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Author: Mecit Ozdemir



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A rhodamine-based colorimetric and fluorescent probe for dual sensing of  $Cu^{2+}$  and  $Hg^{2+}$  ions

Mecit Ozdemir<sup>a\*</sup>

Department of Food Processing, Vocational High School, Kilis 7 Aralik University, Kilis, TURKEY

\*Corresponding author. Tel.: +90 348 0 348 814 26 66; fax: +90 348 814 26 69. E-mail address: <u>mecitozdemir@kilis.edu.tr</u>

## **Graphical Abstract**



A highly selective and sensitive 'off-on' fluorescent sensor RhAsP based on rhodamine derivative for the detection of Hg<sup>2+</sup> and Cu<sup>2+</sup> in aqueous medium

## Highlights

- Synthesis and characterization of '**RhAsP**'.
- The spectroscopic studies were carried out for investigating the selectivity of '**RhAsP**' toward Hg<sup>2+</sup>.
- Addition of mercury ion into ethanol-water solution of sensor caused a strong fluorescent emission at 586 nm.
- The limit of detection was found to be 150 nM for  $Hg^{2+}$ .

#### ABSTRACT

A fluorescent sensor '**RhAsP**' prepared from rhodamine hydrazine and 2-hydroxy-acetophenone was designed and synthesised. The characterisation of '**RhAsP**' was also accomplished by using FT-IR, <sup>1</sup>H NMR, <sup>13</sup>C NMR, ESI-MS, elemental analysis, fluorescence and UV/Vis spectroscopy. For the purpose of investigating optical sensing behaviour of **RhAsP**' toward various metal ions including alkali, alkaline earth and transition metal ions (Na<sup>+</sup>, K<sup>+</sup>, Ag<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, Cr<sup>3+</sup>, Fe<sup>3+</sup>, Hg<sup>2+</sup>, Cu<sup>2+</sup>, Pb<sup>2+</sup>, Zn<sup>2+</sup>, Fe<sup>2+</sup>, Co<sup>2+</sup>, Ni<sup>2+</sup>, Cd<sup>2+</sup>, Al<sup>3+</sup> and Mn<sup>2+</sup>) in aqueous medium under physiological pH conditions, fluorescence and absorption spectroscopic techniques were carefully conducted. Thereby, '**RhAsP**' showed both colorimetric and fluorometric selective recognition of Hg<sup>2+</sup> from colorless to purple with a reversible '*off-on*' fluorescence response. In addition, a nearly 100-fold increase in emission intensity was determined upon the addition of two equivalents Hg<sup>2+</sup> ions.

**Keywords**: chemosensor, selective, rhodamine, fluorometric,  $Hg^{2+}$  and  $Cu^{2+}$ .

#### 1. Introduction

In the last few decades, heavy transition metal ions (HTMs) have been an important environmental issue in the global society due to the industrial and agricultural activities that contaminate aquatic ecosystems with various toxic ion species [1]. Copper and Mercury are two important transition metal ions. Beside  $Fe^{2+}$  and  $Zn^{2+}$ , the copper ion—an essential trace element in the human body—is the third most abundant soft transition metal ion and plays crucial roles in various biological processes, including metalloenzymes, superoxide dismutase, cytochrome c oxidase and tyrosinase. Once the exceeding level of  $Cu^{2+}$  ions for the cellular needs, this leads to neurodegenerative disorders, including Alzheimer's, Parkinson's disease, Wilson's disease, prion disease, amyloidal precipitation and toxicity [2–4]. However, unlike this situation, the deficiency of copper in the human body can increase the risk of coronary heart diseases [4].

On the other hand, one of the most poisonous and hazardous contaminations is Mercury that is released through natural events or human activities. A very little amount of  $Hg^{2+}$  can affect the human health with a various aspects. Therefore, a number of severe harmful effects on human health have been related to mercury-induced toxicity, which leads to many health problems in the brain, digestive system, kidneys and central nervous system. Thus, for all these reasons, the synthesis and development of the highly selective and sensitive chemosensors for  $Cu^{2+}$  and  $Hg^{2+}$  ions are still desired [5–8].

There are various conventional detection techniques such as atomic absorption spectrometry (AAS), atomic fluorescence spectrometry (AFS), inductively coupled plasma-mass spectrometry (ICP-MS), electrochemical sensing, and high performance liquid chromatography (HPLC). Among the well-known and widely used many conventional methods, owing to their low cost, applicability, simplicity, low detection limit and useful application in environmental, biological, and industrial processes, the most promising unconventional techniques based on optical detection methods via colorimetric or fluorescence changes have presently attracted the attention of many researchers worldwide [9-11]. Therefore, new approaches in the design and development of synthetic fluorescent receptors that are capable of recognising biologically and environmentally important ion species, especially HTMs in aqueous media, have received a widespread interest by chemists, biologists, clinical biochemists and environmentalists recently.

[12–19]. As is well known, rhodamine-based dyes are useful for selective detection of metal ions leading to strongly fluorescent colorful open ring form [20]. With all these aspects, since their characteristics such as high quantum yields, good photostability, simplicity, low detection limit, capability for special recognition and excellent spectroscopic properties, rhodamine derivatives have commonly been employed as fluorescent sensors for detection of metal cations in water or organic solvents and labelling them in living cells without causing cell damage [21].

In this work, we developed and described a fluorescent sensor '**RhAsP**' based on a rhodamine framework. To the best of our knowledge, rhodamine derivatives with the spirolactam form are colorless and non-fluorescent, whereas the ring-opened amide form induced by metal ions causes an appearance of a purple color and strong fluorescence intensity at a comparatively long wavelength [22-25]. Likewise, the spirocyclic structure of '**RhAsP**' was non-fluorescent and colorless, while the ring-open form of this chemosensor induced by  $Hg^{2+}$  ions was purple and fluorescent. From this point of view, we speculated that '**RhAsP**' may be used as a rapid and highly selective "*off-on*" fluorescent probe for  $Hg^{2+}$  detection with significant changes in color and fluorescence.

