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Two Novel Colorimetric Probes (5-HMBA-FH and 3-HMBA-FH) Based on Fluorescein for Copper(II) ion Detection

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Draft

Abstract

Two novel isomeric colorimetric probes are established for simultaneous determination of copper ions using 2-Hydroxy-5-methoxybenzaldehyde fluorescein hydrazone (5-HMBA-FH) and 2-Hydroxy-3-methoxybenzaldehyde fluorescein hydrazone (3-HMBA-FH). They are synthesized by reacting fluorescein hydrazide with 2-Hydroxy-5-methoxybenzaldehyde and 2-Hydroxy-3-methoxybenzaldehyde, respectively, and then characterized by ^1H -NMR, ^{13}C -NMR and Infrared spectrum. In addition of copper ions to the solutions of two novel colorimetric probes can generate the obviously peaks at 498 nm in UV-vis absorption spectra along with a rapid color change from colorless to dark yellow. The detection limits of the method for Cu^{2+} ion were 3.442×10^{-6} mol/L and 3.682×10^{-6} mol/L separately, for 5-HMBA-FH and 3-HMBA-FH. The additions of other metal ions hardly affect the copper ions determination. The proposed method was successfully applied to the analysis of Cu^{2+} ions in various samples. This method possessing high sensitivity, simplicity and minimized interfere will provide a great advantage in detecting copper ions in the environmental, food, medical applications.

Keywords: Fluorescein, Colorimetric probes, UV-vis spectra, Copper ions detection

Introduction

Due to the industrialization and urbanization, the heavy metal pollution has become a serious and urgent problem for the society^{1,2}. Among these heavy metals, copper and related compounds as fungicides and bactericides are still being intensively used in agricultural production^{3,4}. In many places, the copper content in the soil presents much high level⁵. Although the copper ion is a kind of essential elements for plant growth and human health, the excessive amount of copper may be harmful to plants, animals and humans⁶⁻⁸. Thus, in order to avoid too much copper intake, it is essential to establish an accurate, sensitive, highly effective and low cost method for timely monitoring the concentration of Cu^{2+} ion in environmental and foods samples.

Previously, the numerous types of techniques adopted for Cu^{2+} ion measurement included atomic absorption spectrometry^{9,10}, chemiluminescence, ICP-OES and spectrophotometric methods¹¹⁻¹⁴. Thereinto, the spectrophotometric method combined with colorimetric probes or fluorescent probes, in terms of fast screening, simple operation, high precision and amenability towards visual inspection, have attracted considerable attentions to detect heavy metal ions in water. For example, Chang's group successfully developed a colorimetric sensing method based on the CHA reaction for protein detection¹⁵. These probes based on some special chromogenic or fluorescent compounds are mostly composed of a large rigid π -conjugated system such as coumarins, quinolones, fluoresceins, rhodamines, boron-dipyrromethene (BODIPY) dyes, calixarenes and polythiophene¹⁶⁻²⁰. In addition, compared with other methods, the application of the colorimetric probes are less cost than common commercial methods

due to the visible to naked eye and no large-scale instruments and equipments needed in the whole process of the experiment.

The fluorescein and rhodamine families, as excellent antenna chromophores, own outstanding optical properties, for instance, long emission and absorption wavelength, relatively large fluorescence quantum yield, large extinction coefficient and high stability to light²¹⁻²³. They are frequently used as raw materials synthesizing fluorescent probes and colorimetric probes, such as pyridoxal fluorescein hydrazone, 1-phenyl-3-methyl-5-hydroxypyrazole-4-benzoyl (fluorescein) hydrazone and 2,7-di(2-benzothiazolyl)-fluorescein^{24,25}. In principle, the primitive state of the fluorescein-based probes in aqueous solution have no fluorescence and color. After adding the detection targets (metal ions), the probes are easy to combine with the specific metal ions forming new coordination bonds or new compounds, which leads to significant changes in color or fluorescence. Up to now, many fluorescein derivatives have been designed and synthesized as fluorescent probes or colorimetric probes for the detection of heavy metal ions, such as Cu^{2+} , Pb^{2+} , Hg^{2+} , As^{3+} and Cd^{2+} ²⁶⁻²⁸. However, the comparative experiment of two isomeric probes based on fluorescein have rarely been reported hitherto.

