A Highly Selective and Sensitive Colorimetric Probe for Cu\textsuperscript{2+} Determination in Aqueous Media Based on Derivative of Tryptanthrin

Yawei LIANG,* Xinna WANG,* Huanle FANG,** Ningjuan HAN,** Cuiling WANG,* Zhiqiang XIAO,** Aihua ZHU,** and Jianli LIU†

*Key Laboratory of Resource Biology and Biotechnology in Western China, Ministry of Education, College of Life Science, Northwest University, Xi’an, 710069, China
**School of Medicine, Xi’an Peihua University, Xi’an, 710125, China
***Shaanxi Key Laboratory of Chinese Medicine and Natural Medicine Research and Development, Xi’an, 710075, China

A new colorimetric probe, based on tryptanthrin derivative (TR-A), has been successfully synthesized. The probe shows good selectivity and sensitivity for Cu\textsuperscript{2+} over 12 competing metal ions in a 10 mM Tris–HCl buffer solution (pH 5.5). A significant peak at 623 nm appears in the UV-Vis absorption of TR-A-Cu\textsuperscript{2+}, and a noteworthy color change is observed with the naked eye from aquamarine blue to light orange. The interaction of TR-A and Cu\textsuperscript{2+} are proven to form a 1:1 binding stoichiometry; this identifying is expected to be completed within 1 min. The probe with a limit of detection (16 nM, \(R^2 = 0.9934\)) shows excellent potential to determine Cu\textsuperscript{2+} in analysis systems.

Keywords: Tryptanthrin derivative, colorimetric probe, cupric determination

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transfer (ICT)\textsuperscript{23} and metal-to-ligand charge transfer (MLCT)\textsuperscript{26} of TR-A can quickly form in the presence of copper ions, generating a color change from light orange to aquamarine blue. The compound TR-A displayed good sensitivity for copper ions, the limit of detection (16 nM, $R^2 = 0.9943$) being much less than the maximum permissible content of copper ions in potable water based on the WHO recommended value.

**Experimental**

**Reagents and chemicals**

NaCl, AlCl\textsubscript{3}, CuCl\textsubscript{2}-2H\textsubscript{2}O were obtained from Tianli Chemical Reagent (Tianjin, China). (CH\textsubscript{3}COO\textsubscript{2})\textsubscript{2}Zn, CoCl\textsubscript{2}-6H\textsubscript{2}O were obtained from Tianjin Kemiou Chemical Reagent (Tianjin, China). FeSO\textsubscript{4}-7H\textsubscript{2}O, CH\textsubscript{3}NO\textsubscript{2}-H\textsubscript{2}O, NiCl\textsubscript{2}-6H\textsubscript{2}O, MnSO\textsubscript{4}-H\textsubscript{2}O, CdCl\textsubscript{2}, HgCl\textsubscript{2} were obtained from Xi’an Chemical Reagent Factory (Xi’an, China). FeCl\textsubscript{3}, NaOH, HCl, NaH\textsubscript{2}PO\textsubscript{4}, Na\textsubscript{2}HPO\textsubscript{4}-7H\textsubscript{2}O, EDTA-2Na were obtained from Sino Pharm Chemical Reagent (Shanghai, China). Absolute ethanol was obtained from Guoan Biotechnology (Xi’an, China). All chemicals of analytical grade were used in this work and without further purification.

**Apparatus**

Mass spectra were recorded with an Agilent 1200-G6410B LCQ ion-trap mass spectrometer. \textsuperscript{1}H-NMR spectra were recorded with a Bruker AV-200 spectrometer in CDCl\textsubscript{3} using TMS as an internal standard. UV-visible spectra were measured with a UV2600 spectrophotometer at room temperature. Fourier-transform infrared (FT-IR) spectra were recorded with an L1600400 PerkinElmer Spectrum. The melting points were detected with an X-5 micro melting-point meter. All pH values were measured with a Model pHS-3C pH meter.

**Synthesis of the probe TR-A**

The probe TR-A was successfully synthesized by modifying tryptanthrin with o-aminophenol. Tryptanthrin (1.0 equiv), o-aminophenol (1.0 equiv) and acetic acid (1.0 equiv) in methanol (15 equiv) were heated up to 80°C and refluxed for 3 - 5 h. TLC was used for monitoring the progress of the reaction. After the reaction was completed, the reaction solution was allowed to cool to room temperature. Gradually, the formation of red precipitation was observed, and the crude product was collected via a Buchner funnel and washed with water. The probe TR-A was characterized by melting point, IR, \textsuperscript{1}H-NMR and HRMS data (Figs. S1 and S2, Supporting Information).