#### 2. Experimental

#### 2.1. Materials and methods

All of the chemicals and solvents used in this study were the best commercial reagents and they were used without further purification. Rhodamine hydrazine was synthesised according to the methods outlined in the previous reports [26–30]. Ethanol (EtOH), 2-hydroxy-acetophenone, methanol, dichloromethane (DCM), acetic acid (AcOH) and metal salts (nitrate or chloride) were purchased from Sigma-Aldrich and Merck. The characterisation of the synthesised fluorescent sensor '**RhAsP**' was based on the analysis of FT-IR, <sup>1</sup>H NMR, <sup>13</sup>C NMR, ESI-MS, elemental analysis, fluorescence spectra and UV/Vis spectra. NMR spectra were recorded on a Bruker AVANCE III HD 400 using CDCl<sub>3</sub> as the solvent and tetramethylsilane (TMS) as an internal standard. LC-MS/MS analyses were performed on a Varian 325-MS. The absorption spectra were recorded on a Varian cary 100 bio spectrophotometer at 298 K. Fluorescence spectra were recorded on a Varian cary eclipse fluorescence spectrophotometer at 298 K. The pH

measurements were carried out on a Corning pH meter equipped with a Sigma-Aldrich micro combination electrode calibrated with a standard buffer solution.

To prepare the solutions of metal ions, the nitrate or chloride salts such as Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, Cr<sup>3+</sup>, Fe<sup>3+</sup>, Hg<sup>2+</sup>, Cu<sup>2+</sup>, Pb<sup>2+</sup>, Zn<sup>2+</sup>, Fe<sup>2+</sup>, Co<sup>2+</sup>, Ni<sup>2+</sup>, Cd<sup>2+</sup>, Al<sup>3+</sup>, Mn<sup>2+</sup> and Ag<sup>+</sup> were dissolved in double-distilled water to afford 1 mM aqueous solutions. A 1 mM stock solution of **'RhAsP'** was prepared in ethanol. Once the stock solution of **'RhAsP'** was stored for more than several months under the daylight condition, no change was observed. These solutions were further diluted to the micro-molar ( $\mu$ M) level for fluorescence and absorption spectrometry measurements with an EtOH-water (2:1, v:v) buffer (10 mM, HEPES, pH 7.2).

#### 2.2. Synthesis of the fluorescent sensor 'RhAsP'

The synthetic pathway of '**RhAsP**' was shown in **Scheme 1**. Rhodamine B hydrazide was firstly synthesised according to the literature methods by a one-step reaction of rhodamine B with hydrazine hydrate (80%) in ethanol [31-32]. The fluorescent sensor '**RhAsP**' was prepared by following procedure [33]. Briefly, 2-hydroxy-acetophenone (272 mg, 2 mmol) in 20 mL ethanol with a few drops of acetic acid was slowly added to the stirred ethanolic solution of rhodamine hydrazide (912 mg, 2 mmol). The mixture was stirred and heated to reflux overnight. The crude product evaporated under the reduced pressure, and the mixture was purified by silica gel column chromatography using CH<sub>2</sub>Cl<sub>2</sub>/CH<sub>3</sub>OH (20:1, v/v) as an eluent to obtain '**RhAsP**'. The yield was 823 mg (72 %).

Elemental Analysis (theoretical/actual): % C (75.24/74.12), % H (6.66/6.26), % N (12.51/11.88). FT-IR (v/cm<sup>-1</sup>): 2970 (Ar-H); 2901-2932 (-C-H); 1699 (C=O, amide); 1614 (C=N, azomethine); 1596 (C=N, pyridine); 1548-1413 (C=C); 1306, 1218, 1117 (C-O); 785, 752) (Supplementary Fig. S1). <sup>1</sup>H NMR (CDCl<sub>3</sub>-*d*, 400 MHz)  $\delta$ (ppm): 11.79 (s, 1H), 7.95 (d, 1H), 7.48-7.52 (m, 3H), 6.80-7.20 (m, 4H), 6.29-6.33 (s, 2H), 6.41-6.59 (d, 4H), 3.32 (q, 8H), 2.33 (s, 3H), 1.16 (t, 12H) (Supplementary Fig. S2).<sup>13</sup>C NMR (CDCl<sub>3</sub>-*d*, 400 MHz)  $\delta$ (ppm): 173 (amide C=O), 161.8 (azomethine C=N), 160.3, 153, 151, 149, 133, 132, 130, 129, 128.5, 128.3, 124, 123, 119, 118, 108, 105, 98, 77 (solvent), 44, 18, 12 (Supplementary Fig. S3). LC-MS/MS: The tandem mass spectrum of the '**RhAsP'** was represented in Supplementary Fig. S4. Fragment at m/z = 575 (base peak) was attributed to molecular ion peak [C<sub>36</sub>H<sub>38</sub>N<sub>4</sub>O<sub>3</sub> + H]<sup>+</sup>. The other one at m/z = 597 that is appeared in the mass spectra was related to [C<sub>36</sub>H<sub>38</sub>N<sub>4</sub>O<sub>3</sub> + Na]<sup>+</sup>. In addition to those

peaks, there are also other fragmentation products of molecular ion such as 497, 326, 179, and 101.

# 2.3. Determination of complexation ratio (S:M), binding constant (K<sub>a</sub>) and limits of detection (LODs)

The stoichiometry of '**RhAsP**' with  $Hg^{2+}$  and  $Cu^{2+}$  was determined by employing Job's plot and mole ratio plot methods. The absorption enhancement observation at 560 nm was plotted against the molar value of the  $[Hg^{2+}]/([Hg^{2+}] +$ **RhAsP**) (Job's plot) and  $[Hg^{2+}]/$ **RhAsP** (mole ratio plot) and also  $[Cu^{2+}]/([Cu^{2+}] +$ **RhAsP**) (Job's plot) and  $[Cu^{2+}]/$ **RhAsP** (mole ratio plot). The binding constants of the fluorescent sensor '**RhAsP**' were determined by the Benesi-Hildebrand equation by using the UV-vis and fluorescence titration data (Figs. 2 and 3). For the practical applicability of '**RhAsP**', limits of detection (LODs) of this chemical probe to  $Hg^{2+}$  and  $Cu^{2+}$  ions were calculated using the equation given by Zheng et al. (Figs. 4 and 5) [34].

