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Research Article

A Turn OFF Fluorescent Probe For Selective Detection Of Hg^{2+} Ions

Duygu Aydin[1]

[1] *Karamanoglu Mehmetbey University, Kamil Ozdag Science Faculty, Department of Chemistry, Karaman, 70100 Turkey, (ORCID: 0000-0003-0436-1869)*

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Abstract

In this study, we prepared a novel fluorescent chemosensor containing an imidazole molecule and the chemosensor characterized utilizing ^{1}H-NMR, ^{13}C-NMR spectroscopy, FT-IR spectrometer and elemental analyzer. Prepared sensor was utilized as an effectively selective and a fastly responsive chemical fluorescent sensor for ‘’turn off’’ determination of mercury (II) ions in EtOH. A clear complex between 2-((4-(1H-phenanthro[9,10-d] imidazol-2-yl)benzylidene)amino) phenol **(PENIM)** and Hg^{+} ions was determined and calculated employing the Job's method and also the limit of detection value was found to be 2.1 nM on the basis of $3\sigma/k$. Furthermore, the sensor-Hg^{2+} displayed and reversible property for mercapto containing cysteine molecules. Also, the fluorescence enhancement and quenching studies were supported by computational experiments based on the density functional theory (DFT) calculations.

Keywords: Fluorescence sensor, Mercury ions.

Öz

Bu çalışmada imidazol içeren yeni bir floresan sensör sentezlenmiş ve sentezlenen sensörün karakterizasyonu ^{1}H-NMR, ^{13}C-NMR spektroskopisi, FT-IR spektroskopisi ve elemental analiz cihazı kullanılarak yapılmıştır. Hazırlanan sensör etanol içerisinde civa iyonlarına hem hızlı hem de seçici olarak floresanı söndürerek cevap vermiştir. Sentezlenen 2-((4-(1H-Fenantreimidazol [9,10-d] imidazol-2-)benziliden)amino) fenol **(PENIM)** and Hg^{+} iyonları arasında kompleksleşme gerçekleştirilmiş ve bu komplesleşme JOB metodu kullanılarak da kompleksleşme oranı teorik olarak hesaplanmıştır. Sentezlenen probun deteksiyon limiti $3\sigma/k$ formülü kullanılarak 2.1 nM olarak tespit edilmiştir. Ayrıca, elde edilen civa (II)-**PENIM**, merkapto molekülü içeren sistein amino asidine karşı tersinir olarak cevap vermiştir. PENIM molekülünün civa iyonlarına karşı cevap vermesinin sonucu olarak floresan şiddetinin artırılması ve söndürülmesi ile ilgili çalışma, yoğun fonksiyonel hesaplama(DFT) yapılarak teorik olarak desteklenmiştir.

Anahtar Kelimeler: Floresan sensör, Civa iyonları

1. Introduction

During the past decade, development of practical chemosensors has been taken attention of researchers since they allow the selective and sensitive detection of heavy metal ions causing serious environmental disasters and health problems. Among these metal ions, mercury ions and most of their compounds exist in water, soil, and food. Even in small concentrations, they are extremely poisonous neurological toxins and the most ubiquitous pollutants in ecosystem. The human body may receive mercury ions through contaminated natural water which is a major source of mercury ions and skin contact to mercury containing natural and anthropogenic sources. The accumulation of mercury ions in the body can cause a number of severe permanent health problems for example brain damage, and various cognitive and motion disorders, mitosis impairment, coronary heart disease and kidney failure(J. F. Chen et al., 2017; Jiao, Zhang, & Zhou, 2016; G. Li, Ma, Liu, Fan, & Pu, 2017; Q. Li et al., 2016). The Environmental Protection Agency (EPA) and The World Health Organization (WHO) have reported that the maximum allowable concentration for mercury (II) in drinking water is 2.0 and 6.0 ppb, respectively (M. Li, Zhou, Ding, Guo, & Wu, 2013).

[1] **Corresponding Author:** Karamanoglu Mehmetbey University, Kamil Ozdag Science Faculty, Department of Chemistry, Karaman, 70100 Turkey, ORCID: 0000-0003-0436-1869, duyguaydin@kmu.edu.tr

Thus, the development of efficient methodologies for rapid and sensitive detection of Hg^{2+} ions in contaminant natural water is undoubtedly an important step to monitor their toxic impacts on both the human health and environment(G. Chen, Guo, Zeng, & Tang, 2015; L. Chen et al., 2011; Han, Yuan, & Wang, 2009; Karuk Elmas & Yilmaz, 2018).

