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# An Indolizine-derived Chemodosimeter with Enhanced Emission in Micellar Environment for ppb-level Detection of Mercury Ions

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**Abstract:** Mercury is a harmful heavy metal that gravely threatens the environment and organisms. In the current study, probes 2-(1,3-oxathiolan-2-yl)-1,3-diphenylindolizine (**DPIC-OS**) and 2-(1,3-dithiolan-2-yl)-1,3-diphenylindolizine (**DPIC-SS**) are designed by protecting a 1,3-diphenylindolizine-2-carbaldehyde (**DPIC-CHO**) with a mercury-recognizable 1,3-dithiolane and 1,3-oxathiolane moieties and utilized for the selective detection of Hg<sup>2+</sup> ions in micellar medium. Hydrophobic probes **DPIC-OS** and **DPIC-SS** exhibit intense fluorescence in the confined environment of micellar solution. A significant enhancement in fluorescence intensity was seen for these probes upon switching from organic to aqueous micellar media. Upon the incremental addition of Hg<sup>2+</sup> ions, it displays a shift with a gradual increase, exhibiting a bluish-green emission at 505 nm through the release of fluorescent **DPIC-CHO** in the working solution by the spontaneous cleavage of thioacetal linkage. **DPIC-OS** was more efficient than **DPIC-SS**, and further analytical studies were conducted with this probe. The **DPIC-OS** displayed no or insignificant response towards numerous common anions, cations, and small molecules, affirming its selectivity to Hg<sup>2+</sup> ions and offering a low limit of detection (LOD) of 3.2 ppb (16.2 nM). The real sample analysis by spiking mercury ions in water showed excellent percent recoveries.

## 1. Introduction

The presence of toxic metal ions like mercury, lead, cadmium, chromium, and arsenic in the environment is a major source of various human health disorders.<sup>1</sup> Of these, mercury is notably one of the most toxic heavy metals that are abundantly distributed in air, soil, and water.<sup>2,3</sup> Mercury emissions are primarily associated with anthropogenic activities, such as oil refining, coal and gold mining, and chemical manufacturing, which together cause severe ecological damage.4-6 Once released into the environment, mercury undergoes chemical transformations that enhance its bioaccumulation in aquatic ecosystems, especially in fish and seafood, thereby increasing the intake by humans through dietary consumption.<sup>7–9</sup> Human exposure to mercury due to contaminated food or industrial activities results in severe health complications like neurological disorders, renal failure, cardiovascular disease, impaired cognitive and motor functions, etc.<sup>10-13</sup> These issues are specifically prevalent in highly populated countries such as India, where water-based food items form an integral part of dietary practices.14 Indian aquatic systems, including coastal waters, have shown significant mercury contamination according to environmental studies, as elevated levels of mercury are present in sediments and suspended

particulates.<sup>15-17</sup> Due to the severe toxicity associated with mercury, regulatory agencies such as the U.S. Environmental Protection Agency (USEPA) have implemented a maximum allowable mercury concentration of 2 ppb in drinking water, whereas, the World Health Organization (WHO) permits up to 50 ppb in agricultural soils, which underscores the urgent need for advanced analytical methods capable of detecting mercury ions with high sensitivity and selectivity.<sup>18,19</sup> Over the years, significant progress has been made in the development of analytical techniques for mercury detection, ranging from conventional methods to emerging technologies, such as atomic absorption spectroscopy (AAS),<sup>20</sup> cold vapor atomic fluorescence spectrometry,<sup>21</sup> inductively coupled plasma mass spectrometry (ICP-MS),<sup>22</sup> electrochemical,<sup>23</sup> and anodic stripping voltammetry (ASV).<sup>24</sup> While these methods offer high precision and quantitative detection capabilities, they are often associated with significant limitations like expensive instrumentation, skilled operators, and extensive sample pretreatment, which collectively restrict their accessibility and practical applications in routine monitoring scenarios.