Hence, the main objective of this study aims to design and synthesize two novel isomeric probes, that is, 2-Hydroxy-5-methoxybenzaldehyde fluorescein hydrazone (5-HMBA-FH) and 2-Hydroxy-3-methoxybenzaldehyde fluorescein hydrazone (3-HMBA-FH), to detect Cu^{2+} ions. Next, several influence factors containing standing time, solution acidity, Cu^{2+} ion concentration and other metal ions are investigated on the

detection of Cu^{2+} ions by using 5-HMBA-FH and 3-HMBA-FH as colorimetric probes. The possible reaction mechanisms and detection processes of above two probes in detection of Cu^{2+} ions are also studied in detail. In the end, the comparative experiments of the two probes are carried out. When 5-HMBA-FH and 3-HMBA-FH are simultaneously used as the colorimetric probes to detect Cu^{2+} ions, the former is more selective than the latter. Therefore, 5-HMBA-FH was built for quantitatively detecting Cu^{2+} ions in lake water and food samples.

Experimental

Reagents and chemicals

Fluorescein (98 %, Sinopharm Chemical Reagent Co., Ltd. Shanghai, China), hydrazine hydrate (85 %, Sinopharm Chemical Reagent Co., Ltd. Shanghai, China), 2-Hydroxy-5-methoxybenzaldehyde (98 %, Aladdin Chemical Co., Ltd. Shanghai, China), 2-Hydroxy-3-methoxybenzaldehyde (99 %, Sinopharm Chemical Reagent Co., Ltd. Shanghai, China) and other materials for syntheses of 2-Hydroxy-5-methoxybenzaldehyde fluorescein hydrazone (5-HMBA-FH) and 2-hydroxy-3-methoxy-benzaldehyd fluorescein hydrazone (3-HMBA-FH) were purchased and directly used without further purification. Other used chemicals were all analytical grade. Ultrapure water was used throughout the work. The pH value of aqueous solution was adjusted with Britton-Robinson (B-R) buffer solution and measured by Global digital pH meter. Phosphoric acid, Boric acid, Acetic acid and NaOH were used to prepare the Britton-Robison ($\text{pH} = 9.00$ and $[\text{B-R buffer}] = 40$

mmol·L⁻¹) buffer solution in order to maintain the ionic strength and adjust the solution acidity.

Apparatus

Infrared spectrums were taken in KBr disks on a spectrometer (5700 FT-IR, Nicolet Company, USA). NMR spectra were conducted with a Nuclear Magnetic Resonance Spectrometer (Technologies Plus-400MR, Agilent Company, USA) with DMSO-*d*₆ as the solvent and tetramethylsilane (TMS) as internal standard. Fluorescence enhancing experiments were carried out by fluorophotometer (Cary 300, Varian Company, USA) and UV-vis absorption spectra were recorded with an UV-Vis spectrophotometer (Cary 50, Varian Company, USA).

Procedure

The synthesis procedure of the intermediate product fluorescein hydrazide is summarized in [Scheme 1](#). Fluorescein (8.00 g, 24.00 mmol) was dissolved in 120 mL of ethanol and excess hydrazine (25 mL, hydrazine content > 80 %) was added by drop. Then the mixed solution was stirred for 9.0 h under heat-refluxing. In this process the suspended particles were consumed and a clear solution was obtained. Afterwards, when the reaction mixture was cooled down to the room temperature (about 25 °C) the ensuing solvent was added into 600 mL ultrapure water and a pale yellow precipitate quickly formed. After two hours of settlement, the resulting precipitate was filtered and washed several times with ethanol. Finally, the residue was recrystallized using methanol and dried to give 5.85 g off white solid (70 %).

The synthesis procedures of 2-Hydroxy-5-methoxybenzaldehyde fluorescein hydrazone (5-HMBA-FH) and 2-hydroxy-3-methoxy-benzaldehyd fluorescein hydrazone (3-HMBA-FH) are also summarized in Scheme 1. 5-HMBA-FH was synthesized from fluorescein hydrazine and 2-Hydroxy-5-methoxybenzaldehyde in ethanol containing catalytic amount of acetic acid. Fluorescein hydrazide (2.76 g, 8.00 mmol) was dissolved in 100 mL ethanol as base under anhydrous condition. Subsequently, 2-Hydroxy-5-methoxybenzaldehyde (2.00 g, 13.14 mmol) was added to the mixed solution. The mixed solution was heated to reflux for 9.0 h with stirring. After the reaction, the mixture was cooled down to the room temperature (about 25 °C) and an off-white solid appeared. The precipitate was separated by filtration and the crude product was recrystallized using methanol to provide 2.53 g of 5-HMBA-FH as an off-white powder in 66 % yield. ¹H-NMR (DMSO-d₆, 300 MHz, 25 °C): δ(ppm): 10.76 (s, 1H), 9.97 (s, 2H), 9.22 (s, N=C-H, 1H), 7.93 (d, J = 6.1 Hz, 1H), 7.65 (s, 1H), 7.14 (d, J = 7.0 Hz, 2H), 6.94 (d, J = 8.0 Hz, 1H), 6.76 (s, J = 7.9 Hz, 2H), 6.66 (d, J = 2.1 Hz, 2H), 6.49 (dd, J₁ = 8.6, J₂ = 5.4, 4H) and 3.73 (s, 1H). ¹³C NMR (DMSO-d₆, 75 MHz, 25 °C): δ (ppm): 163.61, 158.82, 152.30, 150.68, 150.48, 147.93, 147.00, 134.24, 129.32, 128.95, 128.27, 123.98, 123.38, 120.45, 119.23, 119.18, 113.94, 112.66, 109.60, 102.67, 65.42, 55.79.

