Tb-metal organic frameworks-referenced bathocuproine disulfonate
enable fluorescence distinguishing Cu$^+$ from Cu$^{2+}$

Ya-Nan Zuo $^a$, Shuyi Liu $^a$, Xian-En Zhao $^{a,b}$, Shuyun Zhu $^{a,b,*}$, Guobao Xu $^c$

$a$ Key Laboratory of Catalytic Conversion and Clean Energy in Universities of Shandong Province, School of Chemistry and Chemical Engineering, Qufu Normal University, Qufu City, 273165, Shandong, China

$b$ Institute of Medical Healthy and New Materials Applied Technologies, Qufu Normal University, Qufu City, 273165, Shandong, China

$c$ State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Science, Changchun, Jilin, 130022, China

* Corresponding Author: shuyunzhu1981@163.com (S. Zhu).
Abstract

As the main existing form of copper, Cu\(^+\) plays vital roles in human health. It is highly significant to detect Cu\(^+\) selectively in biological matrixes especially in the coexistence of Cu\(^{2+}\). Herein, a ratiometric fluorescence sensor has been fabricated to discriminate Cu\(^+\) from Cu\(^{2+}\) and realize ratiometric detection of Cu\(^+\) by using Tb-MOFs and bathocuproine disulfonate (BCS). The sensor exhibits two fluorescence emissions at 400 nm from BCS as the response signal and 548 nm from Tb-MOFs as the reference signal. BCS can chelate with Cu\(^+\) selectively to form a stable adduct, inducing the fluorescence quenching of BCS through static quenching. When Cu\(^+\) is added, the intensity at 400 nm decreases significantly while that at 548 nm changes negligibly. Notably, the presence of Cu\(^{2+}\) exhibits no effect on the emissions both at 400 nm and 548 nm. Thus, Tb-MOFs-BCS system enable discrimination of Cu\(^+\) from Cu\(^{2+}\) and fast ratiometric detection of Cu\(^+\) within 1 min. The fluorescence ratio \(F_{548}/F_{440}\) shows a good linear relationship in Cu\(^+\) concentrations ranging from 1-200 nM with a low limit of detection of 0.3 nM, which is 1-3 orders magnitude lower than those of other methods. The high selectivity and sensitivity of this method enables the detection of Cu\(^+\) in human serum successfully.

Keywords: Ratiometric sensor; Cuprous ion; Fluorescence; Metal-organic framework; Static quenching

1. Introduction

Copper participates in numerous essential physiological processes including cell
respiration, growth and differentiation, and antioxidant defense [1]. In living system, copper possesses two oxidation states, namely Cu\(^{+}\) and Cu\(^{2+}\), of which Cu\(^{+}\) is the main existing form [2]. The Cu\(^{+}\)/Cu\(^{2+}\) transition affects their biological functions. Abnormal Cu\(^{+}\)/Cu\(^{2+}\) equilibrium disrupts copper homeostasis, resulting in some genetic disorders including Menkes disease, Wilson’s disease and so on [3,4]. Although it plays a vital role in human activities, excess Cu\(^{+}\) has critical toxicity to human body. In addition, the serum Cu\(^{+}\) levels are evaluated in various malignancies [5]. Thus, it is of importance to design a sensitive strategy for identification of Cu\(^{+}\) from Cu\(^{2+}\).

