

# Highly Selective and Sensitive Detection of Hg(II) from HgCl<sub>2</sub> by a Simple Rhodamine-Based Fluorescent Sensor

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**Abstract** N-acryloyl rhodamine B hydrazide, a non-responsive control of a colorimetric Cu<sup>2+</sup> sensor, was used as a turn-on fluorescent sensor for Hg(II) from HgCl<sub>2</sub> in the presence of AgNO<sub>3</sub>. The detection was highly selective and sensitive, and a large number of environmentally and biologically relevant metal ions, such as Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, Fe<sup>3+</sup>, Cu<sup>2+</sup>, Zn<sup>2+</sup>, Cr<sup>3+</sup>, Pb<sup>2+</sup>, Ni<sup>2+</sup>, Fe<sup>2+</sup>, Mn<sup>2+</sup>, Co<sup>2+</sup>, Cd<sup>2+</sup>, including Hg(II) from the easy dissociated salts, did not show significant interference. The fluorescence of the sensor (10 μM) was enhanced 74 folds by 10 equiv. of Hg(II) from HgCl<sub>2</sub> in THF/HAc-NaAc (1/1, v/v, pH=6) aqueous buffer solution containing 20 equiv. of AgNO<sub>3</sub>. The maximal fluorescence intensity increased linearly with the concentration of Hg(II) in the range of 0–70 μM. The detection limit of Hg(II) was 0.59 μM. The sensing mechanism was explored by Job's plot experiment, reversible experiment, mass spectrum analysis, spectroscopic analysis, and thin-layer chromatography.

**Keywords** Rhodamine · Fluorescent · Sensor · Hg(II) · AgNO<sub>3</sub>

## Introduction

Mercury is one of the most toxic heavy metals and its divalent compounds and cations exist widely in all kinds of waste in the environment. Hg(II) in both inorganic and organic compounds can easily penetrate biological membranes and seriously damage the organism, even at a low concentration. [1–3]. HgCl<sub>2</sub> is the most common form of Hg(II) compounds. Therefore the detection of Hg(II) from HgCl<sub>2</sub> is more practical and meaningful. However, HgCl<sub>2</sub> is a covalent compound. Most of HgCl<sub>2</sub> existed in molecular state, only a small amount of HgCl<sub>2</sub> dissociated into HgCl<sup>+</sup> and Cl<sup>-</sup> in aqueous solution. Hg<sup>2+</sup> is also rare in the HgCl<sub>2</sub> aqueous solution [4]. If HgCl<sub>2</sub> can be ionized into Hg(II) ions (HgCl<sup>+</sup> and Hg<sup>2+</sup>), it can be detected by heavy metal ion (HMI) fluorescent sensors.

Fluorescent sensors are very active in the field of HMI detection owing to being highly selective and sensitive, maneuverable, low-cost, and suitable for in-situ and real-time monitoring [5, 6]. Compared to other sensors [7–9], rhodamine-based sensors own the merits of high molar absorption coefficient, long excitation and emission wavelength, high fluorescence quantum yield, easy structure modification and tunable spectral properties [10–13]. A large number of rhodamine-based Hg(II) fluorescent sensors have been reported [14–21], in which the sensors generally bind Hg(II) to N, O, and S sites, and signal Hg(II) by ion-induced rhodamine spirolactam ring-opening mechanism. It is worth noting that most of the Hg(II) sources used in the literatures are environmentally and biologically impossible Hg(ClO<sub>4</sub>)<sub>2</sub>. Now rhodamine-based fluorescent sensors with new complicated structures are very popular [22–24]. However, the research on new sensing performance of rhodamine derivatives with simple structures is also of great significance due to the easy availability of the sensors.

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*N*-acryloyl rhodamine B hydrazide (ARB) was reported as a colorimetric  $\text{Cu}^{2+}$  sensor's control which was non-responsive to metal ions [25]. However, ARB has two features beneficial to being a fluorescent sensor for Hg(II): (1) a rhodamine spirolactam ring; (2) one O atom, two N atoms, and a C = C binding sites. In addition, it can be known from the references and our study [26–29] that the testing conditions, such as the solvent, the sensor's concentration, and the excitation wavelength, have crucial effects on the sensor's properties. The motive of this work is to develop the already known non-responsive rhodamine derivative (ARB) with simple structure into a highly selective and sensitive fluorescent sensor for Hg(II) from the most common Hg(II) salt ( $\text{HgCl}_2$ ) through carefully studying the testing conditions.

