



Detection of Hg²⁺ in Aqueous Media by A New Xanthene Based Schiff Base Sensor

Kaan KARAOĞLU

Recep Tayyip Erdogan University, Vocational School of Technical Sciences, Department of Chemistry and Chemical Process Technology, 53100 Rize, Turkey

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*[ID](https://orcid.org/0000-0003-3265-8328): <https://orcid.org/0000-0003-3265-8328>

***Corresponding author's:**

Kaan KARAOĞLU
Recep Tayyip Erdogan University,
Vocational School of Technical Sciences,
Department of Chemistry and Chemical
Process Technology, 53100 Rize, Turkey
✉: kaan.karaoglu@erdogan.edu.tr
Mobile telephone : +90 (553) 425 86 08
Telephone : +90 (464) 228 00 22
Fax : +90 (464) 228 00 25

Abstract: A xanthene-based colorimetric sensor, 2-((5-chloro-2-oxoindolin-3-ylidene)amino)-3',6'-bis(diethylamino)spiro[isindoline-1,9'-xanthen]-3-one, was designed and its metal sensing properties was evaluated in aqueous solutions. The sensor showed colorimetric response toward Hg²⁺ from colorless solution to pink among various metal ions such as Na⁺, K⁺, Mg²⁺, Ca²⁺, Mn²⁺, Fe³⁺, Co²⁺, Ni²⁺, Cu²⁺, Zn²⁺, Al³⁺, Cd²⁺ and Pb²⁺. The addition of Hg²⁺ exhibits an absorption enhancement of the sensor based on a spirocycle ring-opening process and a subsequent hydrolysis reaction. The detection limit of the sensor for Hg²⁺ was found to be 7.88×10⁻⁸ M.

Keywords: Isatin, Mercury sensor, Rhodamine B hydrazide.

Yeni Bir Ksanten Bazlı Schiff Bazı Sensörü ile Sulu Ortamda Hg²⁺ Tespiti

***Sorumlu yazar:**

Kaan KARAOĞLU
Recep Tayyip Erdoğan Üniversitesi,
Teknik Bilimler Meslek Yüksekokulu,
Kimya ve Kimyasal Proses Teknolojisi
Bölümü, 53100 Rize, Türkiye
✉: kaan.karaoglu@erdogan.edu.tr
Cep telefonu : +90 (553) 425 86 08
Telefon : +90 (464) 228 00 22
Faks : +90 (464) 228 00 25

Öz: Bir ksanten bazlı kolorimetrik sensör, 2-((5-kloro-2-oksoindolin-3-iliden)amino)-3',6'-bis(diethylamino)spiro[izoindolin-1,9'-ksanten]-3-on, bileşiği tasarlanmış ve sulu ortamda metal tespit özellikleri incelenmiştir. Sensör bileşiği Na⁺, K⁺, Mg²⁺, Ca²⁺, Mn²⁺, Fe³⁺, Co²⁺, Ni²⁺, Cu²⁺, Zn²⁺, Al³⁺, Cd²⁺ ve Pb²⁺ iyonları arasında Hg²⁺ iyonuna renksizden pembeye değişen kolorimetrik karşılık vermiştir. Hg²⁺ iyonlarının ilavesi ile sensör bileşiği spirohalka açılması ve ardından hidroliz tepkimesi yaparak absorpsiyon artışı sergilemiştir. Sensörün Hg²⁺ iyonu için tespit limitinin 7,88×10⁻⁸ M olduğu belirlenmiştir.

Anahtar kelimeler: Cıva sensör, izatin, rhodamine B hidrazit.

INTRODUCTION

Ion content of water is essentially crucial for humans because deficiency or excess intake of metal ions to body causes the metabolic disorder. Bioaccumulation of mercury, one of the neurotoxic metal, causes immune

system failure (Voutsadaki et al., 2010, Verrep et al., 2018). Because of its possible damage to the ecosystem, mercury should be monitored effectively in water sources. Some sophisticated systems such as inductively coupled plasma

mass/emission spectrometry (ICP-MS and ICP-AES), atomic absorption spectrometry (AAS) which require expensive instrumentation and pretreatments have been applied to detect trace amount of mercury (Cope et al., 1982; Jarzyńska & Falandysz, 2011; Yuan et al., 2014). On the other hand, optical techniques enable real-time and high precisely online analysis of metal ions (Yoon et al., 2007). Therefore, design fluorescent chemosensors for the detection of mercury ion at the nano-molar level is of great current interest (Aksuer et al., 2011; Cammann et al., 1991; Farruggia et al., 2006; Zhao et al., 2011).