#### 3. Result and discussion

The chemosensor '**RhAsP**' was synthesised according to the literature procedures in ethanol with a good yield as given in Scheme 1 [26, 33]. Then, this fluorescent sensor was successfully characterised by elemental, FT-IR, <sup>1</sup>H NMR, <sup>13</sup>C NMR and LC-MS/MS spectroscopic methods. Therefore, all spectroscopic studies confirmed the structure of 'RhAsP', which is a colorless powder that is highly stable in ethanol. Furthermore, the geometry of 'RhAsP' was optimised by the density functional theory (DFT) method with the 6-31G (d, p) basis set in the ground state. Molecular electrostatic potential (MEP) and frontier molecular orbitals (FMOs) of fluorescent molecule synthesised in this work were investigated by using the theoretical calculations as shown in Figs. S5 and S6 [16, 35]. To better understand the sensing behaviour of 'RhAsP' toward mercury and copper ions in an EtOH-water (2:1, v:v) buffer (10 mM, HEPES, pH 7.2), fluorescence and UV/vis spectroscopy experiments were carried out according to the literature reports [36-38]. The stock solution of 'RhAsP' and metal ions were prepared at a concentration of 1 mM and diluted to the micro-molar (µM) level [39-41]. An EtOH-water (2:1, v:v) buffer solution (10 mM, HEPES, pH 7.2) of 'RhAsP' was used as a testing system to conduct the UVvis and fluorescence spectral studies. As shown in Fig. 1, upon the addition of Hg<sup>2+</sup> and Cu<sup>2+</sup> ions that are HTMs to the fluorescent sensor in HEPES buffered solution, apparent changes in

color from colorless to purple could be observed, permitting by direct observation with "nakedeye" detection. However, the addition of other competing metal ions did not lead to changes in the solution color of "**RhAsP**" in an EtOH-water (2:1, v:v) buffer (10 mM, HEPES, pH 7.2) during the experimental studies.

#### 3.1. Possible sensing mechanism and Effect of pH

To further investigation of the sensing performance of chemosensor, fluorometric and colorimetric titration of Hg<sup>2+</sup> and Cu<sup>2+</sup> ions were conducted using a solution of 'RhAsP' in an EtOH-water (2:1, v:v) buffer (10 mM, HEPES, pH 7.2). Upon the addition of Hg<sup>2+</sup> or Cu<sup>2+</sup> to the colorless solution of 'RhAsP', the fluorescent and colorimetric characteristics of rhodamine appeared as shown in Scheme 2, and Fig. 1. This indicated that the oxygen atom of amide group on the chemosensor 'RhAsP' plays an important role in the course of binding with Hg<sup>2+</sup> and Cu<sup>2+</sup>. Likewise, these changes may also be related to the structural transformation of '**RhASP**' from spirolactam (non-fluorescent) to ring-opened (fluorescent) form due to its complexation behaviour with  $Hg^{2+}$  and  $Cu^{2+}$  ions [42-43]. The optimum pH conditions for the successful application of the fluorescent sensor 'RhAsP' and 'RhAsP-Hg2+' complex were also investigated. For the purpose of this assay, as depicted in Fig. 2, the effects of pH on the fluorescence intensity of the sensor were measured in the absence and presence of Hg<sup>2+</sup> in an EtOH-water (2:1, v:v) buffer (10 mM, HEPES, pH 7.2). As is well known, rhodamine-based spirolactam fluorescent sensors generally display pH-dependent response to Hg<sup>2+</sup> ion. Similar to many reported rhodamine spirolactam-based fluorescent chemosensors, it was found that the fluorescence intensity of **RhAsP**-Hg<sup>2+</sup> complex decreased with the acidic conditions (pH<6). Besides, there were no important observations of emission intensity of free 'RhAsP' in a wide range value of pH from 4 to 11. However, in the presence of  $Hg^{2+}$  (1 equiv.), there was an obvious difference in fluorescence intensity between pH=6 and pH=11 [26, 34]. Meanwhile, as depicted in Fig. S7, the effects of pH on the absorbance of sensor 'RhAsP' in the absence and presence of Cu<sup>2+</sup> were also investigated. The experiments were carried out at a pH range from 4.0 to 11.0. The free chemosensor in EtOH-water (2:1, v:v) buffer (10 mM, HEPES) did not show characteristic color of rhodamine between pH 7.0 and 11.0. But, there were observed both color changes and increases in the absorption of free 'RhAsP' between pH 4.0 and 7.0. Also, the RhAsP-Cu<sup>2+</sup> complex solutions exhibited strong absorbance enhancements with apparent color

changes from colorless to purple at different pH values ranging from 4.0 to 11.0. Thereby, the experimental results revealed that the fluorescent chemosensor '**RhAsP**' may further be used for high-selectivity detecting  $Hg^{2+}$  ions under physiological pH conditions.