The probe TR-A: red solid, yield, 61%, mp = 218 - 219°C, IR (KBr): 3365, 1699, 1648, 1588, 1461, 1242, 774, 750 cm\textsuperscript{-1}. \textsuperscript{1}H-NMR (400 MHz, CDCl\textsubscript{3}) $\delta$ 9.49 (s, 1H), 8.58 (d, $J = 8.0$ Hz, 1H), 8.35 (d, $J = 8.0$ Hz, 1H), 7.95 (d, $J = 5.8$ Hz, 2H), 7.74 - 7.63 (m, 2H), 7.22 (t, $J = 8.1$ Hz, 1H), 7.12 (dd, $J = 8.3$, 4.2 Hz, 1H), 7.05 - 6.89 (m, 1H). ESI-mass (m/z) calculated for C\textsubscript{21}H\textsubscript{13}N\textsubscript{3}O\textsubscript{2} [M+H]+, 339; found, 339.

**Sensitivity and selectivity measurements**

For the colorimetric detection of Cu\textsuperscript{2+}, a Tris–HCl buffered solution (pH 5.5) was collected and filtered with a 0.2-mm membrane. These samples were spiked with a Cu\textsuperscript{2+} aqueous solution of known concentrations of Cu\textsuperscript{2+}. After that, TR-A was added to the above-mentioned samples for detection.

**Results and Discussion**

**The selectivity of the colorimetric probe TR-A for Cu\textsuperscript{2+}**

The color of a TR-A solution (50 μM) is light orange. When Cu\textsuperscript{2+} (50 μM) is added to TR-A in a 10 mM Tris-HCl buffered solution (pH 5.5), it becomes aquamarine blue in seconds. The chelation between TR-A and Cu\textsuperscript{2+} could cause a color change of the system as well as a change in the absorption spectrum. As shown in Fig. 1, after Cu\textsuperscript{2+} was added into the TR-A solution a wide absorption band appeared at 550 - 700 nm, and the maximum absorption was 623 nm. It was noted that there was no absorption band in that region for individual TR-A or Cu\textsuperscript{2+}.

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To demonstrate the selectivity of the detecting system for Cu\textsuperscript{2+}, the ultraviolet absorption spectra of TR-A added other metal ions (Cr\textsuperscript{3+}, Na\textsuperscript{+}, Fe\textsuperscript{3+}, Fe\textsuperscript{2+}, Al\textsuperscript{3+}, Co\textsuperscript{2+}, Cd\textsuperscript{2+}, Hg\textsuperscript{2+}, Mn\textsuperscript{2+}, Pb\textsuperscript{2+}, Co\textsuperscript{2+}, Ni\textsuperscript{2+}, Zn\textsuperscript{2+} and Cu\textsuperscript{2+}) were recorded as shown in Fig. 2a. Interestingly, no response was found toward Na\textsuperscript{+}, Al\textsuperscript{3+}, Fe\textsuperscript{3+}, Fe\textsuperscript{2+}, Hg\textsuperscript{2+} and Mn\textsuperscript{2+} at 623 nm. An small UV absorption was observed with the naked eye and recorded using a digital camera. The absorption spectrum of the system was obtained by using a UV2600 spectrophotometer. The concentration of Cu\textsuperscript{2+} was determined based on the maximum absorption peaks. To estimate the interferences of 12 competing metal ions, Cr\textsuperscript{3+}, Na\textsuperscript{+}, Al\textsuperscript{3+}, Fe\textsuperscript{3+}, Fe\textsuperscript{2+}, Hg\textsuperscript{2+}, Cd\textsuperscript{2+}, Mn\textsuperscript{2+}, Zn\textsuperscript{2+}, Pb\textsuperscript{2+}, Co\textsuperscript{2+}, Ni\textsuperscript{2+} and Cu\textsuperscript{2+} were added, respectively, to TR-A solution and the UV-Vis spectra were recorded.

**Determination of copper ion in real water samples**

To confirm the practical applicability of TR-A as a colorimetric probe for copper ion, real water samples (tap and drinking water) were collected and filtered with a 0.2-mm membrane. These samples were spiked with a Cu\textsuperscript{2+} aqueous solution of known concentrations of Cu\textsuperscript{2+}. After that, TR-A was added to the above-mentioned samples for detection.
due to the better stability of the compound formed between two tertiary nitrogens and phenolic hydroxyl groups with Cu\(^{2+}\), as compared with 12 competing metal ions. Figure 2b shows that Cu\(^{2+}\) was the only ion generated according to an observable color change from light orange to aquamarine blue.