Colorimetric sensors such as triazine, coumarin, quinolone, rhodamine, and chromenyl-cyanine have been designed for detection of the transition metal ions in aqueous media (Aksuer et al., 2011; Du et al., 2017; Voutsadaki et al., 2010; Wei et al., 2016). Hydroxybenzaldehyde Schiff bases derived from Rhodamine B, one of the most used probe structure, enables Cu^{2+} sensing via coordination of transition metal ion through phenolic oxygen and imine nitrogen atoms of the sensor because of its large association constant (Li et al., 2015; Tang et al., 2011; Xiang et al., 2006). Copper sensing properties of the rhodamine B hydrazide and its Schiff base derivatives have been investigated extensively, while few reports have focused on mercury sensing properties of this class of compounds (Jiao et al., 2016; Wanichacheva et al., 2012).

In this work, a new xanthene based Schiff base chemosensor was designed and characterized. Rhodamine B based probe synthesized by condensation reaction between rhodamine B hydrazide and 5-chloroisatin. The molecular structure of the sensor was characterized by IR, UV-vis., LC-MSMS, ^1H NMR, ^{13}C NMR and mass analysis. Spectrophotometric Hg^{2+} -sensing properties of the sensor among various metal ions such as Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Mn^{2+} , Fe^{3+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Zn^{2+} , Al^{3+} , Cd^{2+} and Pb^{2+} have been investigated at physiological pH.

MATERIAL AND METHOD

The aqueous solutions of Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Mn^{2+} , Fe^{3+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Zn^{2+} , Al^{3+} , Cd^{2+} , Hg^{2+} , and Pb^{2+} ions were freshly prepared from their nitrate salts except for Mn^{2+} was prepared acetate salt. Hydrazine hydrate was obtained from Merck and rhodamine B was obtained from Sigma Aldrich. All the solvents were of reagent grade and used as received.

The infrared (FT-IR) spectra of the compounds were recorded on a Perkin Elmer Spectrum 100 spectrometer equipped with an ATR apparatus. UV/vis experiments were performed on a Spectrocan DV 60 spectrophotometer. Heated-electrospray mass spectra (H-ESI) were recorded using Thermo Sci. TSQ Quantum

Access MAX Triple Stage Quadrupole mass spectrometer and ^1H and ^{13}C NMR spectra were recorded on an Agilent Technologies 400/54 spectrometer at the Central Research Laboratory of Recep Tayyip Erdogan University.

Synthesis of sensor: Rhodamine B hydrazide (**1**) was prepared according to the procedure described by Zhang et al. (2017). A round bottom flask was charged with an ethanolic solution of rhodamine B (2.0 g) under nitrogen atmosphere. The solution was stirred at room temperature, and then hydrazine hydrate (8 mL, 85%) was added dropwise to the solution. The reaction was carefully controlled by TLC monitoring. The mixture was cooled to room temperature, and the microcrystalline product was filtrated under vacuum. Microcrystalline product was treated by 1 M hydrochloric acid then the pH of the solution was adjusted to 8 by NaOH solution. The resulting precipitate was filtered, washed three times with pure water, and then purified by column chromatography (CH_2Cl_2 :EtOH, 100:2.5, v/v). 5-Chloroisatin (0.182 g, 1.0 mmol) was added to an ethanolic solution of **1** (0.503 g, 1.1 mmol) under nitrogen atmosphere. The reaction mixture was heated under reflux for 4 hours and then the solvent removed by rotary under reduced pressure. The obtained solid product was purified by column chromatography, CH_2Cl_2 /EtOH (100:2.5, v/v) (Figure 1).

