Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

An Organic Chromophore-based Optical Chemo-Sensor Exhibits Promise in Detecting Hg(II) Ions in Aqueous Medium

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Abstract

The current study involved the development of a new optical sensing system that utilizes a fluorescent probe DTT to detect Hg (II) ions in nanomolar levels. The synthesized probe *5-((4-(dimethylamino) benzylidene) amino)-1,3,4-thiadiazole-2-thiol* (DTT*)* exhibits weak fluorescence due to the photoinduced electron transfer (PET) process. The emission of the DTT probe was seen to be enhanced during the titration reaction with Hg (II) ions. This enhancement can be attributed to forming a 1:1 complex between DTT and the Hg (II) ions that inhibit the PET process and cause chelation-enhanced fluorescence (CHEF). The system's fluorescence intensity was not significantly affected by other metal ions, such as alkali, alkaline metal ions, and some transition metal ions, demonstrating its exceptional selectivity. Job's plot and Benesi-Hildebrand-based fluorescence method were applied to confirm 1:1 binding stoichiometry between DTT and Hg (II) ions and calculate the binding constant value of 2.26×10^{5} M^{-1}, respectively. Determining the limit of detection (LOD) and limit of quantification (LOQ) yielded values of 21 nM and 70 nM, respectively. With its exceptional selectivity and ultra-sensitivity, as seen by its exceptionally low detection limit, The sensing efficiency towards Hg(II) was examined with a dynamic range of 0.070 to 1 µM, even in the presence of other metal ions, demonstrating its high degree of selectivity. The system was rendered reversible by employing an EDTA solution with a concentration of 1 µM. Additionally, the DTT probe exhibits potential as an optical chemical for detecting Hg (II) ions in environmental samples.

Keywords: Mercury; fluorescence; sensor; coordination reaction; water contamination; environmental monitoring

1. Introduction

Many analytical experts were detecting heavy metal ions because of their significant influence on the natural world and human life. The human body's ingestion of mercury and copper ions harms genomes, nerve cells, and the immune system [1,2]. The existence of these metal ions is linked to the development of potentially fatal disorders such as Alzheimer's and Wilson's [3]. Because the prevalent traditional analytical techniques that have been tried and exhausted to identify trace metal ions often require a financial investment in apparatus and complex sample preparations, there is a need for an alternative.

Among the most admired research areas are those that focus on developing and manufacturing sensitive, applicable, reversible, and selective alternative approaches. In recent years, luminescence-based dyes, also known as chromophores, have garnered much attention because of their extraordinary sensitivity and selectivity across broad dynamic ranges. Direct and ratiometric fluorescence intensity measurements are involved in these methodologies [4-7]. On the other hand, a wide variety of fluorescent nanoparticles and biomolecules have been employed extensively in the design and development of sensitive optical sensors for tracking a variety of fundamental analyses, such as metal ions, pesticides, notable gases, and biological

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Received date 09 June 2024; revised date 07 July 2024; accepted date 25 July 2024
DOI: 10.21608/ejchem.2024.296369.9837

molecules [8-12]. These sensors have been used greatly to design and develop optical sensors.

Additionally, the functionalized compounds resulted from the condensation reactions of an aryl- or heteroaryl-amine or hydrazide with carbonyl compounds and incorporating polar groups with efficient structural features, such as the imine group, have found wide applications in chelation applications providing the route of versatile, environmental and multidisciplinary interests [13-16]. Such structural features allowed their derived structure as attractive ligands to achieve the best efficient construction of complexation with various metals due to the interesting stereo-electronic features [17] in their structural skeletons [18-21], which prompted selected derivatives to be investigated as fluorescent chemosensor detecting of metals such as Hg^{2+} [22]. In such context, these functionalized compounds may also act as multifunctional proper substrates as coordination ligands of various ions with various structures [23]. The latter significances prompt our proposed strategy for investigating the ability of such structural types to study their behavior towards Hg(II) ions in varied aqueous systems. When mercury ions come into contact with the ligand, the reaction changes the ligand's optical characteristics, which may be measured, including a change in color or fluorescence [24-27]. These sensors have many benefits compared to more conventional methods. They can be implemented with signified tools or staff and are quite easy to use. They also provide quick and accurate measurements of Hg(II) ions in solution. However, depending on the task, they may not be as sensitive or accurate as conventional methods.

The fluorescence detection technique involves altering the intensity, wavelength, or duration of fluorescence emitted by a fluorescent probe upon interacting with a metal ion [28-33]. Certain probes can produce distinct fluorescence hues based on the specific kind and concentration of metal ions present. The fluorescence probes may exhibit varying selectivity and sensitivity towards different metal ions [34-37]. The utilization of fluorescence detection is notable due to its exceptional sensitivity, minimum interference, easy operation, and real-time monitoring ability. Fluorescence detection has the potential to yield significant advantages in several domains, including environmental monitoring, food safety, medical diagnostics, and biological research. Fluorescence detection can be employed to observe the migration of metal ions within viable cells and tissues. Fluorescence probes for metal ions encompass a range of substances, including organic dyes, fluorescent proteins, and nanomaterials [38-41]. However, most fluorescent probes applied for Hg(II) sensing are based on a fluorescence quenching mechanism rather than fluorescence enhancement. The extreme advantages of the fluorescence turn-on sensing mechanism are the specified analyte's low detection limit and the chemo-sensor's relative dark fluorescence background. These factors decrease the probability of pseudo signal and increase both sensitivity and selectivity of the chemosensor.

Multiple fluorescence-based methods were employed to detect metal ions. Photo-induced electron transfer (PET) is a phenomenon that entails the transfer of electrons between the fluorophore of a probe and the binding unit of a metal ion [42-43]. The fluorophore's fluorescence is suppressed by electron transfer when the probe is not attached to a metal ion. The electron transfer process is impeded by the binding of the probe to a metal ion, resulting in the restoration or augmentation of fluorescence. The probe's fluorescence is suppressed by electron transfer in the absence of a metal ion coupling. The interaction between the probe and the metal ion inhibits the transport of electrons, hence reinstating or amplifying the fluorescence.