The detection of Cu\(^{+}\) may be available with a series of detection technology including UV-Vis spectroscopy [6,7], voltammetry [8], liquid chromatography [9], atomic spectroscopy [10], and fluorescent spectrometry [11]. Considering that Cu\(^{+}\) and Cu\(^{2+}\) possess similar physical and chemical properties and they coexist in biological systems, selective detection of Cu\(^{+}\) especially in the presence of Cu\(^{2+}\) is still a challenging endeavour. Fluorescence technique has drawn great interest for selective sensing Cu\(^{+}\) because of high sensitivity, high selectivity, rapid response and easy operation [12,13]. For example, polyaromatic amphiphilic probes [14], zinc porphyrin derivative [15], BODIPY-based optical probe [16], alkynyl Eu(III) complex [17] and metal-organic frameworks (MOFs) encapsulating carbon dots [18] have been designed and used for discriminating Cu\(^{+}\) from Cu\(^{2+}\). However, these intensity-based detection methods employed single signal response, which is usually affected by external circumstances such as excitation intensity, light source or detector drift, and instrument efficiency, resulting in low detection accuracy [19].
Ratiometric fluorescence sensors can solve the above problems [20,21]. They allow for accurate and quantitative readouts via recording two emission intensities at different wavelengths with a single excitation [21,22]. Up to now, few ratiometric probes have been designed and used to detect Cu$^{+}$ selectively. For instance, Yu et al. designed a ratiometric chemosensor for Cu$^{+}$ with coumarin-tris[(2-pyridyl)-methyl]amine derivative [24]. Satriano et al. prepared a naphthalimide derivative containing tetrathia-azacrown for ratiometric detection of Cu$^{+}$ owing to its selective binding with Cu$^{+}$ [25]. Chung et al. assembled a ratiometric probe using fluorescein and Rhodamine dyes to monitor Cu$^{+}$ [26]. Lee’s group synthesized a fluorescent peptidyl probe for ratiometric detection of Cu$^{+}$ [27]. However, these organic probes need complex and time-consuming synthesis procedures, and long response time owing to ring opening reactions caused by Cu$^{+}$.

MOFs have boomed as a fascinating luminescence material due to their excellent fluorescence [28]. As a significant branch of luminescent MOFs, lanthanide MOFs (Ln-MOFs) have drawn wide concern because of their outstanding features of large Stokes shifts, high quantum yield, long fluorescence life time and narrow emission band. Typically for Tb-MOFs, terbium (Tb$^{3+}$) severs as the metal center and $\pi$-conjugated organic ligands act as antenna, which enhances the emission of Tb$^{3+}$ ions through "antenna effect" [29]. Various Tb-MOFs-based ratiometric fluorescence probes have been designed including luminescent ligand modified Tb-MOFs [30-32], bi-lanthanide metal ions doped Tb-MOFs [33-35] and luminescent nanomaterials modified Tb-MOFs [36-39]. These dual-emissive fluorescence probes have been designed to construct
ratiometric sensors for pH [40], temperature [34], cations [38,39], small organic molecules [33, 35, 36] and so on. Up to date, there has been no attempt for ratiometric monitoring of Cu\(^+\) based on Tb-MOFs.

Bathocuproine disulfonate (BCS) can bind with Cu\(^+\) selectively to form a stable chelate with an absorption peak at 480 nm, which has been the basis for Cu\(^+\) colorimetric quantification [41]. However, the fluorescence properties of BCS are often ignored. Herein, we assemble Tb-MOFs and BCS together for discriminating Cu\(^+\) from Cu\(^{2+}\) and ratiometric sensing Cu\(^+\) (Scheme 1). Cu\(^+\) can chelate with BCS to form a specific 1:2 complex, inducing the fluorescence quenching of BCS significantly. The assembled probe exhibits two characteristic emissions at 400 nm of BCS as the response signal and 548 nm of Tb-MOFs as the reference signal. Notably, the presence of Cu\(^{2+}\) cannot interfere the detection of Cu\(^+\). Thus, a sensitive and selective ratiometric sensing platform has been constructed to detect Cu\(^+\) with \(F_{548}/F_{400}\) as readout. The ratiometric fluorescence sensor exhibits strong anti-interference and satisfactory accuracy to detect Cu\(^+\) in human serum.

2. Experimental

2.1. Materials and Apparatus

The materials and apparatus were provided in the Supporting Information.

2.2. Preparation of the Tb-MOFs

According to a previous method [42], Tb-MOFs was prepared as follows: 30 mL Tb(NO\(_3\))\(_3\) \cdot 5H\(_2\)O aqueous solution (0.02 M) was added into 30 mL ethanol containing 126 mg H\(_3\)BTC followed by stirring vigorously at room temperature. After 1 h, the
white precipitation was obtained by centrifugation and washing with ethanol and ultrapure water successively. The obtained MOF was finally dried at 35 °C for use.

