijing, China). Double-distilled water was used throughout all experiments.

UV-Vis absorption measurements were preceded on a UV2365 spectrometer (Shimadzu). The temperature of a porphyrin aqueous solution was controlled by an electric-heated thermostatic water bath. During the experiment, a 1-cm path length quartz cuvette was used to measure the absorption.

Syntheses of H₂TEHPPS

There were three steps used to synthesize H₂TEHPPS from 3-ethoxy-4-hydroxy-benzaldehyde and pyrrole. The first step was to synthesize meso-tetrakis-(3-ethoxy-4-hydroxy)phenyl porphyrin (H₂TEHPPy) with 3-ethoxy-4-hydroxy-benzaldehyde and pyrrole under reflux using propionic acid as a reagent. The second step was to protect the hydroxyl group of H₂TEHPPy using chloromethyl methyl ether. The last step was to synthesize H₂TEHPPS with the hydroxyl group protected H₂TEHPPy and chlorosulfonic acid. Please refer to our recently published work for the detailed synthesis procedure.

Absorbance determination procedure

H₂TEHPPS was dissolved in double-distilled water to obtain a 17 μmol L⁻¹ standard solution. H₂TEHPPS and Cu²⁺ standard solutions were added into 2 mL of acetic acid and sodium acetate buffer (HAc–NaAc, pH 3.4), then the solution was diluted to 4 mL with double-distilled water. The solution was allowed to stand for 10 min at 95°C before performing any absorbance measurement. A quartz cell of 1 cm path length was used.

Results and Discussion

Absorption property of H₂TEHPPS with Cu²⁺

The absorption spectra of H₂TEHPPS (5 μmol L⁻¹) with Cu²⁺ (10 μmol L⁻¹), heated for 10 min at 95°C in a HAc–NaAc buffer, were measured. The results are shown in Fig. 1. The absorption spectrum of H₂TEHPPS has a Soret band at 450 nm and a weak Q band at 673 nm. The peak at 450 nm shows that the structure of the J-aggregates is a stack of monomer units, stacked as a slipped deck of cards, with overlapping between the negative charges on the periphery of one molecule, and positive charges in the center of the neighboring porphyrin.

The addition of Cu²⁺ into the H₂TEHPPS solution induced a remarkable change in its electronic absorption spectrum, Fig. 1. Along with a blue-shift of the Soret band from 450 to 417 nm, the Q band at 673 nm disappeared while two new absorption peaks appeared around 540 and 580 nm. It is considered that the J-aggregation structure of porphyrin molecules dissociates into a monomer along with the interactions of copper ions (Scheme 1).

Job’s plot analysis was applied to the Cu-TEHPPS complex. The observed binding curve could be fitted to a 1:1 binding model, which gave an apparent association constant (Kₐ) of 1.03 × 10⁷ M⁻¹ (see Fig. S1 in Supporting Information). This demonstrated that the sitting-atop (SAT) complex [Cu(H₂TEHPPS)]²⁺ had been produced as an intermediate, and then the protons on two of the pyrrole nitrogen atoms could be lost to form Cu-TEHPPS upon subjecting the complexes to heat (95°C) for 10 min. In order to validate this mechanism, an NMR titration experiment was used. The ¹H NMR spectra of H₂TEHPPS before and after interacting with Cu²⁺ (Fig. S2) showed that the negative shift at –0.46 ppm for pyrrole N–H was gradually disappeared with an increase of the Cu²⁺ concentration, while other shifts were hardly changed, which also indicated that the pyrrole N chelate with Cu²⁺ and the protons would be lost to form Cu-TEHPPS. Otherwise, we also determined the absorption spectra of H₂TEHPPS to different common metal ions. The experiments were carried out by recording the absorption spectra change of H₂TEHPPS after titrating with the metal ions (Cu²⁺, 10 μmol L⁻¹; and other metal ions, 100 μmol L⁻¹; HAc–NaAc buffer, pH 3.4). The results are presented in Fig. S3. It shows that H₂TEHPPS has spectral selectivity for Cu²⁺. Therefore, H₂TEHPPS could be used to detect Cu²⁺ in following experiments.

Effect of different pH Values on Cu²⁺ determination

First, the effect the solution pH on the absorption spectra of free H₂TEHPPS was studied (Fig. S4). When the pH value was between 2.4 to 3.4, the maximum absorption peak of H₂TEHPPS was at 450 nm. When the pH was gradually increased to 6.4, the maximum absorption peak of H₂TEHPPS was blue shifted.
maximum absorption peak of H$_2$TEHPPS was red shifted to 417 nm. It was shown that H$_2$TEHPPS has the highest sensitivity for Cu$^{2+}$ (10 µmol L$^{-1}$) upon heating (95°C) with different reaction times. Inset, the absorbance at 417 nm of H$_2$TEHPPS with respect to the reaction time over the range of 0 - 20 min.