In this regard, researchers have proposed numerous sensitive methods, including graphite flame atomic absorption spectrometry (AAS), Plasmon-resonance Rayleigh scattering spectroscopy (RSS), inductively coupled mass atomic emission spectrometry (ICM–AES), electrochemical assays, capillary electrophoresis and so on, for effectively monitoring mercury (II) ions in the past few years. Although these methods have good sensitivity and selectivity, they are high cost, time consuming and require complicated sample pretreatment procedures. Therefore, fluorescence chemosensors based determination technique is preferred since this technique presents some advantages for example operational homeliness, real-time monitoring, simple instrumentation, quick response time and non–destructive. In this concept, numerous fluorescent groups based on organic dyes such as BODIPY, fluorescein, perylene, pyrene, coumarin, naphthylamide and cyanine etc have been developed and utilized in sensitive detection of Hg^{2+}(Guo & Irudayaraj, 2011; Taki, Akaoka, Iyoshi, & Yamamoto, 2012). However, most of the designed mercury sensors have disadvantages, especially in terms of long response time, sensitivity and selectivity.

In this study, we fabricate a new highly sensitive fluorescent "turn off" chemosensor containing 4-(1H-phenanthro[9,10-d]imidazol-2-yl)benzaldehyde (**PENIM**) for the determination of mercury (II) ions in ethanol. Fabricated sensor was analyzed by ^{1}H-NMR, ^{13}C-NMR, ATR–FTIR spectroscopy and elemental analyzer. It showed a remarkable fluorescence enhancement for mercury (II) the detection in solution with high sensitivity and selectivity. Therefore, fabricated sensor could be utilized as a fluorescence sensor for analyzing of mercury (II) in solutions.

2. Materials and Methos

2.1. General Information

2-hydroxy anyline, terephtalaldehyde, amoniumacetate, 9,10-phenanthroquinone, ammonium acetate, other used reagents and all solvents were obtained from commercial suppliers and they were employed without further purification. Perchlorate salts of metal ions were utilized. FT-IR spectroscopy and NMR spectra were collected employing a Perkin Agilent Cary Eclipse spectrometer and a Varian 400 MHz instrument, respectively. Elemental analysis results for **PENIM** were obtained by using a Leco CHNS 932 instrument. Fluorescent spectra were taken using on a Perkin Agilent Cary Eclipse spectrometer.

2.2. Synthesis of 4-(1H-phenanthro[9,10-d]imidazol-2-yl)benzaldehyde (PEMA)

The synthesis of 4-(1H-phenanthro[9,10-d]imidazol-2-yl)benzaldehyde (**PEMA**) was carried out according to reported previously literature (Lin, Long, Yuan, Cao, Chen&Tan, 2008).

2.3. Synthesis of 2-((4-(1H-phenanthro[9,10-d]imidazol-2-yl)benzylidene)amino)phenol (PENIM)

To synthesize the ligand, 2-((4-(1H-phenanthro[9,10-d]imidazol-2-yl)benzylidene)amino) phenol (**PENIM**) (0.5 g, 1.55 mmol) and 2-hydroxy anyline (0.169 g, 1.55 mmol) were added into a round-bottom flask and dissolved in absolute ethanol (10 mL) and reaction was refluxed for 12 h under nitrogen atmosphere. Afterwards, precipitate was filtered and washed with excess EtOH for removing unreacted species. The precipitate was dried in *vacuo.* Yield: 60%. Anal. Calcd. for $C_{28}H_{19}N_3O$ (Mw: 413.47): requires C, 81.97; H, 4.38; N, 8.69 % Found: C, 81.21; H, 4.45; N, 8.95%. ^{1}H NMR (400 MHz, d_6-DMSO): δ 6.70 (t, 1H, ArH), 6.90 (d, 1H, ArH), 7.22 (t, 1H, ArH), 7.40 (d, 1H, ArH), 7.60 (m, 2H, ArH), 7.70 (m, 2H, ArH), 8.32 (d, 2H, ArH), 8.45 (d, 2H, ArH), 8.62 (m, 2H, ArH), 9.06 (m, 2H, ArH), 8.89 (s, 1H, CHN), 9.21 (s, 1H, OH), 13.53 (s, 1H, NH). ^{13}C NMR (100 MHz, d_6-DMSO): δ 152.51, 151.99, 140.57, 137.78, 135.06, 134.61, 131.70, 131.50, 130.68, 128.24, 127.43, 127.21, 125.98, 125.86, 125.47, 125.05, 125.01, 124.52, 124.45, 124.34, 124.29, 120.98, 120.29, 116.76 (Fig1 and 2).