**Scheme 1.** Discrimination of Cu$^{+}$ from Cu$^{2+}$ based on Tb-MOFs-BCS system.

2.3. Ratiometric detection of Cu$^{+}$

Tetrakis-(acetonitrile) copper(I) hexafluorophosphate was dissolved in acetonitrile to obtain Cu$^{+}$ stock solution. A series of different concentrations of Cu$^{+}$ were mixed with Tb-MOFs and BCS solutions with a final concentration of 7 μg/mL Tb-MOF solution and 400 nM BCS solution. After incubation for 1 min, the fluorescence emission spectra were recorded with excitation wavelength of 280 nm.
2.4. Detection of Cu\(^+\) in human serum samples

Acetonitrile was added into serum samples and then centrifugated to eliminate the interference of protein. The obtained supernatant was diluted 1000 times with ultrapure water prior to analysis with this ratiometric strategy.

3. Results and discussion

3.1. Characterization of Tb-MOFs

A series of analytical techniques were performed to prove the successful preparation of Tb-MOFs. Firstly, the morphology of Tb-MOFs was investigated by SEM and TEM, respectively. The obtained Tb-MOFs are uniformly dispersed with smooth and neat surface (Fig. 1A). TEM indicates that Tb-MOFs exhibit a regular and uniform rod structure (Fig. 1B), which is consistent with previous reports [24]. The XRD pattern of Tb-MOFs agrees well with the simulated (Fig. 1C), demonstrating the high crystallinity of prepared MOFs. In addition, the IR spectra was collected to confirm the coordination interaction between the organic ligand and Tb\(^{3+}\) ions. Fig. 1D shows that the characteristic peaks corresponding to carboxylic group (-COOH) from free ligand (3090, 1720 and 537 cm\(^{-1}\)) shift to 3409, 1614-1559, 1435-1376 and 530 cm\(^{-1}\) for Tb-MOFs. These results prove that the -COOH group of ligand coordinates to Tb\(^{3+}\) to form Tb-MOFs. XPS analysis was also performed to demonstrate the element composition of Tb-MOFs (Fig. 1E). The characteristic peaks at 150 eV, 284 eV, 532 eV and 1240 eV, demonstrating the existence of Tb, O and C element. These results prove the successful preparation of Tb-MOFs.
Fig. 1. SEM image (A), TEM image (B), XRD patterns (C), the full XPS (D), FT-IR spectrum (E), the excitation and emission spectra of Tb-MOFs (F). Inset of F: the digital photos of Tb-MOFs under UV light (left) and daylight (right).

The optical properties of Tb-MOFs were also studied. When excited at 280 nm, four characteristic emission peaks at 490 nm, 548 nm, 588 nm and 623 nm were observed (Fig. 1F), which are derived from $^5\text{D}_4$ excited states to ground states of $^7\text{F}_J$ ($J = 6, 5, 4,$...)}
3) transitions of Tb$^{3+}$, respectively. The colorless solution of Tb-MOFs under daylight emits bright green fluorescence (inset of Fig. 1F). This is because the hypersensitive transition of $^5\text{D}_4 \rightarrow ^7\text{F}_5$ transition results in the strongest emission at 548 nm, which is subsequently selected for the later ratiometric study. Next, we investigated the luminescence stability of MOFs through measuring their fluorescence intensities after storage at room temperature. Fig. S1 shows that the fluorescence intensity changed negligibly during 30 days, indicating the good stability of Tb-MOFs.

3.2. Feasibility study and possible mechanism

Considering that Cu$^+$ needs to be dissolved in acetonitrile [43], we investigated the influence of acetonitrile on the emission spectra of both BCS and Tb-MOFs prior to analysis. The fluorescence intensities at 400 nm corresponding to BCS increase greatly after the addition of 2% acetonitrile and then remain basically unchanged with the increasing acetonitrile content from 2% to 10% (Fig. S2A). In contrast, the fluorescence of Tb-MOFs changes negligibly upon the addition of 2-10% acetonitrile (Fig. S2B). Moderate 4% acetonitrile was used in the following experiments.
Fig. 2. (A) The fluorescence emission spectra of BCS (a), Tb-MOFs (b), Tb-MOFs-BCS (c), Tb-MOFs-BCS + Cu\(^+\) (d), Tb-MOFs-BCS + Cu\(^2+\) (e) and Tb-MOFs-BCS + Cu\(^+\) + Cu\(^2+\) (f). (B) The absorption spectra of BCS (a), BSC + Cu\(^2+\) (b), BCS + Cu\(^+\) (c) and BCS + Cu\(^+\) + Cu\(^2+\) (d). (C) Decay curves of BCS in the absence and presence of Cu\(^+\). [BCS] = 400 nM; [Tb-MOFs] = 7 μg/mL; [Cu\(^+\)] = 200 nM; [Cu\(^2+\)] = 200 nM. (D) Stern-Volmer plots for Cu\(^+\)-induced BCS fluorescence quenching at three different temperatures.