## Experimental

### Materials and Instruments

#### Materials

Rhodamine B (99 %) was bought from Shanghai SSS Reagent Co., Ltd. Acryloylchloride ( $\geq 99$  %) was supplied by Suzhou Yacoo Chemical Reagent Co., Ltd.  $\text{NH}_2\text{NH}_2 \cdot \text{H}_2\text{O}$  (85 %), NaCl, KCl,  $\text{MgCl}_2$ ,  $\text{CaCl}_2$ ,  $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$ ,  $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ ,  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ ,  $\text{FeCl}_2 \cdot 7\text{H}_2\text{O}$ ,  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ ,  $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ ,  $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ ,  $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ ,  $\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$ ,  $\text{HgCl}_2$ ,  $\text{Hg}(\text{CH}_3\text{COO})_2$ ,  $\text{Pb}(\text{NO}_3)_2$ ,  $\text{AgNO}_3$ ,  $\text{CH}_3\text{COONa}$  (NaAc), and  $\text{CH}_3\text{COOH}$  (HAc), were provided by Sinopharm Chemical Reagent Co., Ltd. The solvents used in synthesis were of analytical grade, others were spectroscopic grade. All the reagents were used as received.

#### Instruments

Infrared (IR) spectra were recorded on a Nicolet Magan-550 spectrometer (Nicolet Co., USA). LC–MS was carried out on an Agilent 1200/6220 spectrometer (Agilent Co., USA) unless otherwise specified.  $^1\text{H}$  NMR spectra were collected on a 400 MHz Varian Unity Inova spectrometer (Varian Co., USA). UV–vis spectra were obtained on a U-3900 spectrophotometer (Perkin–Elmer Co., USA). Fluorescence spectra were taken on a Fluoromax-4 spectrofluorometer (HORIBA Jobin Yvon Co., France). The source of excitation was Xenon lamp (HORIBA 1905-OFR). A wrist-watch was used in the

study of time response of ARB to Hg(II) from  $\text{HgCl}_2$ . The measurements were performed at 25 °C.

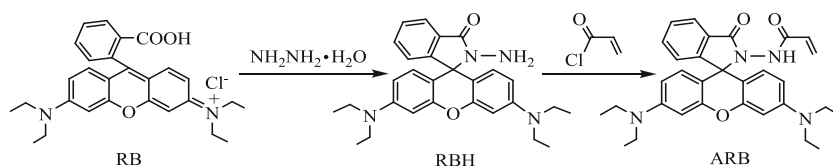
### Synthetic Procedures and Characterization Data

ARB was synthesized from rhodamine B (RB),  $\text{NH}_2\text{NH}_2 \cdot \text{H}_2\text{O}$ , and acryloylchloride, as shown in Scheme 1.

Rhodamine B hydrazide (RBH) was synthesized by a modification of the procedures of the references [30, 31], 3 mL (61.85 mmol) of  $\text{NH}_2\text{NH}_2 \cdot \text{H}_2\text{O}$  was added dropwise to the ethanol solution (15 mL) of RB (1.0005 g, 2.09 mmol) in a three-necked flask equipped with a magnetic stirrer. The mixture was heated to reflux for 6 h. After cooling to room temperature, the solvent was evaporated under reduced pressure. The residue was washed with water and dried in vacuum to afford rhodamine B hydrazide (RBH) 0.8218 g (85.6 %) as a yellow solid. In this step, the measures of RB recrystallized from 1,4-dioxane and ethanol instead of methanol as solvent to complete the reaction simplified the reaction system and resulted in the omission of the complicated purification process described in the references.

ARB was synthesized from rhodamine B hydrazide (RBH) and acryloylchloride with a modified literature method [25]. 0.2 mL (2.46 mmol) of acryloylchloride was added dropwise to the dichloromethane solution (14 mL) of RBH (0.2291 g, 0.48 mmol) at 0 °C under  $\text{N}_2$  atmosphere in a three-necked flask equipped with a magnetic stirrer. The mixture was stirred for 2 h. The reaction mixture was poured into deionized water (20 mL) and fully shaken, then kept stationary for a period of time. The underlying organic layer was collected and concentrated, and the residue was purified by silica-gel column chromatography with  $\text{CH}_3\text{OH}/\text{CHCl}_3$  (1/80, v/v) as an eluent to afford an incarnadine solid (ARB) (0.0697 g, 27.2 %). In this step,  $\text{CH}_3\text{OH}$  was replaced by the mixture of  $\text{CH}_3\text{OH}/\text{CHCl}_3$  (1/80, v/v) as the eluent in order to remove a small amount of impurity from acryloylchloride. The structure of ARB was characterized by  $^1\text{H}$  NMR, IR and LC–MS analysis.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ),  $\delta$  (ppm): 7.98 (d,  $J=7.5$  Hz, 1H, ArH), 7.63–7.53 (m, 1H, ArH), 7.53–7.42 (m, 1H, ArH), 7.12 (d,  $J=7.5$  Hz, 1H, ArH), 6.86 (s, 1H, NH), 6.67 (d,  $J=7.5$  Hz, 2H, ArH), 6.36 (d,  $J=7.5$  Hz, 2H, ArH), 6.29 (s, 2H, ArH), 5.85–6.05 (m, 1H, HC=), 5.61 (d,  $J=2.1$  Hz, 1H, =CH<sub>2</sub>), 5.36 (d,  $J=2.1$  Hz, 1H, =CH<sub>2</sub>), 3.33 (q,  $J=6.8$  Hz, 8H, CH<sub>2</sub>), 1.15 (t,  $J=6.8$  Hz, 12H,

