Journal Pre-proof

Bithiophene-based fluorescent sensor for highly sensitive and ultrarapid detection of Hg2+ in water, seafood, urine and live cells



Chunpeng Li, Qingfen Niu, Jingui Wang, Tao Wei, Tianduo Li, Jianbin Chen, Xuyang Qin, Qingxin Yang

PII:	S1386-1425(20)30186-4					
DOI:	https://doi.org/10.1016/j.saa.2020.118208					
Reference:	SAA 118208					
To appear in:	Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy					
Received date:	25 December 2019					
Revised date:	27 February 2020					
Accepted date:	28 February 2020					

Please cite this article as: C. Li, Q. Niu, J. Wang, et al., Bithiophene-based fluorescent sensor for highly sensitive and ultrarapid detection of Hg2+ in water, seafood, urine and live cells, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*(2020), https://doi.org/10.1016/j.saa.2020.118208

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2020 Published by Elsevier.

Bithiophene-based fluorescent sensor for highly sensitive and ultrarapid detection of Hg²⁺ in water, seafood, urine and live cells

Chunpeng Li, Qingfen Niu*, Jingui Wang, Tao Wei, Tianduo Li*, Jianbin Chen, Xuyang

Qin, Qingxin Yang

Shandong Provincial Key Laboratory of Molecular Engineering, School of Chemistry and Pharmaceutical Engineering, Qilu University of Technology (Shandong Academy of Sciences), Jinan 250353, People's Republic of China

Abstract

Using Hg^{2+} -promoted deprotection reaction, we have developed a new fluorescent turn-on sensor **2TS** based on bithiophene fluorophore for Hg^{2+} detection. The sensing mechanism of **2TS** towards Hg^{2+} was strongly proved by ¹H NMR, FTIR, HRMS, UV-vis and fluorescence spectra. Remarkly, **2TS** towards Hg^{2+} in 100% aqueous solution shows high sensitivity with a low detection limit of 19 nM, superior selectivity and ultra-rapid response of 20 s during a wide sensing pH range from 4 to 10. Taking advantage of the excellent properties, the low-cost sensor **2TS**-based filter paper/TLC test strips were fabricated for visual, immediate and quantitative detection of Hg^{2+} in water, proving its applicability toward sensitive in-situ and on-site detection. Meanwhile, **2TS** showed high analytical performance for Hg^{2+} detection in water, seafood as well as human urine samples. Moreover, thanks to the good water solubility, negligible cytotoxicity, good biocompatibility and cell-membrane permeability, **2TS** was further applied to effectively image Hg^{2+} in live cells. Furthermore, the developed sensor **2TS** acted as good fluorescent display material for Hg^{2+} with obvious color change.

Keywords: Fluorescent sensor; Bithiophene; Hg²⁺; Cell image; Fluorescent display material.

* To whom the correspondence should be addressed E-mail: qf_niu1216@qlu.edu.cn; litianduo@163.com Telephone: +86(531)89631760 Fax number: +86(531)89631208

1. Introduction

On-site quantification and real-time sensing of Hg^{2+} is one of the hot topics of global concern because of its bioaccumulation, biomagnification, persistence in the environment and its high toxicity in the human body [1-6]. Mercury contaminations such as elemental mercury, inorganic mercury (Hg^{2+}) and organic methyl mercury are extensive and exist in numerous effluents. On the basis of the effects of bacteria in the environment, the elemental and ionic mercury can be easily converted into methyl mercury, and then bioaccumulates through the food chain [7-10], which generally causes neurotoxicity prenatal brain damage, DNA lesion, kidney failure, cognitive and motor disorders as well as Minamata disease [11-14]. Considering the extremely hazardous consequences of Hg²⁺, the World Health Organization (WHO) has set the maximum permissible level of mercury concentration in drinking water to be 6 ppb (0.19 µM). For the time being, highly selective and precise sensing of Hg^{2+} at ppb level is very important for environment protection and health care. Considering the important roles of Hg²⁺ in environmental and biological systems, therefore, developing a convenient, rapid and efficient method for sensitive and highly selective detection of Hg^{2+} at ppb level is extremely essential for human health.

Fluorescent sensors have been considerable interest due to their real-time and non-destructive testing, fast response, great sensitivity, high selectivity and wide applications [15–36]. Recently, many Hg²⁺-selective fluorescent sensors have been developed based on the coordination of O,S,N-heteroatom containing ligands [37–40]. However, most of them usually displayed poor selectivity on the basis of their reversible complexation and the interferences from other metal ions ($Fe^{2+}, Pb^{2+}, Ag^{+}, Ag^{+}$ Cd^{2+} , Cu^{2+} , and Fe^{3+}) or anions (CN^{-} , PO_4^{3-} , and Γ), as well as the other ligands (dithiouracil and ethylenediaminetetraacetic acid (EDTA)). Because the chemical reaction between the sensor based on the irreversible reaction and the target forms a substance that produces fluorescence or has a color change [41]. On the other hand, Hg^{2+} is well-known to act as a fluorescence quencher via enhanced spin-orbit coupling [42], a specific fluorescent sensor for Hg²⁺ was designed and synthesized by the sensing mechanism of complex induced fluorescence quenching [43,44], and only a few Hg^{2+} -selective sensors have been reported on the basis of fluorescence enhancement sensing mechanism [45–48]. To date, most of the reported reaction-based Hg²⁺-selective sensors showed some inevitable shortcomings such as poor water solubility [49], sluggish response [50], high detection limit [51], or even application performance deficiency. Thus, developing novel good water-soluble fluorescent "turn-on" sensors for highly selective,