**Effect of pH on the colorimetric response of TR-A to Cu\(^{2+}\)**

To find the optimal pH range for Cu\(^{2+}\) determination, the difference in the pH to the colorimetric response of TR-A to Cu\(^{2+}\) was determined in a 10 mM Tris-HCl buffered solution, as shown in Fig. 3. A wavelength of 623 nm was used for inspecting the aquamarine blue compound. In the absence of Cu\(^{2+}\), no absorption of the probe was observed in a range of pH (2 - 8). At basic pH (9 - 12), the absorption of TR-A increased with increasing pH, and the color changes from light orange to Lyons blue, was mainly due to a disaggregation of the probe’s phenolic hydroxyl (OH) into negative ions (O\(^-\)) in an alkaline condition. Interestingly, at an acidic pH (pH ≤ 5.5), the absorption of the TR-A-Cu\(^{2+}\) complex increased with increasing pH, and reached a maximum at a pH of 5.5. In the pH range of 5.5 - 8.0, the absorbance at 623 nm gradually decreased with increasing pH. The results show that the TR-A-Cu\(^{2+}\) complex is formed by Cu\(^{2+}\) inducing a deprotonation process of the probe’s phenolic hydroxyl under an acidic condition. There is no obvious absorbance of TR-A at 623 nm. Therefore, the response of TR-A to Cu\(^{2+}\) depended on the pH, and a pH of 5.5 was used as the optimal pH for monitoring Cu\(^{2+}\) by using TR-A as a probe.

**Linear range and sensitivity of the detection system for Cu\(^{2+}\)**

To evaluate the potentiality of TR-A as a colorimetric probe for Cu\(^{2+}\), the linear range and sensitivity of TR-A to Cu\(^{2+}\) were investigate based on the colorimetric response. Under the optimum conditions, the color change of the TR-A solution for detecting Cu\(^{2+}\) was monitored by UV-visible spectroscopy. As shown in Fig. 4, with increasing concentration of Cu\(^{2+}\) from 0 to 150 μM, the peak at 312 nm of the TR-A decreased gradually, and a new absorbance peak at 623 nm increased gradually. At the same time, the color change of the system from light orange to aquamarine blue was gradually observed with the naked eye. The aforesaid results imply that higher concentrations of Cu\(^{2+}\) can induce a higher level of deprotonation of the TR-A. Furthermore, 1.0 equiv of Cu\(^{2+}\) was sufficient for saturating the absorbance change, which indicates that the colorimetric probe based on TR-A forms a 1:1 composition with Cu\(^{2+}\). As shown in Fig. 5, calibration graphs were drawn by using the intensities of the absorption at 623 nm versus the concentrations of Cu\(^{2+}\) in the range of 0 to 100 μM. A good linear relationship (R\(^2\) = 0.9934) is found between the intensities of the absorption and the Cu\(^{2+}\) concentrations. The detection limit of TR-A was determined to be 16 nM, based on the standard deviation of the blanks and the slope of the linear relationship, which is much less than the maximum permissible content of Cu\(^{2+}\) in potable water by the WHO recommended value.

**Binding stoichiometry and reversible ability**

A job was proceed to confirm the binding stoichiometry of
TR-A-Cu^{2+} by keeping the total concentration of Cu^{2+} and TR-A at 100 μM. The absorbance at 623 nm against the mole fraction of Cu^{2+} was tested. As shown in Fig. S5 (Supporting Information), at a mole fraction of 0.5, the change of the maximum absorbance was observed. TR-A forms a 1:1 composite with Cu^{2+} is supported by this result.

The reversible sensing ability of TR-A for Cu^{2+} ions was tested in a 10 mM Tris–HCl buffered solution (pH 5.5) (Fig. S6, Supporting Information). The absorption of TR-A (100 μM) was almost negligible at 623 nm (Fig. S6a). However, the addition of Cu^{2+} (50 μM) to the solution of TR-A created a strong absorption at 623 nm (Fig. S6b), suggesting the formation of the composite between TR-A and Cu^{2+}. Then, the addition of EDTA (50 μM) as a chelating agent for Cu^{2+} resulted in a decrease of the absorption at 623 nm; the absorbance did not disappear entirely (Fig. S6c). After the addition of another Cu^{2+} (55 μM) to the aforementioned solution, the absorption at 623 nm increased and was larger than spectrum b (Fig. S6d). Therefore, the complexation reaction of TR-A with Cu^{2+} is reversible and has a potent binding affinity in a 10 mM Tris–HCl buffer solution (pH 5.5).