The feasibility of Tb-MOFs-BCS system for ratiometric fluorescence sensing of Cu\(^+\) was investigated with the results shown in Fig. 2A. When excited at 280 nm, BCS and Tb-MOFs exhibit typical emission at 400 nm (curve a) and 548 nm (curve b),
respectively. For Tb-MOFs-BCS system, the typical emission at 400 and 548 nm can be observed obviously (curve c). When Cu\(^{+}\) is present, the intensity at 400 nm is weakened greatly while that at 548 nm remains unchanged (curve d). In contrast, the intensities at neither 400 nm nor 548 nm change obviously after the addition of Cu\(^{2+}\) (curve e). Notably, the coexistence of Cu\(^{2+}\) cannot influence the response of Tb-MOFs-BCS system towards Cu\(^{+}\) (curve f). To further confirm the recognition unit of Tb-MOFs-BCS system towards Cu\(^{+}\), we investigate the influences of Cu\(^{+}\)/Cu\(^{2+}\) on fluorescence spectra of BCS and Tb-MOFs, respectively. Fig. S3A shows that the presence of Cu\(^{+}\) causes obvious decrease of BCS fluorescence while fluorescence of BCS changes negligibly after the addition of Cu\(^{2+}\). On the contrary, neither Cu\(^{+}\) nor Cu\(^{2+}\) causes no obvious effect on the fluorescence of Tb-MOFs (Fig. S3B). Therefore, ratiometric fluorescence detection of Cu\(^{+}\) in the presence of Cu\(^{2+}\) can be realized with Tb-MOFs-BCS system, in which the emission of BCS and Tb-MOFs as recognition signal and internal reference, respectively.

UV-vis spectra were also used to illustrate the specific recognition of BCS towards Cu\(^{+}\) (Fig. 2B). BCS still exhibits no absorption in the range of 350-800 nm and no color change when Cu\(^{2+}\) is added (curve a,b). However, an absorption peak at 480 nm emerges (curve c) and the solution turns yellow (Fig. S4) when Cu\(^{+}\) is present. All these results indicate the formation of BCS-Cu\(^{+}\) adduct [18]. The coexistence of Cu\(^{2+}\) still cannot change the response of BCS towards Cu\(^{+}\) (curve d).

Then, the quenching mechanism of Cu\(^{+}\) towards BCS was investigated in detail. The quenching mechanism is generally divided into static and dynamic quenching.
Fluorescence lifetime is one of the effective means to distinguish these two quenching modes. Thus, the fluorescent decay curves of BCS and BCS-Cu$^+$ are displayed in Fig. 2C. The fluorescence lifetime of BCS without and with Cu$^+$ is 0.2721 and 0.2531 ns, respectively. The presence of Cu$^+$ does not change the fluorescence lifetime of BCS, which is in accordance with the characteristics of static quenching. The fluorescence quenching efficiency ($F_0/F$) was fitted to Stern-Volmer equation:

$$\frac{F_0}{F} = 1 + K_{sv} [Q],$$

where $F_0$ and $F$ are the fluorescence intensities of BCS without and with Cu$^+$, respectively. $K_{sv}$ is the Stern-Volmer quenching constant and [Q] is the Cu$^+$ concentration. For static quenching, the fluorescence quenching is caused by the formation of nonfluorescent ground-state complex between the fluorophore and quencher. Coincidently, the new absorption peak at 480 nm indicates the formation of BCS-Cu$^+$ complex (curve c, Fig. 2B). The higher temperature leads to the lower stability of the complex. To further prove the occurrence of static quenching, the quenching efficiency at three different temperatures was investigated (Fig. 2D). The $K_{sv}$ was calculated to be $0.539 \times 10^7$, $0.421 \times 10^7$ and $0.298 \times 10^7$ L mol$^{-1}$ at 298 K, 318 K and 328 K, respectively, excluding the dynamic quenching mechanism. So, BCS is quenched by Cu$^+$ through static quenching.