Colorimetric and fluorimetric methods have gained widespread recognition for their simplicity, selectivity, and high sensitivity, making them invaluable tools across diverse fields such as environmental monitoring, analytical chemistry, biology, and medicine.<sup>25,36</sup> Recent advancements have leveraged both Hg-ligand complexes and Hg-induced irreversible chemical reactions to detect Hg<sup>2+</sup> in biological and environmental systems.<sup>27,28</sup> Among these, chelation-based fluorescent probes have proven particularly effective, often employing an on-off fluorescence mechanism in which Hg<sup>2+</sup> quenches fluorescence by enhancing spin-orbit coupling upon analyte binding.<sup>29,30</sup> The

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59 60 strong thiophilic affinity of Hg<sup>2+</sup> has facilitated the development of chemodosimeters incorporating thiourea,<sup>31</sup> thiones,<sup>28</sup> benzothiazole,<sup>32</sup> and thioacetal receptors,<sup>33-37</sup> yielding high sensitivity and robust signal-to-noise ratios for Hg<sup>2+</sup> detection. However, a notable limitation of these small hydrophobic chemosensors is the requirement of some amount of organic solvents in aqueous-organic mixtures during spectroscopic analysis, which greatly compromises the biocompatibility of these sensing systems and often leads to reduced fluorescence intensity.<sup>38–40</sup> Furthermore, as mercury detection is primarily done in aqueous medium, the use of organic solvents proves detrimental to the detection sensitivity of these probes.<sup>41,42</sup> A probable solution to the insolubility of hydrophobic chemosensors lies in nature, which uses only aqueous media for any chemical transformations or biological functions. The use of amphiphilic surfactants in water not only forms micellar nonentities to encapsulate hydrophobic fluorophores,<sup>43-45,</sup> but enhances their fluorescence intensity within the nanoconfined space in a synergistic manner.<sup>46</sup> Continuing our recent work on mercury detection in aqueous systems,<sup>47,48</sup> we focused on developing an indolizine-based chemodosimeter for selective and sensitive detection of mercury ions. The indolizine scaffold offers considerable advantages due to its synthetic versatility, enabling photophysical tunability and large Stokes shifts, which are crucial for chemosensing applications.<sup>49,50</sup> To this end, we designed DPIC-OS and DPIC-SS to specifically target Hg<sup>2+</sup> ions with high efficiency and selectivity. Exploiting the high thiophilic affinity of mercury. The nonpolar hydrophilic nature of DPIC-OS and DPIC-SS limits its solubility in aqueous media, restricting its effectiveness for mercury detection in water. However, a surfactant CTAB-derived microenvironment could overcome this difficulty of the probe.

#### **Results and discussion**

#### 2.1 Design and synthesis

In our endeavor to develop a chemosensor for mercury ions from a barely explored fluorescent indolizine scaffold, we envisaged utilizing the thiophilic nature of mercury. Towards this objective, two probes, DPIC-OS and DPIC-SS, were synthesized via a two-step process. Firstly, DPIC-CHO was prepared from the cycloaddition of cinnamaldehyde and 2benzoylpyridine using a well-reported procedure (Scheme S1, ESI<sup>T</sup>).<sup>51</sup> Following that, DPIC-CHO was treated with 2mercaptoethan-1-ol and ethane-1,2-dithiol separately in the presence of BF<sub>3</sub>.OEt<sub>2</sub>, in a typical protection reaction, to obtain **DPIC-OS** and **DPIC-SS**, respectively (Scheme 1, Scheme S2, ESI<sup>T</sup>). The formation of the probes was characterized by NMR and mass spectrometry. The presence of a peak at  $\delta$  10.01 ppm in <sup>1</sup>H NMR and a peak at  $\delta$  188.0 ppm in <sup>13</sup>C NMR confirms the formation of DPIC-CHO. For DPIC-OS and DPIC-SS, the omission of the signature peaks of the -CHO group indicated the formation of thioacetyl products (DPIC-OS and DPIC-SS). Additionally, the presence of new peaks at  $\delta$  2.5-3.0 ppm in  $^1\text{H}$ NMR and  $\delta$  35-50 ppm in  $^{13}\text{C}$  NMR further confirms the incorporation of the dithiol.



Scheme 1: General procedure for synthesizing the probe DPIC-SS and DPIC-OS.

and mecraptoethanol moieties. The mass spectra of **DPIC-OS** and **DPIC-SS** displayed expected peaks at m/z 358 and 374 corresponding to their [M+H]<sup>+</sup>, explicitly confirming the formation of the desired probes.