2-Hydroxy-3-methoxybenzaldehyde fluorescein hydrazone (3-HMBA-FH) was synthesized in accordance with the above steps. 2.48 g 3-HMBA-FH as an off-white powder was gotten in 65 % yield. ¹H-NMR (DMSO-d₆, 300 MHz, 25 °C): δ (ppm): 12.87 (s, 1H), 9.95 (s, 1H), 9.77 (s, 1H), 9.18 (s, N=C-H, 1H), 7.93 (d, J = 7.2 Hz, 1H),

7.64 (s, 1H), 7.17 (d, $J = 7.0$ Hz, 2H), 6.92 (d, $J = 8.0$ Hz, 1H), 6.89 (d, $J = 8.1$ Hz, 2H), 6.65 (s, $J = 7.9$ Hz, 2H), 6.47 (dd, $J_1 = 17.0$, $J_2 = 5.4$, 4H) and 3.65 (s, 1H). ^{13}C NMR (DMSO- d_6 , 75 MHz, 25 °C): δ (ppm): 163.52, 158.82, 152.43, 152.18, 151.38, 150.26, 149.40, 134.22, 129.35, 129.19, 128.21, 124.04, 123.38, 119.42, 118.75, 117.41, 112.60, 111.62, 109.80, 102.63, 65.49 and 55.52.

Scheme 1.

Results and Discussion

Design ideas of 5-HMBA-FH and 3-HMBA-FH.

Formerly, some metal ions sensors produced by fluorescein family dyes had been reported due to the characteristics, namely long absorption wavelength and long emission wavelength, great extinction coefficient, high stability and strong fluorescence. The mechanisms of these fluorescein-based metal ions probes were based on two kinds of spirocyclic structure and open cyclic forms. Most of the probes were generally analysed separately, however, the isomers of probes had never been studied. As a result, we synthesized two novel isomeric probes and investigated the influences of these different structures on probe detectability.

A chromophore and a ligand are two indispensable parts for colorimetric or fluorescent probes. The 5-HMBA-FH and 3-HMBA-FH were synthesized based on following two reasons. (1) Fluorescein hydrazide can be easily reacted with 2-Hydroxy-5-methoxybenzaldehyde and 2-hydroxy-3-methoxy-benzaldehyd, respectively, forming two isomeric probes which contain nitrogen atoms that are easy to

be combined with Cu^{2+} ions. (2) Fluorescein family dyes are remarkable chromophores. Therefore, 5-HMBA-FH and 3-HMBA-FH as probes for detection of Cu^{2+} ion were both obtained by two-step reactions (Scheme 1).

Infrared spectra of 5-HMBA-FH and 3-HMBA-FH.

5-HMBA-FH and 3-HMBA-FH were characterized by Fourier transform infrared spectrum (FT-IR) as shown in Fig. 1. The formation of $\nu_s(\text{C}=\text{C})$ bonds is evidenced by absorption peaks at 1609 cm^{-1} and 1503 cm^{-1} from the infrared spectrum of 5-HMBA-FH, the peak at 1695 cm^{-1} can be assigned to $\nu_s(\text{C}=\text{N})$, and the $\nu_s(\text{C}=\text{C})$ of fluorescein hydrazone (FHO) display at 1596 cm^{-1} and 1466 cm^{-1} , respectively. Similarly, the $\nu_s(\text{C}=\text{C})$ appear at 1613 cm^{-1} and 1505 cm^{-1} can be seen from the infrared spectrum of 3-HMBA-FH, and the $\nu_s(\text{C}=\text{N})$ appears at 1695 cm^{-1} . These shifts all confirm the formations of 5-HMBA-FH and 3-HMBA-FH, it is also proved that the reaction products are Schiff base compounds which are our target compounds.