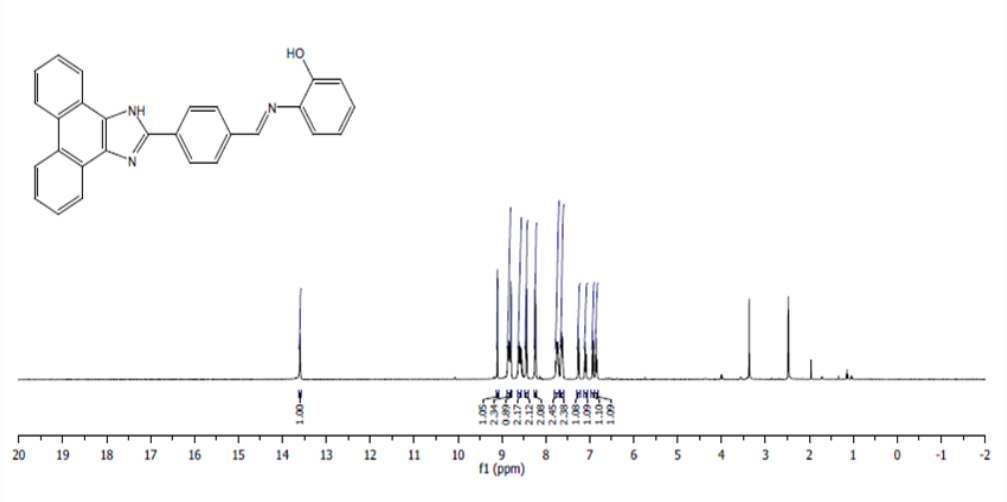


Fig. 1. ^{1}H-NMR spectrum of **PENIM** in DMSO-d_6.

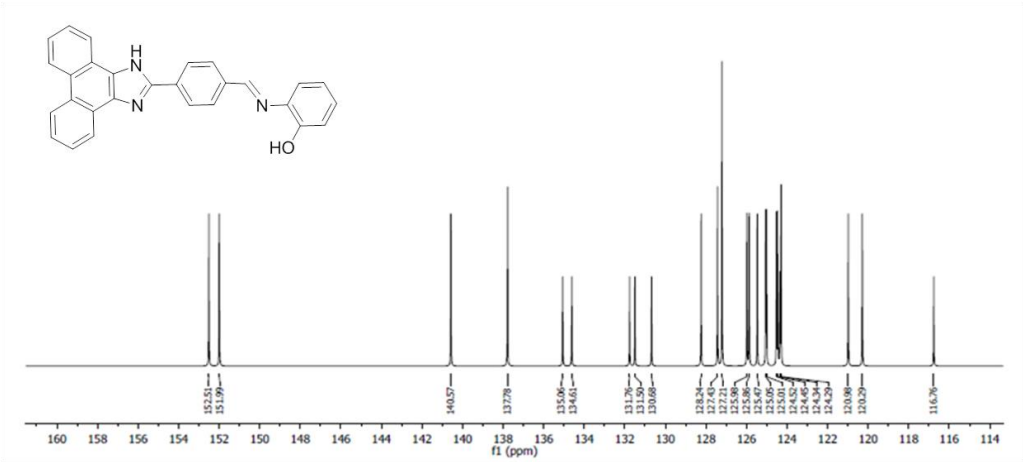


Fig. 2. ^{13}C-NMR spectrum of **PENIM** in DMSO-d_6.

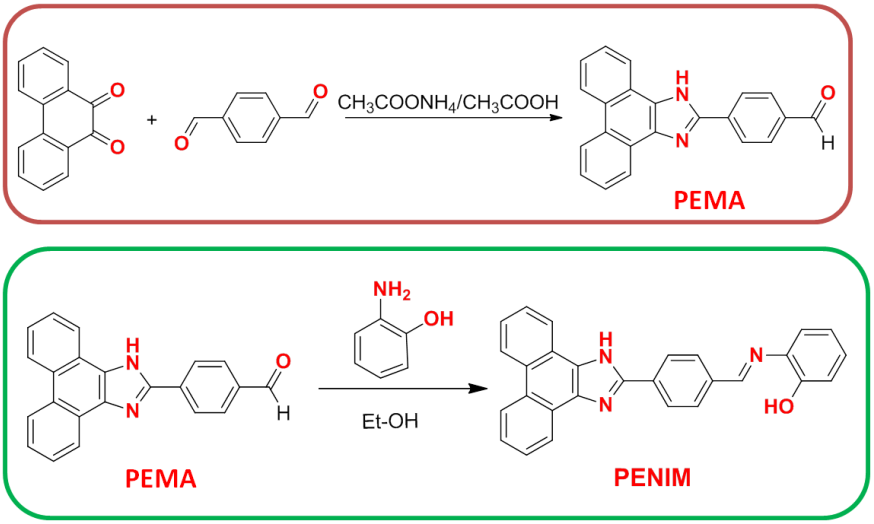
2.4. The procedures of Hg (II) determination

Initially, 10.0 mM ligand solution was dissolved in dimethylsulfoxide (DMSO) and diluted with EtOH and so the concentration of prepared ligand was obtained as 1.0 μM. 3 mL of solution was added to a cuvette to evaluate detection of Hg^{2+}. Afterwards, probe solution (1.0 μM) was mixed to obtain homogenous mixture. Then, the fluorescence measurements of probe solutions was measured in the presence of other cation ions utilizing λ_{ex}=364 nm, λ_{em}=442 nm and slit widhts=10 nm, 10 nm.