10 µmol L$^{-1}$ Cu$^{2+}$ were heated for 10 min at the setting temperature before any measurement. Figure 2 shows the absorption spectra of H$_2$TEHPPS titrated with Cu$^{2+}$ at different temperatures. It is shown that the complex reaction of H$_2$TEHPPS and Cu$^{2+}$ was very slow, and that the absorption at 417 nm did not change significantly when the reaction temperature was below 50°C. During the 50 to 90°C stage, this complex reaction was obviously accelerated, and the absorption at 417 nm increased significantly. When it was higher than 90°C, the absorption at 417 nm hardly changed. This indicates that the complex reaction rate reached the maximum at 90°C. Therefore, 95°C was selected as being the optimal reaction temperature for subsequent experiments.

The effect of the reaction time on the absorption spectra of the Cu-TEHPPS complex was studied at 95°C. The absorbance of free H$_2$TEHPPS at 417 nm was very low. Figure 3 shows that the absorbance of the H$_2$TEHPPS solution (HAc–NaAc, pH 3.4) at 417 nm in the presence of Cu$^{2+}$ increased sharply with the increasing of reaction time. The absorbance reached the maximum value within 10 min, and remained nearly constant thereafter, which demonstrated that the Cu-TEHPPS complex had reached equilibrium. Therefore, a reaction time of 10 min was selected in subsequent experiments.

Effect of common metal ions on Cu$^{2+}$ determination

In addition, the interference of common metal ions on the detection of Cu$^{2+}$ with H$_2$TEHPPS was carried out. Figure 4 (the blue bar portion) illustrates the absorbance of H$_2$TEHPPS to different metal ions of interest at 417 nm; no significant changes were observed with common interferences, such as alkali, alkaline earth and transitional metal ions, indicating that our proposed sensor exhibits high selectivity to Cu$^{2+}$ over other metal ions. To test the practical applicability of our chemosensor for Cu$^{2+}$, competition experiments were also carried out. The 10-times higher concentration of the above-mentioned metal ions (100 µmol L$^{-1}$) and 10 µmol L$^{-1}$ of Cu$^{2+}$ were added into a buffered H$_2$TEHPPS solution (HAc–NaAc, pH 3.4), and the absorbance of the chemosensor was detected and then compared with that of a buffered H$_2$TEHPPS solution (HAc–NaAc, pH 3.4) containing only 10 µmol L$^{-1}$ of Cu$^{2+}$. The results are also given in Fig. 4 (the red bar portion). Figure S6 shows that Fe$^{3+}$ and V$^{5+}$ have an interference effect of about 30%. This indicates

### Table 1: Absorbance of H$_2$TEHPPS and Cu-TEHPPS at 417 nm in different pH buffer solutions (inset, a plot of A/A$_0$ as a function of pH)

| pH | H$_2$TEHPPS $A_c$ (417 nm) | Cu-TEHPPS $A$ (417 nm) | A/A$_0$ |
|----|-----------------|-----------------|------|
| 2.40 | 0.25 | 0.38 | 1.52 |
| 3.40 | 0.27 | 1.24 | 4.59 |
| 4.40 | 0.39 | 1.27 | 3.26 |
| 5.40 | 1.15 | 1.21 | 1.05 |
| 6.40 | 1.16 | 0.99 | 0.85 |
| 7.40 | 0.84 | 0.75 | 0.89 |
| 8.40 | 0.72 | 0.72 | 1.00 |
| 9.40 | 0.64 | 0.72 | 1.12 |
| 10.40 | 0.59 | 0.69 | 1.17 |

Fig. 2 Absorption spectra of H$_2$TEHPPS (5 µmol L$^{-1}$) with Cu$^{2+}$ (10 µmol L$^{-1}$) under different temperatures; Inset, the absorbance at 417 nm of H$_2$TEHPPS with respect to the solution temperature over the range of 16 - 99°C.

The selection of temperature and reaction time

The effect of the solution temperature on the absorption spectra of the Cu-TEHPPS complex was studied. The 5 µmol L$^{-1}$ H$_2$TEHPPS solutions (HAc–NaAc, pH 3.4) with pH values. When the pH value was increased from 3.4 to 4.4, the maximum absorption of Cu-TEHPPS complex was at 417 nm. With the pH subsequently increased, the absorption of Cu-TEHPPS complex gradually descended at 417 nm.