#### Figs. 1 and 2

# 3.2. $K_a$ , limits of detection (LODs) and binding ratio (S:M) calculations of '**RhAsP**' with $Hg^{2+}$ and $Cu^{2+}$

The stoichiometry of **RhAsP**-Hg<sup>2+</sup> and **RhAsP**-Cu<sup>2+</sup> complexes was calculated using the Job's plot and mole ratio plot methods as shown in Figs. S8-11[44]. To determine the association constants, the UV-VIS titrations of different amounts of Hg<sup>2+</sup> and Cu<sup>2+</sup> in the ethanol-water solution of the chemosensor were carried out. As shown in Figs. S10 and S11, upon the addition of various concentrations of Hg<sup>2+</sup> and Cu<sup>2+</sup> ions in the range of 0–100  $\mu$ M caused changes in the absorption spectrum of '**RhAsP**'. Upon addition of Hg<sup>2+</sup> and Cu<sup>2+</sup> ions, the absorbance of chemosensor gradually increased with color changes from colorless to purple [45]. In consequence, we estimated the binding constants by using the absorption titration data and employing the Benesi-Hildebrand equation [16]. According to the Job's plot assay assuming a 1:1 binding ratio between '**RhAsP**' and Cu<sup>2+</sup> or Hg<sup>2+</sup> ions, the association constants '*K<sub>a</sub>*' of the chemosensor with these metal ions were determined using Eqs. 1 and 2 as follows, respectively [46–48].

$$\frac{1}{\Delta(A-A_0)} = \frac{1}{K_a}(A_{max}-A_0)[Cu^{2^+}] + \frac{1}{A_{max}-A_0} Eq. 1$$
  
$$\frac{1}{\Delta(F-F_0)} = \frac{1}{K_a}(F_{max}-F_0)[Hg^{2^+}] + \frac{1}{F_{max}-F_0} Eq. 2$$

A/F and  $A_0/F_0$  are the absorbance or fluorescence intensity of the '**RhAsP**' solution in the presence and absence of Hg<sup>2+</sup> or Cu<sup>2+</sup> ions, respectively; the saturated absorbance or the intensity of the sensor in the presence of too much Cu (II) and Hg (II) is  $A_{max}/F_{max}$ ; [Hg<sup>2+</sup>] and [Cu<sup>2+</sup>] are the concentrations of mercury and copper ions added to the solution of '**RhAsP**' (µM). Based on the Benesi-Hildebrand equation, calculated association constants ' $K_a$ ' for Cu<sup>2+</sup> and Hg<sup>2+</sup> from UV-vis and fluorescence data were 2.2x10<sup>3</sup> L.mol<sup>-1</sup> and 1.3x10<sup>4</sup> L.mol<sup>-1</sup> plotting of  $I/(A-A_0)$  or  $I/(F-F_0)$  versus  $I/[Cu^{2+}]$  or  $I/[Hg^{2+}]$ , respectively, exhibiting a linear relationship as shown in Figs. 3 and 4 [49]. To better understand the selectivity of '**RhAsP**' toward Hg<sup>2+</sup>, protocols for determination of limits of detection were performed using fluorescence titration data with the

equation, based on the definition by IUPAC (LOD=  $3S_b/m$ ), where  $S_b$  is the standard deviation of blank measurements and m represents the slope between fluorescence intensity and mercury metal ion concentrations as shown in Fig. 5. So, the detection limit for Hg<sup>2+</sup> was found to be 150 nM. In addition, the LOD of Cu<sup>2+</sup> was calculated using the absorption titration data of '**RhAsP**' with Cu<sup>2+</sup> (LOD= $3.37 \mu$ M) as shown in Fig. 6. Meanwhile, when the obtained limits of detection for Hg<sup>2+</sup> and Cu<sup>2+</sup> were compared to previous studies, herein, they were quiet acceptable range of results under the similar conditions [31, 41]. Indeed, the standard for the maximum allowable level of Hg<sup>2+</sup> in drinking water is only 10 nM, and the permitted limit for Cu<sup>2+</sup> in drinking water is only < 20  $\mu$ M according to the United States Environmental Protection Agency (EPA) [35, 46–47, and 50–51].

#### Figs. 3, 4, 5, and 6

#### 3.3. Selectivity studies

To shed some light on the metal-binding properties of 'RhAsP', its sensitivity and selectivity for Hg<sup>2+</sup> and Cu<sup>2+</sup> ions were investigated by using UV-vis and fluorescence spectroscopy techniques over various possible interfering metal ions and as shown in Figs. 7 and 8. The free fluorescent sensor 'RhAsP' showed almost no absorption between 450 nm and 600 nm. In fact, this was related to the closed (spirolactam) forms of fluorescent sensor molecules in the solution. Upon the addition of  $Cu^{2+}$  to the solution of '**RhAsP**', a strong absorption band was appeared at 560 nm. When other competing metal ions (Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, Cr<sup>3+</sup>, Fe<sup>3+</sup>, Al<sup>3+</sup>, Hg<sup>2+</sup>, Mn<sup>2+</sup>, Zn<sup>2+</sup>, Pb<sup>2+</sup>, Fe<sup>2+</sup>, Co<sup>2+</sup>, Ni<sup>2+</sup>, Cd<sup>2+</sup> and Ag<sup>+</sup>) were added to the solution of '**RhAsP'**, they did not cause any changes in the absorption spectrum of the chemosensor except there was an absorption increase with  $Hg^{2+}$ , which is smaller than with  $Cu^{2+}$ . Moreover, as shown in Scheme 2 and Fig. 1, only the Cu<sup>2+</sup> and Hg<sup>2+</sup> induced a notable color change in the HEPES buffered from colorless to purple under natural light, which can be assigned to the spirolactam bond (amide group) cleavage of the chemosensor 'RhAsP', while the other metal ions did not proceed any obvious changes under identical conditions [52-57]. Thereby, the finding of the colorimetric recognition of Cu<sup>2+</sup> supported that 'RhAsP' shows a very high sensitivity by important enhancement of absorption band. On the other hand, the addition of Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, Cr<sup>3+</sup>, Fe<sup>3+</sup>, Al<sup>3+</sup>, Mn<sup>2+</sup>,  $Zn^{2+}$ ,  $Cu^{2+}$ ,  $Pb^{2+}$ ,  $Fe^{2+}$ ,  $Co^{2+}$ ,  $Ni^{2+}$ ,  $Cd^{2+}$  and  $Ag^+$  ions exerted little or no effect upon the emission of 'RhAsP', while remarkable fluorescence enhancement was detected only upon the addition of