sensitive and ultrarapid detection of Hg^{2+} in real samples associated with multifunctional practical applications is still a challenge and also is greatly needed.

Herein, by combing the strategies of rational design and screening, we fluorescent "turn-on" report novel reaction-based a sensor 5-(bis(ethylthio)methyl)-2,2'-bithiophene (**2TS**) for Hg^{2+} detection in 100% aqueous solution on the basis of Hg^{2+} -promoted deprotection of dithioacetal to give an aldehyde, which displayed great enhancement of fluorescence with distinct color change from colorless to blue fluorescence. Sensor 2TS showed many attractive advantages: (i) simple and one-step synthesis; (ii) ultra-rapid response (within 20 s) towards Hg^{2+} with high sensitivity (19 nM); (iii) superior selectivity for Hg^{2+} over other common metal ions; (iv) turn-on fluorescent detection of Hg²⁺ within wide pH sensing range (4.0–10.0); (v) good water solubility (100% aqueous solution), ignorable toxicity and good cell-membrane permeability. Based on these satisfactory features, we successfully used **2TS** for Hg^{2+} detection in environmental water, seafood, human urine, test strips and live cells. More importantly, owing to the good fluorometric behavior, the developed sensor **2TS** could be served as a fluorescent display material for conveniently detecting Hg^{2+} in water.

4

2. Experimental Section

2.1. Reagents and Apparatus

All chemicals and reagents in this study were purchased from Aldrich and Alfa Aesar, which were of analytical grade and used without further purification. Anhydrous dichloromethane was obtained using CaH₂. All aqueous solutions were prepared using deionized water. Deionized water was used throughout the test process. [2,2'-Bithiophene]-5-carbaldehyde (2T-CHO) was synthesized using the previous method [52]. NMR spectra were conducted using Bruker Avance 400 MHz instrument with tetramethylsilane (TMS) as an internal reference. A Bruker ALPHA FT-IR spectrometer was used to identify the functional group of the samples. An Agilent 6510 Accurate-Mass Q-TOF LC-MS spectrometer was used to measure the high resolution mass spectra (HRMS). A UV-Visible (UV-Vis) spectrophotometer (Shimadzu UV-2600) was used for the measurement of absorbance and a Hitachi F-4600 fluorescence spectrometer was used for all fluorescence measurements. A Model PHS-3C pH meter was used for the measurement of the pH. A microplate reader (MultiskanTM FC Microplate Photometer, Thermo Scientific, USA) was used to measure the absorbance values in a MTT assay. A Leica TCS SP8 confocal laser scanning microscope (CLSM) with a 63x magnification target oil lens was used to test the cellular fluorescence images.

2.2. Synthesis of sensor **2TS**

Under an atmosphere of argon, compound **2T-CHO** (50 mg, 0.25 mmol) and ethanethiol (38 mg, 0.61 mmol) were dissolved in dry dichloromethane (10 mL) with *p*-toluenesulfonamide (PTSA) (20 mg, 0.11 mmol) as the Lewis acid. After the reaction mixture was stirred for 5 h at room temperature, which was evaporated in vacuo, then the product was easily purified by column chromatography to give **2TS** as a pink oily liquid (47.50 mg, 66% yield). FTIR (KBr, cm⁻¹) v = 1045 (C-S-C), 1511 (C=C, thiophene ring); ¹H NMR (400 MHz, DMSO-d₆, ppm): δ = 7.41 (d, *J* = 4.0 Hz, 1H), 7.20 (d, *J* = 4.0 Hz, 1H), 7.02-6.98 (m, 3H), 5.43 (s, 1H), 2.59-2.48 (m, 4H), 1.11 (t, *J* = 8.0 Hz, 6H); ¹³C NMR (100 MHz, DMSO-d₆, ppm): δ = 145.2, 136.9, 128.9, 127.5, 126.1, 124.5, 123.4, 43.0, 26.3, 14.7; HRMS (ESI) m/z calcd for C₁₃H₁₅S₄ [M-H]⁻: 299.0135; Found 299.0047.

2.3. Cytotoxicity assays

The cell cytotoxicity assays were evaluated by the MTT assay. HeLa cells were firstly seeded into a 96-well plate with well growth medium containing 10% FCS and cultured for 24 h. The cells were then incubated with sensor **2TS** (5, 10, 15, 20, and 25 μ M) for 24 h at 37°C. The 10 μ L

MTT (5 mg/mL) was subsequently added into the each well, and when the HeLa cells were incubated for another 4 h, the absorbanceat 492 nm was recorded using a microplate reader (USA).

