A sensitive and selective method for visual chronometric detection of copper (II) ions using clock reaction

Yue Yaling, He Yi *

School of National Defence Science & Technology, Southwest University of Science and Technology, Mianyang, 621010, P. R. China.

*Corresponding author: Dr. Yi He, Email: yhe2014@126.com.
Abstract

We report a visual chronometric method for detection of copper (II) ions (Cu\(^{2+}\)) using the clock reaction between methylene blue (MB) and hydrazine (\(\text{N}_2\text{H}_4\)). The addition of Cu\(^{2+}\) greatly accelerates the clock reaction due to the formation of Cu cluster between Cu\(^{2+}\) and \(\text{N}_2\text{H}_4\). By recording the change of the reaction time and solution color, different concentrations of Cu\(^{2+}\) in buffer and environmental water have been detected. As low as 300 nM Cu\(^{2+}\) can be visually identified by the naked eye without the assistance of any advanced instruments. This visual chronometric assay shows a dynamic range from 0.2 to 16 \(\mu\text{M}\) with a limit of detection of 20 nM. Additionally, it has a good selectivity toward Cu\(^{2+}\) and against other common metal cations.

Keywords: Copper (II), assay, detection, visualization, chronometry
Introduction

The development of sensitive and selective methods for detection of metal ions is of great importance for environmental protection and monitoring. Among these metal ions, copper (II) ions ($\text{Cu}^{2+}$) have high toxicity for human body when the uptake is excessive. For example, high level of $\text{Cu}^{2+}$ is able to induce cell apoptosis, amyotrophic lateral sclerosis, kidney and liver damage, Menkes syndrome, Wilson’s disease, and so on. Because the copper-containing commercial products have been widely used for electronic industry and agriculture, copper contamination is an unneglectable problem. Accordingly, the determination of $\text{Cu}^{2+}$ in environmental water samples is an important issue. To this end, several approaches have been developed for detection of $\text{Cu}^{2+}$, including inductively coupled plasma-atomic emission spectrometry, atomic absorption spectroscopy, voltammetry, and fluorimetry. However, these methods often require sophisticated and expensive instrumentation.

On the contrary, visual detection methods are extremely fascinating for detection of $\text{Cu}^{2+}$ because they can be directly read out with the naked eye. A variety of visual methods based on plasmonic nanoparticles and organic chromogenic reagents have been developed for visual detection of $\text{Cu}^{2+}$. These assays are based on the color change induced by $\text{Cu}^{2+}$. Despite these progresses, there are still some limitations. For example, the methods using organic chromogenic reagents have a low sensitivity, which requires multi-step organic preparations. Although the plasmonic nanoparticle-based assays are able to improve the sensitivity, yet the nanoparticle probes are easily affected by environmental conditions such as salts, temperature, and thiol-containing substances. Moreover, the plasmonic nanoparticle is metastable, which gradually aggregates with time. These drawbacks seriously influence their analytical performances in terms of accuracy, reproducibility, and practicality. Therefore, it is an urgent need to develop a novel $\text{Cu}^{2+}$ detection strategy.

Recently, the clock reaction of methylene blue (MB) and hydrazine ($\text{N}_2\text{H}_4$) is receiving more and more attention, in which a reversible color change from blue to
colorless is observed based on redox reactions after an incubation time\textsuperscript{27-29}. The reaction time of the color change can be used for visual chronometric detection of analytes such as chromium(III) ions, glutathione and cysteine in the presence of nanocatalysts \textsuperscript{30,31}. However, the visual detection of Cu\textsuperscript{2+} based on the clock reaction is not reported to date. Herein, we present a visual chronometric assay for ultrasensitive and selective detection of Cu\textsuperscript{2+} without the assistance of nanocatalysts. As illustrated in Scheme 1, the reduction of MB by N\textsubscript{2}H\textsubscript{4} can not be effectively carried out in the absence of Cu\textsuperscript{2+}. The color change requires a long time. However, after introduction of Cu\textsuperscript{2+}, an obvious color change from blue to colorless is observed during a short incubation time since the blue MB is reduced to the colorless leucomethylene blue (LMB). When shaking the reaction solution, the color of the solution becomes blue again due to the reoxidation of the LMB by oxygen (clock reaction). The variation of the reaction time (it is the time when the blue color of the test sample in the solution has completely despaired) upon addition of Cu\textsuperscript{2+} provides a visual chronometric detection strategy. This method shows a nanomolar sensitivity, and the feasibility for determination of Cu\textsuperscript{2+} in real environmental water is also studied.