3. Results and Discussion

3.1. Synthesize

To synthesize our designed sensor **(PENIM),** we first prepared **PEMA** according to previous literature. Afterwards, **PEMA** was reacted with hydroxy anyline using reflux system in dry EtOH at high yield. The prepared compound was analyzed utilizing ^{1}H-NMR, ^{13}C-NMR, FT-IR spectroscopy and elemental analyzer. The general procedure for synthesis is presented in **Scheme 1**.



Scheme 1. Synthesis routes of **PEMA** and **PENIM**.

3.2. Fluorescence studies

The fluorescence ability of the prepared **PENIM** (1.0 μM) in EtOH was evaluated in the presence of 10.0 equiv of a series of metal ions (K^+, Mg^{2+}, Ni^{2+}, Ba^{2+}, Mn^{2+}, Sr^{2+}, Fe^{2+}, Fe^{3+}, Cu^{2+}, Zn^{2+}, Cd^{3+}, Hg^{2+}, Al^{3+}, Ca^{2+} and Pb^{2+}) employing fluorescence spectroscopy. Initially, the probe in EtOH controlled and displayed the highest fluorescence intensity at 442 nm in the absence of metal ions (Fig 3a). Afterwards, as depicted in Fig 3b, addition of Hg^{2+} to prepared **PENIM** in EtOH (1.0 μM) leads to the fluorescence complete quenching of emission band at 442 nm, but none of the other metal ions except Hg^{2+} displayed a remarkable change of the fluorescence intensity of fabricated probe at 442 nm. Furthermore, the fluorescence intensity of the designed sensor was tested in various solvents, including N,N-dimethyl formamide (DMF), ethanol (EtOH) and water, compared with acetonitrile (CH_3CN) (Fig. 3b). Consequently, fabricated probe displayed the strong interaction with Hg (II) ions compared to other metal ions and this is as a result of the formation of the stable chelation between **PENIM** and Hg^{2+} through the phenolic oxygen and N atoms.