2.4. Fluorescence imaging

For fluorescence imaging, HeLa cells were incubated with 10 μ M **2TS** for 1 h. Next, after being washed 3 times with PBS buffer, HeLa cells were incubated with 10 μ M Hg²⁺ for 30 min. Finally, HeLa cells were further washed 3 times with PBS, and imaged using a CLSM under blue channel (430–470 nm) with an excitation at 405 nm.

3. Result and Discussion

3.1. Design and synthesis of **2TS**

As described in **Scheme 1**, sensor **2TS** was facilely synthesized by compound **2T-CHO** with ethanethiol in 66% overall yield. The whole synthetic route is quite simple and Post-treatment purification is easy. The structure of **2TS** was well characterized and strongly confirmed by NMR, FTIR and HRMS spectra (**Figs. S1–4**). Our design was based on the well-known deprotection reaction that bithiophene-based dithioacetal could easily be transformed into bithiophene aldhyde promoted with Hg^{2+} . Thus, this desulfurization reaction was conveniently used for the design of an efficient optical sensor for Hg^{2+} .

Scheme 1

3.2. Spectral responses of 2TS to Hg^{2+}

Firstly, in order to select the best test condition for sensing of Hg^{2+} , the UV-Vis absorption and fluorescence spectral responses of **2TS** to Hg^{2+} were studied. A series of solutions with different ratios between EtOH and H_2O with increasing water content (0~100%) were chosen for the test, and finally we found that the sensor **2TS** showed highly fluorimetric response for sensing Hg^{2+} in 100% aqueous solution (**Fig. S5**). However, with increasing water content, no obvious change was found in UV-Vis spectral response of **2TS** towards Hg^{2+} . Thus, the 100% aqueous solution is the best test condition for sensing of Hg^{2+} .

An important feature of **2TS** is the excellent selectivity towards Hg^{2+} . The UV-Vis absorption and fluorescence spectral response of **2TM** towards Hg^{2+} was investigated in 100% aqueous solution. As displayed in **Fig. 1a**, the **2TS** (10 μ M) showed a maximal absorption band at 334 nm, ascribing to the absorption of bithiophene moiety. The addition of 2.0 equiv. Hg^{2+} induced a drastic spectral change: the appearance of a new absorbance band at 370 nm with a large red-shift (36 nm), which substantiates that **2TS** reacted with Hg^{2+} to generate a new species. However, other tested ions (Ca²⁺, Cu²⁺, Co²⁺, Cd²⁺, Mg²⁺, Al³⁺, Cr³⁺, Fe³⁺, Fe²⁺, Ni²⁺, Zn²⁺, Pb²⁺, Na⁺, K⁺, Ag⁺, NO₂⁻, and ClO⁻) have no visible effect on the **2TS** in 100% aqueous media. As shown in **Fig. 1b**, **2TS** showed almost no fluorescence, and no noteworthy change was found when adding to other tested species. In particular, an instant fluorescence color change from colorless to blue and a great fluorescence enhancement at 470 nm were induced only by Hg²⁺. In addition, the Hg²⁺ led to the fluorescence quantum yield (Φ_f) of **2TS** increased significantly from 0.055 to 0.460 (**Table 1**). These results revealed that the method is very simple and low-cost in terms of the important feature of naked-eye observation, and also highlighted **2TS** was an ultra-selective fluorescent turn-on sensor for Hg²⁺.

Fig. 1

Table 1

To further investigate the interaction of **2TS** and Hg^{2+} , the optical spectra of **2TS** with various concentrations of Hg^{2+} (0–2.0 equiv.) were recorded in 100% aqueous solution. As can be clearly seen from **Fig. 2a**, by treatment with Hg^{2+} , the absorption peak located at 334 nm was gradually

reduced and a new absorption appeared at 370 nm was gradually increased. The ratio (A_{370}/A_{334}) of the absorbance at two spectral peaks shows a gradual enhancement with the increased concentrations of Hg^{2+} , and the absorbance ratio (A_{370}/A_{334}) of **2TS** reaches a saturation state when the concentration of Hg^{2+} is above 10 μ M (Fig. S6). A clear well-formed isobestic point at 355 nm of the Hg²⁺-elicited absorbance changes suggested the formation of the new species by the deprotection reaction of **2TS** and Hg^{2+} . As shown in **Fig. 2b**, the fluorescence titration of **2TS** with Hg^{2+} (0–2.0 equiv.) results in a gradual enhancement of the emission at 470 nm. The emission intensity at 470 nm experienced a great change with 10-fold emission enhancement with increasing Hg²⁺ concentration and arrived at the platform after treatment with Hg^{2+} over 10 μ M (Fig. 2c). The distinct fluorescent enhancement signal could be ascribed to the formation of new species (compound 2T-CHO) via Hg²⁺-promoted dethioacetalization on 2TS, which induced the intramolecular charge transfer (ICT) process from bithiophene moiety to aldehyde group switched on. A preferable linearity ($R^2 = 0.99336$) between the Hg²⁺ concentration and fluorescence intensity at 470 nm was observed with sensitive solution color changes from colorless to blue under UV lamp, indicating that **2TS** was a Hg²⁺-specific fluorescent "turn-on" sensor and could detect Hg²⁺ both quantitatively and qualitatively. The detection limit (DL) of **2TS** is determined to be 19 nM (DL = $3\sigma/k$). Such a low DL indicated that **2TS** owns high sensitivity for Hg^{2+} detection in environmental and biological systems.