**Experimental section**

**Materials and instruments**

MB was purchased from Shanghai Aladdin Bio-Chem Technology Co., LTD. N\textsubscript{2}H\textsubscript{4}, copper(II) sulfate pentahydrate (CuSO\textsubscript{4}·5H\textsubscript{2}O), and other metal salts were obtained from Chengdu Kelong Reagent Co., Ltd. All the chemical reagents were directly used without further purification. Deionized water was employed to prepare various water solutions. Ultraviolet-visible (UV-vis) absorption spectra were recorded with a Shimadzu UV-1800 spectrophotometer.

**Visual chronometric detection protocol for Cu\textsuperscript{2+}**

All the operations were carried out at room temperature. 2.6 mL of of Britton-Robinson (BR) buffer (pH 10.4) was mixed with 0.1 mL of Cu\textsuperscript{2+} aqueous solution with different concentrations (0-0.5 mM). Then, 0.2 mL of 0.5 mM MB was
added to the above solution. Finally, 0.1 mL of 1 M N$_2$H$_4$ was introduced to induce the color change, which was recorded with a video camera of a smartphone.

To demonstrate the practicality of this assay, river and lake water were collected, which is diluted 10-fold with deionized water. Subsequently, different concentrations of Cu$^{2+}$ (0.5, 0.1, 0.01 mM) were added to these water samples. The spiked water samples were analyzed by using the same experimental procedures as mentioned above.

**Results and Discussion**

*Cu$^{2+}$ promoted MB-N$_2$H$_4$ clock reaction*

The Cu$^{2+}$ promoted MB-N$_2$H$_4$ clock reaction is investigated by UV-vis absorption. As shown in Fig. 1, the MB solution shows a maximum absorption wavelength at 664 nm. The reduction reaction between MB and N$_2$H$_4$ is relatively slow, and a slight change in absorbance is observed as shown in Fig. 1a. In contrast, the introduction of 16 μM Cu$^{2+}$ remarkably accelerates the reaction speed of MB and N$_2$H$_4$, the characteristic absorption peak of MB at 664 nm completely disappears within in 90 s thanks to the generation of the colorless LMB$^{31}$, indicating that Cu$^{2+}$ can effectively promote the reaction between MB and N$_2$H$_4$. Moreover, the reaction speed of MB and N$_2$H$_4$ is dependent on the Cu$^{2+}$ concentration (Fig. S1 and Fig. S2). With the increase of Cu$^{2+}$ concentration, the reaction speed increases as shown in Fig. 1c. Importantly, the MB is easily to be reproduced by directly shaking the reaction solution as depicted in Fig. 1d. The absorbance value at 664 nm gradually increases with the shaking time, suggesting that the colorless LMB is oxidized by oxygen from air. Whereafter, the MB is reduced by the excess N$_2$H$_4$ again. Therefore, a periodic color change between MB and LMB is found, confirming the clock reaction.

It has been demonstrated that the Cu cluster is capable of catalyzing the reduction of MB by N$_2$H$_4$ due to the formation of the Cu cluster-MB complex.$^{32}$ In our reaction system, Cu$^{2+}$ and N$_2$H$_4$ coexist the solution, in which a reduction reaction may occur to in-situ produce the Cu cluster. To prove the production of the Cu cluster, the UV-vis absorption spectra of Cu$^{2+}$, N$_2$H$_4$, and their mixture are measured as displayed in Fig.
S3. The UV-vis absorption results show that Cu²⁺ and N₂H₄ have no obvious absorption in the wavelength range of 400-600 nm, while the mixture of Cu²⁺ and N₂H₄ give rises to a strong absorption band, which is attributed to the Cu cluster³². In addition, a strong fluorescence emission is observed in the mixture of Cu²⁺ and N₂H₄ (Fig. S4), which further demonstrates the production of Cu cluster. Consequently, the Cu²⁺ promoted MB-N₂H₄ clock reaction involves three steps as shown in Fig. S5. Cu²⁺ is firstly reduced by N₂H₄ to generate Cu cluster which further interact with MB to produce the Cu cluster-MB complex. The resulted complex reacts with N₂H₂ to yield colorless LMB.

