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Multichannel Detection of Cu\textsuperscript{2+} Based on a Rhodamine-Ethynylferrocene Conjugate\textsuperscript{†}

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A novel multichannel chemosensor DR3 juxtaposed with a rhodamine chromophore and the electrochemical characterization of an ethynylferrocene group was developed. This chemosensor could selectively recognized Cu\textsuperscript{2+} in the presence of other competing ions in a wide pH range, which exhibits the multi-response of UV/vis absorption, fluorescence emission, and electrochemical parameters.

Introduction

Recently, due to its innate advantages such as high sensitivity, selectivity and real-time monitoring, luminescent chemosensors for detection of transition metal have attracted increasing attention. It usually contain the reaction sites and an obvious change in characteristics upon host-guest interactions.\textsuperscript{1} Among the transition metal, copper is the third most abundant essential heavy metal in the human body after zinc and iron and plays a crucial role in biological process. It forms as an important catalytic cofactor in redox chemistry for proteins.\textsuperscript{2} However, the abnormal level of Cu\textsuperscript{2+} in living system may lead to various neurodegenerative diseases including Menkes, Wilson, and Alzheimer’s diseases.\textsuperscript{3,4} Thus, it is of significant importance to develop a luminescent chemosensor for monitoring Cu\textsuperscript{2+} in biological process. As far as we know, a large number of Cu\textsuperscript{2+}-chemosensors based on fluorescence enhancement have been reported.\textsuperscript{6-19} Most of them are based on the changes of UV/vis absorption and fluorescence emission. But few multichannel Cu\textsuperscript{2+}-selective chemosensors have been developed so far through multiple responses including chromaticity, fluorescence, electrochemistry et. al. Compared with single signal detection, multichannel have higher sensitivity, better excellent selectivity and anti-interference ability. More importantly, multichannel detection system can make self-calibration measurements come true through different analytic methods.

Zade et. al. reported a thiophene-based salphen-type chemosensor for detection of Cu\textsuperscript{2+} and Zn\textsuperscript{2+} with electrochemical properties.\textsuperscript{20} It is expected to introduce an excellent group to multichannel chemosensors which have electrochemical characteristics, realizing to establish multichannel analytic systems. Ferrocene has the brilliant ability to store electrons strengthening the coordination between the chemosensor and the metal ion.\textsuperscript{21} It is well known that ferrocene-containing chemosensors exhibit the electrochemical response upon complexation of a suitable guest ion. Zhang and Zapata et. al also observed a significant potential shift of the Fe\textsuperscript{II}/Fe\textsuperscript{III} on coordination of an analytic, which exhibits a multi-responsive signaling.\textsuperscript{22} The expanded work relied on rhodamine-based multichannel chemosensors for Cr\textsuperscript{3+} and Hg\textsuperscript{2+} et. al linked with ferrocene have been reported.\textsuperscript{23} However, the multichannel Cu\textsuperscript{2+}-chemosensors have been barely mentioned to the best of our knowledge.\textsuperscript{24}

Herein, we synthesized a novel multichannel chemosensor DR3 for the detection of Cu\textsuperscript{2+} (Scheme 1). It is expected to achieve three-channel Cu\textsuperscript{2+}-selective chemosensor through introducing ferrocene group to the rhodamine chromophore. We designed the acetylene group linked with the electrochemical properties of a ferrocene group and chromatic, fluorescent rhodamine with ring-opening process, increasing multichannel output signaling upon interaction with Cu\textsuperscript{2+}. The chemosensor was introducing a hydrazide functional group to act as the potential reaction site for Cu\textsuperscript{2+}. Upon reacts with copper ion, the fluorescence and absorption intensity evidently increased due to the process of spirolactam ring-opening and hydrolysis. This multichannel chemosensor DR3 exhibits suitable variations of absorption spectrum, fluorescence emission and electrochemical parameters.