# 2.2 Photophysical properties and optimization of the sensing assembly

To establish a sensing system for mercury ions, the photophysical properties of three compounds, DPIC-CHO, DPIC-**OS**, and **DPIC-SS**, were evaluated via UV-Vis and fluorescence spectroscopy. In UV-Vis studies, DPIC-CHO exhibited a prominent absorption band at 400 nm, while DPIC-OS and DPIC-SS displayed absorption bands at 290 nm with a shoulder at 380 nm in organic solvents (Figure S1a,b,c, ESI<sup>T</sup>). Upon excitation at 400 nm, **DPIC-CHO** exhibited a green fluorescence with a  $\lambda_{max}$ at 505 nm, while the protected probes displayed fluorescence intensity at a slightly lower wavelength (at  $\lambda_{max}$  455 nm) (Figure S1d,e,f, ESI<sup>1</sup>). To gain a deeper insight into the optical properties of DPIC-OS and DPIC-SS, a preliminary solvent study was performed to assess their absorbance and emission behavior in various organic solvents (Table S1, ESI<sup>1</sup>). Among organic solvents, both probes DPIC-OS and DPIC-SS displayed better emission properties in DMSO with a broad peak at around 455 nm with quantum yields of 36% and 39%, respectively, taking quinine sulfate as standard, while other solvents displayed lower quantum yields.52 Mercury ion contamination is largely prevalent in water bodies, particularly sea water; therefore, pure aqueous media is ideal for the detection of Hg<sup>2+</sup> ions. As DPIC-OS and DPIC-SS are insoluble in water an organic-aqueous mixed medium was initially chosen for the detection of mercury. Therefore, stock solutions of the probes (1 mM) in DMSO and ACN were prepared separately and utilized for mercury detection. 10  $\mu$ L of DPIC-OS and DPIC-SS (1 mM) was added to water and mercury ions were gradually added, and its fluorescence spectra were recorded (Figure 1a). The organic-aqueous system displayed poor fluorescence intensity, with minimal changes upon the addition of mercury ions (Figure 1b). Presumably, as chemodosimeters undergo a chemical reaction to generate a fluorimetric change, the hydrophobic DPIC-OS and DPIC-SS require an organic environment to react with mercury ions. The organic-aqueous mixed media simply could not provide sufficient solvation of the probes for the reaction to occur. <sup>43,53</sup> Thus, we were encouraged to use micellar media for chemodosimetric detection of Hg<sup>2+</sup>

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ions using DPIC-OS and DPIC-SS. Three surfactants with contrasting head groups, viz. cetyltrimethylammonium bromide (CTAB) (cationic), sodium dodecyl benzene sulfonate (SDBS) (anionic), and Triton X-100 (neutral) were chosen as the additives for the sensing systems. Firstly, the UV-Vis spectra of DPIC-OS and DPIC-SS were recorded in a micellar medium, revealing minimal spectral changes (Figure S2a, ESI<sup>†</sup>). As expected, the addition of CTAB and Triton X-100 (above CMC) led to a significant increase in the fluorescence of the DPIC-OS and DPIC-SS, with the fluorescence intensity in the surfactant solutions being comparable to that in organic solvents, indicating that the probes are in a relatively hydrophobic environment (Figure S2b,c, ESI<sup>†</sup>). Interestingly, in the case of SDBS, the probes displayed minimal changes in fluorescence output. Although the probes displayed relatively higher quantum yield in Triton X-100 than other surfactants, it was noticed that the probes displayed a better response to mercury ions with greater Stokes shift when CTAB was chosen as the micelle forming agent (Figure 1b, Table S2, ESI<sup>T</sup>). A study with varying concentrations of CTAB, below CMC, at CMC, and above CMC was carried out to understand the role of micellar microenvironment on fluorescence output. As expected, the CTAB concentration at and above CMC displayed similar or better fluorescence enhancement as that of organic solvents, while below CMC, no enhancement is noted (Figure S3, ESI<sup>†</sup>). Before proceeding further, a time-dependent response study of both DPIC-OS (10  $\mu$ M) and DPIC-SS (10  $\mu$ M) with mercury ions (25  $\mu$ M) in CTAB was conducted. It was observed that the maximum fluorescence output was achieved within just 2 min for **DPIC-OS** but took 15 min for **DPIC-SS** (Figure S4,  $ESI^{T}$ ). The seemingly faster FL output of the DPIC-OS probe can be attributed to the labile hemiacetal bond formed after the cleavage of the C-S bond by Hg<sup>2+</sup> ions. In order to establish the pH stability, the emissive properties of DPIC-CHO, DPIC-OS and DPIC-SS were evaluated over a broad range of pH (3-11). The pH study revealed that all three compounds showed no changes in the fluorescence output in the neutral pH, with the best results