**Scheme 1** Synthetic route of ARB



CH<sub>3</sub>). IR (KBr pellet, cm<sup>-1</sup>): 3447 (NH), 2927, 2968 (CH<sub>3</sub>, CH<sub>2</sub>), 1729 (C=O), 1685 and 1515 (Ar-H), 1615 (C=C or C=N), 1119 (C-O-C). LC-MS [M+H]<sup>+</sup>: *m/z*, 511.20, Calcd. 511.26; [M+2H]<sup>2+</sup>: *m/z*, 256.20, Calcd. 256.13.

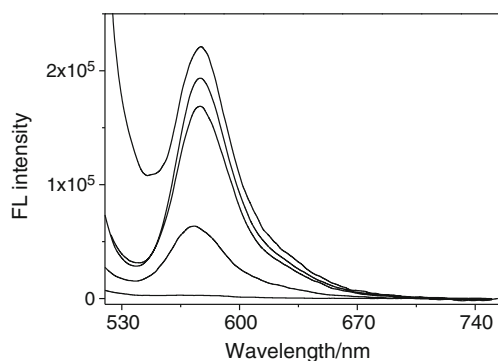
## Methods

ARB was dissolved in tetrahydrofuran (THF) to form 5 mM stock solutions. Metal salts were dissolved in de-ionized water to get 10 mM stock solutions. When studying the fluorescence sensing behaviors of the sensor to metal ions, 20  $\mu$ L stock solution of the sensor was put into 10 mL volumetric flasks, mixed with 100  $\mu$ L one of the stock solutions of the metal ions and 200  $\mu$ M AgNO<sub>3</sub>, then diluted to volume with THF/HAc-NaAc (1/1, v/v, pH=6) aqueous buffer solution. The concentration of the sensor and metal ions was 10  $\mu$ M and 100  $\mu$ M, respectively. In the fluorescence titration, the mixed stock solutions of Hg(II) varied from 0 to 130  $\mu$ L and the concentration of Hg(II) was 0–130  $\mu$ M. The used excitation and emission wavelengths of measurement were 500 nm and 574 nm, and the slit width was 5 nm. The measurement was performed after 30 min.

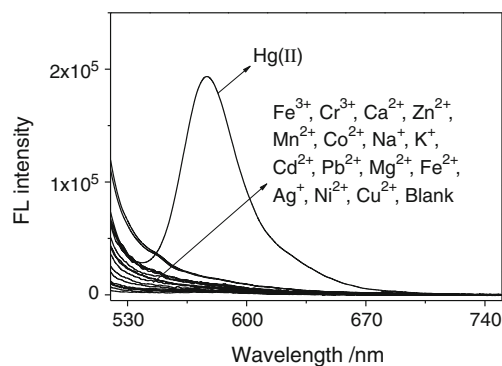
## Results and Discussion

### Determination of the Testing Conditions

The rhodamine derivatives are usually pH sensitive, which interfere with the detection of the analytes. Based on the literature [25] as well as to get rid of the interferences of pH, THF/HAc-NaAc (1/1, v/v, pH=6) aqueous buffer solution was selected from several solvents tried by us as the medium for the study of ARB as a fluorescent sensor for metal ions.



**Fig. 1** Effects of the AgNO<sub>3</sub> concentration on the fluorescence spectra of ARB/Hg(II). Solvent: THF/HAc-NaAc (1/1, v/v, pH=6), c: 10  $\mu$ M for ARB, 100  $\mu$ M for Hg(II). From bottom to top: 0, 50, 100, 200, 300  $\mu$ M for AgNO<sub>3</sub>.  $\lambda_{\text{ex}}$ : 500 nm, slit width: 5 nm

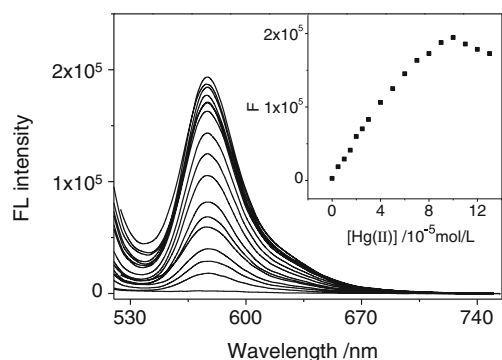


**Fig. 2** Fluorescence spectra of ARB in the absence and presence of various metal ions. Solvent: THF/HAc-NaAc (1/1, v/v, pH=6), c: 10  $\mu$ M for ARB, 100  $\mu$ M for metal ions, and 200  $\mu$ M for AgNO<sub>3</sub>.  $\lambda_{\text{ex}}$ : 500 nm, slit width: 5 nm