Interference study in the presence of other metal ions
To investigate the interference of 12 competing metal ions in the detectability, the colorimetric response of TR-A for Cu^{2+} was tested in the presence of other competing ions, for example, Na^{+}, Al^{3+}, Fe^{3+}, Fe^{2+}, Hg^{2+}, Cd^{2+}, Mn^{2+}, Zn^{2+}, Pb^{2+}, Ni^{2+}, Cr^{3+}, Co^{2+}. A masking agent (NaF) was used to eliminate the interference of Fe^{2+}, Fe^{3+}, Cr^{3+}, Co^{2+}, and there was no obvious absorbance of NaF, itself, at 623 nm. As shown in Fig. S7 (Supporting Information), the interference of Fe^{3+} and Fe^{2+} could be basically eliminated by NaF. Although Fe^{2+} has little influence on the determination of Cu^{2+}, compared with the reaction of TR-A and Cu^{2+}, the interference is not big because their reaction rates are relatively low. Despite the fact that NaF could not completely eliminate the interference of Cr^{3+} and Co^{2+}, it could reduced this impact. When the concentration of Cr^{3+} and Co^{2+} was 30 μM, respectively, it could be found that Cr^{3+} and Co^{2+} had no big influence, as shown in Fig. 6.

Application of TR-A in real water samples
To validate the practicability and reliability of TR-A as a colorimetric sensor, Cu^{2+} aqueous solutions of known concentrations (0, 20 and 40 μmol/L) were added to real water samples (tap, drinking) and analyzed by the aforesaid procedure. The analytical results are shown in Table 1 (n = 3), which reveal that the aforementioned-method procedure shows satisfactory recoveries in the range of 93.59 – 100.10%, with % RSD values of 0.64 – 2.68% for Cu^{2+} in tap and drinking water. Therefore, the new method could be used for the detection of Cu^{2+} in environmental samples with good precision and accuracy.

Reaction mechanism of TR-A for Cu^{2+}
The complexing mechanism of TR-A and Cu^{2+} was studied by analyzing the FT-IR and MS spectra of TR-A and TR-A-Cu^{2+}. For TR-A (Fig. S1, Supporting Information), a broad band at 3365.8 cm^{-1} was due to –OH stretching vibration, and 1699.9 cm^{-1} was due to C =O vibration. The ligand exhibited absorptions due to aromatic ring bands at the 1648.8, 1588, 1461.2, 1356 cm^{-1}. The C –N and C –O bands were observed in the region of 1326.8, 1242 cm^{-1}. The bands at 774.56 and 750.17 cm^{-1} were due to aromatic C –H out-of-plane bending vibration. In the MS spectra of TR-A-Cu^{2+} (Fig. S4, Supporting Information), some new bands with weak intensity in the region of 620 – 450 cm^{-1}, tentatively assigned to (Cu –N) and (Cu –O) vibration. The complex also showed some new bands with weak intensity in the range of 620 – 450 cm^{-1}, tentatively assigned to (Cu-N) and (Cu-O) vibration.27 In the MS spectra of TR-A-Cu^{2+} (Fig. S4, Supporting Information),
the peak was observed at $m/z$ 402.42, which revealed the formation of the complex TR-A-Cu$^{2+}$. In addition, TR-A degraded into TR under the MS condition, and TR combined with Na$^+$ formed $m/z$ 362.42. Therefore, it is speculated that the complexing mechanism of TR-A with Cu$^{2+}$ most likely undergoes the mechanism shown in Scheme 2.

**Conclusions**

In summary, a new colorimetric probe based on the tryptanthrin derivative (TR-A) was synthesized for the detection of Cu$^{2+}$. TR-A could specifically chelate with Cu$^{2+}$ at a pH of 5.5 with a much less than the maximum allowance of Cu$^{2+}$ in potable water ($2.0$ mg L$^{-1}$). The method disclosed in this study is simple, fast and cost effective, and does not require any complicated apparatus, which makes it a promising sensor for the quick and on-site determination of copper ions in aqueous environments by naked-eye observation and UV-Vis absorption at 623 nm.

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**Supporting Information**

$^1$H NMR and FT-IR spectra of TR-A; FT-IR and MS spectra of TR-A-Cu$^{2+}$; binding stoichiometry, reversible affinity and interference study in the presence of other metal ions are listed in Supporting Information. This material is available free of charge on the Web at [http://www.jsac.or.jp/analsci/](http://www.jsac.or.jp/analsci/).

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