Fig. 1.

Effect of standing time in the presence of Cu^{2+} ion.

As shown in Fig. 2, the influence of standing time on the UV-vis absorption spectra was measured for three concentrations of Cu^{2+} ion solutions including $8.00 \times 10^{-6}\text{ mol/L}$, $14.00 \times 10^{-6}\text{ mol/L}$ and $20.00 \times 10^{-6}\text{ mol/L}$ in B-R buffer aqueous solution. From Fig. 2(a, b), for 5-HMBA-FH+ Cu^{2+} and 3-HMBA-FH+ Cu^{2+} solutions of three different concentrations, the maximum absorbance values at 498 nm were almost no longer increased after 1.0 min response time, which indicating that both these two synthesized organic compounds as probes began to

rapidly react with Cu^{2+} ions, then the stable complexes were generated, that is, 5-HMBA-FH- Cu^{2+} complex and 3-HMBA-FH- Cu^{2+} complex. It meant that these two probes could be applied as a real-time detection and 1.0 min was used as a standard test time to carry out the Cu^{2+} ions detection experiment.

Fig. 2.

Effect of Cu^{2+} ion concentration.

The UV-vis spectra of 5-HMBA-FH and 3-HMBA-FH solutions with various concentrations of Cu^{2+} ions in ultrapure water at room temperature were depicted in Fig. 3. From Fig. 3(a, b). Results showed that the 5-HMBA-FH and 3-HMBA-FH solutions had hardly any absorption peaks in the visible region, thus, the solutions were colorless. After adding Cu^{2+} ions, the new peaks appeared obviously at 498 nm in UV-vis absorption spectra of 5-HMBA-FH and 3-HMBA-FH solutions. Furthermore, their absorption peaks gradually increased along with the increase of Cu^{2+} ions concentration. At the same time, for 5-HMBA-FH and 3-HMBA-FH solutions, their colors both changed from colorless to dark yellow, which are macroscopic. Indicating that the 5-HMBA-FH and 3-HMBA-FH both could form stable complexes with Cu^{2+} ions in aqueous solution due to the presence of coordinate atoms (O and N), which mainly forming the specific bonding between Cu^{2+} and the O and N atoms, and the suitable space configuration of ligands.

Fig. 3.

Further more, the UV-vis absorption of 5-HMBA-FH and 3-HMBA-FH with Cu^{2+} ions are shown in Fig. 4(a-1) and Fig. 4(b-1), respectively. As expected, the fluorescence intensities of

5-HMBA-FH and 3-HMBA-FH solutions both increased with addition of Cu^{2+} ions. The change of fluorescence intensities displays a good linear relationship in permitted detection concentration range of Cu^{2+} ions from 2.00×10^{-6} mol/L to 20.00×10^{-6} mol/L. The corresponding linear regression equations of 5-HMBA-FH and 3-HMBA-FH are $\Delta A = 2.54 \times 10^4 [\text{Cu}^{2+}] - 0.058$ ($R^2 = 0.9876$) and $\Delta A = 3.58 \times 10^4 [\text{Cu}^{2+}] - 0.031$ ($R^2 = 0.9891$), respectively. Apparently, the high linearities are satisfactory for the detection of Cu^{2+} ions. The absorbance differences at 498 nm $\Delta A = A - A_0$, A and A_0 are the absorbances of 5-HMBA-FH and 3-HMBA-FH solutions in the presence and absence of Cu^{2+} ions. Subsequently, the calculated limits of detection (LOD) of Cu^{2+} ion could be obtained by the formula ($\text{LOD} = 3\sigma/s$) based on the slopes which were mentioned in the above straight lines and the calculated results were 3.442×10^{-6} mol/L and 3.682×10^{-6} mol/L separately, for 5-HMBA-FH and 3-HMBA-FH. Apparently, the low limits of detection (LOD) were sufficient to prove that the 5-HMBA-FH and 3-HMBA-FH were both qualified probes for the detection of Cu^{2+} ions. By comparison, the 5-HMBA-FH gave a slightly LOD than the 3-HMBA-FH. The former showed more advantages in the area of Cu^{2+} ions testing.