Fig. 2

3.3. Mechanism of sensing

To explore the mechanism, ¹H NMR titration was firstly conducted in DMSO-d₆ in Fig. 3. When adding 1.0 equiv. of Hg^{2+} to the 2TS (10 μ M), the methane characteristic proton signals (H_a, H_c and H_b) were observed at 5.43 and 2.53–1.16 ppm accompanied with the disappearance of methylene protons of the thioacetal group. In addition, the occurance of the -CHO signal (Ha₁) was also observed at 9.80 ppm, indicating that the successful deprotection reaction of **2TS** promoted by Hg²⁺. What is more, the spectral similarity to that of compound **2T-CHO** further confirmed the production of **2T-CHO** from the reaction between **2TS** and Hg^{2+} . Subsequently, the reaction product of the **2TS** with Hg^{2+} was also proved by FTIR spectra (**Fig. S7**). The FTIR spectrum of **2TS** treated with Hg^{2+} shows that the saturated hydrocarbon (CH) characteristic peak at 2917 cm⁻¹ disappeared, accompanied by a new typical and outstanding tensile absorption peak at 1660 cm⁻¹, which corresponded to an aldehyde group (C=O). Meanwhile,

the characteristic stretching bands at 3281 and 2820 cm^{-1} for the unsaturated aldehyde hydrogen (H-C=O) group occurred, which has a similar signal patterns as the compound **2T-CHO**, suggesting that the desulfurization reaction has taken place and successfully converted to aldehyde. The HRMS spectrum showed that a new strong peak at m/z = 194.9936 was ascribed to the product **2T-CHO** $[2T-CHO + H]^+$ (194.9860) (Fig. S8). Additionally, in the presence of 1.0 equiv. of Hg^{2+} , the optical spectra of **2TS** along with a distinct fluorescence color change are the same as that of 2T-CHO (Figs. S9, S10), strongly demonstrated the successful deprotection reaction of 2TS promoted by Hg^{2+} [53–56]. The alkyl thiols show high affinity toward Hg^{2+} $(\log K_a = 22.1)$ [57], and the thioacetal in **2TS** can be successfully deprotected in the presence of Hg^{2+} . Therefore, the huge changes highlighted that the **2TS** for selectively sensing Hg^{2+} is on the basis of Hg^{2+} -induced desulfurization reaction, causing the typical ICT on and thus resulted in a great fluorescence enhancement (Scheme 2).

Fig. 3

Scheme 2

3.4. Anti-interference of **2TS** for detecting Hg^{2+}

The selectivity and anti-interference ability of a fluorescent sensor is important for its practical application. As shown in **Fig. 4**, upon mixing with representative tested ions, the fluorescence intensity at 470 nm of **2TS** (10 μ M) is almost unaltered. However, subsequent addition of 2.0 equiv. Hg²⁺ resulted in a prominent fluorescence enhancement, revealing that the fluorescence turn-on signal response induced by Hg²⁺ is not interfered. All results indicated that **2TS** has an excellent anti-interference ability and can be acted as an ultra-selective-Hg²⁺ fluorescent sensor.

Fig. 4

3.5. pH effects and response time studies

It is quite significant to ensure that a designed sensor is suitable for testing withnin the physiological pH range, thus, the pH-dependent experiment was carried out (**Fig. 5a**). No dramatic change in the emission intensity at 470 nm of **2TS** was observed over the pH range 1.0–13.0, indicating that the fluorescence properties of **2TS** was stable at a wide pH range. Upon treatment with Hg^{2+} (2.0 equiv.), the fluorescence emission intensity at 470 nm dramatically enhanced within the pH range (4.0–10.0),

Journal Pre-proof

which covered the physiological pH range perfectly, revealing 2TS could act as a turn-on fluorescent sensor for Hg^{2+} under physiological pH conditions.

In additon, short response time is another vital condition for **2TS** to dynamically detect Hg^{2+} in actual samples. Therefore, the time-dependent fluorescence response of **2TS** to Hg^{2+} (2.0 equiv.) was examined [54]. Surprisingly, the **2TS** shows a quick fluorescence response to Hg^{2+} . The emission intensity at 470 nm increased instantly as the time prolong and reached a plateau within 20 s, and then remained stable (**Fig. 5b**). The results show that **2TS** has great potential in application for Hg^{2+} in environmental and biological systems.

