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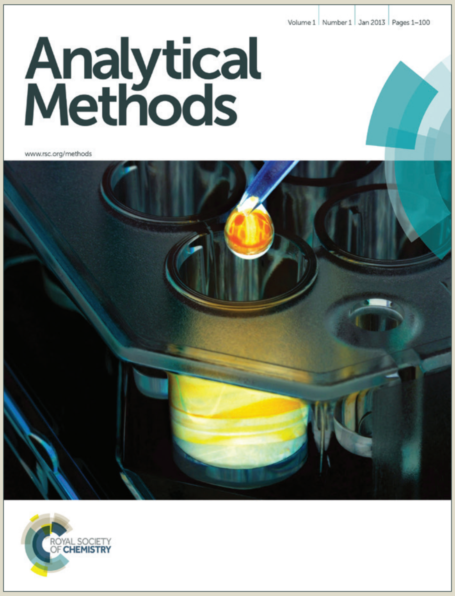

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Trace Copper Ion Detection by the Suppressed Decolorization of Chromotrope 2R Complex

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Chromotrope 2R (CR) is a monoazo dye, which can be easily degraded under ultraviolet C (UVC) light irradiation. However, the degradation extent of CR is suppressed after it is chelated with Cu^{2+} ions, forming a coordination complex (Cu^{2+}-CR). This phenomenon was developed as a novel method for the quantitative detection of Cu^{2+} ions, which is based on determining the change of the absorbance (ΔA, the absorbance of Cu^{2+}-CR complex subtracted by that of CR after UVC light irradiation) by UV–visible absorption spectrum. Under the optimal detection conditions, the ΔA at 509 nm highly depends on the concentration of Cu^{2+} ions in the range from 5.0×10^{-9} to 1.0×10^{-6} M as expressed by the following equation: $\Delta A = 0.3066 + 0.03605 \lg c$ with the correlation coefficient of $r = 0.9912$. The limit of detection (*ld*) is 3.4 nM as calculated by the formula $3\sigma = 0.3066 + 0.03605 \lg ld$. This method provides affordable and selective detection of Cu^{2+} ions and was used to detect the Cu^{2+} ions in a human hair sample.

1. Introduction

Copper ion (Cu^{2+}) is one of the essential transition metal ions in the human body, and its deficiency or excess may cause health problems [1]. The deficiency of Cu^{2+} may lead to hematological manifestations [2] and diverse neurological problems [3]. Excess amount of Cu^{2+} may cause gastrointestinal disturbance and neurotoxicity, commonly known as Parkinson's disease and Alzheimer's disease, through the deposition of Cu^{2+} in the lenticular nucleus of the brain and liver [4, 5]. Therefore, the analysis and detection of Cu^{2+} in environmental and/or biological samples is of great importance. Various sensors and techniques have been developed for the determination of Cu^{2+} [6-9], such as organic fluorophores [10] or chromogenic sensors [11], DNAzyme-based method [12], colorimetric detection of Cu^{2+} [13], absorbance spectrophotometry [14], atomic absorption spectroscopy (AAS) [15], inductively coupled plasma mass spectroscopy (ICP-MS) [16]. However, a sensitive and selective method for Cu^{2+} detection is still needed.

Chromotrope 2R [17] (abbreviated as CR, the structure is shown in Scheme 1) is a type of azo dye [18, 19]. CR and/or its homologs have been used to detect nitrate [20], formaldehyde [21], methanol [22], titanium [23], and unsymmetrical dimethylhydrazine [24]. Moreover, they were used as the chelating agents for metal ions, particularly Cu^{2+} ions [25-27]. For example, the chromotropic acid-intercalated Zn−Al layered double hydroxides have been used to remove Cu^{2+} ions [28], and polyurethane foam/2-(6′-ethyl-2′-benzothiazolylazo) chromotropic acid has been used to preconcentrate Cu^{2+} ions in water samples [29].

Herein, we found that UVC light induced decolorization of CR could be suppressed after coordinating with Cu^{2+} ions. Furthermore, the decolorization extent of CR depends on the concentration of Cu^{2+} ions. Thus, a novel method for the detection of Cu^{2+} ions was established using UV–visible spectroscopy. The content of Cu^{2+} ions in a human hair sample was detected successfully, indicating its potential application in other environmental and biological samples.