Next, according to linear Benesi-Hildebrand expression (as shown in Fig. 4(a-2) and Fig. 4(b-2), the reciprocals of absorbance differences ($1/(A_{5\text{-HMBA-FH+Cu}} - A_{5\text{-HMBA-FH}})$ and $1/(A_{3\text{-HMBA-FH+Cu}} - A_{3\text{-HMBA-FH}})$) at 498 nm could be changed as a function of $1/[\text{Cu}^{2+}]$, and the obtained corresponding correlation coefficients (R^2) are 0.9509 and 0.9932, respectively, for 5-HMBA-FH and 3-HMBA-FH. It demonstrated that their high linearities are satisfactory for the speculation of compositions and calculation of stability constants of 5-HMBA-FH- Cu^{2+} and 3-HMBA-FH- Cu^{2+} complexes. Then, the good linear relationship indicated that the

compositions of 5-HMBA-FH-Cu²⁺ and 3-HMBA-FH-Cu²⁺ complexes were both 1:1, there was little change in the signal intensity when the ratio was greater than 1.0, therefore the ratio of 1.0 was chosen for subsequent experiments. And the stability constants of 5-HMBA-FH-Cu²⁺ and 3-HMBA-FH-Cu²⁺ complexes calculated by the slopes of the straight lines (from Benesi-Hildebrand method) were 2.54×10^4 mol/L and 3.58×10^4 mol/L, respectively, for 5-HMBA-FH and 3-HMBA-FH solutions. Through talking different outcomes, it could be demonstrated that, in the presence of Cu²⁺ ions, both of 5-HMBA-FH and 3-HMBA-FH form stable complexes with Cu²⁺ ions. Not only that, the 3-HMBA-FH-Cu²⁺ complex was slightly more stable than the 5-HMBA-FH-Cu²⁺ complex.

Fig. 4.

Effect of pH values in the presence of Cu²⁺ ion.

In addition, due to the presences of oxygen (O) and nitrogen (N) atoms in 5-HMBA-FH and 3-HMBA-FH as ligands, the acidity of the solution which is a major influence factor of great concern to the formation and stability of 5-HMBA-FH-Cu²⁺ and 3-HMBA-FH-Cu²⁺ complexes. In order to apply the 5-HMBA-FH and 3-HMBA-FH as probes to detect the Cu²⁺ ions in more complicated environmental conditions, the effects of pH values on the detection of Cu²⁺ ions were studied by UV-vis absorption spectroscopy with pH values between 3.00 and 12.00. As shown in Fig. 5(a, b), for both 5-HMBA-FH-Cu²⁺ and 3-HMBA-FH-Cu²⁺ solutions, the changes of acidity only affected the intensity of absorption peak but not shift the absorption peaks. All of these were obvious, the acidity changes only altered the amount of 5-HMBA-FH-Cu²⁺ and

3-HMBA-FH-Cu²⁺ complexes, their composition and structure in the given pH value range were not transformed.

In Fig. 5c, in the presence of Cu²⁺ ions, the absorption peaks were very low in weak acidic conduction (pH = 3.00 - 5.00), which makes the solutions of 5-HMBA-FH and 3-HMBA-FH almost colorless. It suggested that the 5-HMBA-FH and 3-HMBA-FH were both difficult to form stable complexes with Cu²⁺ ions and both of them were inappropriate to detect Cu²⁺ ions under acidic condition. Afterwards, from Fig. 5c, it could be seen that, for 5-HMBA-FH-Cu²⁺ and 3-HMBA-FH-Cu²⁺ solutions, the absorption peaks both increased along with the increase of pH values and reached their maximums at pH = 9.00. Next, they decreased with further increase of pH value from 9.00 to 12.00. It also could be seen that the colors of 5-HMBA-FH-Cu²⁺ and 3-HMBA-FH-Cu²⁺ solutions both changed from colorless (in weak acidity solution) to dark yellow (in weak basicity solution), and then to pale yellow (in basicity solution). According to our analysis, it implied that the 5-HMBA-FH-Cu²⁺ and 3-HMBA-FH-Cu²⁺ complexes may be destroyed partly in alkaline solution. To sum up, the weak basicity condition was necessary for both 5-HMBA-FH and 3-HMBA-FH in detection for Cu²⁺ ions and the pH 9.00 were selected as optimal condition for target detection.

Fig. 5.

Job's plot analyses.