Moreover, the chelation-enhanced fluorescence (CHEF) process includes the transfer of electrons within the probe molecule [44,45]. The CHEF modifies the fluorophore's electronic structure and polarity, altering the fluorescence intensity spectrum. The probe changes two fluorophores' fluorescence intensity or spectrum by attaching to a metal ion, resulting in their combination or rearrangement [46,47]. Herein, this investigation used a method that proved effective, including PET in conjunction with the CHEF procedure. When the PET process is present in aqueous solutions, the fluorescence of the chemical probe DTT is extremely faint. This is because of the presence of the enzyme. As a result of the inhibited PET process, which results in strong fluorescence, the DTT can bond with Hg(II) and exhibits a considerable rise in fluorescence intensity upon binding. When detecting Hg(II), the DTT probe possesses remarkable selectivity, sensitivity, and photostability.

Additionally, it has been demonstrated that the Hg(II)-DTT complex is effective as a fluorescence turn-on probe for detecting Hg(II).

2. Materials and Methods

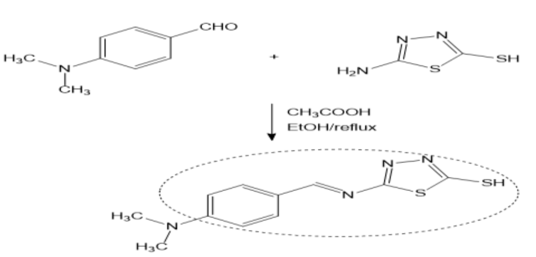
2.1. Instruments and Materials

The melting points of the *5-((4-(dimethylamino)-benzylidene) amino)-1,3,4-thiadiazole-2-thiol* molecu-le (*DTT)* were measured in a preserved capillary using Stuart-SM/P3. NMR spectra were measured by Bruker Avance DRX200 (200 MHz-^{1}H) spectrometer calibrated to the residual proton solvent signal of DMSO-d_6. The UV–vis spectra were collected using a 1-cm quartz cell utilizing (Evolution™_200-series/UV-Visible) spectrophotometer. The emission and excitation spectra of the DTT chemosensor were measured in a 1 cm quartz cell using a JASCOFP6300-spectrofluorometer. All Chemicals were provided by Sigma–Aldrich and used without further purification.

2.2. Preparation of the DTT chemical probe

The chemosensor probe was synthesized using the method previously reported (see Scheme 1) [48]. A catalytic amount of glacial acetic acid (0.3 mL) was provided to a well-stirred solution of 4-N,N-dimethylaminobenzaldehyde (10 mmol), and the amine, namely 3-amino-1,3,4-thiadiazole-5-thiol (10 mmol) in absolute ethanol (30 mL) while stirring. The reaction mixture was heated under a reflux system at the reflux temperature for 7 hours. The completion of the reaction was checked for consumption of the starting materials and complete formation of the produced Schiff base by TLC (pet. Ether-ethyl acetate; 3:1). The resulting product content was standing for 14 hours at room temperature to afford a pale yellow precipitated. The precipitated solid was filtered, washed with cold ethanol, and recrystallized from ethanol to give the benzylidene) amino)-1,3,4-thiadiazole-2-thiol derivative as a yellow powder.

Yield: 76%; m.p. 212–214 °C; IR (KBr) cm^{-1}, ν: 3292 (NH), 3050 (C-H), 2950 (C-H), 1610 (C=N); ^{1}H-NMR (DMSO-d_6) δ/ppm: 2.07 (s, 6H, $N(CH_3)_2$), 6.68 (d, 2H, J = 8.8 Hz, Ar-H), 7.81 (d, 2H, J = 8.8 Hz, Ar-H), 8.24 (s, 1H, N=C*H*), 14.40 (s, 1H, NH).



Scheme 1. Synthesis of *DTT*

2.3. Optical Measurements

A stock solution of DTT (1x10^{-3} M) in DMSO: H_2O (1:9) mixture using HEPPS buffer 20 mM was prepared for all optical measurements. To study the effect of other metal ions on the optical properties of DTT, 1x10^{-3} M solutions of different metal nitrate (Hg(II), Fe(II), Cr(III), Cd(II), Zn(II), Sr(II), Na(I), Pb(II), Mn(II), Cu(II), K(I), Fe(III), Ag(I), Mg(II), Ba(II), Ni(II), and Hg(II)) were prepared. The fluorescence spectra were collected with a slit width of 5 nm.

2.4. Quantum Yield measurements

The free ligand DTT's quantum yields and its complex with Hg(II) ions were calculated utilizing fluorescein as a standard reference dye in ethanol. To avoid the influence of the inner filter effect, the absorbance and fluorescence spectra were measured in 1 cm quartz cells, and the absorbance value was obtained below 0.1 for all wavelength ranges.

$$Q_X = Q_R \frac{A_R\, I_s n_s^2}{A_X\, I_R\, n_s^2}$$

While

- X and R refer to ligand and reference solutions, respectively.
- η is a refractive index at room temperature
- I the integrated area under the peak
- A is the maximum absorbance peak.

3. Results and discussion

3.1. The optical features of the chemical probe DTT

The emergency need for new materials for sensing applications has increased. Many new chemical sensors have been developed to monitor different toxic chemicals. Organic probe-system-based sensors exhibited advanced features over other materials. They provide high sensitivity, selectivity, and easy processing. Here, we introduce a new chemical probe, DTT, to monitor one of the most important toxic elements, Hg(II) ions. Most developed organic chemical probs for sensing Hg(II) ions are based on fluorescence quenching. Worthy here, we introduce a new chemical probe for monitoring Hg(II) ions in nano levels based on more favorable fluorescence enhancement. To investigate the sensing strategy, the optical properties of the synthesized DTT probe were examined in the absence and presence of Hg(II) ions.

The absorption spectrum of 1 µM of the DTT was studied. The DTT was dissolved in a mixture of DMSO: H_2O (1:9) using 20 mM of HEPPS buffer solution at pH 7.5. Two main absorption peaks for the DTT solution appeared at 239 and 316, as shown in Figure 1a, corresponding to π-π* and n-π*, respectively. The fluorescence emission spectra of DTT were also measured under the same condition with excitation of 360 nm; DTT exhibits a maximum emission peak centered at 470 nm, as shown in Figure 1b.