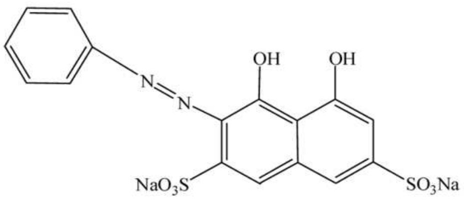
2. Experiment

2.1 Instrument

The UV–visible absorption spectra were recorded using a Lambda-35 UV–visible spectrophotometer (Perkin Elmer Instruments Inc., USA) and a quartz cell (1 × 1 cm^2). Mass spectroscopy (MS) were recorded using a ACQUITY UPLC/Xevo Q-TOF (Waters, USA) instrument. A UVC lamp with 110 W power (wavelength range 200–275 nm, Shanghai Yanguang Electronic Technology Co. Ltd., China) was used as the UVC light source. A QL-901 Vortex (Qilinbeier Instrument Manufacturing Co. Ltd., Haimen, China) was used to mix the reaction solution.

2.2 Reagents

CR was purchased from Shanghai Chemical Co., and used as received without further purification. The concentration of the CR stock solution is 4.0×10^{-4} M. A Cu^{2+} stock solution with a concentration of 1.0×10^{-3} M was freshly prepared by dissolving pure $Cu(NO_3)_2 \cdot 3H_2O$ (purchased from Tianjin Municipality Kemi'ou Chemical Reagent Co. Ltd.) with redistilled water and then diluted to appropriate concentrations with water. The Britton–Robinson (BR) buffer solution was used to adjust the pH value of the reaction system from 1.81 to 8.69. All the chemicals were analytical reagents, and redistilled water was used.



Scheme 1. Chemical structure of CR

2.3 Irradiation procedure

Irradiation experiments were carried out in a 4.0 mL centrifuge tube with the CR solution (0.1 mL, initial concentration 4.0×10^{-4} M). Then, appropriate concentrations of Cu^{2+} ions or sample solutions (3.5 mL) were added to the centrifuge tube and shaken for 30 s. Next, the BR buffer solution (400 μL, pH = 4.78) was added. The mixtures were illuminated under UVC irradiation for 50 min. Finally, the absorbance values of these samples were measured using a UV–visible spectrophotometer.

2.4 Pretreatment of real samples

The hair sample from a barber shop was pretreated by following a procedure reported previously [30, 31]. The hair sample was first cut into pieces with a length <1.0 cm using stainless steel scissors, and then it was immersed in a detergent solution with a concentration of 1.0% for 0.5 h. Next, the sample was washed four times with water and dried for 3 h in an oven at 105 °C. Then, 1.0 g of the hair sample and 2.0 mL of a mixture of $HNO_3/HClO_4$ (3:1 *v/v*) were added into a quartz beaker, covered with a watch glass, and stand overnight. The next day, the hair sample was heated until it dissolved completely. Finally, the sample was diluted to 50.0 mL with water and used as the stock solution.

3. *Results and discussion*

3.1 Degradation of CR with and without Cu^{2+}

The UV–visible absorption spectra of CR (1.0×10^{-5} M) before and after UVC light irradiation are shown in Fig. 1A (curves 1 and 2). The absorbance (A) of the three absorption peaks at 300, 375, and 509 nm clearly decreased after the irradiation for 50 min. The reduction in the A was 84.1% at 509 nm. However, in the presence of a trace amount of Cu^{2+} ions (1.0×10^{-6} M), the photodegradation was significantly suppressed, and the A decreased by only 35.4% at 509 nm (curve 3 in Fig. 1A), which can be ascribed to the formation of a coordination complex between CR and Cu^{2+} ions (CR-Cu^{2+}).

To identify the complexation of CR with Cu^{2+} ions, the UV–visible absorption and MS spectra of CR-Cu^{2+} in the presence of different concentrations of Cu^{2+} ions (from 0 to 1.0×10^{-3} M) were investigated. As shown in Fig. 1B, the absorbance of CR (curve 1, Fig. 1B) decreased slightly over the wavelength range of 450–550 nm in the presence of Cu^{2+} ions (curves 2–4, Fig. 1B). Two new absorption bands appeared in the wavelength ranges of 200–300 nm and 550–630 nm. Simultaneously, the color of the solution deepened with Cu^{2+} addition (Fig. S1 in the Supporting Information). These results can be attributed to the complexation between Cu^{2+} ions and the amine groups in CR. The result of MS showed that the molecular weight of the CR-Cu^{2+} mixture is 958.9 (Fig. S2 A and B in the Supporting Information), indicating that the coordination ratio between CR and Cu^{2+} was 2:1, and the possible structure of the complex is shown in Fig. S3.