![Scheme 1. Design of Cu\textsuperscript{2+} multichannel chemosensor DR3](image_url)

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Experimental section

Materials and measurements

All reagents were purchased from commercial suppliers and used without further purification. 1H and 13C NMR spectra were measured on a Bruker Avance 500 or Bruker Avance 400 spectrometer in CDCl3. Electrospray ionization mass spectra (ESI-MS) were measured on a Micromass LCTTM system. UV–visible spectra were recorded on a Perkin–Elmer 35 spectrometer and Fluorescence measurements were performed on a Perkin-Elmer LS 50B fluorescence spectrophotometer. Electrochemical measurements were performed with an Eco Chemie Autolab. The pH measurements were made with a PHS/3C Precision Ph/mV Meter. TLC analysis were made with a PHS-3C Precision Ph/mV Meter. TLC analysis was conducted on silica gel plates and chromatography was performed over silica gel (mesh 300-400).

UV/vis and fluorescence experiments

A stock solution of 1.0 mM DR3 was prepared in acetonitrile. Cu(NO3)2·3H2O was dissolved in doubly distilled water to form a 5.0 mM stock solution. For detecting metal ions, various metal ions solutions of NaNO3, Co(NO3)2, KNO3, Zn(NO3)2, 6H2O, Ni(NO3)2·6H2O, Mg(NO3)2·6H2O, Ca(NO3)2·4H2O, MnCl2·4H2O, Pb(NO3)2·HgCl2·2AgNO3·Ba(NO3)2 were used. Before fluorescence and UV/vis titration investigation was conducted, the stock solution was mixed with the stock solutions of metal salts in a 10 ml volumetric flask and diluted with H2O and CH3CN to volume. Spectral data were recorded at 2 min after the addition. For fluorescence measurements, excitation was provided at 550 nm, and emission was collected from 546 to 700 nm. The wide pH was adjusted by HCl or NaOH solutions.

Electrochemical test

Electrochemical characteristics was tested according to a literature.

Synthesis of compound DR1

Compound DR1 was synthesized according to a literature. JR1: 1H NMR (400 MHz, CDCl3) δ: 7.85 (d, J = 8.1 Hz, 1H, Ar-H), 7.70 (dd, J = 8.1, 1.6 Hz, 1H, Ar-H), 7.32 (d, J = 1.5 Hz, 1H, Ar-H), 6.66 - 6.57 (m, 2H, Ar-H), 6.48 (d, J = 2.5 Hz, 2H, Ar-H), 6.41 (dd, J = 8.8, 2.6 Hz, 2H, Ar-H), 2.99 (s, 12H, -CH3). DR1: 1H NMR (400 MHz, CDCl3) δ: 8.12 (d, J = 1.7 Hz, 1H, Ar-H), 7.83 - 7.66 (m, 1H, Ar-H), 7.05 (t, J = 8.8 Hz, 1H, Ar-H), 6.67 - 6.55 (m, 2H, Ar-H), 6.48 (d, J = 2.5 Hz, 2H, Ar-H), 6.41 (dd, J = 8.9, 2.6 Hz, 2H, Ar-H), 2.99 (s, 12H, -CH3).

Synthesis of compound DR2

Compound DR2 was synthesized according to a literature with some modification. A mixture of DR1 (0.5 mmol, 233 mg), ethynylferrocene (0.5 mmol, 157 mg), 35 mg (0.05 mmol) of PdCl2(PPh3)2, and PPh3 (26 mg, 0.1 mmol), 4.8 mg (0.025 mmol) of CuI, THF (20 mL), NEt3 (5 mL) under nitrogen, upon the temperature reached 95 °C and refluxed 12 h after completion of the reaction by TLC, evaporated the solvent, the crude product was purified by column chromatography with CH2Cl2/NEt3 (v/v = 200/4) and afforded the target product DR2 as a purplish red (247 mg, 83%). 1H NMR (400 MHz, CDCl3) δ: 8.08 (d, J = 0.7 Hz, 1H, Ar-H), 7.75 – 7.66 (m, 1H, Ar-H), 7.13 (d, J = 7.9 Hz, 1H, Ar-H), 6.63 (d, J = 8.8 Hz, 2H, Ar-H), 6.48 (d, J = 2.5 Hz, 2H, Ar-H), 6.40 (dd, J = 8.9, 2.6 Hz, 2H, Ar-H), 4.55 (s, J = 1.8 Hz, 2H, ferrocene-H), 4.30 – 4.29 (m, 2H, ferrocene-H), 4.27 (d, J = 4.5 Hz, 5H, ferrocene-H), 2.99 (s, 12H, -CH3). 13C NMR (101 MHz, CDCl3): 169.19, 152.09, 151.91, 137.38, 128.75, 127.93, 127.34, 125.55, 124.08, 108.66, 106.40, 98.53, 90.75, 84.31, 71.62, 70.08, 69.19, 64.30, 46.15, 40.26. ESI-MS m/z 595.2 [M]+.