Yield: 42%. Color: Pale yellow. $\text{C}_{36}\text{H}_{34}\text{ClN}_5\text{O}_3$ (M= 620.13), ESI-MS, m/z (%): 619.67 (91%) $[\text{M}+\text{H}]^+$, 641.78 (44%) $[\text{M}+\text{Na}]^+$. FT-IR (cm^{-1}): 1748 $\nu(\text{C}=\text{O})$, 1704 $\nu(\text{C}=\text{O})$, 1613 $\nu(-\text{C}=\text{N}-)$, 1544–1449 $\nu(\text{Ar-H})$, 1214 $\nu(\text{C}-\text{O})$.

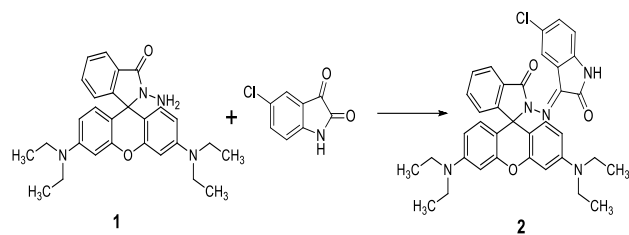


Figure 1. Synthesis reaction of the sensor.

RESULTS AND DISCUSSION

Structural Characterization: The compounds were characterized by spectroscopic techniques such as IR, UV-Vis, ^1H and ^{13}C NMR, mass and elemental analysis. Characteristic FT-IR peaks corresponding to C=O and NH groups for 5-chloroisatin were observed at 1747 and 3179 cm^{-1} , respectively (blue line in Figure 2). FT-IR spectra of **1** give a characteristic peak at 3326, 3198, and 1657 cm^{-1} , which are assignable to a N-H stretching band and a C=O stretching bands. The disappearance of amine peaks at 3326 and 3198 cm^{-1} (red line in Figure 2) and appearance of a new peak at 1704 cm^{-1} supports the condensation of 5-

chloroisatin with **1**. IR spectrum of the sensor shows two absorption band at 2970 and 2929 cm^{-1} corresponding to CH stretching. ^1H and ^{13}C NMR spectra of the compounds have been recorded in d_6 -dimethylsulfoxide, and spectra are in accordance with suggested structures (Figure 3 and Figure 4) (Aires-de-Souza et al., 2002; Banfi&Patiny, 2008; Binev & Aires-de-Souza, 2004; Binev et al., 2004; Castillo et al., 2011). The characteristic proton signals corresponding to NH, CH_3 and CH_2 signals were observed at 8.670 (a broad singlet), 3.328 (a quartet) and 1.164 ppm (a triplet), respectively. The characteristic ^{13}C NMR signals of C_{10} and C_{17} corresponding spirocycle closed-form were observed at 166.175 and 65.957 ppm, respectively (see **Hata! Başvuru kaynağı bulunamadı.** for detailed information). The mass spectrum of compound **2** showed a high intensity protonated molecular ion $[\text{M}+\text{H}]^+$ peak at m/z 619.67 (91%) and $[\text{M}+\text{Na}]^+$ peak at m/z 641.78 (44%) (Figure 5)

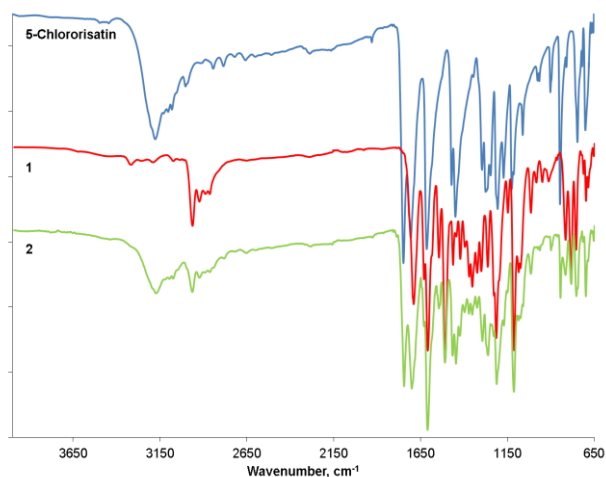


Figure 2. FTIR spectra of Rhodamine B hydrazide (**1**) and sensor (**2**).