Fig. 5

3.6. Practical applications of 2TS

3.6.1. Application in water, seafood and human urine samples

The usefulness of **2TS** for recognizing Hg^{2+} in practical water, seafood and human urine samples for quantitative detection was firstly evaluated using a standard addition method, and the analytical results obtained as summerized in **Table 2**. The **2TS** (10 µM) was added to the these samples and then two concentrations of Hg^{2+} (5.0/10.0 µM) were added, respectively. The results obtained in these real samples were satisfactory with good percentage fluorescent recoveries and low relative standard deviation (RSD) values, indicating the evident practicability of **2TS** in environmental water, seafood and biological samples.

Table 2

3.6.2. Visual detection on test strips and used as fluorescent display material

Based on the outstanding fluorescent color change of 2TS after Hg^{2+} 100% aqueous with in solution, a colorimetric treament paper/TLC-based test strips were subsequently prepared to further explore its practical application. Primitively, we immersed the test strips into the **2TS** (1.0 mM) EtOH solution and then completely dried in air. Next, the **2TS**-loaded test strip was immersed into an aqueous solution containing an increased amount of Hg²⁺ for several minutes, a significant visual color change was detected under 365 nm UV lamp (Fig. S11), suggesting the **2TS**-based detection kit can effectively detect trace amounts of Hg^{2+} in water with the naked eye sensitivity. In addition, the Fig. S12 displayed the fluorescent patterns and characteristics on the filter paper under 365 nm UV

light, indicating that the **2TS** served as a good fluorescent display material for conveniently sensing Hg^{2+} in 100% aqueous solution.

3.6.3. Application in bio-imaging

To study the applicability of **2TS** in honeycomb imaging, the cell-imaging experiments were done. Firstly, the MTT assay proved that the cell viability after 24 hours was over 90% (**Fig. S13**), meaning the neglected cytotoxicity and good biocompatibility of **2TS**. Subsequently, the HeLa cells incubated with **2TS** (10 μ M) alone for 1 h did not show any fluorescence (**Fig. 6a,b**). To prove the high sensitivity of **2TS** towards Hg²⁺ in HeLa cells, Hg²⁺ (10 μ M) was added to the HeLa cells and co-incubated with 10 μ M of **2TS**, no fluorescence was initially detected (**Fig. 6c**). After incubation of **2TS**-loaded HeLa cells with Hg²⁺ for 30 and 60 min, it can be clearly noticed that blue fluorescence and a stronger blue fluorescence were respectively imaged (**Fig. 6d,e**). These observations implied that **2TS** has outstanding cell-membrane permeability and thus was capable of imaging Hg²⁺ in living cells.

Fig. 6

3.7. Comparison of **2TS** with other selective Hg^{2+} sensors

Compared with other reported Hg^{2+} -sensitive fluorescent sensors [54,58–65], our designed sensor **2TS** shows obvious advantages including (a) simple and one-step synthesis procedure with low cost; (b) ultra-rapid response (within 20 s) towards Hg^{2+} with high sensitivity (19 nM); (c) superior selectivity for Hg^{2+} over other tested ions; (d) turn-on fluorescent detection of Hg^{2+} with distinct color change in a wide sensing pH range (4–10); (e) good water solubility in 100% aqueous solution, ignorable cytotoxicity, good cell-membrane permeability and biocompatibility; (f) good fluorescent display for Hg^{2+} with obvious and instant color change; (g) high application performances for Hg^{2+} detection in environmental water, seafood and human urine samples, test strips and the fluorescence imaging in living cells (**Table S1**).

4. Conclusions

In summary, a new bithiophene-based water-soluble fluorescent turn-on sensor **2TS** for Hg²⁺ was developed. The low cost sensor **2TS** exhibits high sensitivity and excellent selectivity to Hg²⁺ in a suitable working pH range. The **2TS**-based filter paper/TLC test strip was fabricated and used for ultra-rapid and quantitative on-site detection of Hg²⁺. Interestingly, **2TS** could serve as a good fluorescent display material for detecting Hg²⁺. In addition, the **2TS** was successfully applied to detect Hg²⁺ in real water, seafood and

Journal Pre-proof

human urine samples with satisfactory results. More importantly, in terms of its excellent features, the **2TS** was used successfully to image Hg^{2+} in HeLa cells. Based on these investigations, **2TS** could be very useful as a powerful molecular tool for ultrafast, ultra-selective and highly sensitive detection of Hg^{2+} in both environmental and biological systems.

Acknowledgments

We greatly appreciate financial support from the National Natural Science Foundation of China (21776143, 21376125, 51702178 and 21801144), the Natural Science Foundation of Shandong Province (ZR2017LB009), the Program for College Students' Innovation and Entrepreneurship Training of Shandong Province (S201910431035), the Youth Innovative Talents Recruitment and Cultivation Program of Shandong Higher Education, and the Program for Scientific Research and the Program for Scientific Research Innovation Team in Colleges and Universities of Shandong Province.