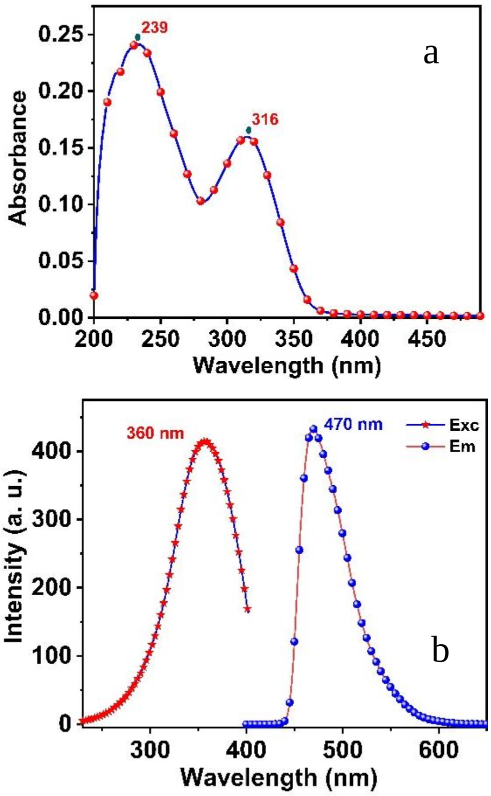


Figure 1. (a) DTT absorbance spectrum, (b) Fluorescence emission spectra of DTT probe, in DMSO: H_2O (1:9) mixture using HEPPS buffer 20 mM.

3.2. Testing the optical properties of the DTT in response to Hg(II)ions

The same previous experiments were carried out in the same conditions but in the presence of Hg(II) ions. The absorbance spectrum of the DTT exhibits significant enhancement in the absorption peaks at 316 and 239 nm, and the enhancement increased with the gradual increase in the addition of Hg(II) ions. When the metal concentrations reach the same concentration of the DTT (1 µM), the absorption starts to be constant. No enhancement was observed at the Hg(II) concentration of 1.4 µM, as shown in Figure 2a. This observation helped us to predict the stoichiometry ratio of the Hg(II)-DTT complex to be 1:1. The calibration curve of the absorbance response of DTT with different Hg(II) concentrations is shown in Figure 2b.

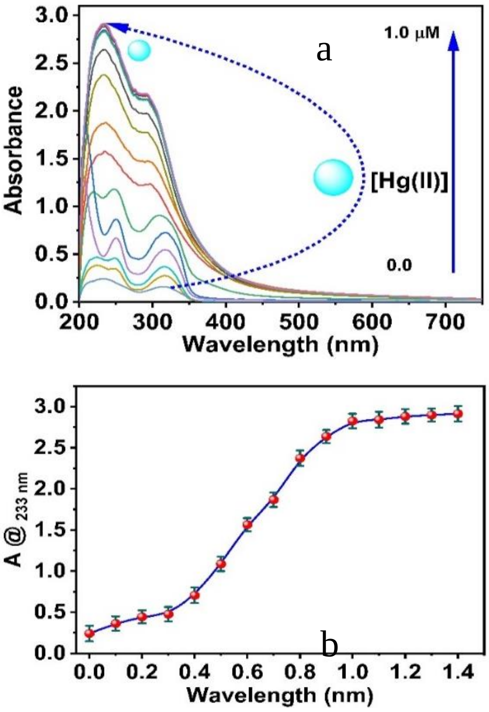


Figure 2. (a) The DTT absorption spectra in different concentrations of Hg(II) ions in the range (0 to 1.4 µM), (b) the calibration curve of DTT with Hg(II).

The Fluorescence of DTT was studied at 470 nm in the presence of Hg(II) to examine the ability of DTT to detect the toxic ion Hg(II). Different concentrations of Hg(II) ions were regularly added in the range (0 to 1.3 µM). As shown in Figure 3a, the fluorescence spectrum of the DTT ligand starts to increase significantly with the addition of Hg(II) ions. After reaching the ratio of approximately (1:1) metal to a ligand, the fluorescence at 470 nm becomes constant. This confirms the same stoichiometry of the metal-ligand complex to be fitted to 1:1, which agrees with the result obtained from absorbance spectra. Compared to the fluorescence of the DTT in the absence of Hg(II) ions, a strong turn-on can be seen in the fluorescence after adding 1.3 µM of Hg(II) ions under a 360 nm UV lamp, as seen in the situ image in Figure 3a. The calibration curve for the response of the emission peak at 470 nm with the change in the concentration of Hg(II) ions is shown in Figure 3b. The emission spectra enhancement is about ≈10 fold, providing high sensitivity towards Hg(II) ions. The chemosensor responds to the Hg(II) ions within the 0-1.3 μM concentration with massive fluorescence enhancement.

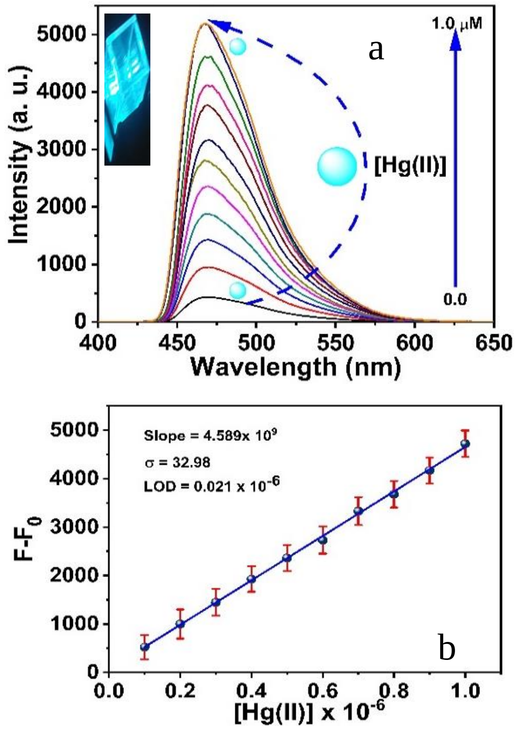


Figure 3. (a) Fluorometric titration of DTT (1 μM) with Hg (II) ions in the range 0-1.3 μM under excitation with 360 nm. The photo in Fig shows DTT's strong turn-on in fluorescence under a 360 nm UV lamp with 1.3 μM of Hg(II). (b) The response in the emission of DTT at 470 nm as a function of Hg(II) concentration.

The limit of detection (LOD) of the Hg (II) using the chemosensor DTT was estimated from the calibration curve in Figure 3b. Based on the relation 3σ/K, the LOD was calculated to be 21 nM, where σ is the standard deviation, and K is the slope of the calibration curve. The limit of quantification (LOQ) was determined at the nanomolar level based on the relation 10σ/K to be 70 nM. As a result, the chemosensor can estimate the Hg (II) ions concentration in a dynamic range from 70 nM to 1.3 μM [49].