According to Figs. S4 and S5, the absorbances of H$_2$TEHPPS and Cu-TEHPPS at 417 nm in different pH buffer solutions (inset, a plot of $A/A_0$) with different reaction times. Inset, the absorbance of Cu-TEHPPS at 417 nm in the presence of Cu$^{2+}$ increased sharply with the increasing of reaction time. The absorbance reached the maximum value within 10 min, and remained nearly constant thereafter, which demonstrated that the Cu-TEHPPS complex had reached equilibrium. Therefore, a reaction time of 10 min was selected in subsequent experiments.

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that these metal ions can also form a complex with H$_2$TEHPPS. When the absorbance of Cu-TEHPPS was detected with these coexisting ions, these ions would consume a certain concentration of H$_2$TEHPPS. Thus, the absorbance of H$_2$TEHPPS to Cu$^{2+}$ with these coexisting ions is lower than H$_2$TEHPPS containing only Cu$^{2+}$. In this part, tartaric acid (1 mmol L$^{-1}$) was chosen as a masking agent to eliminate any interference of these ions. A 10-fold excess of Fe$^{3+}$ and V$^{5+}$ could be masked by tartaric acid (Fig. S7, Supporting Information).

These experimental results showed that the response of the sensor to Cu$^{2+}$ was unaffected by the presence of other potentially contaminating metal ions. These results implied that the selectivity of H$_2$TEHPPS toward Cu$^{2+}$ was remarkable, suggesting that H$_2$TEHPPS is a viable selective chromogenic sensor for Cu$^{2+}$.

**Sensitivity and response range of H$_2$TEHPPS for Cu$^{2+}$ determination**

In this part, we wanted to detect the Cu$^{2+}$ range from 0.05 to 30 μmol L$^{-1}$. The absorbance of Cu$^{2+}$ with H$_2$TEHPPS would be very high if the concentration of H$_2$TEHPPS was 5 μmol L$^{-1}$. This means that only a small amount of the light detected by the detector and low signal measured could increase the error of the measurement. Therefore, the concentration of H$_2$TEHPPS would be adjusted to 1 μmol L$^{-1}$ during the determination. Under the optimized conditions, the absorption spectra of H$_2$TEHPPS increased with the Cu$^{2+}$ concentration increasing in the range of 0.05 to 30 μmol L$^{-1}$; the absorbance of H$_2$TEHPPS at 417 nm was 5 μmol L$^{-1}$. The limit of detection of Cu$^{2+}$ was 15 nmol L$^{-1}$ (based on S/N = 3). Therefore, the presented method provides a sensitive chemical sensor that may be used for the detection of Cu$^{2+}$ in actual samples.

**Analysis of the target element in water samples**

To test the reliability of this method, we collected four different water samples from South lake and Yitong river in Changchun, China. In order to reduce the pH influence in the detection, 2 mL of a HAc-NaAc buffered solution containing H$_2$TEHPPS was added to 1 mL of the sample to keep the pH value at 3.4. The solution was then diluted to 4 mL with double-distilled water. Its absorbance at 417 nm was measured. Cu$^{2+}$ in four samples was not found, and recovery experiments for varying the added amounts of Cu$^{2+}$ were carried out. The experimental results are given in Table 2. These results confirmed the validity of the proposed method.

**Table 2  Results of Cu$^{2+}$ detection in water samples**

| Water sample | Cu$^{2+}$ added/μmol L$^{-1}$ | Cu$^{2+}$ found/μmol L$^{-1}$ | Recovery, % |
|--------------|-------------------------------|-------------------------------|-------------|
| 1            | 1.00                          | 0.966                         | 96.5        |
|              | 5.00                          | 5.25                          | 105         |
| 2            | 1.00                          | 0.988                         | 98.8        |
|              | 5.00                          | 5.10                          | 102         |
| 3            | 1.00                          | 0.993                         | 99.3        |
|              | 5.00                          | 5.20                          | 104         |
| 4            | 1.00                          | 0.976                         | 97.6        |
|              | 5.00                          | 4.90                          | 98          |

**Conclusions**

We have designed and synthesized a novel water-soluble porphyrin H$_2$TEHPPS, which shows a remarkable enhancement of the absorbance in the presence of Cu$^{2+}$ at 417 nm. Moreover, the compound showed excellent selectivity for Cu$^{2+}$ over other metal ions. Under the optimized conditions of aqueous media buffered by 2 mL of HAc-NaAc at pH 3.4, the determination of Cu$^{2+}$ with H$_2$TEHPPS (1 μmol L$^{-1}$) has a satisfactory linear response range of 0.05 to 2.5 μmol L$^{-1}$, with a detection limit of 15 nmol L$^{-1}$. The H$_2$TEHPPS as a selective and sensitive Cu$^{2+}$ sensor was used to detect several actual water samples. The results are satisfactory.

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