References

- [1] D.W. Boening, Chemosphere 12 (2000) 1335–1351.
- [2] A.P. de Silva, H.Q.N. Gunaratne, T. Gunnlaugsson, A.J.M. Huxley, C.P. McCoy, J.T. Rademacher, T.E. Rice, Chem. Rev. 97 (1997) 1515–1566.
 [3] G.P. Guzzi, C.A.M.L. Porta, Toxicology 244 (2008) 1–12.
- [4] J.S. Kim, D.T. Quang, Chem. Rev. 107 (2007) 3780–3799.

- [5] S.K. Kim, S.H. Lee, J.Y. Lee, J.Y. Lee, R.A. Bartsch, J.S. Kim, J. Am. Chem. Soc. 126 (2004) 16499–16506.
- [6] I. Onyido, A.R. Norris, E. Buncel, Chem. Rev. 104 (2004) 5911–5929.
- [7] J. Gasparik, D. Vladarova, M. Capcarova, P. Smehyl, J. Slamecka, P. Garaj, R. Stawarz, P. Massanyi, Part A: Toxic/Hazard. Subst. Environ. Eng. 45 (2010) 818–823.
- [8] E.M. Nolan, S.J. Lippard, Chem. Rev. 108 (2008) 3443–3480.
- [9] A. Renzoni, F. Zino, E. Franchi, Environ. Res. 77 (1998) 68–72.
- [10] H.H. Harris, I.J. Pickering, G.N. George, Science 301 (2003) 10 1203–1203.
- [11] S.A. Counter, L.H. Buchanan, Toxicol. Appl. Pharm. 198 (2004) 209–230.
- [12] M. Gochfeld, Ecotox. Environ. Safe. 56 (2003) 174–179.
- [13] C.M.L. Carvalho, E.H. Chew, S.I. Hashemy, J.Lu, A. Holmgren, J. Biol. Chem. 283 (2008) 11913–11925.
- [14] T.W. Clarkson, L. Magos, G.J. Myers, New Engl. J. Med. 349 (2003)1731–1737.
- [15] T. Ueno, T. Nagano, Nat. Methods. 8 (2011) 642–645.
- [16] Y.M. Yang, Q. Zhao, W. Feng, F.Y. Li, Chem. Rev. 113 (2013) 192–270.
- [17] Q.Q. Wan, S.M. Chen, W. Shi, L.H. Li, H.M. Ma, Angew. Chem. Int.Ed. 53 (2014) 10916–10920.

- [18] H. Zhu, J.L. Fan, J.J. Du, X.J. Peng, Acc. Chem. Res. 490 (2016) 2115–2126.
- [19] Z. Guo, Q. Niu, T. Li, Spectrochim. Acta A 200 (2018) 76–84.
- [20] Y. Li, Q. Niu, T. Wei, T. Li, Anal. Chim. Acta 1049 (2019) 196–212.
- [21] Q. Niu, T. Sun, T. Li, Z. Guo, H. Pang, Sens. Actuators B 266 (2018) 730–743.
- [22] Z. Guo, T. Hu, T. Sun, T. Li, H. Chi, Q. Niu, Dyes Pigm. 163 (2019) 667–674.
- [23] Z. Guo, Q. Niu, Q. Yang, T. Li, H. Chi, Anal. Chim. Acta 1065 (2019) 113–123.
- [24] L. Lan, T. Li, T. Wei, H. Pang, T. Sun, E. Wang, H. Liu, Q. Niu, Spectrochim. Acta A 193 (2018) 289–296.
- [25] X. Wu, Q. Niu, T. Li, Y. Cui, S. Zhang, J. Lumin. 175 (2016) 182–186.
- [26] Q. Niu, L. Lan, T. Li, Z. Guo, T. Jiang, Z. Zhao, Z. Feng, J. Xi, Sens. Actuators B 276 (2018) 13–22.
- [27] T. Sun, Q. Niu, Y. Li, T. Li, T. Hu, E. Wang, H. Liu, Sens. Actuators B 258 (2018) 64–71.
- [28] Z. Guo, Q. Niu, T. Li, T. Sun, H. Chi, Spectrochim. Acta A 213 (2019) 97–103.
- [29] T. Sun, Y. Li, Q. Niu, T. Li, Y. Liu, Spectrochim. Acta A 195 (2018) 142–147.