Fig. 1. a) Fluorescence intensity output of DPIC-SS, DPIC-OS, and DPIC-CHO (10  $\mu$ M) before and after surfactant (CTAB) addition ( $\lambda_{ex}$  = 400 nm). b) The change in

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fluorescence intensity of **DPIC-SS** and **DPIC-OS** in the presence of **DPIC-SE** and after surfactant addition 1 mM CTAB ( $\lambda_{ex} = 400 \text{ Mm}$ ): 10.1039/D5AN00502G at a pH of 7.4, indicating its potential to be used in physiological conditions (Figure S5, ESI<sup>†</sup>). Presumably, due to the acid-sensitive nature of thioacetals, **DPIC-OS** and **DPIC-SS** showed higher fluorescence intensity under acidic conditions, indicating their cleavage to the parent aldehyde group. As **DPIC-OS** displayed better fluorescence output in a shorter time, this combination was chosen for further analytical studies.

#### 2.3 Detection of Hg<sup>2+</sup> ions

Initially, an organic aqueous system containing 10  $\mu$ L DPIC-OS (1 mM in DMSO) in 5% DMSO-HEPES buffer was utilized for the detection of mercury ions. The fluorescence output of DPIC-OS displayed a gradual increase with a slight bathochromic shift from 445 nm to 494 nm upon the gradual addition of mercury ions (Figure 2a). The shift in peak is presumably due to the generation of DPIC-CHO formed after the cleavage of the oxathiolane moiety. The relative fluorescence intensity displayed a gradual increase with saturation after the addition of 80  $\mu$ M of Hg<sup>2+</sup> ions upon incubation for 30 min. The high amount of mercury ions required and higher response time are possibly due to the phase separation in the microenvironment, the preference of Hg<sup>2+</sup> to remain in the aqueous phase, and also the low dispersibility of the probe DPIC-OS in aqueous solutions (Figure 2b). A linear relationship between the relative fluorescence output and concentration of mercury ions with a good regression coefficient ( $R^2 = 0.9185$ ) was observed at the lower concentration range (0-5  $\mu$ M). Following the linear plot, a mediocre detection limit of 0.55  $\mu$ M (109 ppb) was calculated



Fig. 2 a) Fluorescence output of **DPIC-OS** (10  $\mu$ M in 5% DMSO-HEPES buffer) upon gradual addition of Hg<sup>2+</sup> (0–95  $\mu$ M) ( $\lambda_{ex}$  = 400 nm;  $\lambda_{em}$  = 455 nm, 495 nm). b) The relative fluorescence output of **DPIC-OS** (10  $\mu$ M in 5% DMSO-HEPES buffer) upon gradual addition of Hg<sup>2+</sup> ions [inset: linear correlation between relative fluorescence intensity and concentration of Hg<sup>2+</sup> ions in lower range]. a) Fluorescence output of **DPIC-OS** (10  $\mu$ M in micellar media using 1.0 mM of CTAB) upon gradual addition of Hg<sup>2+</sup> ions (0–16  $\mu$ M) ( $\lambda_{ex}$  = 400 nm;  $\lambda_{em}$  = 455 nm, 505 nm). b) Plot of the ratio of fluorescence intensities against Hg<sup>2+</sup> concentrations (0–

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16  $\mu M)$  [inset: ratio of the fluorimetric response of  $\mbox{DPIC-OS}$  in a lower concentration of mercury ions.]