To further understand the mechanism of this photodegradation of CR, we carried out the experiments by deoxygenating the solution. The UV–visible absorption spectra are shown in Fig. S4 in the Supporting Information, indicating that the degradation extent decreased significantly without dissolved oxygen (curve 1 of Fig. S4) compared to that in the presence of dissolved oxygen (curve 2 of Fig. S4). The MS spectra showed that the intensity of the peak at m/z of 468.9 for the CR molecule significantly decreased under UVC light irradiation in the presence of dissolved oxygen (Fig. S2 C in the Supporting Information). Thus, we deduced that the photodegradation of CR involves oxidation by oxygen under UVC light irradiation.

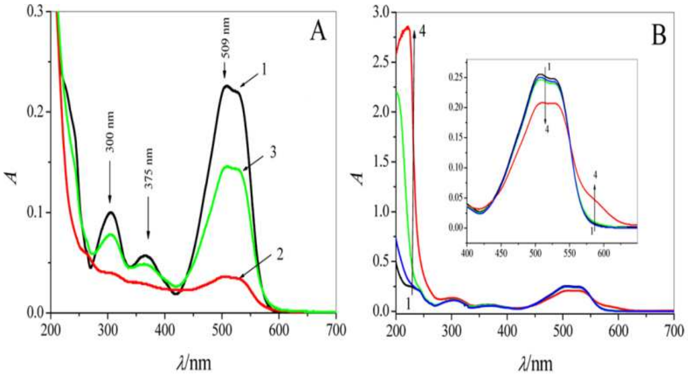


Fig.1. The UV-visible absorption spectra (A) of CR before (curve 1) and after (curve 2) UVC light irradiation, and the mixture of CR with 1.0×10^{-6} mol L^{-1} Cu^{2+} before (curve 3) and after (curve 4) UVC irradiation for 50min. (B) the mixture of CR with different concentrations of Cu^{2+} 0, 1.0×10^{-5}, 1.0×10^{-4}, 1.0×10^{-3} mol L^{-1} (curves 1–4) without UVC light irradiation and the insert is the amplified graph. pH=4.78, c_{CR} (1.0×10^{-5} mol L^{-1}).

3.2 Optimization of reaction conditions

Many studies showed that CR is sensitive to UV light. First, we confirmed that the natural light has a negligible effect on CR at room temperature (Fig. S5 in the Supporting Information). We also investigated the effect of different powers of UVC light on the degradation of CR. It was found that CR could not be degraded within 50 min under low-power (30 W, 45 W) light irradiation. High-power (110 W) UVC light irradiation was efficient for this study.

To study the effect of the concentration of CR on the detection sensitivity, we measured the ΔA values (the absorbance of the CR solution in the presence of Cu^{2+} subtracts that in the absence of Cu^{2+}) under different concentrations of CR solutions (Fig. S6 in the Supporting Information). The results indicate that the ΔA values are 0.142 for 1.0×10^{-4} M CR, 0.148 for 5.0×10^{-5} M CR, and 0.11 for 1.0×10^{-5} M CR respectively, which is increased with increasing concentration of CR. However, the ratios of signal to noise (S/N), which was calculated using the equation $S/N = (A - A_O)/A_O$, were 0.0796 for 1.0×10^{-4} M CR, 0.2016 for 5.0×10^{-5} M CR, and 3.056 for 1.0×10^{-5} M CR respectively. I.e., the value of S/N is the highest when the concentration of CR is 1.0×10^{-5} M.

The pH of the solution is another key factor affecting the sensing sensitivity. Fig. 2 shows the absorbance values at the maximum absorption peak of CR in the presence (curve a) and absence (curve b) of Cu^{2+} ions (1.0×10^{-6} M) at different pH values in the range from 1.81 to 8.69. Curve c in Fig. 2 shows the change of ΔA with different pH values. The ΔA values increased (curve c, Fig.2) with the increase pH value for pH $\leq$4.78. The ΔA values decreased with the increase pH value for pH>4.78 (curve c in Fig. 2). This indicates that the weakly acidic media (pH =4.78) are suitable for sensing Cu^{2+}ions.

Fig. 3 shows the absorbance at the maximum absorption peak of CR in the presence (curve a) and absence (curve b) of Cu^{2+} ions (1.0×10^{-6} M) with different irradiation times in the range from 30 to 90 min at the optimum pH value of 4.78. Curve c in Fig. 3 shows that when the reaction time was 50 min, the ΔA reached the maximum value. Therefore, the optimum irradiation time was 50 min for the determination of Cu^{2+} ions.