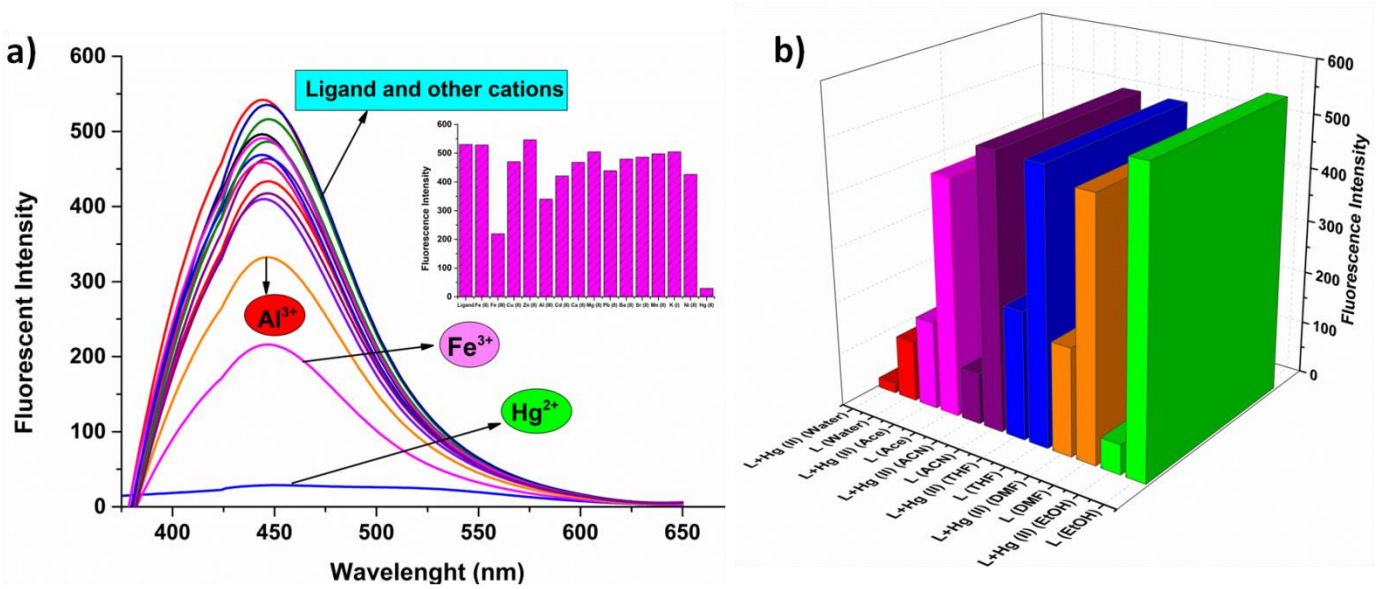


Figure 3. (a) Fluorescence spectra of **PENIM** in EtOH solution before and after addition of 10.0 equiv of various metal ions (K^+, Mg^{2+}, Ni^{2+}, Ba^{2+}, Mn^{2+}, Sr^{2+}, Fe^{2+}, Fe^{3+}, Cu^{2+}, Zn^{2+}, Cd^{3+}, Hg^{2+}, Al^{3+}, Ca^{2+} and Pb^{2+}) and (b) fluorescence spectra of **PENIM** and **PENIM-Hg^{2+}** in various solvents.

The chemosensing properties of the proposed sensor toward Hg^{2+} in EtOH were determined utilizing titration experiments. The fluorescence intensity of fabricated **PENIM** at 442 nm gradually decreased and quenched by the addition of varying amount of Hg^{2+} concentration from 0.0 up to 35.0 equiv. This quenching results from the complexation between **PENIM** and mercury (II) complex and this may be attributed due to heavy atom effect of transition metals (Fig. 4).

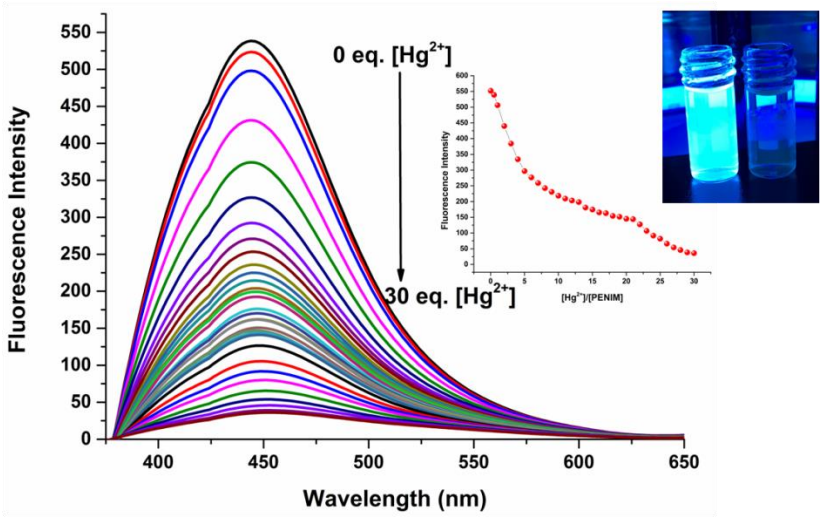


Figure 4. The fluorescence spectrum of **PENIM** with of increasing concentration of Hg^{2+} in EtOH (ex= 364 nm).

The binding constant of **PENIM** with mercury (II) has been determined employing Benesi–Hildebrand plot based on the fluorescence titration data and calculated to be 1.119×10^{5} M^{-1} (Fig. 5a). Moreover, according to a plot of the concentration of mercury ions versus fluorescence intensity at 442 nm, the detection limit (LOD) of for the detection of **PENIM** for Hg^{2+} based on $3\sigma/k$ equation was found to be 2.1 nM and this value was much lower than the maximum allowed levels of mercury (II) in drinking water of U.S. EPA (2.0 ppm) and WHO (6.0 ppm) (Fig. 5b)(M. Li et al., 2013). To examine the binding stoichiometry between **PENIM** and Hg^{2+}, the Job's plot was conducted and 2:1 stoichiometric complexation between **PENIM** and Hg^{2+} was obtained as a result of the maximum relative fluorescence intensity was around 0.66 (Fig. 6a).