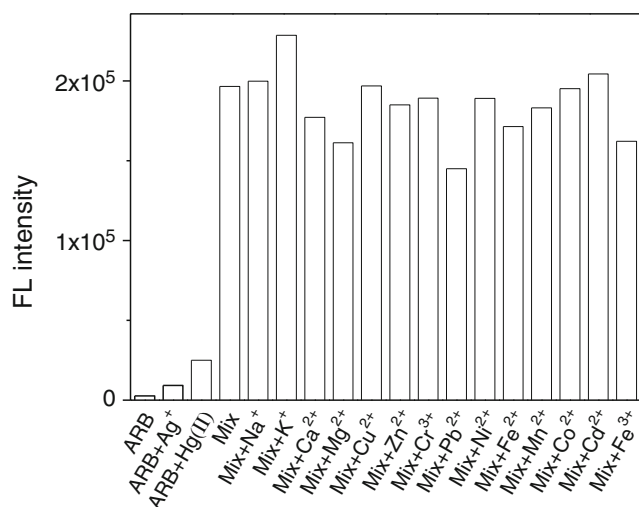
ARB (10  $\mu$ M) in THF/HAc-NaAc (1/1, v/v, pH=6) buffer solution was almost non-emissive. Upon addition of various metal ions (100  $\mu$ M) individually, the fluorescence of the solutions showed no apparent changes. HgCl<sub>2</sub> could only cause a slight fluorescence enhancement. The results indicated that ARB was almost not responsive to HgCl<sub>2</sub> and other metal ions under the present conditions.

AgNO<sub>3</sub> was reported to avail the dissociation of HgCl<sub>2</sub> [32] by producing poorly water soluble precipitate (AgCl) and may help the detection of Hg(II) from HgCl<sub>2</sub> by fluorescent sensor. Hence, 50  $\mu$ M of AgNO<sub>3</sub> stock solution was introduced to the THF/HAc-NaAc (1/1, v/v, pH=6) buffer solution of ARB (10  $\mu$ M) and HgCl<sub>2</sub> (100  $\mu$ M). As expected, the mixed solution emitted strong fluorescence with an 11-fold enhancement at 574 nm (Fig. 1). The result showed that AgNO<sub>3</sub> can help ARB respond to Hg(II) from HgCl<sub>2</sub>.

The optimal concentration of AgNO<sub>3</sub> was further sought by variation of AgNO<sub>3</sub> amount from 0 to 300  $\mu$ M. The result was shown in Fig. 1. It can be seen that the fluorescence of the



**Fig. 3** Fluorescent spectra of ARB with various concentration of Hg(II). Solvent: THF/HAc-NaAc (1/1, v/v, pH=6), c: 10  $\mu$ M for ARB, 200  $\mu$ M for AgNO<sub>3</sub>, from bottom to top, the concentration of Hg(II): 0, 5, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, and 130  $\mu$ M.  $\lambda_{\text{ex}}$ : 500 nm, slit width: 5 nm. Inset: The relationship between the maximal fluorescence intensity and the concentration of Hg<sup>2+</sup>

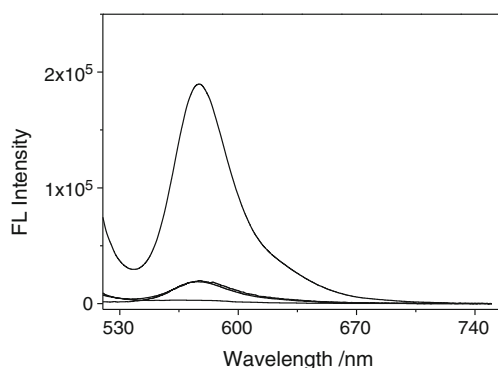


**Fig. 4** Effects of coexisting ions on the fluorescence maxima of ARB/Hg(II). Solvent: THF/HAc-NaAc (1/1, v/v, pH=6); c: 10  $\mu$ M for ARB, 200  $\mu$ M for AgNO<sub>3</sub>, 100  $\mu$ M for Hg(II) and other ions. Mix=ARB+Ag<sup>+</sup>+Hg(II),  $\lambda_{\text{ex}}$ : 500 nm, slit width: 5 nm