Hg<sup>2+</sup> ions by excitation of the chemosensor at 530 nm. However, the colorful solution of '**RhAsP**' induced by Cu<sup>2+</sup> did not show any significant fluorescence emission band around 586 nm because of fluorescence quenching by the paramagnetic effect of the d<sup>9</sup> electronic system of Cu<sup>2+</sup> as shown in Fig. 8 [58]. Unlike copper metal ions, 'RhAsP' displayed high fluorescence selectivity toward Hg<sup>2+</sup> over other metal ions, except for a very small enhancement of fluorescence intensity with  $Cr^{3+}$  and  $Al^{3+}$ . For better understanding the sensing property of '**RhAsP**' to  $Hg^{2+}$ , fluorescence titration experiments of the sensor solution with  $Hg^{2+}$  were gradually carried out. As shown in Fig. S10, the absorption band and fluorescence intensity steadily increased with the progressive addition of  $Hg^{2+}$  (in the range of 0-200  $\mu$ M) to the '**RhAsP**' solution (100  $\mu$ M). From the titration profiles, the binding constants 'K<sub>a</sub>' and LODs of 'RhAsP' for mercury and copper ions were determined as depicted in Figs. 4 and 5. For practical applications, reversibility that is the ability to reproduce the free sensors from the complex is a significant parameter for fluorescent sensors. As depicted in Fig. S12, and Scheme 2, once the colorful solution of the fluorescent sensor-Hg<sup>2+</sup> complex was finally treated with 0.1 mM Na<sub>2</sub>S, the fluorescence turned off gradually with changes in color from purple to colorless form. Therefore, these results demonstrated that coordination occurring between 'RhAsP' and Hg<sup>2+</sup> was reversible by chemical processes.

In addition to the above-mentioned studies, the competitive experiments of mercury over other various metal ions were investigated by performing the fluorescence response of '**RhAsP**' toward  $Hg^{2+}$  in the presence of metal ions such as Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, Ag<sup>+</sup>, Cr<sup>3+</sup>, Fe<sup>3+</sup>, Al<sup>3+</sup>, Mn<sup>2+</sup>, Zn<sup>2+</sup>, Cu<sup>2+</sup>, Pb<sup>2+</sup>, Fe<sup>2+</sup>, Co<sup>2+</sup>, Ni<sup>2+</sup> and Cd<sup>2+</sup> as shown in **Fig. S13**. A reduced selectivity for  $Hg^{2+}$  showed no changes with the addition of the other metal ions; only Fe (II) and Cu (II) showed a very small decrease in fluorescence intensity because of the their paramagnetic effects. Moreover, as shown in Fig. S13, some alkaline, alkaline-earth or transition metal ions used in this study did not interfere with the  $Hg^{2+}$ -induced fluorescence increase, showing that '**RhAsP**' may also serve as a selective fluorescent probe for  $Hg^{2+}$  detection.

#### Figs. 1, 7 and 8

#### 4. Conclusion

In this paper, we report a novel rhodamine hydrazone derivative as a fluorescent "*off-on*" sensor '**RhAsP**' for  $Hg^{2+}$  in aqueous medium. The experimental results revealed that '**RhAsP**'

behaves both as a colorimetric and selective fluorescent sensor for  $Hg^{2+}$ , whereas it displays colorimetric and absorbance (at 560 nm) responses toward  $Cu^{2+}$  as colorless to purple. In addition, '**RhAsP**' showed high fluorescent selectivity toward mercury in the coexistence of various metal ions as depicted in Fig. S12, representing a nearly 100-fold increase in fluorescence emission intensity in an EtOH-water buffer under physiological pH conditions. Meanwhile, the binding mode of the sensor to  $Hg^{2+}$  ion was found to be at a 1:1 ratio (**RhAsP**/Hg^{2+}). Similarly, the LOD was calculated as 150 nM. Consequently, a highly selective and sensitive fluorescent sensor '**RhAsP**' may be further performed a promising candidate for detecting of  $Hg^{2+}$  species for real time applications in drinking water and human serums.

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### References

[1] M. Saleem, K.H. Lee, Selective fluorescence detection of Cu<sup>2+</sup> in aqueous solution and living cell, J. Lumin. 145 (2014) 843-848.

[2] N. Kumari, N. Dey, S. Bhattacharya, Remarkable role of positional isomers in the design of sensors for the ratiometric detection of copper and mercury ions in water, *RSC Adv.* 4 (2014) 4230.

[3] J. Wang, H. Li, L. Long, G. Xiao, D. Xie, Fast responsive fluorescence turn-on sesnsor for  $Cu^{2+}$  and its application in live cell imaging, J. Lumin. 132 (2012) 2456-2461.

[4] M. Wang, F. Yan, Y. Zou, L. Chen, N. Yang, X. Zhou, Recognition of Cu<sup>2+</sup> and Hg<sup>2+</sup> in physiological conditions by a new rhodamine based dual channel fluorescent probe, Sens. and Actuators B: Chem. 192 (2014) 512-521.

[5] W. Wang, Y. Zhang, Q. Yang, M. Sun, X. Fei, Y. Song, Y. Zhang, Y. Li, Fluorescent and colorimetric magnetic microspheres as nanosensors for Hg<sup>2+</sup> in aqueous solution prepared by a sol-gel grafting reaction and host-guest interaction, Nanoscale 5 (2013) 4958.

[6] T. Li, Z. Yang, Y. Li, Z. Liu, G. Qi, B. Wang, A novel fluorescein derivative as a colorimetric chemosensor for detecting copper (II) ion, Dyes Pigm. 88 (2011) 103-108.

[7] Y. Zhou, J. Zhang, H. Zhou, Q. Zhang, T. Ma, J. Niu, A new rhodamine B-based "off-on" fluorescent chemosensor for  $Cu^{2+}$  in aqueous media, J. Lumin. 132 (2012) 1837-1841.