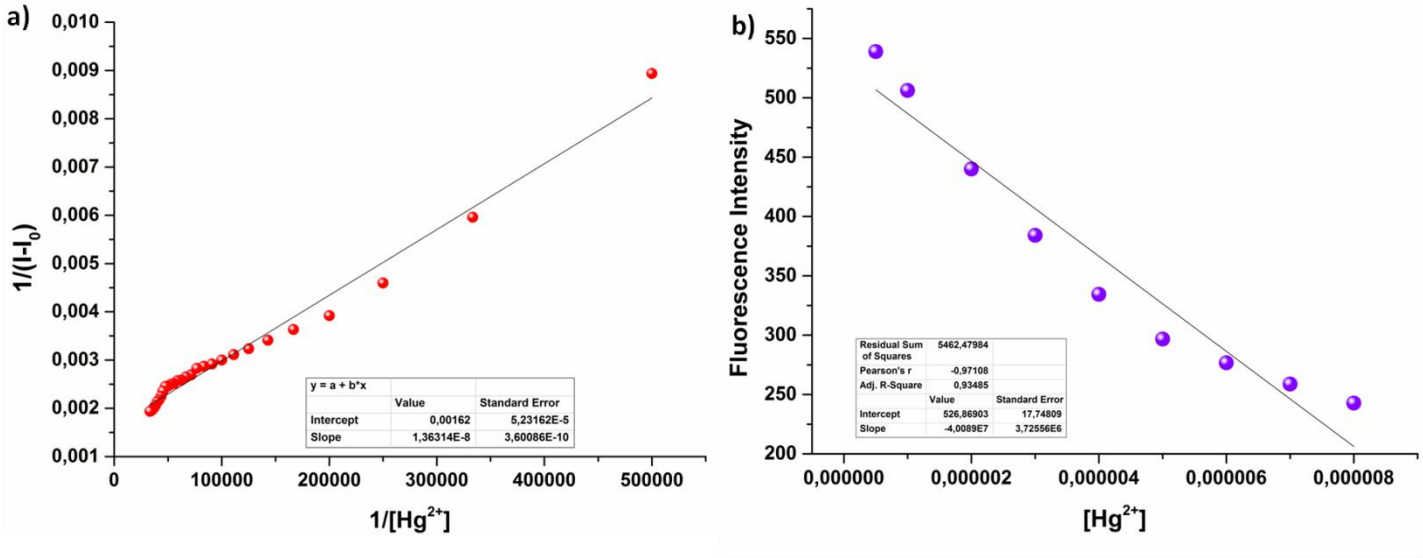


Figure 5. (a) Benesi–Hildebrand plots from fluorometric titration data of **PENIM** (1.0 μM) with Hg^{2+} and (b) The plot of emission intensities of **PENIM** at 412 nm versus with various Hg^{2+} concentrations.

The effects of other cations on the interaction of **PENIM** (1.0 μM) in EtOH with mercury (II) ions were examined by comparison experiments. These experiments were carried out by Hg (II) ions in the presence of 10.0 equiv of other metal ions. Other metal ions, except for Fe^{3+} and Al^{3+}, did not interfere with mercury (II) ions (Fig. 6b). It appears that **PENIM** is an effective fluorescence sensor for the detection of Hg^{2+} cations, even in the presence of many other metals.