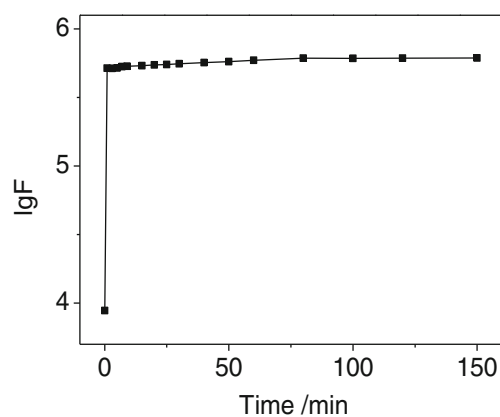
THF/HAc-NaAc (1/1, v/v, pH=6) solution of ARB/Hg(II) strengthened along with the increase of the AgNO<sub>3</sub> concentration. But when the concentration of AgNO<sub>3</sub> was more than 100  $\mu$ M, the fluorescence increment became small. Therefore, 200  $\mu$ M of AgNO<sub>3</sub> was adopted in the next study.

#### Selectivity of ARB to Hg(II) from HgCl<sub>2</sub>

To investigate the selectivity of ARB to Hg(II) from HgCl<sub>2</sub> in THF/HAc-NaAc (1/1, v/v, pH=6) containing 200  $\mu$ M of AgNO<sub>3</sub>, various of metal ions, Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, Fe<sup>3+</sup>, Cu<sup>2+</sup>, Zn<sup>2+</sup>, Cr<sup>3+</sup>, Pb<sup>2+</sup>, Ni<sup>2+</sup>, Fe<sup>2+</sup>, Mn<sup>2+</sup>, Co<sup>2+</sup>, Cd<sup>2+</sup>, Ag<sup>+</sup>, and HgCl<sub>2</sub> (10 equiv.), were added to the ARB (10  $\mu$ M) solution, and the fluorescent spectra were recorded (Fig. 2). It could be seen clearly that only Hg(II) from HgCl<sub>2</sub> enhanced the fluorescent intensity of ARB dramatically over other metal



**Fig. 5** Fluorescence spectra of ARB under different conditions. Solvent: THF/HAc-NaAc (1/1, v/v, pH=6). c: 10  $\mu$ M for ARB, 100  $\mu$ M for HgCl<sub>2</sub> and Hg(CH<sub>2</sub>COOH)<sub>2</sub>, and 200  $\mu$ M for AgNO<sub>3</sub>. From bottom to top: ARB/HgCl<sub>2</sub>, ARB/Hg(CH<sub>3</sub>COO)<sub>2</sub>, ARB/Hg(CH<sub>3</sub>COO)<sub>2</sub>/AgNO<sub>3</sub>, and ARB/HgCl<sub>2</sub>/AgNO<sub>3</sub>.  $\lambda_{\text{ex}}$ : 500 nm, slit width: 5 nm

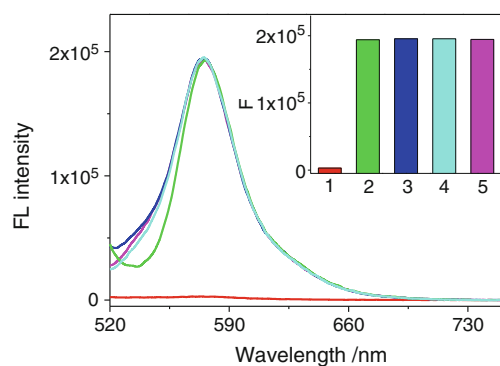


**Fig. 6** Time response of ARB to Hg(II). Solvent: THF/HAc-NaAc (1/1, v/v, pH=6); c: 10  $\mu$ M for ARB, 100  $\mu$ M Hg(II), and 200  $\mu$ M for AgNO<sub>3</sub>.  $\lambda_{\text{ex}}$ : 500 nm, slit width: 5 nm

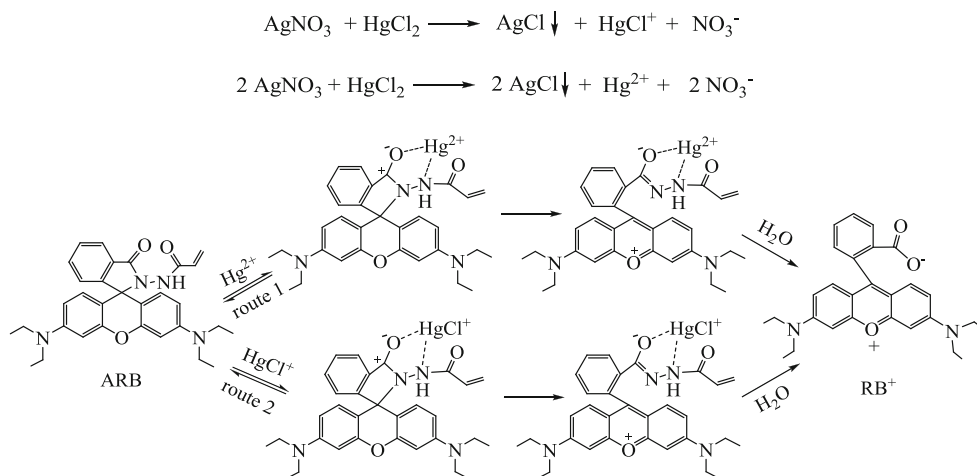
ions including Ag<sup>+</sup>. The results implied that ARB may be a potential “turn-on” selective fluorescent sensor for Hg(II) from HgCl<sub>2</sub> in THF/HAc-NaAc (1/1, v/v, pH=6) in the presence of AgNO<sub>3</sub>.