using the formula  $3\sigma/k$ , where  $\sigma$  is the standard deviation and k is the slope of the calibration curve. Next, the sensing was carried out in the micellar media containing 1 mM of CTAB. Upon addition of mercury ions to DPIC-OS, a bathochromic shift from 455 nm to 505 nm, similar to that of the aqueous-organic mixture, was noted (Figure 2c). However, the increase in fluorescence output after the addition of increasing concentrations of mercury ions was greatly enhanced with almost a four-fold increase and the response time was significantly decreased to 2 min. The enhancement in fluorescence response was proportional to the concentration of Hg<sup>2+</sup> ions added and reached saturation upon the addition of about one equiv of Hg<sup>2+</sup> ions. The FL output displayed a linear response in the lower concentration range of 0.1–1.0  $\mu$ M Hg<sup>2+</sup>, with a high regression coefficient of 0.9906 (Figure 2d). Based on this data, the limit of detection (LOD) was determined to be 16.2 nM (3.2 ppb) using the same formula as above. This ensures the advantage of using a micellar microenvironment for analyte detection in the aqueous phase.

As both **DPIC-OS** and **DPIC-CHO** exhibited a strong absorption band in the UV-vis spectrum at 290 nm and 400 nm, investigations were conducted to monitor absorption changes in the presence and absence of Hg<sup>2+</sup> ions. Upon incubation with Hg<sup>2+</sup> ions (0–17  $\mu$ M), a gradual increase in the absorption peak at around 400 nm was noted, with saturation again occurring at 12  $\mu$ M of Hg<sup>2+</sup> ions (Figure S6a, ESI<sup>†</sup>). The corresponding increase displayed a linear trend from 1–10  $\mu$ M, and from the graph, the detection limit was determined to be 94 ppb or 0.47  $\mu$ M (Figure S6b, ESI<sup>†</sup>). Similarly, the absorption spectrum of **DPIC-SS** was recorded with Hg<sup>2+</sup> ion additions (0–22  $\mu$ M), also showing a linear response in the 1–10  $\mu$ M range (Figure S5c,d, ESI<sup>†</sup>). Thus, both probes can work as fluorimetric and colorimetric dual-output chemodosimeters for mercury ions.

#### 2.4 DFT studies

In order to gain better insights into the observed optical properties of the probes, DFT studies were conducted.<sup>54</sup> Both **DPIC-OS** and **DPIC-SS** displayed similar results in DFT analysis with the electron population spread across the entire molecular structure with higher density on the indolizine moiety (Figure 3). However, in **DPIC-CHO**, a shift was noticed, with the electron density delocalized in the aldehyde group. Presumably due to the delocalizing effect of the oxygen atom, **DPIC-CHO** displayed a lower energy gap than **DPIC-OS** and **DPIC-SS** which indicates the emission maxima for **DPIC-CHO** at a relatively higher wavelength, as experimentally observed.

#### 2.5 Selectivity of DPIC-OS towards Hg<sup>2+</sup>

Following the successful detection of mercury ions (Hg<sup>2+</sup>), the next essential step was to assess and mitigate any interference from other species in the detection process. A comprehensive study was performed to identify potential competitors to Hg<sup>2+</sup>, including different anions, metal ions, and neutral molecules (Figure 4). The system displayed excellent selectivity against



Figure 3. Optimized structures, visualized electron distribution and energy levels of ground state HOMO and LUMO of **DPIC-SS, DPIC-OS** and **DPIC-CHO**.

acids, urea,  $H_2O_2$ , etc., indicating the feasibility of the **DPIC-OS** as a selective fluorimetric sensor for mercury ions. The high specificity is attributed to the strong thiophilic affinity of  $Hg^{2+}$ , which selectively cleaves the oxathiolane moiety in **DPIC-OS**, triggering the release of **DPIC-CHO** and the subsequent fluorescence shift. These results confirm that **DPIC-OS** functions