[8] D. Guo, Z. Dong, C. Luo, W. Zan, S. Yan, X. Yao, A rhodamine B-based "turn-on" fluorescent sensor for detecting  $Cu^{2+}$  and sulfur anions in aqueous media, RSC Adv. 4 (2014) 5718-5725.

[9] Z. Sun, H. Li, D. Guo, Y. Liu, Z. Tian, S. Yan, A novel piperazine-bis(rhodamine-B)-based chemosensor for highly sensitive and selective naked-eye detection of Cu<sup>2+</sup> and its application as an INHIBIT logic device, J. Lumin.167 (2015) 156–162.

[10] F. Wang, S.W. Nam, Z. Guo, S. Park, J. Yoon, A new rhodamine derivative bearing benzothiazole and thiocarbonyl moieties as a highly selective fluorescent and colorimetric chemodosimeter for Hg<sup>2+</sup>, Sens. and Actuators B: Chem. 161 (2012) 948-953.

[11] J. Ni, Q. Li, B. Li, L. Zhang, A novel fluorescent probe based on rhodamine B derivative for highly selective and sensitive detection of mercury (II) ion in aqueous solution, Sens. and Actuators B: Chem. 186 (2013) 278-285.

[12] D.S. Tira, M. Focsan, S. Ulinici, D. Maniu, S. Astilean, Rhodamine B-Coated Gold Nanoparticles as Effective "Turn-on" Fluorescent Sensors for Detection of Zn II Ions in Water, Spectrosc Lett. 47 (2014) 153-159.

[13] R. Balamurugan, C.C. Chien, K. M. Wu, Y.H. Chiu, J.H. Liu, A depropargylation-triggered fluorescence "turn-on" probe for the detection of Pd2+ based on a bispropargylamine-rhodamine conjugate, Analyst 138 (2013) 1564.

[14] T. Hirayama, K. Okuda, H. Nagasawa, A highly selective turn-on fluorescent probe for iron(II) to visualize labile iron in living cells, Chem. Sci. 4 (2013) 1250.

[15] Y. Wei, Z. Aydin, Y. Zhang, Z. Liu, M. Guo, A Turn-on Fluorescent Sensor for Imaging Labile Fe<sup>3+</sup> in Live Neuronal Cells at Subcellular Resolution, ChemBioChem. 13 (2012)1569 – 1573.

[16] L. Zhang, J. Fan, X. Peng, X-ray crystallographic and photophysical properties of rhodamine-based chemosensor for Fe<sup>3+</sup>, Spectrochim. Acta A 73 (2009) 398-402.

[17] K. Dai, B. Xu, J. Chen , A rhodamine-based "off-on" colorimetric and fluorescent

chemosensor for Cu (II) in aqueous and non-aqueous media, J Fluoresc, 24 (2014) 1129-1136.

[18] Y. Kai, S. Yang, X. Gao, Y. Hu, Colorimetric and "turn-on" fluorescent for Hg<sup>2+</sup> based on rhodamine-3,4-etylenedioxythiophene derivative, Sens. and Actuators B: Chem. 202 (2014) 252-256.

[19] N. Wanichacheva, O. Hanmeng, S. Kraithong, K. Sukrat, Dual optical Hg<sup>2+</sup>-selective sensing through FRET system of fluorescein and rhodamine B fluorophores, J. Photochem. Photobiol. A Chem. 278 (2014) 75-81.

[20] Z. Sun, D. Guo, H. Li, L. Zhang, B. Yang, S. Yan, Multifunctional Fe3O4@SiO2 nanoparticles for

selective detection and removal of  $Hg^{2+}$  ion in aqueous solution, RSC Adv. 5 (2015) 11000.

[21] R.P. Haugland, Handbook of Fluorescent Probes and Research Chemicals, 2002, 9<sup>th</sup> ed., Molecular Probes, Eugene, OR.

[22] S. Erdemir, O. Kocyigit, S. Malkondu, Detection of  $Hg^{2+}$  ion in aqueous media by new fluorometric and colorimetric sensor based on triazole–rhodamine, J. Photochem. Photobiol. A Chem. 309 (2015) 15–21.

[23] J.C. Qin, Z.Y. Yang, G.Q. Wang, A novel ratiometric fluorescent probe for detection of Fe<sup>3+</sup> by rhodamine–quinoline conjugate, J. Photochem. Photobiol. A Chem. 310 (2015) 122–127.

[24] N. Wanichacheva, O. Hanmeng, S. Kraithong, K. Sukrat, Dual optical Hg<sup>2+</sup>-selective sensing through FRET system of fluorescein and rhodamine B fluorophores, J. Photochem. Photobiol. A Chem. 278 (2014) 75–81.

[25] C.Y. Li, Y. Zhou, Y.F. Li, C.X. Zou, X.F. Kong, a fluorescent chemosensor for Hg<sup>2+</sup> based on a rhodamine derivative in an aqueous solution, Analytical Sciences 29 (2013) 899-903.

[26] V. Dujols, F. Ford, A.W. Czarnik, A Long- Wavelength Fluorescent Chemodosimeter Selective for Cu(II) Ion in Water , Chem Soc. 119 (1997) 7386.

[27] L. Xu, Y. Xu, W. Zhu, B. Zeng, C. Yang, B. Wu, X. Qian, Versatile trifunctional chemosensor of rhodamine derivative for  $Zn^{2+}$ ,  $Cu^{2+}$  and His/Cys in aqueous solution and living cell, Org. Biomol. Chem. 9 (2011) 8284-8287.

[28] Y. Zhao, B. Zheng, J. Du, D. Xiao, L. Yang, A fluorescent "turn-on" probe for the dualchannel detection of Hg (II) and Mg (II) and its application of imaging in living cells, Talanta 85 (2011) 2194-2201.

[29] Y. Mao, M. Hong, A. Liu, D. Xu, Highly selective and sensitive detection of Hg (II) from HgCl<sub>2</sub> by a simple rhodamine-based fluorescent sensor, J. Fluoresc, 2015; DOI 10.1007/s10895-015-1564-7.