#### Sensitivity of ARB to Hg(II) from HgCl<sub>2</sub>

Furthermore, the relationship between the fluorescence intensity of the ARB/Hg(II) solution and the concentration of Hg(II) was studied (Fig. 3). The results showed that the fluorescence intensity of the ARB/Hg(II) solution first increased then decreased gradually with the raise of Hg(II) from 0 to 130  $\mu$ M, and got a 74-fold fluorescence enhancement with 100  $\mu$ M of Hg(II). The reason of the gradual decrease of fluorescence after the concentration of HgCl<sub>2</sub> over 100  $\mu$ M may be the heavy atom effect from the excessive unbounded Hg(II) or HgCl<sub>2</sub>. The maximal



**Fig. 7** Fluorescent spectra of ARB, ARB/Hg(II), and ARB/Hg(II)/EDTA in THF/HAc-NaAc (1/1, v/v, pH=6) with 200  $\mu$ M of AgNO<sub>3</sub>. c: 10  $\mu$ M for ARB, 100  $\mu$ M for Hg(II). 1: ARB, 2: ARB/Hg(II), 3: ARB/Hg(II)/EDTA (100  $\mu$ M), 4: ARB/Hg(II)/EDTA (200  $\mu$ M), and 5: ARB/Hg(II)/EDTA (300  $\mu$ M).  $\lambda_{\text{ex}}$ : 500 nm, slit width: 5 nm. Inset: the maximal fluorescence intensity (F) of the five spectral lines. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article)

**Scheme 2** Proposed mechanism of ARB for Hg(II)

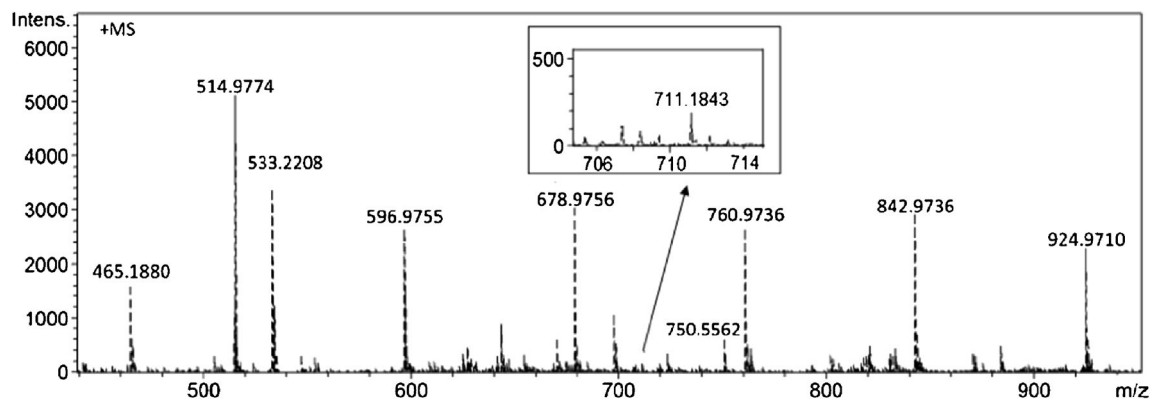
fluorescence intensity ( $F$ ) and the concentration of Hg(II) ( $[\text{Hg(II)}]$ ) showed good linear relationship in the range of 0–70  $\mu\text{M}$   $[\text{Hg(II)}]$ . The computationally linear fitting equation was  $F = 8752.02586 + 23021.77586 [\text{Hg(II)}]$  with a correlation coefficient of 0.99242 [the concentration of Hg(II) for the linear fitting was 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, and  $7 \times 10^{-5}$  M ( $n=11$ )]. The results showed that ARB could be used to quantitatively detect Hg(II) from HgCl<sub>2</sub> with high sensitivity. The detection limit evaluated by  $3\sigma/k$  based on the fluorescence titration was  $5.9 \times 10^{-7}$  M (0.59  $\mu\text{M}$ ), where  $\sigma$  is the standard deviation of the fluorescence intensity from five times measurement of the ARB solution without Hg(II) and  $k$  is the slope in the linear fitting equation ( $23021.77586 \times 10^5 \text{ M}^{-1}$ ).