3. 3. The stoichiometry and binding constant of the Hg(II)-DTT complex

The enhancement in the fluorescence of DTT in the presence of Hg(II) ions was studied using Job's plot to confirm the stoichiometry of Hg(II): DTT [50,51]. The fluorescence intensities were measured for solutions of different mole fractions of the Hg(II) where Hg(II) concentrations were changed in the range of 0.1 to 1.0 μM where the initial concentration of DTT was constant at 1 μM. The relation between the fluorescence intensities versus different mole fractions of Hg(II) ions was drawn as F_0 and F are the fluorescence intensities measured in the absence and the presence of Hg(II), respectively see Figure 4. The fluorescence intensity reached a maximum value at 0.5 mole fraction, which provides more evidence that the stoichiometry of DTT and Hg(II) ions is 1:1.

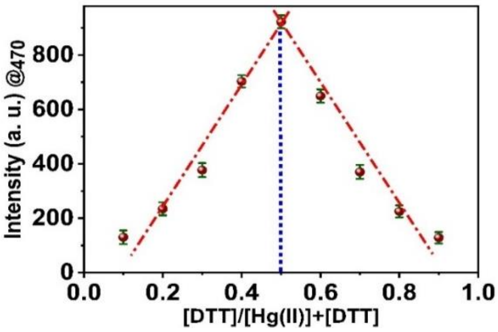


Figure 4. Job's plot for measuring the stoichiometry of Hg (II)-DTT complex.

The binding constant (K_b) of the interaction of the DTT as a ligand and the Hg(II) ions was calculated based on the Benesi-Hildebrand fluorescence method [52,53]. By plotting the relation between $1/(F_o-F)$ against 1/[Hg(II)], where F_o is the fluorescence intensity of DTT in the absence of Hg(II) ions, F is the fluorescence intensity of DTT after adding different concentrations of Hg(II). A linear fit was obtained, as shown in Figure 5. The binding constant K_b value was calculated to be 2.26 X 10^5 M^{-1} with R^2=99.92. This indicates a strong affinity between the ligand and the binding site. The high value suggests efficient complex formation. Additionally, the high (R^2) value (99.92%) indicates an excellent fit to the experimental data, supporting the reliability of the determination. This result suggests a robust interaction between the ligand and the binding partner.

The graph's slope and intercept are obtained from the figure, where F_{max} is the fluorescence intensity of DTT in the presence of a maximum concentration of Hg(II). According to the Benesi-Hildebrand equation, the linear fit of the relation confirms the stichometry of DTT to Hg(II) to be 1:1.

$$1/(F-F_0)=1/\{K(F_{\max}-F_0)\,[\mathrm{M}]^n\}+1/[F_{\max}-F_0]$$

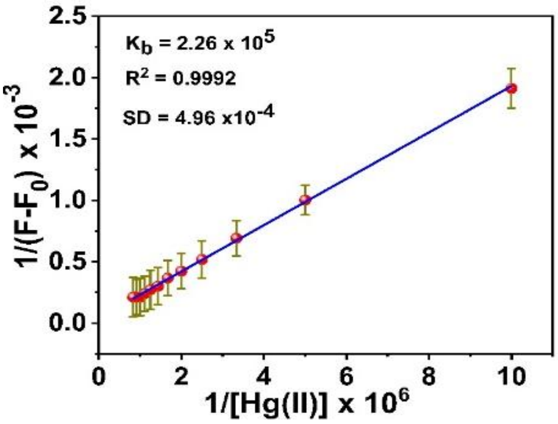
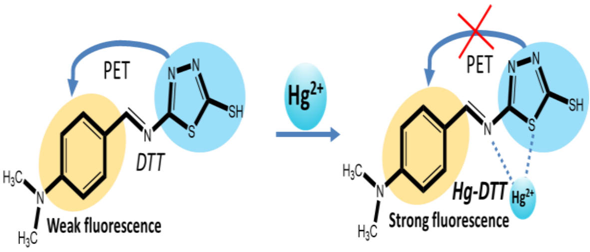


Figure 5. Benesi-Hildebrand relation of Hg(II) chemical detection using DTT chemical probe

3.4. Binding mechanism of Hg(II)-DTT complex

To the best of our knowledge, the binding mechanism between the chemical probe DTT and metal ions Hg(II) is based on Job's plot, which predicts the stoichiometry of Hg(II)-DTT complex to be 1:1. For the free probe, DTT has two organic moieties of benzylidene and 1,3,4-thiadiazole. This molecule exhibited a photoinduced electron transfer (PET) process between the two moieties, leading to weak fluorescence. When the Hg(II) ions chelate to the DTT, the complexation prohibits the PET process, causing a strong fluorescence enhancement and a turn-on switching. DTT is likely chelate with Hg(II) via 1,3,4-thiadiazole S atom and benzylidene N atom, as shown in Scheme 2. The enhancement of the absorbance peaks may be attributed to the chelation-enhanced fluorescence (CHEF) process between the metal ions Hg(II), in which the ligand molecule acts as a receptor and the free ligand *DTT* as the donor [54].



Scheme 2. Representation of the mechanism of chelation between the Hg(II) ions and DTT

3.5. The effect of the pH on Hg(II) sensing based on DTT

The pH of the sensing solution is significant to the response and sensitivity of the chemical sensor based on organic probes. The complexation between metal ions and free ligand is always accompanied by hydrogen proton transfer that can cause alteration in the fluorescence of the chemical probe. The pH effect of the surrounded solution in the pH range of 2-13 was studied on the sensing of Hg(II) based on DTT using 20 mM universal buffer solutions. The Hg(II) ion concentration was 1 µM, and DTT was 1 µM. As shown in (Fig. 6) the fluorescence intensity of DTT has no significant change in the range between 7-8. The decrease in intensities at pH>8 is due to the precipitation of Hg(II) as a hydroxide. At low pH values, the sensor's response changes are related to the proton binding by imine nitrogen, preventing complex formation to some extent. As a result, the high affinity and selectivity of the Hg(II)sensing system was adjusted to pH 7.5.