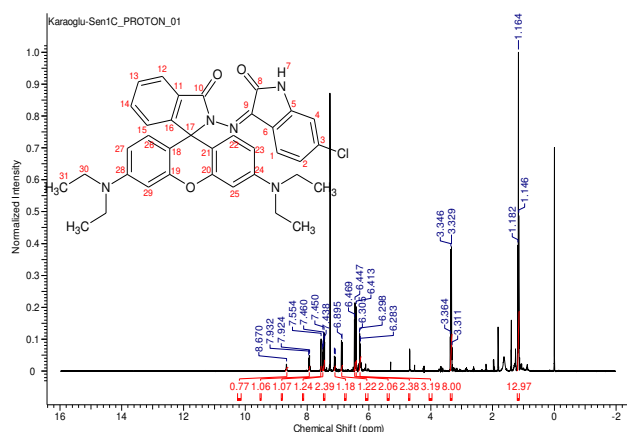


Figure 3. ^1H NMR spectrum of **2**.

Selectivity of Sensor: The selectivity experiments were carried out by using a 1×10^{-5} M sensor and a series of

mono-, di- and trivalent metal ions solutions in EtOH/water mixture (1:1) at pH 7.02. Optical responses of the sensor toward Li^+ , Na^+ , Mg^{2+} , Al^{3+} , K^+ , Ca^{2+} , Mn^{2+} , Fe^{3+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Zn^{2+} , Sr^{2+} , Cd^{2+} , Ba^{2+} , Hg^{2+} and Pb^{2+} ions were examined at visible region, Figure 6A. An absorption enhancement was observed at 557 nm after the Hg^{2+} addition. Other metal ions did not cause any significant changes, except for Cu^{2+} showed a considerably low optical response to sensor solution. The present chemosensor based on 5-chloroisatin Schiff base showed selectivity toward Hg^{2+} , while isatin based analog generates optical response Cd^{2+} and Pb^{2+} (Xu et al., 2012).

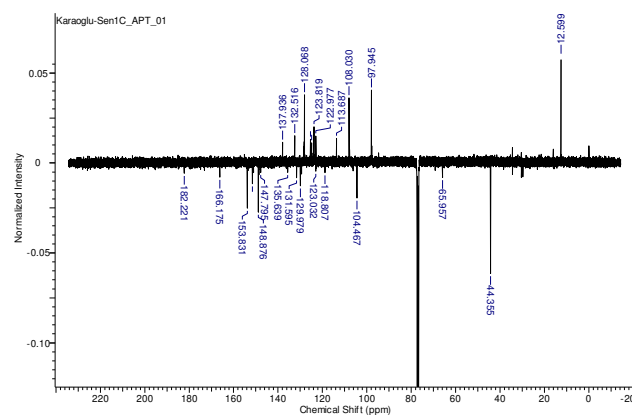


Figure 4. ^{13}C NMR spectrum of **2**.

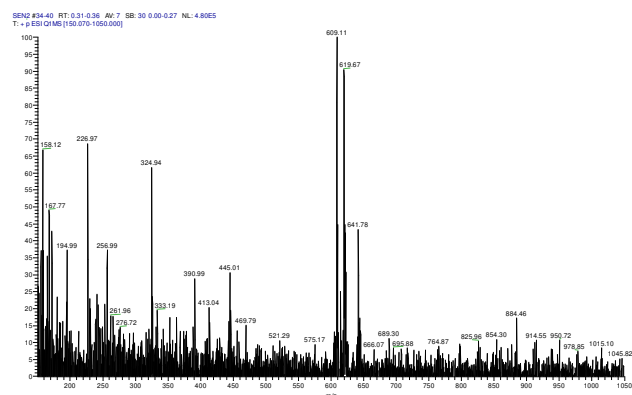


Figure 5. H-ESI mass spectra of the sensor.