#### Anti-Interference of ARB for Detecting Hg(II) from HgCl<sub>2</sub>

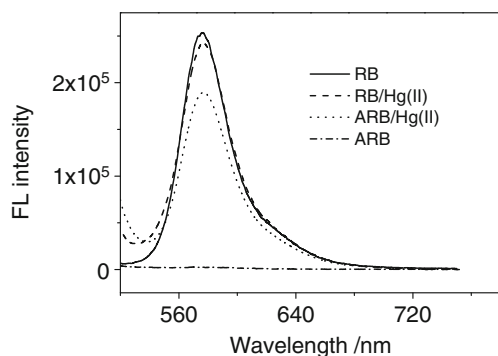
To examine the effects of coexistent metal ions on the detection of Hg(II) from HgCl<sub>2</sub>, Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, Fe<sup>3+</sup>, Cu<sup>2+</sup>, Zn<sup>2+</sup>, Cr<sup>3+</sup>, Pb<sup>2+</sup>, Ni<sup>2+</sup>, Fe<sup>2+</sup>, Mn<sup>2+</sup>, Co<sup>2+</sup>, and Cd<sup>2+</sup> (100  $\mu\text{M}$ ) were added to the THF/HAc-NaAc (1/1, v/v, pH=6) solution of ARB (10  $\mu\text{M}$ )/Hg(II)

(100  $\mu\text{M}$ ) including 200  $\mu\text{M}$  of AgNO<sub>3</sub> and fluorescence spectra were tested. As shown in Fig. 4, the competitive ions showed no pronounced influence on the fluorescence intensity of ARB/Hg(II).

To investigate the effects of Hg(II) from the easy dissociated salts on the detection of Hg(II) from HgCl<sub>2</sub>, the recognition experiments were repeated at the same conditions except HgCl<sub>2</sub> was replaced by another environmentally and biologically possible Hg(II) salt, Hg(CH<sub>3</sub>COO)<sub>2</sub>. As shown in Fig. 5, Hg(CH<sub>3</sub>COO)<sub>2</sub> could enhanced the fluorescence of ARB in THF/HAc-NaAc (1/1, v/v, pH=6) aqueous buffer solution a little because of its stronger dissociated ability (compared to that of HgCl<sub>2</sub>) [32]. However, when 200  $\mu\text{M}$  of AgNO<sub>3</sub> was added, the fluorescence of the ARB/Hg(CH<sub>3</sub>COO)<sub>2</sub> solution did not changed obviously. The reason may be interpreted that CH<sub>3</sub>COOAg is water soluble so that AgNO<sub>3</sub> has no contribution to the dissociation of Hg(CH<sub>3</sub>COO)<sub>2</sub>. This result indicates that the Hg(II) from the easy dissociated salts has very little effects on the detection of Hg(II) from HgCl<sub>2</sub>. Hence, ARB is an effective turn-on fluorescent sensor for Hg(II) from HgCl<sub>2</sub>.



**Fig. 8** LC-MS spectrum of ARB (10  $\mu\text{M}$ )/Hg(II) (100  $\mu\text{M}$ ) in THF/HAc-NaAc (1/1, v/v, pH=6) with AgNO<sub>3</sub> (200  $\mu\text{M}$ ) recorded on a Bruker micro TOF-QIII LC/MS spectrometer (Bruker Daltonics Co., German)



**Fig. 9** Fluorescence spectra of ARB and RB in the absence and presence of Hg(II) in THF/HAc-NaAc (1/1, v/v, pH=6) with 200  $\mu\text{M}$  of  $\text{AgNO}_3$ . From top to bottom: RB, RB/Hg(II), ARB/Hg(II), and ARB. c: 10  $\mu\text{M}$  for ARB, 0.25  $\mu\text{M}$  for RB, 10 equiv. for Hg(II),  $\lambda_{\text{exc}}$ : 500 nm, slit width: 5 nm

### Time Response of ARB to Hg(II) from $\text{HgCl}_2$

Time response of ARB (10  $\mu\text{M}$ ) to Hg(II) from  $\text{HgCl}_2$  (100  $\mu\text{M}$ ) in THF/HAc-NaAc (1/1, v/v, pH=6) with  $\text{AgNO}_3$  (200  $\mu\text{M}$ ) was studied and the result was exhibited in Fig. 6. Upon addition of  $\text{HgCl}_2$ , the fluorescence intensity of the ARB solution almost achieved the maximum within 1 min, and generally kept stable during the testing time. The result suggests that ARB is a quickly responsive “turn-on” fluorescent sensor for Hg(II) from  $\text{HgCl}_2$ .