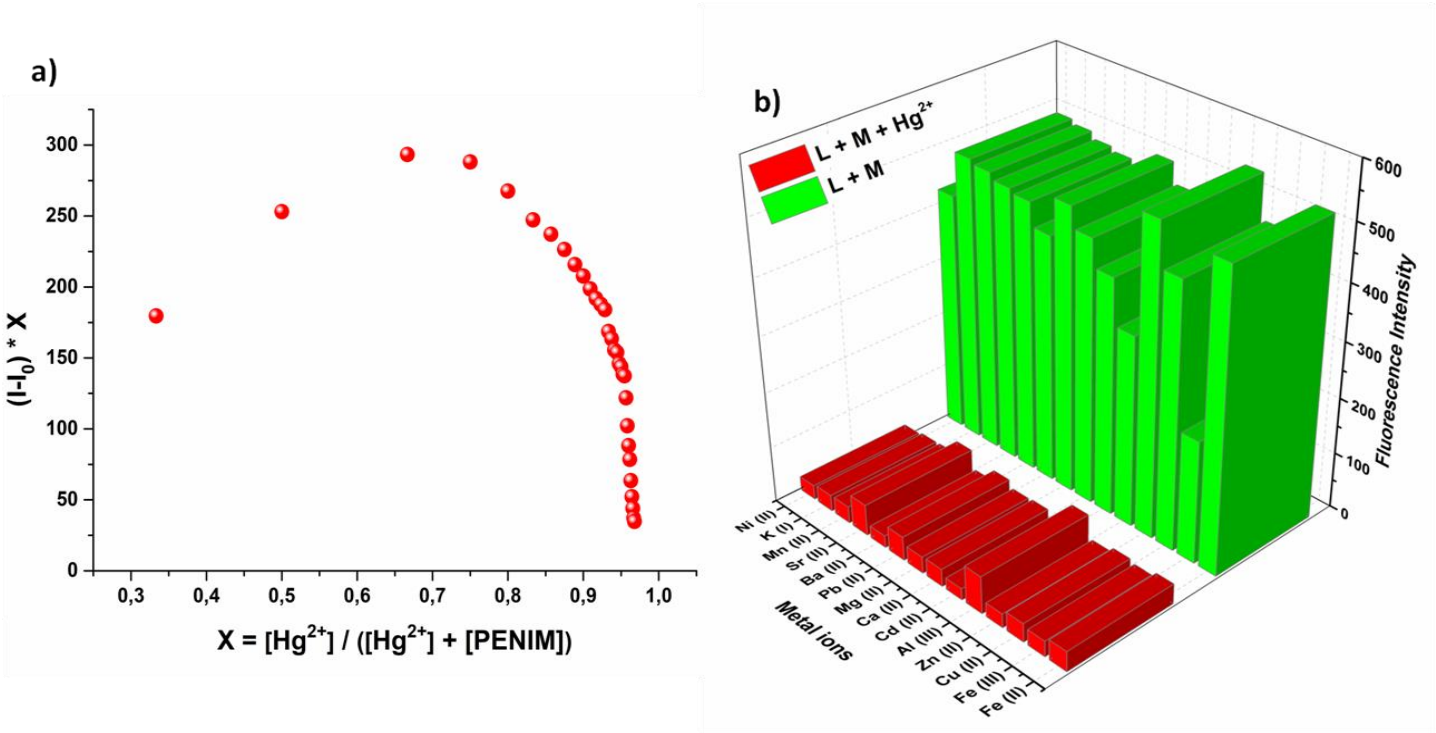


Figure 6. (a) Job's plot study of **PENIM** (1.0 μM) with Hg^{2+} and (b) competition studies of **PENIM** with Hg^{2+} in the presence of various metal ions at 412 nm in EtOH.

To confirm the optimized structure of the complexation, the density functional theory (DFT) calculations for obtaining computational experiments were carried out with B3LYP/6-31G(d) basis sets utilizing a suite of Gaussian 09 programs. The gaps of bands between HOMO (highest occupied molecular orbital) and LUMO (highest occupied molecular orbital) of **PENIM** and **PENIM**-Hg^{2+} were found to be 3.4 eV and 2.76 eV respectively. These results revealed that the interaction between PENIM and Hg^{2+}decreases the HOMO-LUMO energy gap of the complex and so the stabilized complex formation between **PENIM** and Hg^{2+} was obtained (Fig. 7).