Synthesis of compound DR3.

A solution of DR2 (0.25 mmol, 160 mg), excess 98% N2H4·H2O (1 ml) was resolved in 10 mL of ethanol and refluxed 6 h, evaporated the solvent the crude product was purified by column chromatography with EtOAc/P/E (v/v = 3/2) get the desired product DR3 (110 mg, 73%). 1H NMR (400 MHz, CDCl3) δ: 8.06 (s, 1H, Ar-H), 7.55 (dd, J = 7.9, 1.4 Hz, 1H, Ar-H), 7.03 (d, J = 7.9 Hz, 1H, Ar-H), 6.52 (d, J = 8.8 Hz, 2H, Ar-H), 6.48 (d, J = 2.3 Hz, 2H, Ar-H), 6.39 – 6.36 (m, 2H, Ar-H), 4.52 (t, J = 1.7 Hz, 2H, ferrocene-H), 4.26 (s, 2H, ferrocene-H), 4.25 (s, 5H, ferrocene-H), 3.63 (s, 2H, -NH3), 2.97 (s, 12H, -CH3). 13C NMR (101 MHz, CDCl3): 165.56, 153.48, 151.55, 150.33, 135.50, 130.21, 127.94, 124.94, 124.30, 123.79, 108.81, 105.22, 99.01, 89.69, 84.90, 71.55, 70.05, 69.35, 69.03, 65.89, 40.31. ESI-MS m/z 609.2 [M+ H]+.

Results and discussion

DR3 was prepared based on a two steps route shown in Scheme 2. Firstly, compound DR2 was achieved in 83% yield by a Sonogashira reaction in catalyst of PdCl2(PPh3)2, PPh3, and CuI. Then compound DR2 reacts with hydrazine hydrate to obtain DR3 in 73% yield by 6 h refluxing. These compounds were characterized and confirmed by 1H NMR, 13C NMR, ESI-MS (ESI, Fig. S1-6).
As shown in Fig. 1a, the influence of pH on the fluorescence of DR3 was investigated first. Under acidic conditions (pH < 5), ring opening of rhodamine occurred due to the protonation of chemosensor. When the pH ranged from 5.0 to 9.0, no obvious characteristic fluorescence emission of rhodamine was observed. However, the addition of Cu$^{2+}$ led to the fluorescence enhancement over a comparatively range (5.0-9.0), which is attributed to the ring-opening process of rhodamine and hydrosis. Multichannel Chemosensor DR3 and DR3-Cu$^{2+}$ remained unaffected in fluorescence intensity in the pH 6.0-8.0, suggesting that it was insensitive to pH around 7.0 and could be suitable for physiological conditions. This result was also same to the UV/vis spectra in range of pH 5-9, as illustrated in Fig.1b.

![Figure 1](image1.jpg)

**Fig. 1.** (a) The fluorescence response of DR3 (10 µm) at 585 nm before and after addition of 10 equiv Cu$^{2+}$ with different pH condition. (b) The absorbance response of DR3 (10 µm) at 555 nm before and after addition of 10 equiv Cu$^{2+}$ with different pH condition.

The UV/Vis spectrum of DR3 shows an off(on) luminescence chemosensor for Cu$^{2+}$. As an excellent luminescence chemosensor, DR3 was insensitive to pH around 7.0 and could be suitable for physiological conditions. This result was also same to the UV/vis spectra in range of pH 5-9, as illustrated in Fig.1b.

