Rhodamine-based multianalyte colorimetric probe with potentialities as on-site assay kit and in biological systems

Tahir Rasheed[a], Chuanlong Li[a], Yinglin Zhang[a], Faran Nabeel[a], Jiaxin Peng[a], Jie Qi[a], Lidong Gong[b], Chunyang Yu[a],*

[a] The School of Chemistry & Chemical Engineering, State Key Laboratory of Metal Matrix Composites, Shanghai Jiao Tong University, 800 Dongchuan Road, Shanghai, 200240, China
[b] School of Chemistry and Chemical Engineering, Liaoning Normal University, 850 Huanghe Road, Dalian, 116029, China

A R T I C L E   I N F O

A B S T R A C T

Herein, we report a new rhodamine-based multianalyte (Cu^{2+} and Hg^{2+}) fluorescent sensor **RH-FP**. The synthesized probe acts as a dual chemosensor for recognition of Cu^{2+} and Hg^{2+} in acetonitrile-water (4:1 v/v, pH 7.2 with 10 mM, HEPES) in the presence of other miscellaneous metal cations *i.e.* Ag^+, Al^{3+}, Ba^{2+}, Ca^{2+}, Cd^{2+}, Fe^{3+}, Fe^{2+}, K^+, Li^+, Mg^{2+}, Mn^{2+}, Cu^{+2}, Pb^{2+}, Ni^{2+}, Na^+ and Zn^{2+}. A highly selective and sensitive colorimetric (colorless to pink) response accompanied by a significant increase in absorption and emission maxima (250-folds) was observed by the interaction of **RH-FP** and Cu^{2+} or Hg^{2+}. This makes **RH-FP** a suitable "bare-eye" detector. The Job's plot and ESI–MS confirm 1:1 binding stoichiometry between sensor **RH-FP** and metal cations. The calculated binding constant for the complexes (**RH-FP-Cu^{2+} and RH-FP-Hg^{2+}**) are 3.37×10^5 mol^{-1} and 7.6×10^5 mol^{-1}, with a detection limit of 3.9 nm and 2.36 nm respectively. The binding mechanism was confirmed by Fourier transform infra-red spectroscopy (FT-IR) ^1H, ^{13}C NMR and density functional theory (DFT) calculations. Moreover, the fluorescent sensor **RH-FP** displayed potential in-field applications as detection kit and in fluorescent imaging of biological systems to detect Cu^{2+} or Hg^{2+}.

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1. Introduction

In recent years, extensive use of heavy transition metals in industry and crop cultivation has become a serious environmental issue for humans, wild and aquatic life [1,2]. Among heavy transition metals, Cu^{2+} and Hg^{2+} are considered to be the most important transition metal ions. In human body, copper is the third most abundant element after zinc and iron that plays vital role in a number of bodily reactions [3]. On contrary, an excess of Cu^{2+} can exert adverse effects on normal physiological processes [4]. The acute exposure of Cu^{2+} can create stomach problem, a neurodegenerative disorder, Wilson's, prion, and Parkinson's disease *etc.* The worst among all is amyloidal precipitation, liver, and kidneys failure [5,6]. Contrary to Cu^{2+} heave in the body, its deficiency increases heart-related disease risks. On the other hand, Hg^{2+} is the most toxic and hazardous element released by natural means and human deeds.

The transformation of Hg^{2+} to methylmercury by bacterial species accumulates in the body through the food chain, which is lethal and affects central nervous system [7,8]. However, the concentration of Hg^{2+} higher than 5 $\mu g\,L^{-1}$, can affect kidney, nervous disorder, stomach problem, heart diseases, nose bleeding and renal failure *etc.* [9]. Therefore, the detection, quantification, and exploration of heavy metal ions are of prominent significance.

Commonly used techniques for the sensing of heavy metals include inductively coupled plasma mass spectrometry electrochemical sensors, inductively coupled plasma atomic emission spectrometry and cold vapor atomic absorption spectrometry [10–13]. These traditional methods have high selectivity and sensitivity and can calculate contents of the analyte species. But these methods are expensive, complex and time intensive, have limits of hiring skilled personnel, complex apparatus, high operating expenditures and difficult sample preparation processes become hard for real-time evaluations [14,15]. In order to overcome these issues, simple and low-cost sensors are preferred that can detect and calculate the metal ions in-line real-time with little time consumption for environment, aquatic and biotic life surveillance. Over the last