The Job's plots were drawn and the results were given in Fig. 6 for further confirmation of the stoichiometric ratio of 5-HMBA-FH-Cu²⁺ and 3-HMBA-FH-Cu²⁺

complexes. The total concentrations of $[5\text{-HMBA-FH}]+[\text{Cu}^{2+}]$ and $[3\text{-HMBA-FH}]+[\text{Cu}^{2+}]$ were both fixed as 20.00×10^{-6} mol/L and the absorbance values at 498 nm were used as the analyses of Job's plots. As can be seen from Fig. 6a and Fig. 6b, for both $[5\text{-HMBA-FH}]+[\text{Cu}^{2+}]$ and $[3\text{-HMBA-FH}]+[\text{Cu}^{2+}]$ solutions, the absorbance increased along with the increase of Cu^{2+} ions concentration (or with the decrease of 5-HMBA-FH or 3-HMBA-FH concentration) at the beginning. The absorbance value got the plateau when the concentration of Cu^{2+} ions equalled to that of 5-HMBA-FH or 3-HMBA-FH ($[\text{Cu}^{2+}] = [5\text{-HMBA-FH}] = [3\text{-HMBA-FH}] = 10.00 \times 10^{-6}$ mol/L). Subsequently, the absorbance values decreased along with the further increase of Cu^{2+} ions concentration (or with the further decrease of 5-HMBA-FH or 3-HMBA-FH). It was further proved that the 5-HMBA-FH and 3-HMBA-FH both formed 1:1 molar ratio complexes with Cu^{2+} ion, which was the same as the results from linear Benesi-Hildebrand expression mentioned previously.

Fig. 6.

Fluorescence spectra of 5-HMBA-FH and 3-HMBA-FH solutions in the presence of Cu^{2+} ion.

The fluorescence spectra of 5-HMBA-FH and 3-HMBA-FH solutions were displayed in Fig. 7. It could be found that, in the absence of Cu^{2+} ion, the fluorescence emission peaks of 5-HMBA-FH and 3-HMBA-FH solutions were appeared at 516 nm and 519 nm, respectively, under respective excitation wavelength of lights at 481 nm and 492 nm. From Fig. 7a, after adding Cu^{2+} ions, the fluorescence emission peak of

5-HMBA-FH solution did not change, but the position of fluorescence emission peak shifted a little from 516 nm to 519 nm. It is implied that the effect of Cu^{2+} ions on the fluorescence emission spectrum of 5-HMBA-FH solution was not significant in terms of fluorescence intensity.

However, as shown in Fig. 7b, the fluorescence emission peak of 3-HMBA-FH solution increased obviously after adding Cu^{2+} ions, but the position of fluorescence emission peak was still not change, which showed that the addition of Cu^{2+} ion sensitized the fluorescence of 3-HMBA-FH solution. It could be confirmed that the Cu^{2+} ions had little influences on fluorescence of 5-HMBA-FH solution, but had a great effect on the fluorescence intensity of 3-HMBA-FH solution.

Fig. 7.

To determine the specificity and selectivity of the developed colorimetric probes. We investigated the sensitivity and selectivity of 5-HMBA-FH and 3-HMBA-FH for Cu^{2+} ions, other environmentally and physiologically active metal ions may coexist such as Fe^{2+} , K^+ , Zn^{2+} , Cr^{3+} , Ni^{2+} , Mn^{2+} , Al^{3+} , Ba^{2+} , Ca^{2+} , Fe^{3+} , Mg^{2+} , Cd^{2+} and Cr^{6+} as influencing factors were researched. As shown in Fig. 8(a-1) and Fig. 8(b-1), adding moderate above-mentioned metal ions, respectively, into the 5-HMBA-FH or 3-HMBA-FH solutions displayed different influences on the absorption spectra. When the Cu^{2+} , Fe^{2+} , Ni^{2+} and Fe^{3+} ions were added into the 5-HMBA-FH or 3-HMBA-FH solutions, as shown in Fig. 8(a-1) and Fig. 8(b-1), the relevant ultraviolet absorption peaks increased significantly. The corresponding color change could also be observed by

the naked eye, as the yellow color deepened with increasing concentrations of ions. Especially, the adding Cu^{2+} ions gave the highest absorption peak, which laid a solid foundation as the Cu^{2+} ions probes. This may be attributed to the high affinity between the target and aptamers. By comparing the absorption spectra of 5-HMBA-FH and 3-HMBA-FH solutions in Fig. 8(a-2) and Fig. 8(b-2), for 5-HMBA-FH, the difference between the absorption peaks containing Cu^{2+} ions and Fe^{2+} ions were bigger than that of 3-HMBA-FH. Indicating that, the selectivity of 5-HMBA-FH is more excellent than 3-HMBA-FH.