![Figure 2](image2.jpg)

**Fig. 2.** (a) Absorption spectra of DR3 (10 µM) in HEPES/CH$_3$CN (2:8, v/v, pH 7.0) solutions upon addition of increasing concentrations of 0-24 equiv Cu(NO$_3$)$_2$·3H$_2$O. Insets: the absorbance at 555 nm as a function of Cu$^{2+}$ concentrations. (b) Color changes upon adding different cations in HEPES/CH$_3$CN (2:8, v/v, pH 7.0) solutions upon addition of increasing concentrations of 0-8 equiv Cu(NO$_3$)$_2$·3H$_2$O. (c) Absorption spectra of DR3 in HEPES/CH$_3$CN (2:8, v/v, pH 7.0) solutions with 8 equiv of metal ions: free, Cu$^{2+}$, Hg$^{2+}$, Pb$^{2+}$, Ba$^{2+}$, Mn$^{2+}$, Ca$^{2+}$, Mg$^{2+}$, Ni$^{2+}$, Ag$^+$, Zn$^{2+}$, K$^+$, Cr$^{3+}$, Co$^{2+}$, Na$^+$.

The UV/Vis spectrum of DR3 in a solvent system of CH$_3$CN/HEPES 80:20 with titration of Cu$^{2+}$ was shown in Fig. 2a. It exhibits almost no absorption in the visible wavelength range, indicating that chemosensor DR3 is predominantly in the form of spirolactam. Upon addition of Cu$^{2+}$, a new absorption peak at 555 nm was observed, and the intensity gradually increased with increasing Cu$^{2+}$ ion concentration. This could be attributed to the formation of ring-opened amide form of DR3 upon interaction with Cu$^{2+}$. The inset in Fig. 2a showed the absorbance at 555 nm as a function of Cu$^{2+}$ concentrations. The ring-opening mechanism was also confirmed by the color change of 10 µM DR3 upon adding different cations (Fig. 2b). Among the metal ions, only Cu$^{2+}$ can induce an obvious color change from colorless to pink in the solution of DR3, allowing colorimetric detection of Cu$^{2+}$ by the naked eye. Fig. 2c shows the absorption response of DR3 towards various metal ions in HEPES/CH$_3$CN (2:8, v/v, pH 7.0) solutions. Under identical condition, DR3 exhibits almost no absorbance enhancement at around 555 nm on addition of Hg$^{2+}$, Pb$^{2+}$, Ba$^{2+}$, Mn$^{2+}$, Ca$^{2+}$, Mg$^{2+}$, Ni$^{2+}$, Ag$^+$, Zn$^{2+}$, K$^+$, Cr$^{3+}$, Co$^{2+}$, Na$^+$. free. These results indicate that chemosensor DR3 has the high selectivity towards Cu$^{2+}$.

![Figure 3](image3.jpg)

**Fig. 3.** (a) Fluorescence spectra of DR3 in HEPES/CH$_3$CN (2:8, v/v, pH 7.0) solutions upon addition of increasing concentrations of 0-24 equiv Cu$^{2+}$. Inset: fluorescence intensity at 585 nm as a function of Hg$^{2+}$ concentrations. (b) Fluorescence intensity at 585nm of DR3 (10 µM) in HEPES/CH$_3$CN (2:8, v/v, pH 7.0) solutions as a function of Cu$^{2+}$ concentration (0-3.5 µM). (c) Fluorescence intensity of DR3 (10 µM) in HEPES/CH$_3$CN (2:8, v/v, pH 7.0) solutions upon addition of 24 equiv metal ions (blank, Cu$^{2+}$, Hg$^{2+}$, Pb$^{2+}$, Ba$^{2+}$, Mn$^{2+}$, Ca$^{2+}$, Mg$^{2+}$, Ni$^{2+}$, Ag$^+$, Zn$^{2+}$, K$^+$, Cr$^{3+}$, Co$^{2+}$, Na$^+$).

![Figure 4](image4.jpg)

**Fig. 4.** (a) Fluorescence spectra of DR3 in HEPES/CH$_3$CN (2:8, v/v, pH 7.0) solutions with the addition of Cu$^{2+}$. Chemosensor DR3 shows a very weak emission at around 585 nm upon excitation at 530 nm. When Cu$^{2+}$ was added to the DR3 buffer solution, fluorescence intensity at 585 nm was observed, attributed to the ring-opening process of rhodamine derivatives. The solution showed an approximately 274-fold enhancement in the fluorescence intensity. This fact means that DR3 could be act as an off-on luminescence chemosensor for Cu$^{2+}$. The fluorescence intensity finally levelled off until the amount of added Cu$^{2+}$ was 2.2 × 10$^{-4}$ M (Fig. 3a inset). As an excellent luminescence chemosensor, it is important to have high selectivity. As illustrated in Fig. 3c,
additions of other metal ions including Hg^{2+}, Pb^{2+}, Ba^{2+}, Mn^{2+}, Ca^{2+}, Mg^{2+}, Ni^{2+}, Ag^{+}, Zn^{2+}, K^{+}, Cr^{3+}, Co^{2+}, Na^{+} and free induced no obvious fluorescence enhancement under the same condition. These observations indicated that chemosensor DR3 could selectively recognize Cu^{2+} in HEPES/CH_{3}CN (2:8, v/v, pH 7.0) solutions. For practical application the detection limit was also calculated as 6.85 × 10^{-6} M for Cu^{2+} (3σ/slope), which is sufficiently low for the detection of many chemical systems for Cu^{2+} (Fig. 3b).²⁶