The limit of detection (LOD) by the method adopted by UIPAC was determined from the following equation (Currie, 1995):

$$\text{Limit of detection} = 3 \times \frac{S_d}{S}$$

where S_d is the standard deviation of the blank measurements ($n=10$), and S is the slope of the calibration curve (Figure 6B). LOD value for **2** toward Hg^{2+} ion by colorimetry was calculated as 7.88×10^{-8} M. Colorimetric experiments showed that the analytical performance of the sensor is sufficient for the detection of Hg^{2+} ion in water samples.

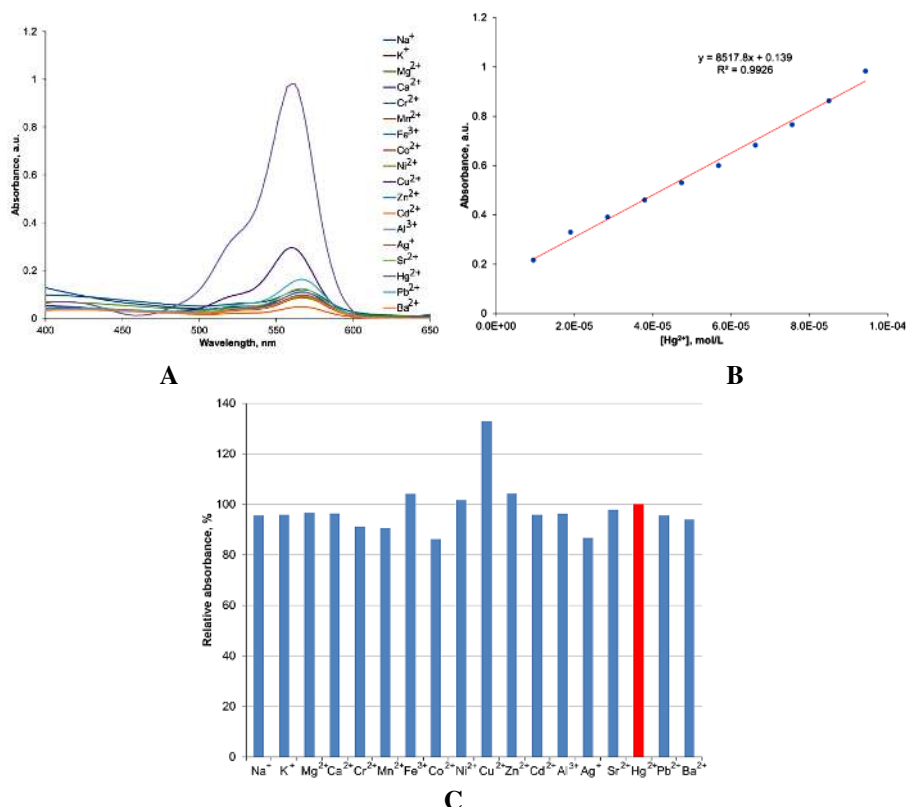


Figure 6. UV/vis. experiments of **2** (1.0×10^{-5} M) in MeCN/H₂O (4:1); **A**) absorption spectra of sensor with 5×10^{-5} M metal ions **B**) titration with Hg²⁺ **C**) titration curve derived from titration with Hg²⁺ at 561 nm **D**) Relative absorbance of **2** (1.0×10^{-5} M) with Hg²⁺, followed by 5 equivalent of other metal ions.

The competition experiments in the presence of 5 equivalent of mono-, di-, trivalent metal ions were also conducted and the results are shown in Figure 6C. The competition experiments showed that while Cu²⁺ ions cause interference, the detection of mercury ions is not interfered by Li⁺, Na⁺, Mg²⁺, Al³⁺, K⁺, Ca²⁺, Mn²⁺, Fe³⁺, Co²⁺, Ni²⁺, Zn²⁺, Sr²⁺, Cd²⁺, Ba²⁺, Hg²⁺ and Pb²⁺ ions. It is expected that the binding of the Hg²⁺ ion could take place through electrostatic interactions between the carbonyl oxygen of isatin and spirocycle, nitrogen atom of

the Schiff base and Hg²⁺ ions (Wanichacheva et al., 2012). Sensing features of the sensor toward Hg²⁺ were also investigated by ESI mass analysis in methanol. A mass peak corresponding rhodamine B methyl ester was observed at 456.98 m/z (Figure 7). The mass study showed that the sensor/mercury(II) complex is not stable at sensing media, and spirocycle undergoes Hg²⁺ mediated hydrolysis that resulted in the formation of rhodamine B methyl ester, as seen in Figure 8.