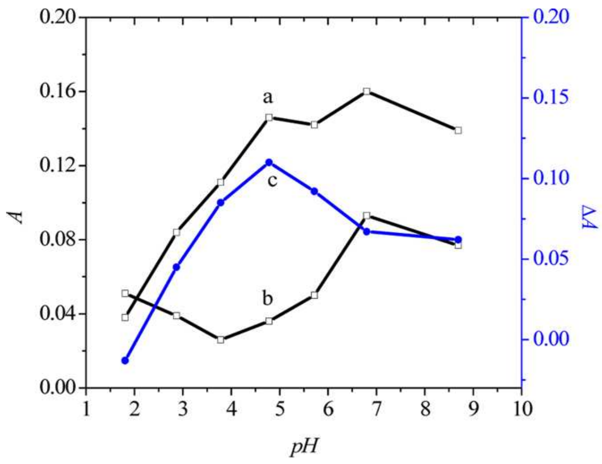


Fig.2. The absorbance of CR (1.0×10^{-5}M) in the presence (curve a) and absence (curve b) of Cu^{2+}(1.0×10^{-6} mol L^{-1}) at different pH medium, including 1.81, 2.87, 3.78, 4.78, 5.72, 6.80. The curve c represented the change of ΔA with different pH medium.

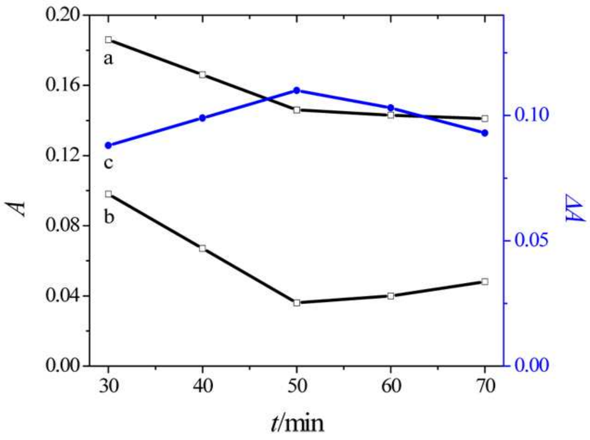


Fig. 3. The absorbance of CR (1.0×10^{-5} mol L^{-1}) in the presence (curve a) and absence (curve b) of Cu^{2+} (1.0×10^{-6} mol L^{-1}) in different irradiation time (30 min, 40 min, 50 min, 60 min, and 70 min). The curve c represented the ΔA (the difference value of the absence and presence of Cu^{2+} under UVC light irradiation).

3.3 Selectivity of Cu^{2+} ion detection

The selectivity of CR for the detection of Cu^{2+} ions over other ions is shown in Fig. 4. Besides Cu^{2+} (1.0×10^{-6} M), the effects of other 11 types of cations, including K^+, Na^+, Co^{2+}, Ni^{2+}, Hg^{2+}, Mg^{2+}, Pb^{2+}, Al^{3+}, Zn^{2+}, Mn^{2+}, and Ca^{2+} ions at the concentration of 5.0×10^{-6} M, on the UV–visible absorbance of CR were investigated under UVC irradiation for 50 min. The results indicate that Mn^{2+} and Ni^{2+} ions slightly suppressed the CR degradation, and the other ones slightly accelerated the CR degradation. The signals responded by Cu^{2+} ions are the most pronounced among other cations. Therefore, the sensing system shows high selectivity for Cu^{2+} ion detection.

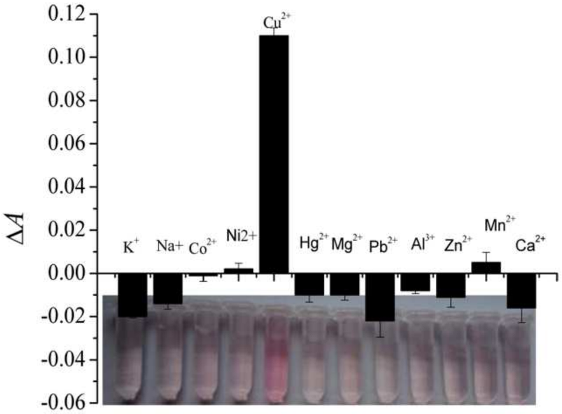


Fig. 4. Selectivity for Cu^{2+} ions detection over other ions in pH 4.78 BR solution under UVC light irradiation for 50min. The concentrations of CR, Cu^{2+} and other metal ions were 1.0×10^{-5}mol L^{-1}, 1.0×10^{-6}mol L^{-1}, and 5.0×10^{-6}mol L^{-1}, respectively.