Fig. 4 (a) DPV of DR3 (100 µM) in CH_{3}CN solution in the absence and presence of 1.6 equiv of Cu^{2+} with n-BuNPF_{6} as supporting electrolyte. (b) CV of DR3 (100 µM) in CH_{3}CN solution in the absence and presence of Cu^{2+} with n-BuNPF_{6} as supporting electrolyte.

As designed, DR3 shows an evident change in its reversible ferrocene/ferricinium redox cycles upon complexation. Differential pulse voltammetry (DPV) curves of DR3 were recorded in CH_{3}CN solution containing 0.1 M n-tetraethylammonium hexafluorophosphate (n-BuNPF_{6}) as supporting electrolyte in the absence and presence of Cu^{2+}.²²a As shown in Fig. 4a, a significant displacement was observed upon addition of Cu^{2+}. The oxidation peak was shifted in CH_{3}CN from 0.728 to 0.54 V (ΔE_{1/2} = 188 mV). For cyclic voltammetry (CV), a significant shift of the redox potential of the ferroceny group was also observed (Fig. 4b). The CV behavior of chemosensor DR3 was measured in CH_{3}CN, suggesting a reversible one-electron redox process. The addition of Cu^{2+} induces a positive shift of the ferrocene/ferricinium couple, which is attributed to the redox process of Cu^{2+}. This fact indicated that DR3 could be a multi-signal chemosensor for Cu^{2+} with electrochemical measurements.

Fig. 5 Fluorescence spectra in HEPES/CH_{3}CN (2:8, v/v, pH 7.0) solutions: (a) DR3 (10 µM); (b) DR3 (10 µM) with Cu^{2+} (240 µM); (c) DR3 (10 µM) with Cu^{2+} (240 µM) and then addition of EDTA (500 µM).

In order to explore mechanism, the reaction product of DR3 and Cu^{2+} was detected by ESI spectra analyses (ESI, Fig. S7). The ion peak was detected at m/z 609.2, which was corresponding to [DR3 + H]^+ . In addition, the main ion peak at m/z 595.2 corresponding to intermediate DR2 was detected after the addition of Cu^{2+} to DR3 aqueous solution, which suggested that Cu^{2+} induces the hydrolysis and spirolactam ring-opening of rhodamine. To further confirm Cu^{2+} mediated hydrolysis, we have carried out the chemical reversibility experiment in the CH_{3}CN-water solution. Upon addition of 500 µM chelating agent EDTA to the DR3-Cu^{2+} complex in HEPES/CH_{3}CN (2:8, v/v, pH 7.0) solutions, the color and fluorescence intensity discovered no obvious changes (Fig. 5). These findings indicated that DR3 is an irreversible luminescence chemosensor for Cu^{2+}. According to the obtained results, we proposed that the reaction process may proceed as the route depicted in Scheme 3.

![Scheme 3](image)

Scheme 3 The proposed reaction mechanism of DR3 with Cu^{2+}.

Conclusions

In summary, we have designed a multichannel chemosensor DR3 for Cu^{2+} with the chromatic, fluorescent rhodamine derivatives and the electrochemical characterization of a ferrocenyl group. The novel chemosensor exhibits the multisensitive, colorimetric, fluorescent and electrochemical detection for Cu^{2+}. Multichannel chemosensor DR3 could detect micromole level of Cu^{2+} and in a wide pH range (5.0-9.0). Above these results, we believe that DR3 could be further used for monitoring intracellular Cu^{2+} ions in biological systems.

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