- [30] T. Sun, Q. Niu, T. Li, Z. Guo, H. Liu, Spectrochim. Acta A 188 (2018)411–417.
- [31] S. Zhang, T. Sun, D. Xiao, F. Yuan, T. Li, E. Wang, H. Liu, Q. Niu, Spectrochim. Acta A 189 (2018) 594–600.
- [32] Z. Zuo, X. Song, D. Guo, Z. Guo, Q. Niu, J. Photoch. Photobio. A 382 (2019) 111876.
- [33] J. Wang, T. Wei, F. Ma, T. Li, Q. Niu, J. Photoch. Photobio. A 383 (2019) 111982.
- [34] Z. Guo, T. Hu, X. Wang, T. Sun, T. Li, Q. Niu, J. Photoch. Photobio. A 371 (2019) 50–58.
- [35] Z. Guo, Q. Niu, T. Li, E. Wang, Tetrahedron 75 (2019) 3982–3992.
- [36] P. Yin, Q. Niu, T. Wei, T. Li, Y. Li, Q. Yang, J. Photoch. Photobio. A 389 (2020) 112249.
- [37] A. Aliberti, P. Vaiano, A. Caporale, M. Consales, M. Ruvo, A. Cusano, Sens. Actuators B 247 (2017) 727–735.
- [38] I. Kim, N.E. Lee, Y.J. Jeong, Y.H. Chung, B.K. Cho, E. Lee, Chem. Commun. 50 (2014) 14006–14009.
- [39] S. Zhang, Q. Niu, L. Lan, T. Li, Sens. Actuators B 240 (2017) 793–800.
- [40] L.N. Neupane, E.T. Oh, H.J. Park, K.H. Lee, Anal. Chem. 88 (2016)3333–3340.
- [41] M.E. Jun, B. Roy, K.H. Ahn, Chem. Commun. 47 (2011) 7583–7601.

- [42] A.B. Descalzo, R. Martinez-Manez, R. Radeglia, K. Rurack, J. Soto, J. Am. Chem. Soc. 125 (2003) 3418–3419.
- [43] Y. Zhao, Z. Zhong, J. Am. Chem. Soc. 128 (2006) 9988–9989.
- [44] Q. Niu, X. Wu, S. Zhang, T. Li, Y. Cui, X Li, Spectrochim. Acta Part A 153 (2016) 143–146.
- [45] E.M. Nolan, S.J. Lippard, J. Am. Chem. Soc. 125 (2003) 14270–14271.
- [46] A. Caballero, R. Martines, V. Lloveras, I. Ratera, J. Vidal-Gancedo, K. Wurst, A. Tarraga, P. Molina, J. Veciana, J. Am. Chem. Soc. 127 (2005) 15666–15667.
- [47] S. Qiu, Q. Niu, T. Sun, T. Li, Tetrahedron Lett. 57 (2016) 4297–4301.
- [48] L. Lan, Q. Niu, T. Li, Anal. Chim. Acta. 1023 (2018) 105–114.
- [49] Y. Zhou, X.F. He, H. Chen, Y. Wang, S.Z. Xiao, N.N. Zhang, D.J. Li, K.B. Zheng, Sens. Actuators B 247 (2017) 626–631.
- [50] M.M. Hong, X.Y. Lu, Y.H. Chen, D.M. Xu, Sens. Actuators B 232 (2016) 28–36.
- [51] C.J. Wu, J.B. Wang, J.J. Shen, C. Bi, H.W. Zhou, Sens. Actuators B 242 (2017) 678–683.
- [52] C.G. Wu, M.F. Chung, H.H. G. Tsai, C.J. Tan, S.C. Chen, C.H. Chang,T.W. Shih, ChemPlusChem 77 (2012) 832–843.
- [53] B. Gu, L. Huang, W. Su, X. Duan, H. Li, S. Yao, Anal. Chim. Acta 954 (2017) 97–104.

- [54] P. Yin, Q. Niu, Q. Yang, L. Lan, T. Li, Tetrahedron 75 (2019) 130687.
- [55] X. Cheng, Q. Li, J. Qin, Z. Li, ACS Appl. Mater. Inter. 2 (2010) 1066–1072.
- [56] Z. Ruan, Y. Shan, Y. Gong, C. Wang, F. Ye, Y. Qiu, Z. Liang, Z. Li, J. Mater. Chem. C 6 (2018) 773–780.
- [57] M. Ravichandran, Chemosphere 55 (2004) 319–331.
- [58] S. Malkondu, S. Erdemir, Dyes Pigm. 113 (2015) 763–769.
- [59] W.C. Lin, C.Y. Wu, Z.H. Liu, C.Y. Lin, Y.P. Yen, Talanta 81 (2010) 1209–1215.
- [60] A.L. Luo, Y.J. Gong, Y. Yuan, J. Zhang, C.C. Zhang, X.B. Zhang, W. Tan, Talanta 117 (2013) 326–332.
- [61] C. Wu, J. Wang, J. Shen, C. Bi, H. Zhou, Sens. Actuators B 243 (2017) 678–683.
- [62] H. Wang1, P. Zhang, J. Che, Y. Li, M.L. Yu, Y.F. Long, P.G. YiKey, Sens. Actuators B 242 (2017) 818–824.
- [63] L.Y. Zong, Y.J. Xie, Q.Q. Li, Z. Li, Sens. Actuators B 238 (2017) 735–743.
- [64] C.G. Chen, N.J. Vijay, N. Thirumalaivasan, S. Velmathi, S.P. Wu, Spectrochim. Acta. A 219 (2019) 135–140.
- [65] M. Hong, S. Lu, F. Lv, D. Xu, Dyes Pigm. 127 (2016) 94–99.