[30] J. Ni, B. Li, L. Zhang, H. Zhao, H. Jiang, A fluorescence turn-on probe based on rhodamine derivative and its functionalized silica material for Hg<sup>2+</sup>-selective detection, Sens. and Actuators B: Chem. 215 (2015) 174-180.

[31] Y. Kai, S. Yang, X. Gao, Y. Hu, Colorimtric and "turn-on" fluorescent for Hg2+ based on rhodamine-3,4-ethylenedioxythiophene derivative, Sens. and Actuators B: Chem. 202 (2014) 252–256.

[32] J. Zhang, B. Li, L. Zhanga, H. Jiang, An optical sensor for Cu(II) detection with upconverting luminescent nanoparticles as an excitation source, Chem. Commun. 48 (2012) 4860–4862.

[33] L. Liu, P. Guo, L. Chai, Q. Shi, B. Xu, J. Yuan, X. Wang, X. Shi, W. Zhang, Fluorescent and colorimetric detection of pH by a rhodamine-based probe, Sens. and Actuators B: Chem. 194 (2014) 498–502.

[34] A. Zheng, J. Chen, G. Wu, G. Wu, Y.G. Zhang, A novel fluorescent distinguished probe for Cr(VI) in aqueous solution, Spectrochim. Acta A 74 (2009) 265-270.

[35] M. Shellaiah, Y.C. Rajan, P. Balu, A. Murugan , Apyrene based Schiff base probe for selective fluorescence turn-on detection of  $Hg^{2+}$  ions with live cell application, New J. Chem 39 (2015) 2523-2531.

[36] F. Yan, D. Cao, M. Wang, N. Yang, Q. Yu, L. Dai, L. Chen, A new Rhodamine-Based "Off-On" Fluorescent Chemosensor for Hg (II) Ion and its Application in Imaging Hg (II) in Living Cells, J Fluoresc 22 (2012) 1249-1256.

[37] X. Chen, X. Meng, S. Wang, Y. Cai, Y. Wu, Y. Feng, M. Zhu, Q Guo, A rodamine-based fluorescent probe for detecting Hg<sup>2+</sup> in a fully aqueous environment, Dalton Trans. 42 (2013) 14819-14825.

[38] S. Mandal, A. Banerjee, S. Lohar, A. Chattopadhyay, B. Sarkar, S.K. Mukhopadhyay, A. Sahana, D. Das, Selective sensing of  $Hg^{2+}$  using rhodamine-thiophene conjugate: Red light emission and visual detection of intracellular  $Hg^{2+}$  at nanomolar level, J. Haz. Mat. 261 (2013) 198-205.

[39] M. P. Walkees, Cadmium carcinogenesis, Mutat. Res. 533 (2003) 107-120.

[40] M. Waisberg, P. Joseph, B. Hale, D. Beyersmann, Molecular and cellular mechanisms of cadmium carcinogenesis, Toxicology. 192 (2003) 95-117.

[41] C. Wang, D. Zhang, X. Huang, P. Ding, Z. Wang, Y. Zhao, Y. Ye, A ratiometric fluorescent chemosensor for  $Hg^{2+}$  based on FRET and its application in living cells, Sens. and Actuators B: Chem. 198 (2014) 33-40.

[42] S. Tong, L. Zhang, L. Bing, L. Yi, Two rhodamine derived fluorescence turn-on chemosensors for Hg(II) recognition and sensing: synthesis, characterization and sensing performance, Sens. and Actuators B: Chem. 203 (2014) 157–164.

[43] J. Xu, Y. Hou, Q. Ma, X. Wu, S. Feng, J. Zhang, Y. Shen, A highly selective fluorescent probe for Cu2+ based on rhodamine B derivative Spectrochim. Acta A 124 (2014) 416–422.

[44] B. Valeur, S. Berberan, N. Mário, Molecular Fluorescence: Princeples and Applications, 2. Edition, 2002, Wiley-VCH. Weinheim.

[45] J.T. Hou, Q.F. Zhang, B.Y. Xu, Q. Liu, J. Zhang, X.Q. Yu, A novel BINOL-based cyclophane via click chemistry: synthesis and its applications for sensing silver ions, Tedrahedron Lett., 52 (2011) 4927-4930.

[46] Y. Sun, S. Fan, D. Zhao, L. Duan, A Fluorescent Turn-On Probe Based on Benzo [E] Indolium for Cyanide Ion in Water With High Selectivity, J Fluoresc, 23 (2013) 1255-1261.

[47] K.C. Tayade, U.A. Kuwar, A.S. Fegade, H. Sharma, N. Singh, U.D. Patil, S.B. Atarde, Dessign and Synthesis of a Pyridine Based Chemosensor: Highly Selective Fluorescent Probe For Pb<sup>2+</sup>, J Fluoresc 24 (2014) 19-26.

[48] R. Tang, K. Lei, K. Chen, H. Zhao, J. Chen, A Rhodamine-Based Off-On Fluorescent Chemosensor for Selective Sensing Cu(II) in Aqueous Solution, J Fluoresc 21 (2011) 141-148.

[49] R. Tang, K. Lei, K. Chen, H. Zhao, A Rhodamine-Based Off-On Fluorescent Chemosensor for Selectively Sensing Cu(II) in Aqueous Solution, J Fluoresc 21 (2011) 141-148.

[50] Z. Wang, J.H. Lee, Y. Lu, Highly sensitive "turn-on" fluorescent sensor for Hg<sup>2+</sup> in

aqueous solution based on structure-switching DNA, Chem Commun 45 (2008) 6005-6007.

[51] L. Tang, J. Guo, N. Wang, A new rhodamine B hydrazide hydrazone derivative for colorimetric and fluorescent "off-on" recognition of copper (II) in aqueous media, Bull. Korean Chem. Soc. 34 (2013) 159.