Figure 4. Selective fluorescence output of the probe against cations (1. Hg<sup>2+</sup>, 2. Ca<sup>2+</sup>, 3. Na<sup>+</sup>, 4. Mg<sup>2+</sup>, 5. K<sup>+</sup>, 6. Ag<sup>+</sup>, 7. Al<sup>3+</sup>, 8. Cd<sup>2+</sup>, 9. Co<sup>2+</sup>, 10. Cu<sup>2+</sup>, 11. Cr<sup>3+</sup>, 12. Fe<sup>3+</sup>, 13. Mn<sup>2+</sup>, 14. Ni<sup>2+</sup>, 15. Pb<sup>2+</sup> 16. Zn<sup>2+</sup>, 17. Ba<sup>2+</sup> 18. Sn<sup>2+</sup>), anions (1. Hg<sup>2+</sup>, 2. F<sup>-</sup>, 3. Cl<sup>-</sup>, 4. Br<sup>-</sup>, 5. I<sup>-</sup>, 6. SCN<sup>-</sup>, 7. CO<sub>3</sub><sup>2-</sup>, 8. NO<sub>2</sub><sup>-</sup>, 9. NO<sub>3</sub><sup>-</sup>, 10. AcO<sup>-</sup>, 11. SO<sub>4</sub><sup>2-</sup>, 12. S<sub>2</sub>O<sub>3</sub><sup>2-</sup>, 13. S<sub>2</sub>O<sub>4</sub><sup>2-</sup>, 14. CN<sup>-</sup>, 15. N<sub>3</sub><sup>-</sup>, 16. PO<sub>4</sub><sup>3-</sup>, 17. HS<sup>-</sup>, 18. S<sup>2-</sup>, and small molecules (1. Hg<sup>2+</sup>,

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2. Ala, 3. Arg, 4. Gly, 5. Leu, 6. Lys, 7. glucose, 8. urea, 9. H<sub>2</sub>O<sub>2</sub> 10. NH<sub>2</sub>NH<sub>2</sub>, 11. Phe, 12. Pro, 13. Spm, 14. Spd, 15. Mel, 16. His, 17. Tet, 18. Cip).

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Figure 5. a) The sensing mechanism of detection of mercury ions by DPIC-OS in the micellar media. The changes in b) 1H NMR and c) LCMS spectra of DPIC-OS before and after the addition of mercury ions

as a highly selective chemodosimeter for Hg<sup>2+</sup> detection without cross-reactivity from other potential interfering agents (Figure 4).

#### 2.6 Sensing Mechanism

The sensing mechanism capitalizes on the thiophillicity of Hg<sup>2+</sup> ions. Upon addition of mercury ions in the presence of DPIC-OS in micellar media, DPIC-OS reacts to mercury ions, which leads to the cleavage of the C-S bond. This results in the formation of a hemiacetal, which quickly breaks to generate DPIC-CHO, showing a bluish-green fluorescence (Figure 5). Micelles are not rigid entities and remain in the dynamic equilibrium with their monomeric form.<sup>55</sup> An organic probe prefers to stay inside the hydrophobic interior of micelles but may have access to the water sink. Presumably, DPIC-OS hops from one micellar core to another via the water sink in a dynamic transition and interacts with mercury ions close to the CTAB-derived micelles near its head group or in the water sink to facilitate the cleavage of the oxathiolane group. Once DPIC-CHO is formed, it goes inside the micellar microenvironments and fluoresces with enhanced (Figure 5a). In order to confirm the sensing mechanism, a bulk-scale reaction was kept by interacting DPIC-OS with 2 equiv of mercury ions in a micellar media at room

temperature, keeping the reaction condition similar to the sensing conditions. After 30 min of stirring, the product was isolated with ethyl acetate and subjected to NMR spectroscopy. The <sup>1</sup>H NMR spectra matched well with the original NMR of **DPIC-CHO** (Figure 5b). Also, in a mass sample taken out from the sensing solution, the spectrum showed a peak m/z 298 in LC-MS, which corresponds to [M+H]<sup>+</sup> of DPIC-CHO (Figure 5c). The above results firmly established the proposed mechanism of detection of mercury ions in a chemodosimetric approach via the cleavage of the oxathiolane moiety in micellar media.

#### 2.7 Real sample analysis

Numerous studies have shown mercury contamination in natural water sources, highlighting the critical need for its detection and removal. To demonstrate the indolizine-based probe's real-world applications, water samples from ponds, rivers, the sea, tap water, lakes, and rain were collected and spiked with known concentrations of Hg2+ ions. The spiked samples were incubated with the probe, and their fluorescence was measured. Each test was performed in triplicate, with a relative standard deviation (RSD) of 3-4%, indicating the reproducibility of the results. The %recovery for all real samples

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using DPIC-OS was above 95% (Table 1), indicating the efficient functioning of the sensing assay in detecting mercury ions in real water samples, while the %recovery for all real samples using **DPIC-SS** was above 88% (Table S3, ESI<sup>T</sup>), indicating that DPIC-OS is better than DPIC-SS functioning of the sensing assay in detecting mercury ions in real water samples.