Fig. 8.

Futhermore, the interference experiments were performed by adding other metal ions solutions used above into the 5-HMBA-FH and 3-HMBA-FH solutions containing Cu^{2+} ions, respectively, in which the concentrations of other metal ions were the same as that of Cu^{2+} ions, to examine the influence of coexistent other metal ions on the detection of Cu^{2+} ions. The corresponding results were given in Fig. 9. As shown in Fig. 9a and Fig. 9b, the absorption spectra of the 5-HMBA-FH+ Cu^{2+} and 3-HMBA-FH+ Cu^{2+} solutions had little change after adding these metal ions (Fe^{2+} , K^+ , Zn^{2+} , Cr^{3+} , Ni^{2+} , Mn^{2+} , Al^{3+} , Ba^{2+} , Ca^{2+} , Fe^{3+} , Mg^{2+} , Cd^{2+} and Cr^{6+} ions). For both 5-HMBA-FH+ Cu^{2+} and 3-HMBA-FH+ Cu^{2+} solutions, only when the Zn^{2+} ions were added, the absorption peaks displayed a slight decrease. It demonstrated that the 5-HMBA-FH and 3-HMBA-FH as probes also showed very high sensitivity, selectivity and anti-interference performance in the detection of Cu^{2+} ions. Moreover, because the 5-HMBA-FH possessed much high

selectivity, it also displayed much better probe characteristics than the 3-HMBA-FH.

Fig. 9.

Analyses of Cu^{2+} ions in water and food samples.

In view of high selectivity and good probe characteristics, the 5-HMBA-FH was selected as probe for Cu^{2+} ion detection in actual samples. For checking the practicality of 5-HMBA-FH in factual Cu^{2+} ion detection, the standard recovery experiments were performed for diverse samples, such as lake water and food samples. After adding different concentrations of Cu^{2+} ion into lake water, milk and tea samples, respectively, the analyzed results were confirmed by the standard addition method. The results showed that the satisfactory recoveries between 91.20 % and 107.10 % were obtained for lake water, milk and tea samples (see Table 1). The results are in perfect agreement with the linear regression equation of $\Delta F = 1.9103C + 0.5024$. It suggested that this method can be straight forwardly implemented in the detection of Cu^{2+} ion in real samples, showing a good accuracy and reproducibility.

Table 1.

Conclusion

In summary, two highly sensitive colorimetric probes for detection of Cu^{2+} ion were reported, 2-Hydroxy-5-methoxybenzaldehyde fluorescein hydrazone (5-HMBA-FH) and 2-hydroxy-3-methoxy-benzaldehyd fluorescein hydrazine (3-HMBA-FH), which had been successfully synthesized by a simple two-step method. The detection performances of these two kinds of probes for Cu^{2+} ions were studied by UV-vis spectra and fluorescence spectra. The 5-HMBA-FH and 3-HMBA-FH could completely combine

with Cu^{2+} ions in a minute whose stoichiometric ratios were both 1:1 and their UV-vis absorption spectra varied greatly along with clear color changed from colorless to dark yellow. 5-HMBA-FH and 3-HMBA-FH also exhibited the excellent selectivity and anti-interference in the detection of Cu^{2+} ions over other alkali, alkaline earth and transition metal ions. In addition, the fluorescence of 3-HMBA-FH solution could be strongly sensitized after adding Cu^{2+} ions, which made it have potential to be a fluorescent probe. The limits of detection (LOD) were 3.442×10^{-6} mol/L and 3.682×10^{-6} mol/L, respectively, for 5-HMBA-FH and 3-HMBA-FH, indicating that the probes were sufficiently sensitive and relatively stable for real-time Cu^{2+} ions detection. What's more, the experimental results provided two good candidates for rapid, selective and sensitive detection of Cu^{2+} ions and a great potential application of the proposed method in real samples, thus paving the way for wide use of fluorescein in design of colorimetric probes.

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Content of Scheme, Figures and Table:

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Scheme 1. Synthetic routes for 5-HMBA-FH (I) and 3-HMBA-FH (II).

Figures:

Fig. 1. FT-IR spectra of FHO, 5-HMBA-FH and 3-HMBA-FH in KBr disk

Fig. 2. Absorbance of 5-HMBA-FH+Cu²⁺ and 3-HMBA-FH+Cu²⁺ solutions along with standing time.

Fig. 3. UV-vis absorption spectra of 5-HMBA-FH and 3-HMBA-FH solutions with various Cu²⁺ ion concentrations.