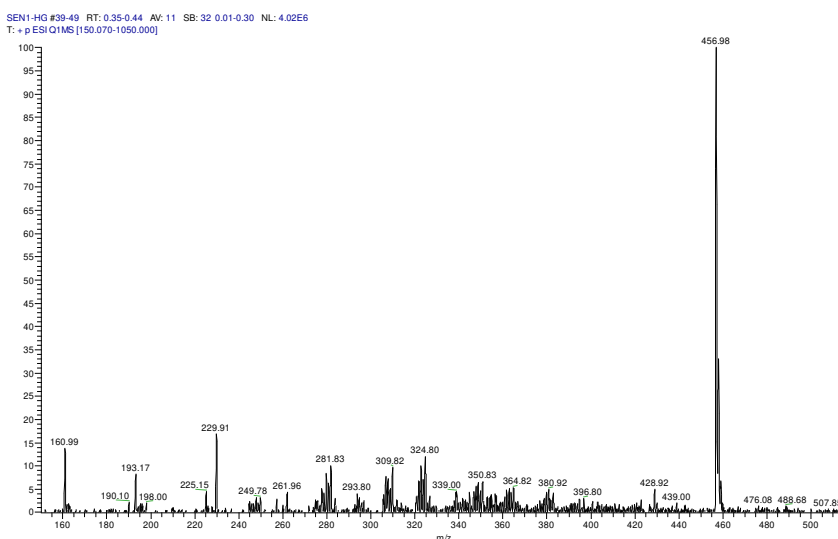


Figure 7. Mass spectra of rhodamine B methyl ester by Hg²⁺ mediated hydrolysis of the sensor.

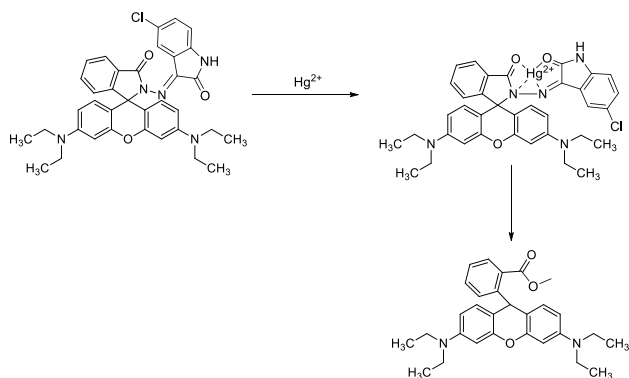


Figure 8. The proposed hydrolysis reaction of the sensor.

CONCLUSIONS

In summary, synthesis, characterization, and metal sensing properties of a new rhodamine B-based sensor have been reported. The metal ion detection ability of the sensor was investigated by colorimetric assays. The spectrophotometric experiments showed that the sensor enables selective and sensitive recognition of Hg²⁺ ion over competitive metal ions, such as Li⁺, Na⁺, Mg²⁺, Al³⁺, K⁺, Ca²⁺, Mn²⁺, Fe³⁺, Co²⁺, Ni²⁺, Cu²⁺, Zn²⁺, Sr²⁺, Cd²⁺, Ba²⁺ and Pb²⁺. The sensor showed linear optical response to Hg²⁺ ion between 1×10⁻⁵ to 1×10⁻⁴ M. LOD value for the sensor toward Hg²⁺ were calculated as and 7.88×10⁻⁸ M. According to mass data, optical signals could be produced by two step mechanism. First, Hg²⁺ ion coordinates to sensor through two carbonyl oxygen and nitrogen atom of imine bond and then complex structure undergoes Hg²⁺ ion mediated hydrolysis reaction.

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