3.4 Cu^{2+} ion detection in a human hair sample

Fig. 5 shows that different concentrations of Cu^{2+} ions resulted in different degradation degrees of CR with a good linear relationship. Under the optimum conditions, this approach provides a linear response to Cu^{2+} ions in spiked samples at concentrations in the range from 5.0×10^{-9} to 1.0×10^{-6} M ($\Delta A = 0.3066 + 0.03605 \log c$, r = 0.9912, n = 6, the inset of Fig. 5). Compared to the published spectrometry for Cu^{2+} ion detection (Table S1 in the Supporting Information), the method has comparable sensitivity with the fluorescence method [32-34] and has relatively higher sensitivity than the absorption method [35, 36]. To evaluate the potential application of this method for detecting Cu^{2+} ions in a real sample, we tested its absorbance response for Cu^{2+} ions in a human hair sample. The content of Cu^{2+} ions in the human hair sample (n = 3) detected using this new approach was 3.72 μg g^{-1} (Table S2 in the Supporting Information), and the detection result agreed well with the Cu^{2+} content of healthy human hair [37] with an excellent relative standard deviation (RSD, 3.62%). These results confirmed the reliability of this CR sensing method for the detection of Cu^{2+} ions in real samples.

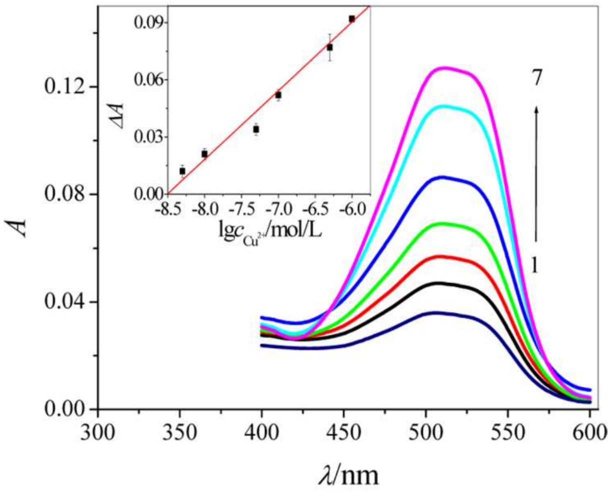


Fig.5. The UV-visible absorption spectra of CR under UVC light irradiation in the presence of different concentrations of Cu^{2+} 0, 5.0×10^{-9}, 1.0×10^{-8}, 5.0×10^{-8}, 1.0×10^{-7}, 5.0×10^{-7}, 1.0×10^{-6}mol L^{-1} (1~7). The calibration curve for the detection of Cu^{2+} by UV-vis absorption spectrum. Linear range of the plot of ΔA against the $\lg c_{Cu2+}$. Error bars represent standard deviations from three repeated measurements. Other conditions were the same as mentioned above.

Conclusions

In this study, we found that CR could be degraded easily under UVC light irradiation. Cu^{2+} ions reacted with CR to form a stable complex, suppressing the CR degradation under UVC irradiation. After the optimization of the experimental conditions, an UV–visible absorption spectrum based sensing system was developed for the detection of Cu^{2+} ions. The results show that CR is an excellent UV–visible absorption spectrum probe for Cu^{2+} ion detection. The sensing system shows many advantages such as good selectivity and high sensitivity with the lowest detection concentration of 5.0×10^{-9} M. It was used for the detection the content of Cu^{2+} ions in a real sample (human hair) with acceptable results.

Acknowledgements

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Notes and references

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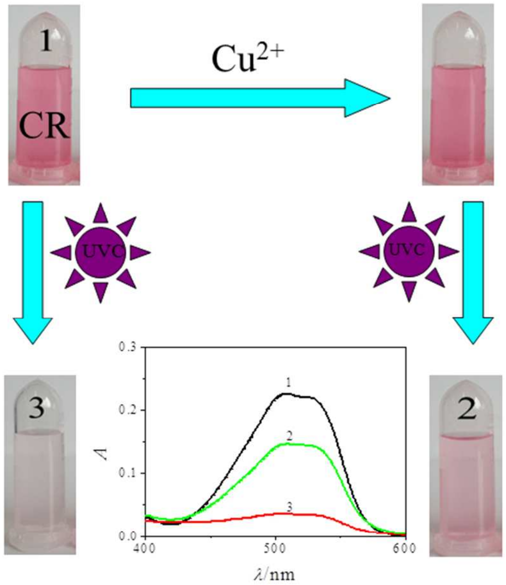

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