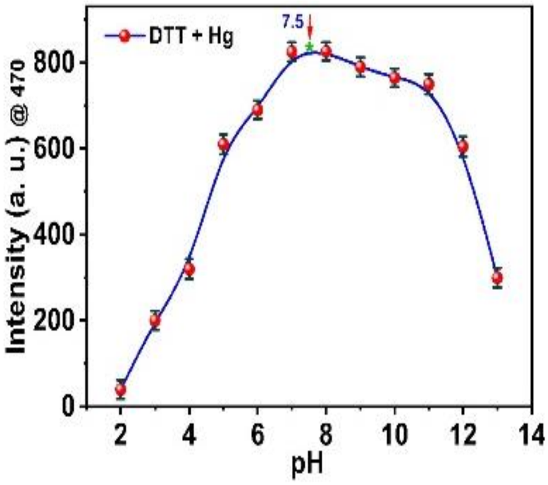


Figure 6. The pH effect on the Hg(II) sensing based on DTT

3.6. The Competitive Metal Ions Study

The fluorescence intensity of the DTT was recorded in the presence of other significant metal ions under the same conditions to determine the selectivity of the DTT probe towards Hg(II). Different metal ions were used in this study, such as Fe(II), Cr(III), Cd(II), Zn(II), Sr(II), Na(I), Pb(II), Mn(II), Cu(II), K(I), Fe(III), Ag(I), Mg(II), Ba(II), Ni(II) with a concentration of 1 µM. Most of the studied metal ions have no significant effect on the fluorescence intensity of the DTT see Figure 7a. The study provides strong evidence of the high specificity and selectivity of the new sensor towards the Hg(II) ions.

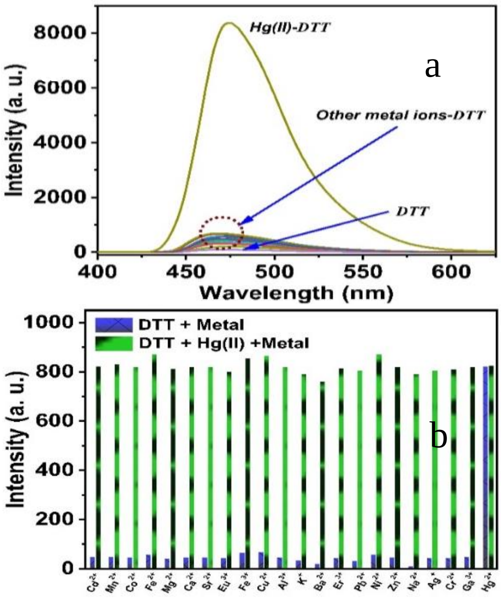


Figure 7. The study of the response of the DTT towards different metal ions.

The same study was carried out in the presence of 1μM of Hg(II) and 1μM of different metal ions as an interference to investigate the response of the DTT probe towards Hg(II). As shown in (Figure 7b), no considerable change in the fluorescence intensity was observed in the presence of different metal ions compared to the fluorescence intensity in the absence of the interfering metal ions. The results provide excellent confirmation of the selectivity of the DTT sensor toward Hg(II) ions.

The reversibility of the Hg(II) chemical sensor was studied using EDTA as a strong chelating agent. By adding 25 μL of 1 x 10^{-4} M EDTA for each 2.5 mL of Hg(II)-DTT complex solution, the enhancement of the fluorescence intensity caused by Hg(II)-DTT formation is omitted in the presence of EDTA due to the formation of an Hg(II)-EDTA complex in the solution and the liberated DTT free ligand. As shown in Figure 8, the intensity decreased in the presence of EDTA and increased again after adding new aliquots of Hg(II).

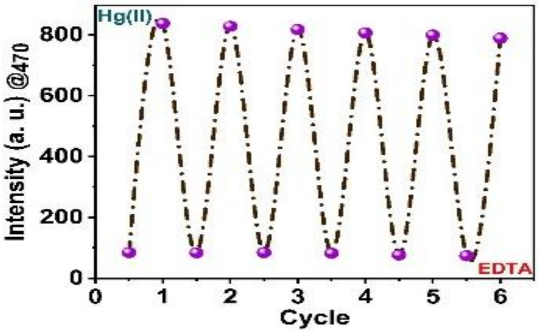


Figure 8. The study of the reversibility of chemical probes in the presence of EDTA.

3.7. Measuring the Quantum Yield

For the determination of the quantum yield (QY) of the DTT free ligand and Hg(II)-DTT complex, quinine sulfate (QY of 55%) in 0.05M H_2SO_4 was used as a reference dye [55]. The integrated fluorescence intensities of quinine sulfate and the DTT in the absence and presence of 1 μM of Hg(II) were compared under 360 nm excitation. The free ligand DTT and the metal complex Hg(II)-DTT exhibited quantum yield of 0.15 and 0.39, respectively.

3.8. Applications

The performance of the DTT as an optical nanosensor for detecting Hg(II) was examined in real environmental samples, including tap water and mineral water. The recovery values for tap water's Hg(II) detection ranged from 98.6% to 99.0%, while those for mineral water ranged from 97.0% to 98.77%. This result proves the chemical probe DTT is highly competent in quantitatively measuring Hg(II) in environmental samples.

Table 1. Detection of Hg(II) in tap and mineral water samples using DTT probe (n=3).

Sample	added Hg(II) (μM)	ICP-MS (μM)	Found (μM)	RSD (n=3)	Recovery (%)
Tap	0	Non	0	0	-
Water	0.1	0.102	0.099	1.72	99.00
	0.5	0.503	0.493	1.89	98.60
	0.9	0.902	0.891	1.57	99.00
Mineral	0	Non	0	0	-
Water	0.1	0.101	0.097	1.56	97.00
	0.5	0.502	0.488	1.65	97.60
	0.9	0.903	0.889	1.12	98.77

3.9. Comparison

The DTT chemosensor designed for fluorescence detection of Hg(II) is quite impressive. Table 2 presents the results of a comparison that we carried out between the current sensor and chemosensors for Hg(II) that have been described in the past. In comparison to other sensors, the chemosensor DTT showed a significantly better responsiveness.

Table 2. Comparative analysis of chemosensor DDT with previously reported sensors.