Table 1: Real sample analysis of DPIC-OS probe in water samples.

S. No.	Sample	Spiked concentration (μΜ)	Obtained concentration (μM)	%Reco very	%RSD (n = 3)
1	Rain Water	0.116	0.114	98	3.2
2	Tap Water	0.064	0.061	95	3.5
3	Pond Water	0.064	0.061	95	2.7
4	Sea Water	0.062	0.059	95	3.1
5	River Water	0.046	0.044	96	2.9

## 2.8 Comparative study

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Finally, a comparative analysis of DPIC-OS against other conventional and water-soluble fluorescent probes for mercury ions was conducted, focussing on key differences in sensitivity, selectivity, photostability, and practical applicability (Table S4, S5, ESI<sup>1</sup>). Compared to other probes, **DPIC-OS** displays a higher selectivity and quicker response time owing to its oxathiolane group and showed LOD in ppb level. Notably, several existing probes use significant amounts of organic solvents in aqueous media, which is sometimes as high as 50%, and even if they show low LOD, they often require a longer incubation period. In contrary, in the case of **DPIC-OS**, the detection is carried out in micellar medium ensuring that the sensing system is biocompatible.

# Conclusions

In this study, we developed two efficient fluorescent probes, DPIC-OS and DPIC-SS, derived from a less-explored indolizine scaffold, as highly selective and sensitive turn-on sensing of mercury ions (Hg<sup>2+</sup>) exhibiting a distinct blue-to-green fluorescence transition in aqueous micellar media. The probes were obtained by protecting 1,3-diphenylindolizine-2carbaldehyde (DPIC-CHO) with a mercury-recognizable 1,3dithiolane and 1,3-oxathiolane moieties. Although these hydrophobic probes demonstrated strong fluorescence in organic solvents, their fluorescence was significantly reduced in mixed aqueous-organic solvents due to the poor solubility of the probe, and they were found less effective for mercury detection. The addition of CTAB to provide a micellar microenvironment aided the solubility that proved effective as the fluorescence of both DPIC-CHO, DPIC-OS, and DPIC-SS was considerably enhanced. Presumably, the micelles can encapsulate the probe and provide a hydrophobic environment improving the fluorescence emission and sensing efficiency. DPIC-OS showed better sensing ability than DPIC-SS. In CTABderived micellar media, DPIC-OS could detect mercury ions through the release of fluorescent DPIC-CHO exhibiting a bluishgreen emission at 505 nm. The probe demonstrated excellent selectivity towards mercury ions in the presence of various competing cations (e.g., Na<sup>+</sup>, Mg<sup>2+</sup>, Al<sup>3+</sup>, Ag<sup>+</sup>), anions (e.g., Cl<sup>-</sup>,

Br<sup>-</sup>, I<sup>-</sup>), and neutral molecules (e.g., amino acids, glucose, urea). This exceptional specificity is attributed to the chernodosina etc. sensing mechanism, which leverages the strong thiophillicity of mercury to trigger the deprotection of DPIC-OS. The probe exhibited a considerably low limit of detection of 3.2 ppb (16.2 nM) and 94 ppb (0.47  $\mu$ M) in fluorimetry and colorimetry, respectively. The successful application of DPIC-OS in real sample analysis, including spiked water, with excellent recovery rates, underscores its potential for practical environmental monitoring and public health applications.

# Author contributions

A. A. P.: Investigation, analysis, validation, writing- original draft. A. T.: Validation, visualization, writing - original draft & editing. S. S.: Synthesis of the probe; M. B.: Project administration, Supervision, writing–original draft, review & editing; Amrita Chatterjee: Conceptualization; supervision, project administration, writing review & editing.

# Conflicts of interest

There are no conflicts of interest to declare.

# Data availability

The data supporting this article have been included as part of the Supplementary Information.

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## **Data availability**

The data supporting this article have been included as part of the Supplementary Information.