Feasibility and optimization of the visual chronometric method

The feasibility of the visual chronometric method for detection of Cu²⁺ is verified in BR buffer solution by chronometric measurements. As shown in Fig. 2, the color change of the MB-N₂H₄ reaction from blue to colorless requires about 1002 s. Nevertheless, the introduction of Cu²⁺ induces a fast reduction process, and the MB solution becomes colorless after reaction for 43 s. The alteration of the reaction time in the absence and presence of Cu²⁺ illustrates that the method is feasible to determine Cu²⁺.

In order to obtain better analytical performance, some key experimental parameters including N₂H₄ concentration and the pH of the buffer solution were optimized. The reaction time of the Cu²⁺ promoted MB-N₂H₄ clock reaction is related to the N₂H₄ concentration (Fig. S6a). The high concentration of N₂H₄ results in a short reaction time. When the N₂H₄ concentration exceeds 0.03 M, the reaction time is maintained constant. From the effect of the pH on the reaction time as shown in Fig. S6b, it can be seen that the acidic medium is adverse to the Cu²⁺ promoted MB-N₂H₄ clock reaction because N₂H₄ is apt to interact with proton to yield N₂H₅Cl, leading to a weak reducing activity, while a weak alkali environment is advantageous, and the reaction speed reaches a maximum value at pH of 10.4. Hence, 0.03 M N₂H₄ and pH 10.4 were selected as optimized experimental conditions for determination of Cu²⁺.

Sensitivity, selectivity, and reproducibility of the visual chronometric method

In order to evaluate the sensitivity of the visual chronometric method, the reaction
time of the MB-N$_2$H$_4$ clock reaction is recorded after adding different concentrations of Cu$^{2+}$. Fig. 3a-3c show the typical optical image of the clock reaction at different reaction time points. Interestingly, the distinct color change allows visual detection of Cu$^{2+}$, and the lowest detection concentration by the naked eye is as low as 300 nM. The reaction time against Cu$^{2+}$ concentrations are displayed in Fig. 3d. The reaction time gradually decreases with the increasing of the Cu$^{2+}$ concentration from 0.2 to 16 μM, and a non-linear fitting method (ExpAssoc model) is utilized to fit the calibration curve with a correlation coefficient of 0.996. The limit of detection (LOD) is calculated to be 20 nM based on a signal-to-noise ratio of 3.2. This LOD is typically one to three orders of magnitude better than those of widely used electrochemistry, colorimetric and fluorescence techniques (table 1). Additionally, this sensitivity is comparable to the lateral flow method that employs expensive DNA and complicated surface modification procedures for gold nanoparticles (AuNPs). Oppositely, our method is extremely simple and low-cost without involving any advanced instrument.

The selectivity of this assay toward Cu$^{2+}$ is further demonstrated in the presence of other metal cations, including Na$^+$, Mg$^{2+}$, Cr$^{3+}$, Ba$^{2+}$, Mn$^{2+}$, Sr$^{2+}$, Zn$^{2+}$, Al$^{3+}$, Fe$^{3+}$, Pb$^{2+}$, Ag$^+$, K$^+$, Co$^{2+}$, and Hg$^{2+}$. Remarkably, as shown in Fig. 4, the introduction of other metal cations does not cause significant change of the reaction time, and only Cu$^{2+}$ results in dramatic change of the reaction time. This result confirms the good selectivity of the visual chronometric method, which is ascribed to the specific production of Cu cluster between Cu$^{2+}$ and N$_2$H$_4$.

To examine the reproducibility of this method, three different concentrations of Cu$^{2+}$ (16, 3, and 0.3 μM) were repeatedly measured nine times. The relative standard deviations (RSDs) of the reaction time are calculated to be 1%, 4.1%, and 3.1%, proving the good reproducibility of the visual chronometric method. Taken together, these results suggest that this visual chronometric potentially be applied to replace the traditional methods for detection of Cu$^{2+}$.