Sensing Molecule	LOD (mM)	Sensing Type	Medium	Ref.
N,N-bis(2-(pyridin-2-ylmethoxy)ethyl)aniline	0.181	Turn on	CH_3CN –water (1:1, v/v)	[56]
Indole derivative and 2,4-dihydroxybenzaldehyde	1.08	Turn-off	HEPES buffer	[57]
Naphthalene diimide (1)	3.0	Turn-on	Acetone	[58]
Naphthalene diimide (2)	1.3	Turn-on	Acetone–water (1:1, v/v)	[59]
Azobenzene gelator	7.46	Turn-off	CH_3CN	[60]
Fluorescein-phenylalaninol	0.34	Turn-off	Water	[61]
small-molecular dicarboxylic acid functionalized chemosensor	0.061	Ratiometric	DMF–water (2:3, v/v)	[62]
DTT	0.021	Turn-on	DMSO: H_2O (1:9)-HEBES	Present work

4. Conclusion

In conclusion, a novel optical chemo-sensor was developed and developed with 5-((4-(dimethyl-amino)benzylidene)amino)-1,3,4-thiadiazole-2-thiol DTT. The chemosensor exhibits optical properties, specifically the capacity to generate or emit light at wavelengths of 360 and 470 nm, correspondingly. A 20 mM HEPPS buffer was used to quickly detect Hg(II) ions within a dynamic concentration range of 70 nM to 1 □M at a pH of 7.5. The chemosensor demonstrates a significant capacity to detect Hg(II) ions. The chemosensor's results indicate that the Sulphur atom inside the thiazole unit is involved in the interaction with Hg(II) ions. The observed binding mechanism in this interaction is in accordance with the CHEF mechanism. Hence, DTT molecules can form complexes with Hg(II) ions rapidly. The detection approach utilizing the CHEF mechanism demonstrated fluorescence amplification. The sensor detection limit was determined to be 21 nM. The stability constant of the Hg(II)-DTT complex was determined to be 2.26 X 10^5 M^{-1}. The sensor emissions are rapidly suppressed by injecting an EDTA solution. A study was carried out to assess the specificity of the DTT chemosensor, which exhibited a remarkable affinity for Hg(II) ions. The optical sensor accurately recognized Hg(II) ions in genuine samples.

5. Acknowledgment

“The author(s) gratefully acknowledge Qassim University, represented by the Deanship of Scientific Research, on the financial support for this research under the number (2023-SDG-1-BSRC36693) during the academic year 1445 AH/ 2023 AD.”

6. References

[1] M.A.S. Islam, M.E. Hossain, K. Naharand, N. Majed, Assessment of Environmental Hazard and Heavy Metal Contamination in Dhaleshwari River Sediment: A Toxicity based Study on Pollution. Pollution, 9(1) (2023) 67-83.

[2] H. Deng, Y. Tu, H. Wang, Z. Wang, Y. Li, L. Chai, W. Zhang, Z. Lin, Environmental behavior, human health effect and pollution control of heavy metal (loid) s towards full life cycle processes, Eco-Environment & Health (2022).

[3] M. Babić Leko, M. Mihelčić, J. Jurasović, M. Nikolac Perković, E. Španić, A. Sekovanić, T. Orct, K. Zubčić, L. Langer Horvat, N. Pleić, S. Kiđemet-Piskač, Heavy Metals and Essential Metals Are Associated with Cerebrospinal Fluid Biomarkers of Alzheimer’s Disease, International Journal of Molecular Sciences, 24(1) (2022) 467.

[4] R.F. Elshaarawy, R. Ali, S.M. Saleh, C. Janiak, A novel water-soluble highly selective “switch-on” ionic liquid-based fluorescent chemi-sensor for Ca (II), Journal of Molecular Liquids, 241 (2017) 308-315.

[5] S.M. Saleh, R. Ali, R.F. Elshaarawy, A ratiometric and selective fluorescent chemosensor for Ca (II) ions based on a novel water-soluble ionic Schiff-base, RSC advances, 6(73) (2016) 68709-68718.

[6] R. Ali, F.M. Alminderej, S. Messaoudi, S.M. Saleh, 2021. Ratiometric ultrasensitive optical chemisensor film based antibiotic drug for Al (III) and Cu (II) detection, Talanta, 221 (2021) 121412.[7] S.M. Saleh, R. Ali, I.A. Ali, A novel, highly sensitive, selective, reversible and turn-on chemi-sensor based on Schiff base for rapid detection of Cu (II), Spectrochimica Acta Part A:

Molecular and Biomolecular Spectroscopy, 183 (2017) 225-231.

[8] R. Umapathi, B. Park, S. Sonwal, G.M. Rani, Y. Cho, Y.S. Huh, Advances in optical-sensing strategies for the on-site detection of pesticides in agricultural foods, Trends in Food Science & Technology, 119 (2022) 69-89.

[9] S.M. Saleh, F.M. Alminderej, R. Ali, O.I. Abdallah, Optical sensor film for metribuzin pesticide detection, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 229 (2020) 117971.

[10] N. De Acha,, C. Elosúa,, J.M. Corres, F.J. Arregui,, Fluorescent sensors for the detection of heavy metal ions in aqueous media, Sensors, 19(3) 599.

[11] N.A. Azmi, S.H. Ahmad, S.C. Low, Detection of mercury ions in water using a membrane-based colorimetric sensor, RSC advances, 8(1) (2018) 251-261.

[12] D. Dai, J. Yang, Y. Wang, Y.W. Yang, Recent progress in functional materials for selective detection and removal of mercury (II) ions, Advanced Functional Materials, 31(1) (2021) 2006168.

[13] A. Hameed, M. Al-Rashida, M. Uroos, S. Abid Ali, K.M. Khan, Schiff bases in medicinal chemistry: a patent review (2010-2015), Expert opinion on therapeutic patents, 27(1) (2017) 63-79.

[14] J. Ceramella, D. Iacopetta, A. Catalano, F. Cirillo, R. Lappano, M.S. Sinicropi, A review on the antimicrobial activity of Schiff bases: Data collection and recent studies, Antibiotics, 11(2) (2022) 191.

[15] M. Pervaiz, S. Sadiq, A. Sadiq, U. Younas, A. Ashraf, Z. Saeed, M. Zuber, A. Adnan, Azo-Schiff base derivatives of transition metal complexes as antimicrobial agents, Coordination Chemistry Reviews, 447 (2021) 214128.

[16] P.A. Vigato, S. Tamburini, The challenge of cyclic and acyclic Schiff bases and related derivatives, Coordination Chemistry Reviews, 248(17-20) (2014) 1717-2128.

[17] R.M. Clarke, T. Storr, The chemistry and applications of multimetallic salen complexes, Dalton Transactions, 43(25) (2014) 9380-9391.