[52] H. Sasaki, K. Hanaoka, Y. Urano, T. Terai, T. Nagano, Design and synthesis of a novel fluorescence probe for  $Zn^{2+}$  based on the spirolactam ring-opening process of rhodamine derivatives, Bioorg Med Chem 19 (2011) 1072-1078.

[53] P. Puangploy, S. Smanmoo, W. Surareungchai, A new rhodamine derivative-based chemosensor for highly selective and sensitive determination of  $Cu^{2+}$ , Sens. and Actuators B: Chem. 193 (2014) 679-686.

[54] M. Wang, F. Yan, Y. Zou, L. Chen, N. Yang, X. Zhou, Recognition of Cu<sup>2+</sup> and Hg<sup>2+</sup> in physiological conditions by a new rhodamine based dual channel fluorescent probe, Sens. and Actuators B: Chem. 192 (2014) 512-521.

[55] J. Hu, Z. Hu, Y. Cui, X. Zhang, H.W. Gao, K. Uvdal, A rhodamine-based fluorescent probe for Hg<sup>2+</sup> and its application for biological visualization, Sens. and Actuators B: Chem. 203 (2014) 452-458.

[56] S.K. Kempahanumakkagari, R. Thippeswamy, P. Malingappa, A new rhodamine B based fluorometric chemodosimeter for  $Cu^{2+}$  ion in aqueous and cellular media, J. Lumin. 146 (2014) 11-17.

[57] M. Saleem, K.H. Lee, Selective fluorescence detection of  $Cu^{2+}$  in aqueous solution and living cells, J. Lumin. 145 (2014) 843-848.

[58] J.S. Kim, D.T. Quang, Calixarene-derived fluorescent probes, Chem. Rev. 107 (2007) 3780-3799.

#### **FIGURES**



**Fig. 1** Colorimetric changes of chemical sensor '**RhAsP**' (50  $\mu$ M) in absence and presence of Na<sup>+</sup>, K<sup>+</sup>, Mg<sup>2+</sup>, Ca<sup>2+</sup>, Al<sup>3+</sup>, Ag<sup>+</sup>, Zn<sup>2+</sup>, Hg<sup>2+</sup>, Fe<sup>2+</sup>, Cd<sup>2+</sup>, Mn<sup>2+</sup>, Ni<sup>2+</sup>, Cu<sup>2+</sup>, Fe<sup>3+</sup>, Cr<sup>3+</sup>, Pb<sup>2+</sup>, Co<sup>2+</sup> (100  $\mu$ M) in EtOH-water (2:1, v:v) buffer (10 mM, HEPES, pH 7.2) under natural light (a) and UV lamp at 365 nm (b).



**Fig. 2** Fluorescent intensity (586 nm) of '**RhAsP**' (100  $\mu$ M) in the absence and presence of Hg<sup>2+</sup> ions (200  $\mu$ M) in EtOH-water (2:1, v:v) buffer (10 mM, HEPES,  $\lambda_{ex}$ = 530 nm) with different pH conditions. The pH values of solution were adjusted by using an appropriate volume of 0.1 M HCl or NaOH stock solution.



**Fig. 3** Benesi-Hildebrand plot from absorbance (at 560 nm) of  $1/\Delta A$  versus  $1/[Cu^{2+}]$  to determine the binding constant (K<sub>a</sub>) of Cu<sup>2+</sup> with '**RhAsP**' in EtOH-water (2:1, v:v) buffer (10 mM, HEPES, pH 7.2, absorption method).



**Fig. 4** Benesi-Hildebrand Plot from fluorescence intensity (at 586 nm) of  $1/\Delta F$  versus  $1/[Hg^{2+}]$  to determine the binding constant (K<sub>a</sub>) of '**RhAsP**' (50 µM,  $\lambda_{ex}$ = 530 nm, fluorescence method).



**Fig. 5** Fluorescence intensity of '**RhAsP**' (50  $\mu$ M, at 586 nm) upon continuous addition of increasing amount of Hg<sup>2+</sup> (0-55  $\mu$ M) in EtOH-water (2:1, v:v) buffer (10 mM, HEPES, pH 7.2,  $\lambda_{ex} = 530$  nm).



**Fig. 6** Absorption intensity of '**RhAsP**' (50  $\mu$ M, at 560 nm) upon continuous addition of increasing concentration of Cu<sup>2+</sup> (0-55  $\mu$ M) in EtOH-water (2:1, v:v) buffer (10 mM, HEPES, pH 7.2).



**Fig. 7** UV-vis absorption spectra of chemical sensor '**RhAsP**' (50  $\mu$ M) in the presence of Cu<sup>2+</sup> and Hg<sup>2+</sup> with other metal ions (100  $\mu$ M) (a), and the bar graph represents the changes of absorbance value of '**RhAsP**' upon addition of various competing metal ions (100  $\mu$ M) (b) in EtOH-water (2:1, v:v) buffer (10 mM, HEPES, pH 7.2).



**Fig. 8** Fluorescence response of **RhAsP** (100  $\mu$ M) to various metal ions (200  $\mu$ M) (a), bar graph represents the change of relative emission intensity of **RhAsP** upon addition of various competing metal ions (b), and inset: color changes of **RhAsP**-Hg<sup>2+</sup> complex (100  $\mu$ M) under natural light and portable UV lamp at 365 nm in EtOH-water (2:1, v:v) buffer (10 mM, HEPES, pH 7.2,  $\lambda_{ex} = 530$  nm).



RhAsP

Scheme 1. The synthesis route of fluorescent sensor '*RhAsP*'



**Scheme 2.** Binding mode of **'RhAsP'** (100  $\mu$ M) with Hg<sup>2+</sup> (2 equiv.), "*off-on*" situation: photograph of **'RhAsP'** in absence and presence of Hg<sup>2+</sup> in EtOH-water (2:1, v:v) buffer (10 mM, HEPES, pH 7.2).