### Sensing Mechanism of ARB for Hg(II) from $\text{HgCl}_2$

In order to explore the mechanism of ARB for Hg(II) from  $\text{HgCl}_2$ , Job’s plot analysis was carried out firstly. But there was no satisfactory result obtained. This situation may be led by the complicated chemical recognition process with the participation of  $\text{AgNO}_3$ . Next, reversible experiment was conducted. Remarkable fluorescence enhancement was observed on addition of  $\text{HgCl}_2$  (100  $\mu\text{M}$ ) to the ARB (10  $\mu\text{M}$ ) THF/HAc-NaAc (1/1, v/v, pH=6) buffer solution with  $\text{AgNO}_3$  (200  $\mu\text{M}$ ), while there was almost no change in fluorescence when over capacity of EDTA was added then (Fig. 7). So irreversible chemical reactions between ARB and Hg(II) may happen.

Based on the well-known ring-opening principle of rhodamine spirolactam and the widely accepted hydrolysis mechanism [27, 33–35], as well our experimental results, we speculated the possible mechanism for ARB identifying Hg(II) from  $\text{HgCl}_2$  as Scheme 2.

To confirm the assumptive mechanism, LC-MS analysis was done and the results were displayed in Fig. 8. The peaks at  $m/z$  465.1800, 514.9774, and 533.2208 were corresponding to  $[\text{RB}+\text{Na}^+]$ ,  $[\text{RB}^++\text{THF}]$ , and  $[\text{ARB}+\text{Na}^+]$  respectively. The peaks at  $m/z$  711.1843 and 750.5562 were assigned to  $[\text{ARB}+\text{Hg}^{2+}]$  and  $[\text{ARB}+\text{HgCl}^++2\text{H}^+]$ . The peaks at  $m/z$  596.9755, 678.9756, 760.9736, 842.9736, and 924.9710 were

from  $[\text{RB}^++\text{THF}+\text{NaAc}]$ ,  $[\text{RB}^++\text{THF}+2\text{NaAc}]$ ,  $[\text{RB}^++\text{THF}+3\text{NaAc}]$ ,  $[\text{RB}^++\text{THF}+4\text{NaAc}]$ , and  $[\text{RB}^++\text{THF}+5\text{NaAc}]$  respectively. These data well supported the proposed mechanism. Since the peak of  $[\text{ARB}+\text{Hg}^{2+}]$  is greatly weaker than that of  $[\text{ARB}+\text{HgCl}^++2\text{H}^+]$ , route two in Scheme 2 may be dominant, which is in accordance with the fact that ARB is only a very little responsive to  $\text{Hg}(\text{CH}_3\text{COO})_2$ .

The fluorescent profile of the ARB/Hg(II) solution was very similar to that of the RB/Hg(II) solution (Fig. 9) also supported the deduction of hydrolysis mechanism.

Furthermore, TLC analysis of the THF/HAc-NaAc (1/1, v/v, pH=6) buffer solution of RB (10  $\mu\text{M}$ ), RB (10  $\mu\text{M}$ )/Hg(II) (100  $\mu\text{M}$ ), ARB (10  $\mu\text{M}$ ), and ARB (10  $\mu\text{M}$ )/Hg(II) (100  $\mu\text{M}$ ) in the presence of 200  $\mu\text{M}$   $\text{AgNO}_3$  was done with methanol and chloroform (1/20, v/v) as the eluent. The results for the four solutions were one main point ( $R_f=0.27$ ), one main point ( $R_f=0.27$ ), one point ( $R_f=0.67$ ), and two points ( $R_f=0.27$  and 0.67), respectively. It can be clearly seen that RB was produced in the ARB (10  $\mu\text{M}$ )/Hg(II) (100  $\mu\text{M}$ ) solution, which supported the hypothetical mechanism again.

### Conclusions

We developed non-responsive N-acryloyl rhodamine B hydrazide (ARB) into a highly selective and sensitive turn-on fluorescent sensor for Hg(II) from  $\text{HgCl}_2$  by using  $\text{AgNO}_3$  as a requisite assistant. THF/HAc-NaAc (1/1, v/v, pH=6) aqueous buffer solution is a suitable medium which can screen the effect of pH. ARB responded to Hg(II) from  $\text{HgCl}_2$  rapidly with a working concentration of 0–70  $\mu\text{M}$  [Hg(II)] and a detection limit of 0.59  $\mu\text{M}$ . The sensing mechanism may be that  $\text{AgNO}_3$  helps  $\text{HgCl}_2$  to produce  $\text{HgCl}^+$  and  $\text{Hg}^{2+}$ , the generated cations coordinate with ARB and induce the ring-opening and hydrolysis reaction of the rhodamine spirolactam.

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