[18] H., Kargar, P. Forootan, M. Fallah-Mehrjardi, R. Behjatmanesh-Ardakani, H.A. Rudbari, K.S. Munawar, M. Ashfaq, M.N. Tahir, Novel oxovanadium and dioxomolybdenum complexes of tridentate ONO-donor Schiff base ligand: Synthesis, characterization, crystal structures, Hirshfeld surface analysis, DFT computational studies and catalytic activity for the selective oxidation of benzylic alcohols, Inorganica Chimica Acta, 523 (2021) 120414.

[19] S.A. Aly, A. Eldourghamy, B.A. El-Fiky, A.A. Megahed, W.A. El-Sayed, E.M. Abdalla, H.H. Elganzory, Synthesis, spectroscopic characterization, thermal studies, and molecular docking of novel Cr (III), Fe (III), and Co (II) complexes based on Schiff base: In vitro antibacterial and antitumor activities, Journal of Applied Pharmaceutical Science, 13(2) (2023) 196-210.

[20] M.S. Dastjerdy, N. Monadi, Copper Schiff base complex immobilized on magnetic graphene oxide: Efficient heterogeneous nanocatalyst for treating environmental pollutants and synthesis of chromenes, Applied Organometallic Chemistry, 37(1) (2023) e6931.

[21] E.M. Emara, W.A. El-Sayed, A.S. Khalaf-Allah, F.M. Alminderej, Y.K. Abdel-Monem, A.A. Abd-Rabou, Spectral studies, thermal investigations, and anticancer activity of some divalent metal complexes derived from 2-(4-bromophenylamino) acetohydrazide ligand, Applied Organometallic Chemistry, 36(5) (2022) e6657.

[22] E.K. Inal, A fluorescent chemosensor based on schiff base for the determination of Zn^{2+}, Cd^{2+} and Hg^{2+}, Journal of Fluorescence, 30(4) (2020) 891-900.

[23] C. Boulechfar, H. Ferkous, A. Delimi, A. Djedouani, A. Kahlouche, A. Boublia, A.S. Darwish, T. Lemaoui, R. Verma, Y. Benguerba, Schiff bases and their metal complexes: a review on the history, synthesis, and applications, Inorganic Chemistry Communications, (2023) 110451.

[24] G.M. Escandar, A.C. Olivieri, A critical review on the development of optical sensors for the determination of heavy metals in water samples. the case of mercury (II) ion, Acs Omega, 7(44) (2022) 39574-39585.

[25] Z., Gul, S. Khan, E. Khan, Organic molecules containing N, S and O heteroatoms as sensors for the detection of Hg (II) ion; coordination and efficiency toward detection, Critical Reviews in Analytical Chemistry, (2022) 1-22.

[26] M.J. Culzoni, A.M. De La Peña, A. Machuca, H.C. Goicoechea, R. Babiano, Rhodamine and BODIPY chemodosimeters and chemosensors for the detection of Hg^{2+}, based on fluorescence enhancement effects, Analytical Methods, 5(1) (2013) 30-49.

[27] T. Samanta, R. Shunmugam, Colorimetric and fluorometric probes for the optical detection of environmental Hg (II) and As (III) ions. Materials Advances, 2(1) (2021) 64-95.

[28] L.M. Aroua, R. Ali, A.E. Albadri, S. Messaoudi, F.M. Alminderej, S.M. Saleh, A New, Extremely Sensitive, Turn-Off Optical Sensor Utilizing Schiff Base for Fast Detection of Cu (II), Biosensors, 13(3) (2023) 359.

[29] R. Ali, S. Ghannay, S. Messaoudi, F.M. Alminderej, K. Aouadi, S.M. Saleh, A Reversible Optical Sensor Film for Mercury Ions Discrimination Based on Isoxazolidine Derivative and Exhibiting pH Sensing, Biosensors, 12(11) (2022) 1028.

[30] S.M. Saleh, W.A. El-Sayed, M.A. El-Manawaty, M. Gassoumi, R. Ali, Microwave-Assisted Rapid Synthesis of Luminescent Tryptophan-Stabilized Silver Nanoclusters for Ultra-Sensitive Detection of Fe (III), and Their Application in a Test Strip, Biosensors, 12(6) (2022) 425.

[31] S.M. Saleh, R. Ali, O.S. Wolfbeis, Quenching of the luminescence of upconverting luminescent nanoparticles by heavy metal ions, Chemistry-A European Journal, 17(51) (2011) 14611-14617.

[32] S.M. Saleh, W.A. El-Sayed, M.A. El-Manawaty, M. Gassoumi, R. Ali, An ecofriendly synthetic approach for copper nanoclusters and their potential in lead ions sensing and biological applications, Biosensors, 12(4) (2022) 197.

[33] S.M. Saleh, M.K. Almotiri, R. Ali, Green synthesis of highly luminescent gold nanoclusters and their application in sensing Cu(II) and Hg(II), Journal of Photochemistry and Photobiology A: Chemistry, 426 (2022) 113719.

[34] R. Ali, I.A. Ali, S. Messaoudi, F.M. Alminderej, S.M. Saleh, An effective optical chemosensor film for selective detection of mercury ions, Journal of Molecular Liquids, 336 (2021) 116122.

[35] R. Ali, F.M. Alminderej, S.M. Saleh, A simple, quantitative method for spectroscopic detection of metformin using gold nanoclusters, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 241 (2020) 118744.

[36] M. Saleem, K.H. Lee, Optical sensor: a promising strategy for environmental and biomedical monitoring of ionic species, Rsc Advances, 5(88) (2015) 72150-72287.

[37] S.M. Saleh, R. Ali, M.E.F. Hegazy, F.M. Alminderej, T.A. Mohamed, The natural compound chrysosplenol-D is a novel, ultrasensitive optical sensor for detection of Cu (II), Journal of Molecular Liquids, 302 (2020) 112558.

[38] S. Kollenda, M. Kopp, J. Wens, J. Koch, N. Schulze, C. Papadopoulos, R. Pöhler, H. Meyer, M. Epple, A pH-sensitive fluorescent protein sensor to follow the pathway of calcium phosphate nanoparticles into cells, Acta Biomaterialia, 111 (2020) 406-417.

[39] Y. Wang, Q. Guo, X. Wu, H. Gao, R. Lu, W. Zhou, A facile and total watersoluble fluorescent organic nanoparticles-based sensor for Hg^{2+} detection and its application in tea samples, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 265 (2022) 120358.