**Practical application of the visual chronometric method**

The practical application of the visual chronometric method is estimated by detecting Cu$^{2+}$ spiked environmental water samples. The river and lake water samples
were collected and diluted 10-fold with deionized water without further treatment. As summarized in Table S1, the good recoveries from 96.7% to 104% are obtained in the spiked samples by this method. Besides, the RSDs are less than 3%. These findings authenticate that this visual chronometric method is promising for detection of Cu$^{2+}$ in real water samples.

**Conclusions**

In summary, a visual chronometric assay is developed for detection of Cu$^{2+}$ based on the Cu$^{2+}$ promoted MB-N$_2$H$_4$ clock reaction. The promotion mechanism is attributed to the production of Cu cluster between Cu$^{2+}$ and N$_2$H$_4$. The assay shows a good sensitivity with a LOD of 20 nM, and selectivity against other metal cations, which is successfully applied to determine Cu$^{2+}$ in environmental water samples. Compared with the traditional methods, this assay does not involve any complicated instruments, nanomaterials preparation, surface modification processes, and sample pretreatment, making it has a great potential as a facile, reliable, low-cost technique for on-site and ultrasensitive detection of Cu$^{2+}$, especially in remote regions.

**Supporting Information**

Fig. S1-S6. Table S1.

**Acknowledgements**

We gratefully acknowledge the Undergraduate Innovation Fund Project of SWUST (CX17-024).

**References**

18, 3896.


Scheme 1  Schematic illustration of the visual chronometric detection of Cu\(^{2+}\) using the clock reaction of MB and N\(_2\)H\(_4\).
**Fig. 1** UV-vis absorption spectra of 33 μM MB and 33 mM N₂H₄ in the (a) absence and (b) presence of 16 μM Cu²⁺ at different reaction time. (c) The absorbance values at 664 nm versus reaction time in the presence of different Cu²⁺ concentrations. (d) UV-vis absorption spectra of LMB reoxidation under shaking (the Cu²⁺ concentration is 3 μM).
Fig. 2 Typical photo images of the MB-N₂H₄ clock reaction in the absence and presence of 16 μM Cu²⁺. The concentrations of MB and N₂H₄ are 33 μM and 33 mM, respectively.
Fig. 3 (a-c) Visual detection and (d) calibration curve of the reaction time versus different concentrations of Cu^{2+} (0-16 μM).
Fig. 4 The selectivity of the visual chronometric method for detection of Cu$^{2+}$. The concentrations of Cu$^{2+}$ and other metal ions are 16 μM.
Table 1 Comparison of the sensitivity of various assays for Cu$^{2+}$

<table>
<thead>
<tr>
<th>Method</th>
<th>Detection system</th>
<th>Detection limit (nM)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrochemistry</td>
<td>Carbon dot- TPEA$^a$</td>
<td>100</td>
<td>34</td>
</tr>
<tr>
<td>Fluorimetry</td>
<td>Carbon dot- TPEA</td>
<td>1000</td>
<td>35</td>
</tr>
<tr>
<td>Fluorimetry</td>
<td>Carbon dot-SiO$_2$</td>
<td>35.2</td>
<td>9</td>
</tr>
<tr>
<td>Fluorimetry</td>
<td>Hydrogel</td>
<td>20000</td>
<td>36</td>
</tr>
<tr>
<td>Fluorimetry</td>
<td>YVO$_4$:Eu nanoparticles</td>
<td>570</td>
<td>37</td>
</tr>
<tr>
<td>Fluorimetry</td>
<td>BODIPY$^b$</td>
<td>1000</td>
<td>38</td>
</tr>
<tr>
<td>Lateral flow</td>
<td>Gold nanoparticles-DNA</td>
<td>10</td>
<td>17</td>
</tr>
<tr>
<td>Colorimetry</td>
<td>Gold nanoparticles</td>
<td>20000</td>
<td>16</td>
</tr>
<tr>
<td>Chronometry</td>
<td>MB-$N_2H_4$</td>
<td>20</td>
<td>This work</td>
</tr>
</tbody>
</table>

$^a$TPEA is the abbreviation of N-(2-aminoethyl)-N,N,N’tris(pyridin-2-ylmethyl)ethane-1,2-diamine.

$^b$BODIPY is the abbreviation of 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene.
Graphic abstract