Figure and Table captions

Scheme 1. The synthesis of sensor 2TS.

Fig. 1. Absorption (**a**) and fluorescence (**b**) spectra and of **2TS** (10 μ M) after addition of 20 μ M of various tested ions in 100% aqueous solution; **Inset:** Fluorimetric responses of **2TS** (10 μ M) in 100% aqueous solution after the addition of various tested ions.

Fig. 2. The absorption (**a**) and emission (**b**) spectral changes of **2TS** (10 μ M) exposed to various concentrations of Hg²⁺ (0–2.0 equiv.) in 100% aqueous solution; (**c**) Fluorescence intensity of **2TS** as gradual addition of Hg²⁺ (0–2.0 equiv.); **Inset:** Fluorescence images of **2TS** treated with [Hg²⁺].

Fig. 3. ¹H NMR spectra of **2TS**, **2TS** upon the addition of Hg^{2+} (**2TS+Hg^{2+}**) and **2T-CHO** in DMSO-d₆.

Scheme 2. Proposed complexation model of 2TS with Hg^{2+} .

Fig. 4. Fluorescence intensity of 2TS (10 μ M) exposed to various tested ions (20 μ M) and to the mixture of Hg²⁺ (20 μ M) in 100% aqueous solution.

Fig. 5. The pH (a) and time (b) effects on fluorescence intensity of 2TS (10 μ M) with Hg²⁺ (20 μ M) in 100% aqueous solution.

Fig. 6. Fluorescence confocal images of HeLa cells. (**a**) Fluorescent confocal imaging of HeLa cells with **2TS** for 60 min; (**b**) Bright-field image; (**c**) Fluorescent confocal imaging of HeLa cells co-incubated with **2TS** and Hg²⁺ immediately, (**d**) for 30 min; (**e**) for 60 min.

Table 1. Photophysical parameters of 2TS and 2TS-Hg²⁺ complex.

Table 2. Determination of Hg^{2+} in water, seafood and human urine samples by the **2TS**-based proposed method.

Surral Reality

Scheme 1.



Fig. 1(a)



Fig. 1b







Journal Pre-proof



Table 1

Compound	$\lambda_{ex}(nm)$	Stokes shift (nm)	λ_{em} (nm)	$\Phi^a{}_{ m f}$
2TS	334	136	470	0.055
$2TS+Hg^{2+}$	370	100	470	0.460

^aDetermined by using quinine sulfate in 0.1 M $H_2SO_4(\Phi = 0.55)$ as standard reference.

Fig. 3



Scheme 2



ż

Fig. 5



Sample	Added (µM)	Detect ($\overline{x} \pm SD$)	Recovery (%)	Relative error	
		(µM)		(%)	KSD (%)
Tap water	5.0	4.87±0.14	97.4	2.6	2.8
	10.0	10.22±0.19	102.2	2.2	1.8
River water	5.0	5.05 ±0.11	101	1	2.2
	10.0	9.77±0.29	97.7	2.3	2.9
Distilled Water	5.0	4.85±0.13	97.0	3.0	2.6
	10.0	10.38±0.14	103.8	3.8	1.4
Lake Water of Ji'nan	5.0	5.14±0.15	102.8	2.8	2.9
Garden Expo	10.0	10.22±0.10	102.2	2.2	0.9
Urino	5.0	4.94 ± 0.09	98.8	1.2	1.95
UTille	10.0	9.94±0.07	99.4	0.6	0.74
Fish	5.0	4.91±0.13	98.2	1.8	2.6
	10.0	9.85±0.15	98.5	1.5	1.5
Procambarus clarkii	5.0	4.88±0.18	97.6	2.4	4.3
	10.0	10.13±0.15	101.3	1.3	1.5
kelp	5.0	4.94±0.13	98.8	1.2	2.6
	10.0	9.76±0.16	97.6	2.4	1.6

Table 2

Fig. 6



25 µn

Author contributions

Chunpeng Li: Conceptualization, Data curation, Software, Investigation,

Writing-Original Draft.

Qingxin Yang: Validation, Formal analysis, Visualization.

Qingfen Niu: Resources, Writing-Review & Editing, Supervision, Data Curation.

Jingui Wang: Formal analysis, Review & Editing.

Tao Wei: Formal analysis, Review & Editing.

Tianduo Li: Resources, Formal analysis, Supervision, Review & Editing.

Jianbin Chen: Review & Editing.

Xuyang Qin: Review & Editing.

Declaration of interests

 \Box The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

⊠ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Sonta

Graphical Abstract



Highlights

A new turn-on fluorescent sensor **2TS** for Hg^{2+} in 100% aqueous solution was developed.

► 2TS features good selectivity, ultra-sensitivity, ultra-rapid response towards Hg^{2+} in a wide sensing pH range.

▶ 2TS was used to detect Hg^{2+} in water, seafood, human urine, test strips and living cells.

► 2TS was used as fluorescent display material for conveniently detecting Hg^{2+} .