[40] B. Kumar, K. Malhotra, R. Fuku, J. Van Houten, G.Y. Qu, P.A. Piunno, U.J. Krull, Recent trends in the developments of analytical probes based on lanthanide-doped upconversion nanoparticles, TrAC Trends in Analytical Chemistry, 139 (2021) 116256.

[41] S. Gurusamy, M. Sankarganesh, V. Sathish, P. Thanasekaran, A. Mathavan, A novel colorimetric, selective fluorescent “turn-off” chemosensor and biomolecules binding studies based on iodosalicylimine schiff-base derivative, Journal of Photochemistry and Photobiology A: Chemistry, 425 (2022) 113674.

[42] T.I. Kim, D. Kim, J. Bouffard, Y. Kim, Rapid, specific, and ultrasensitive fluorogenic sensing of phosgene through an enhanced PeT mechanism, Sensors and Actuators B: Chemical, 283 (2019) 458-462.

[43] M. Zhu, L. Sun, X. Liu, X. Pang, F. Fan, X. Yang, R. Hua, Y. Wang, A reversible CHEF-based NIR fluorescent probe for sensing Hg2+ and its multiple application in environmental media and biological systems, Science of The Total Environment, 874 (2023)162460.

[44] Y. Dong, R. Fan, W. Chen, P. Wang, Y. Yang, A simple quinolone Schiff-base containing CHEF based fluorescence ‘turn-on’chemosensor for distinguishing Zn^{2+} and Hg^{2+} with high

sensitivity, selectivity and reversibility, Dalton Transactions, 46(20) (2017) 6769-6775.

[45] H. Lee, H.S. Lee, J.H. Reibenspies, R.D. Hancock, Mechanism of “turn-on” fluorescent sensors for mercury (II) in solution and its implications for ligand design, Inorganic chemistry, 51(20) (2012) 10904-10915.

[46] M. Stanisavljevic, S. Krizkova, M. Vaculovicova, R. Kizek, V. Adam, Quantum dots-fluorescence resonance energy transfer-based nanosensors and their application, Biosensors and Bioelectronics, 74 (2015) 562-574.

[47] A. Miyawaki, Development of probes for cellular functions using fluorescent proteins and fluorescence resonance energy transfer, Annual review of biochemistry, 80 (2011) 357-373.

[48] F.M. Alminderej,, H.H. Elganzory,, M.N. El-Bayaa,, H.M. Awad, and W.A. El-Sayed,, Synthesis and cytotoxic activity of new 1,3,4-thiadiazole thioglycosides and 1,2,3-triazolyl-1,3,4-thiadiazole N-glycosides. Molecules, 24(20), p. 2019. 3738.

[49] T. Pivetta, S. Masuri, M.G. Cabiddu, C. Caltagirone, A. Pintus, M. Massa, F. Isaia, E. Cadoni, A novel ratiometric and turn-on fluorescent coumarin-based probe for Fe (iii), New Journal of Chemistry, 43(30) (2019) 12032-12041.

[50] H. Jia, X. Gao, Y. Shi, N. Sayyadi, Z. Zhang, Q. Zhao, Q. Meng, R. Zhang, Fluorescence detection of Fe^{3+} ions in aqueous solution and living cells based on a high selectivity and sensitivity chemosensor, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 149 (2015) 674-681.

[51] J. Xie, Y. Zheng, J.Y. Ying, Protein-directed synthesis of highly fluorescent gold nanoclusters, Journal of the American Chemical Society, 131(3) (2009) 888-889.

[52] K.M. Vengaian, C.D. Britto, K., Sekar, G. Sivaraman, S. Singaravadivel, Phenothiazine-diaminomalenonitrile based colorimetric and fluorescence “turn-off-on” sensing of Hg^{2+} and S^{2-}, Sensors and Actuators B: Chemical, 235 (2016) 232-240. [53] H. Li, L. Cai, Z. Chen, Coumarin-derived fluorescent chemosensors, Advances in chemical sensors, 1 (2012)121-150.

[54] R. Ali, B. Alfeneekh, S. Chigurupati, S.M. Saleh, Green synthesis of pregabalin stabilized gold nanoclusters and their applications in sensing and drug release, Archiv der Pharmazie, 355(4) (2022) 2100426.

[55] S.M. Saleh, R. Ali, F. Alminderej, I.A. Ali, Ultrasensitive optical chemosensor for Cu (II) detection, International journal of analytical chemistry, (2019) 2019.

[56] A. Maity, A. Sil, S. Nad, S.K. Patra, A highly selective, sensitive and reusable BODIPY based ‘OFF/ON'fluorescence chemosensor for the detection of Hg2+ Ions, Sensors and Actuators B: Chemical, 255 (2018) 299-308.

[57] Y. Zhang, C. Zhang, Y. Wu, B. Zhao, L. Wang, B. Song, A novel water-soluble naked-eye probe with a large Stokes shift for selective optical sensing of Hg2+ and its application in water samples and living cells, RSC advances, 9(40) (2019) 23382-23389.

[58] L. Zong, C. Wang, Y. Song, Y. Xie, P. Zhang, Q. Peng, Q. Li, Z. Li, A dual-function probe based on naphthalene diimide for fluorescent recognition of Hg2+ and colorimetric detection of Cu2+, Sensors and Actuators B: Chemical, 252 (2017) 1105-11.

[59] L. Zong, Y. Xie, Q. Li, Z. Li, A new red fluorescent probe for Hg2+ based on naphthalene diimide and its application in living cells, reversibility on strip papers, Sensors and Actuators B: Chemical, 238 (2017) 735-43.

[60] X. Cao, Y. Li, Y. Yu, S. Fu, A. Gao, X. Chang, Multifunctional supramolecular self-assembly system for colorimetric detection of Hg2+, Fe3+, Cu2+ and continuous sensing of volatile acids and organic amine gases, Nanoscale, 11(22) (2019)10911-20.

[61] R.V. Rathod, S. Bera, P. Maity, D. Mondal, Mechanochemical synthesis of a fluorescein-based sensor for the selective detection and removal of Hg2+ ions in industrial effluents, ACS omega, 5(10) (2020) 4982-4990.

[62] A. Kumar, R. Ananthakrishnan, G. Jana, P.K. Chattaraj, S. Nayak, S.K. Ghosh, An Intramolecular Charge Transfer Induced Fluorescent Chemosensor for Selective Detection of Mercury (II) and its Self-Turn-On Inside Live Cells at Physiological pH, ChemistrySelect, 4(17) (2019) 4810-4819.