Fig. 4. Linear range calibration curve (1) of 5-HMBA-FH (a) and 3-HMBA-FH (b) in determination of Cu²⁺ ion and the corresponding Benesi-Hildebrand plot (2) of $1/(A_{5\text{-HMBA-FH+Cu}} - A_{5\text{-HMBA-FH}})$ versus $1/[\text{Cu}^{2+}]$ and $1/(A_{3\text{-HMBA-FH+Cu}} - A_{3\text{-HMBA-FH}})$ versus $1/[\text{Cu}^{2+}]$.

Fig. 5. UV-vis absorption spectra of 5-HMBA-FH+Cu²⁺ (a) and 3-HMBA-FH+Cu²⁺ (b) solutions and their corresponding absorbances (c) at different pH values.

Fig. 6. Job's plot of 5-HMBA-FH+Cu²⁺ (a) and 3-HMBA-FH+Cu²⁺ (b) solutions (at 498 nm).

Fig. 7. Fluorescence emission spectra of 5-HMBA-FH and 5-HMBA-FH+Cu²⁺ ($\lambda_{\text{ex}} = 481 \text{ nm}$) (a) and 3-HMBA-FH and 3-HMBA-FH+Cu²⁺ ($\lambda_{\text{ex}} = 492 \text{ nm}$) (b) solutions.

Fig. 8. UV-vis absorption spectra (1) of 5-HMBA-FH, 5-HMBA-FH+Cu²⁺ and 5-HMBA-FH+Mⁿ⁺ (a) and 3-HMBA-FH, 3-HMBA-FH+Cu²⁺ and 3-HMBA-FH+Mⁿ⁺ (b) solutions and the corresponding absorbances (at 498 nm) (2). ($[5\text{-HMBA-FH}] = [3\text{-HMBA-FH}] = [\text{Cu}^{2+}] = [\text{M}^{n+}] = 20.00 \times 10^{-6} \text{ mol/L}$, B-R buffer solution, pH = 9.00 and 25 °C. Mⁿ⁺: Fe²⁺, K⁺, Zn²⁺, Cr³⁺, Ni²⁺, Mn²⁺, Al³⁺, Ba²⁺, Ca²⁺, Fe³⁺, Cd²⁺, Cr⁶⁺ and Mg²⁺).

Fig. 9. UV-vis absorption spectra of 5-HMBA-FH+Cu²⁺ and 5-HMBA-FH+Cu²⁺+Mⁿ⁺ (a) and 3-HMBA-FH+Cu²⁺ and 3-HMBA-FH+Cu²⁺+Mⁿ⁺ (b) solutions. ($[5\text{-HMBA-FH}] =$

[3-HMBA-FH] = [Cu²⁺] = [Mⁿ⁺] = 20.00 × 10⁻⁶ mol/L, B-R buffer solution, pH = 9.00 and 25 °C.)

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Table 1. Recoveries of Cu²⁺ ion in lake water, milk and tea samples in the proposed method

Draft

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Table:

Table 1. Recoveries of Cu²⁺ ion in lake water, milk and tea samples in the proposed method

Samples	Spiked (10 ⁻⁶ mol/L)	Detected (10 ⁻⁶ mol/L)	Recovery (%)	RSD (n=3, %)
Water-1	5.00	5.33	106.60	1.87
Water-2	10.00	10.71	107.10	1.35
Water-3	20.00	19.21	96.05	1.72
Milk-1	5.00	4.66	93.20	2.21
Milk-2	10.00	9.12	91.20	2.07
Milk-3	15.00	15.32	102.13	1.42
Tea-1	5.00	4.80	96.00	2.24
Tea-2	10.00	10.52	105.20	1.57
Tea-3	15.00	14.21	94.73	1.03

Draft

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Water-1	5.00	5.33	106.60	1.87
Water-2	10.00	10.71	107.10	1.35
Water-3	20.00	19.21	96.05	1.72
Milk-1	5.00	4.66	93.20	2.21
Milk-2	10.00	9.12	91.20	2.07
Milk-3	15.00	15.32	102.13	1.42
Tea-1	5.00	4.80	96.00	2.24
Tea-2	10.00	10.52	105.20	1.57
Tea-3	15.00	14.21	94.73	1.03

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

Fig. 6.

Fig. 7.

Fig. 8.

Fig. 9.

Graphical Abstract

Two Novel Colorimetric Probes (5-HMBA-FH and 3-HMBA-FH)

Based on Fluorescein for Copper (II) ion Detection

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