3.3. Optimization of experimental parameters

Prior to analysis, the experimental conditions such as time, the amount of Tb-MOFs and pH were optimized. In Fig. 3A, $F_{548}/F_{400}$ changes negligibly within 10 min after the addition of Cu$^+$ into Tb-MOFs-BCS system. The rapid response of Tb-MOFs-BCS system to Cu$^+$ is caused by the fast complexation rate of BCS and Cu$^+$. Considering the
unstability of Cu⁺, a quick reaction is required here. Therefore, 1 min is selected as the reaction time between Tb-MOFs-BCS and Cu⁺.

Fig. 3. (A) The effect of reaction time between BCS and Cu⁺ on the $F_{548}/F_{400}$ of the Tb-MOFs-BCS system. (B) The effect of Tb-MOFs amount on the $F_{548}/F_{400}$ of the Tb-MOFs-BCS system without and with Cu⁺. (C) The fluorescence emission spectra of Tb-MOFs under different pH. (D) The effect of pH on the $F_{548}/F_{400}$ of the Tb-MOFs-BCS system without and with Cu⁺.

Then, we investigated the effect of Tb-MOFs amount on the response of Cu⁺. When Tb-MOFs amount increases, $F_{548}/F_{400}$ of Tb-MOFs-BCS system without and with Cu⁺ increases gradually (Fig. 3B). The maximum difference for $F_{548}/F_{400}$ of TB-MOFs-BCS
system with and without Cu$^+$ is obtained when Tb-MOFs amount is 7 μg/mL, which is selected in the next experiments.

pH is also an essential factor that influences the analytical performance. The stability of Tb-MOFs under different pH was investigated with the results given in Fig. 3C. Tb-MOFs exhibits strong fluorescence emission at pH 4-9 and the intensities remains basically unchanged in the pH range of 4-9, indicating the good stability of Tb-MOFs with such wide pH range. Fig. 3D shows the $F_{548}/F_{400}$ of Tb-MOFs-BCS system without and with Cu$^+$. With the increasing pH, both of them exhibits a volcano plot and the maximum $F_{548}/F_{400}$ is obtained at pH 6. At pH 5-7, the difference for $F_{548}/F_{400}$ of Tb-MOFs-BCS system with and without Cu$^+$ is similar, indicating that Cu$^+$ can be detected with pH 5-7. Considering that the pH of biological fluids is close to 7, pH 7 is chosen as the optimal pH.

3.4. Ratiometric fluorescence determination of Cu$^+$

Fig. 4. (A) Fluorescence emission spectra of Tb-MOFs-BCS system with different concentrations of Cu$^+$ (0, 1, 3, 5, 10, 15, 30, 50, 75, 100, 125, 150, 175, 200, 300, 400, 500 nM). (B) Linear relationship between $F_{548}/F_{400}$ and Cu$^+$ concentration.
Under the optimal conditions, the responses of Tb-MOFs-BCS system towards Cu$^+$ was carried out. Fig. 4A illustrates that intensities at 400 nm decrease obviously while those at 548 nm remain unchanged with the increase in Cu$^+$ content ranging from 1 nM to 500 nM. A linear regression equation of $F_{548}/F_{400} = 0.931 + 0.00532 C$ ($R^2 = 0.995$) was obtained within Cu$^+$ content in a range of 1-200 nM with the detection limit of 0.3 nM (S/N=3). Compared with most fluorescent methods [15, 18, 44, 45], this method realizes ratiometric detection of Cu$^+$ (Table 1). More importantly, the detection limit of this method is 1-3 orders magnitude lower than those of other methods. Such higher sensitivity of this system may be caused by the strong complexation ability of BCS towards Cu$^+$.

Table 1 Comparison of other fluorescence probes for Cu$^+$ detection.