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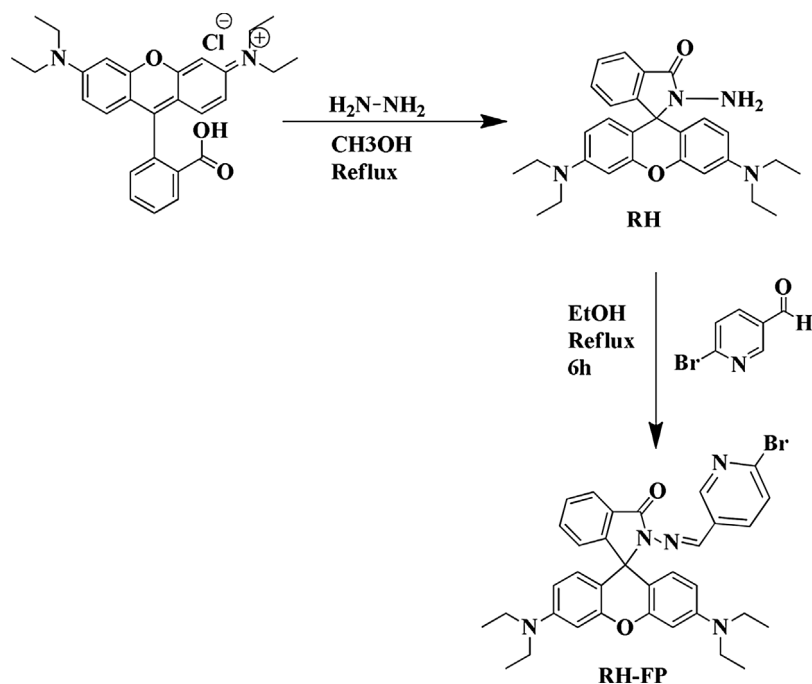
Scheme 1. Synthetic route for the synthesis of **RH-FP**.

twenty years, continuous efforts have been devoted to develop chemosensors for detection and quantification of the heavy metals in real-time without time-taking sample preparation procedures [16–20]. Fluorescent or colorimetric sensors with high selectivity and sensitivity are considered to be the most suitable sensors for low-limit detection of heavy metal ions [21,22].

Recently, several chemical sensors for Cu^{2+} and Hg^{2+} have been reported [23]. Some Cu^{2+} detecting sensors have poor solubility in water. The excitation wavelength of these sensors lies in UV light region. On the other hand, many of the reported Hg^{2+} sensors are based on thiophilic nature of Hg^{2+} which interfere the detection and sensing process [24–27]. These are the challenges for the researchers to design and fabricate the chemical sensors, which can detect Cu^{2+} and Hg^{2+} selectively. The naked eye fluorescent techniques for the recognition of heavy metal ions are being used on regular basis due to their highly sensitive, selective, simple operative, less expensive and nondestructive nature [28–30]. On the bases of excellent photophysical properties rhodamine-based frameworks are ideal candidates for the designing of fluorescent colorimetric chemical sensors. These fluorescent constructs have spirolactam structure and remarkable properties of high molar extinction-coefficient (ε), enhanced quantum fluorescence yield (Φ) and long emission and absorption wavelengths extended up to the visible region. All these parameters are in best agreement to support the visual detection of analyte [31–34]. Several rhodamine-based chemosensors have been successfully devised and employed for recognition of heavy metal ions, utilizing the property of spirocyclic closed ring (nonfluorescent) and ring-opened (fluorescent) form [35,36]. Most of the sensors show enhanced fluorescence with more effective single analyte detection [37–39]. Nevertheless, a few chemosensors have also been reported for the selective recognition of more than one metal ion at the same time [40]. In this study, we devised and prepared a new rhodamine-based chemosensor **RH-FP** as shown in Scheme 1. **RH-FP** is a dual-analyte rhodamine-based chemosensor that detects Cu^{2+} and Hg^{2+} as spirolactam derivative by UV visible/fluorescence spectroscopy. The dual detection is of importance because few constructs are liable to show detecting capacities to bi-cations when using transformed signaling bands.

2. Materials and methods

2.1. Chemicals and reagents

Rhodamine B, 2-bromo-5-formylpyridine and hydrazine hydrate (78–80%) were procured from Adamas beta chemical Co. Ltd, whereas the metal chlorides, nitrates, and perchlorates were provided by the Sigma-Aldrich, USA and Shanghai reagent company, China. All the other solvents were of analytical grade and used as received. De-ionized water was used throughout the experiment.

2.2. Instrumentation

The emission spectra were measured with a PerkinElmer LS 55 fluorescence spectrophotometer with a 10 mm quartz cuvette. All the absorbance experiments were carried out by using 1×1 cm quartz cuvettes on a Shimadzu UV- visible spectrophotometer (UV-2600). Mettler– Toledo Delta 320 pH meter was used for measuring the pH, whereas ^{1}H and ^{13}C NMR spectra were recorded using a mercury plus 400 spectrometer (Varian, USA) operated at 400 MHz with tetramethylsilane (TMS) as an internal standard. Mass spectra were performed on Q-TOF Premier Mass Spectrometer equipped with an electrospray interface (Waters Corporation, Milford, MA). All the analyses were performed at the Instrumental Analysis Center of Shanghai Jiao Tong University, China.

2.3. Spectroscopic analysis

The stock solution (100 mM) of all the metal ions was prepared from their nitrates, chlorides, and perchlorates in deionized water. The stock solution of **RH-FP** (10 mM) was prepared in acetonitrile/water (8:2 v/v). All the solutions and samples were prepared at room temperature and equilibrated for 5 min before UV–visible and fluorescence measurements. The excitation and emission slit width for fluorescence measurements were adjusted at 7.0 nm. The excitation wavelength used for the measurements was 550 nm.