<table>
<thead>
<tr>
<th>Fluorescent probe</th>
<th>Detection mode</th>
<th>Linear range (nM)</th>
<th>Detection limit (nM)</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eu-MOFs@CDs</td>
<td>Turn-off</td>
<td>500-20000</td>
<td>220</td>
<td>18</td>
</tr>
<tr>
<td>BSA-CDs@RHB/BCS$^a$</td>
<td>Turn-off</td>
<td>500-20000</td>
<td>200</td>
<td>44</td>
</tr>
<tr>
<td>Near-infrared probe</td>
<td>Turn-on</td>
<td>0-1000000</td>
<td>91000</td>
<td>45</td>
</tr>
<tr>
<td>Zinc porphyrin derivative</td>
<td>Turn-on</td>
<td>-</td>
<td>8.2</td>
<td>15</td>
</tr>
<tr>
<td>Coumarin-TPA$^b$ derivative</td>
<td>Ratiometric</td>
<td>0-5000</td>
<td>229</td>
<td>20</td>
</tr>
<tr>
<td>Tb-MOFs-BCS</td>
<td>Ratiometric</td>
<td>1-200</td>
<td>0.3</td>
<td>This work</td>
</tr>
</tbody>
</table>

$^a$ decorating carbon dots on BSA encapsulated rodamine hydrazide and conjungating
with bathocuproine disulfonate

\[ \text{tetradentate ligand tris[(2-pyridyl)-methyl]amine} \]

Fig. 5. (A) The \( F_{548}/F_{400} \) of Tb-MOFs-BCS system with interfering compounds. (B) The \( F_{548}/F_{400} \) of Tb-MOFs-BCS system with interfering compounds and \( \text{Cu}^{+} \). The concentration of each analyte is 200 nM.

To test the selectivity of the probe, common metal cations were investigated. Fig. 5A shows that \( F_{548}/F_{400} \) increases obviously in the presence of \( \text{Cu}^{+} \), while \( F_{548}/F_{400} \) changes slightly with the coexisting metal cations. In addition, the anti-interference ability was investigated. Fig. 5B indicates that the coexisting metal cations show negligible interference on the response of \( \text{Cu}^{+} \). The specific recognition of BSC to \( \text{Cu}^{+} \) enables this method excellent selectivity and anti-interference ability, demonstrating good potential in the practical applications.

3.5. Practical samples analysis

To evaluate the practical application of this strategy, the Tb-MOFs-BCS probe was used to detect \( \text{Cu}^{+} \) in human serum samples. Table S1 shows that the favorable spiking recoveries were achieved ranging from 95.2% to 103.3% with RSD less than 3.5%,
demonstrating the great potential of this method in real sample analysis.

4. Conclusion

A ratiometric fluorescence sensor assembled by Tb-MOFs and BCS has been designed to detect Cu$^{+}$ rapidly and selectively without the interference of Cu$^{2+}$. Fluorescence of BCS at 400 nm is selectively quenched by Cu$^{+}$ because of the generation of BCS-Cu$^{+}$ complex while the intensity at 548 nm of Tb-MOFs remains unchanged. Notably, the coexistence of Cu$^{2+}$ cannot affect the response of Cu$^{+}$. The specific chelation between BCS and Cu$^{+}$ and unique quenching of BCS caused by Cu$^{+}$ endowed the sensor with excellent selectivity as well as high sensitivity towards Cu$^{+}$. Importantly, a fast response of 1 min was achieved. This work provides not only a promising strategy for discrimination of Cu$^{+}$ from Cu$^{2+}$ but also ratiometric method for monitoring Cu$^{+}$ in biological systems.

Acknowledgements

This work was financially supported by the National Natural Science Foundation of China (Nos. 22076097 and 22176109) and the Natural Science Foundation of Shandong Province (No. ZR2020MB066).

References


[2] V.A. Rapisarda, S.I. Volentini, R.N. Farias, E.M. Massa, Quenching of


[29] D.M. Chen, N.N. Zhang, C.S. Liu, M. Du, Template-directed synthesis of a luminescent Tb-MOF material for highly selective Fe\(^{3+}\) and Al\(^{3+}\) ion detection and