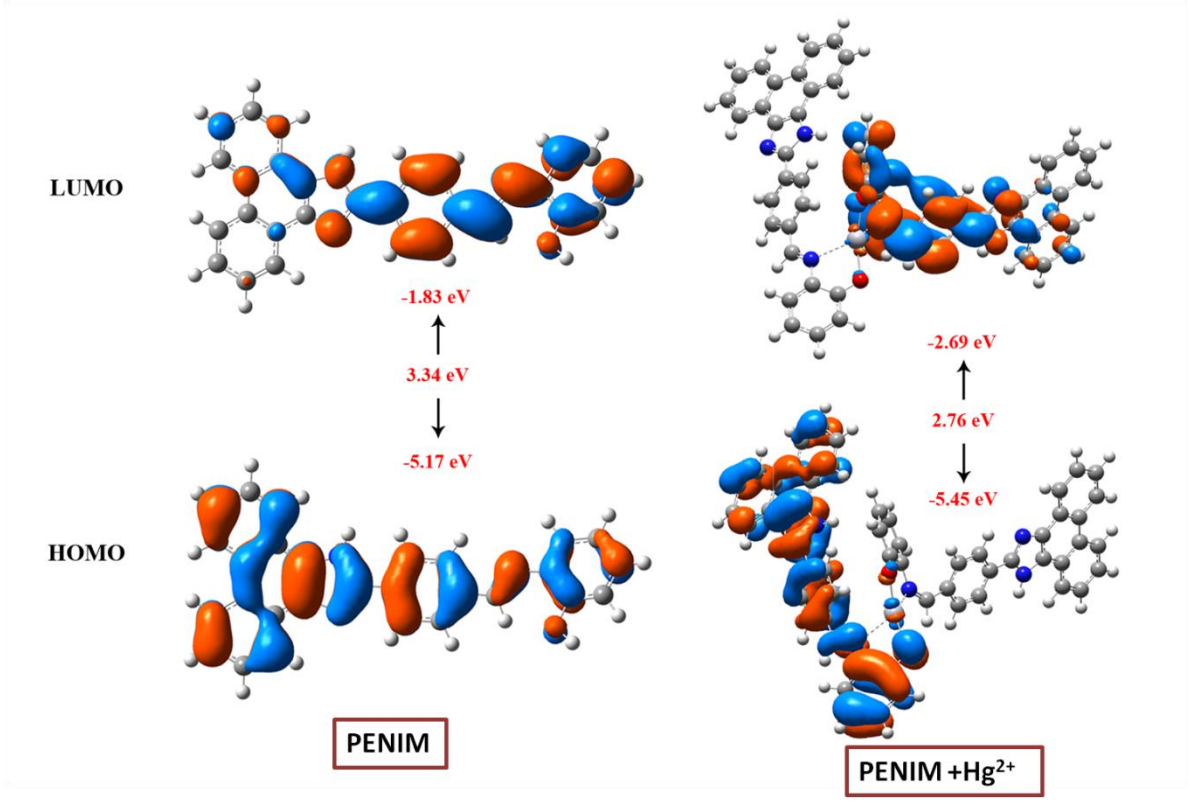


Figure 7. Electron density in HOMO and LUMO of **PENIM** and its complex with Hg^{2+}.

In addition, **PENIM** and **PENIM**-Hg^{2+} were characterized with FT-IR spectroscopy measurements. The band at 3321 cm^{-1} is attributed to the characteristic vibration of the phenolic -OH and this band conforming **PENIM**-Hg^{2+} formation via coordination between -OH and Hg^{2+} disappears. Also, a band at 1585 cm^{-1} proves the vibration of -CH bonding of shift base and shifts to higher frequency to 1643 cm^{-1}. The above results display that the complexation **PENIM**-Hg^{2+} formed via CHN group of shift base and OH group of **PENIM** (Fig 8).

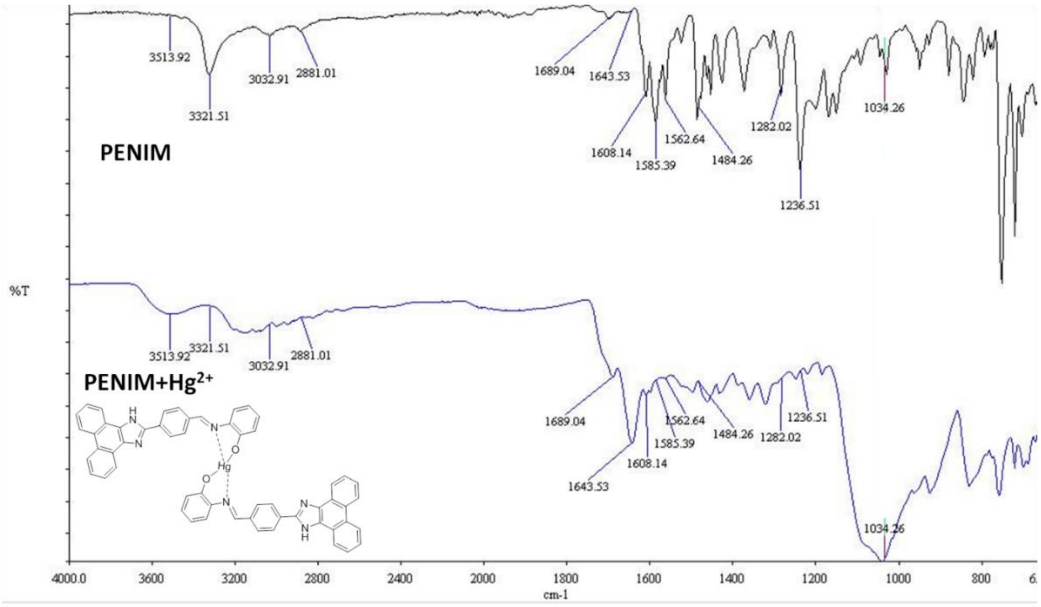


Figure 8. FT-IR spectrum of **PENIM** and **PENIM**-Hg^{2+}

Response time is a very important parameter for various applications and real time monitoring, so low response time has advantages. Therefore, the binding process of mercury (II) ions to prepared **PENIM** was investigated to understand response time of this complexation process as shown in Fig. 9a. Fluorescence intensity of fabricated **PENIM**-Hg^{2+} remains constant within 30s after the addition of Hg^{2+} to the **PENIM** solution (1.0 μM) in EtOH.

Reversibility of a probe plays critical roles in judging its chances for practical applications. The reversibility of the Hg^{2+} complexation with the **PENIM** employed reversible property allowed the detecting of various amounts of thiol containing cysteine amino acid. As shown in Fig 9b, when thiol containing cysteine was added to the Hg^{2+} complexation with the **PENIM**, the fluorescence intensity raised. However, upon adding of the Hg^{2+} solution again, it was observed that the intensity decreased again. These results clearly demonstrated that the reversibility of the Hg^{2+} complexation with the probe can be easily regenerated for repeated use and gives a chance for utilizing practical applications.

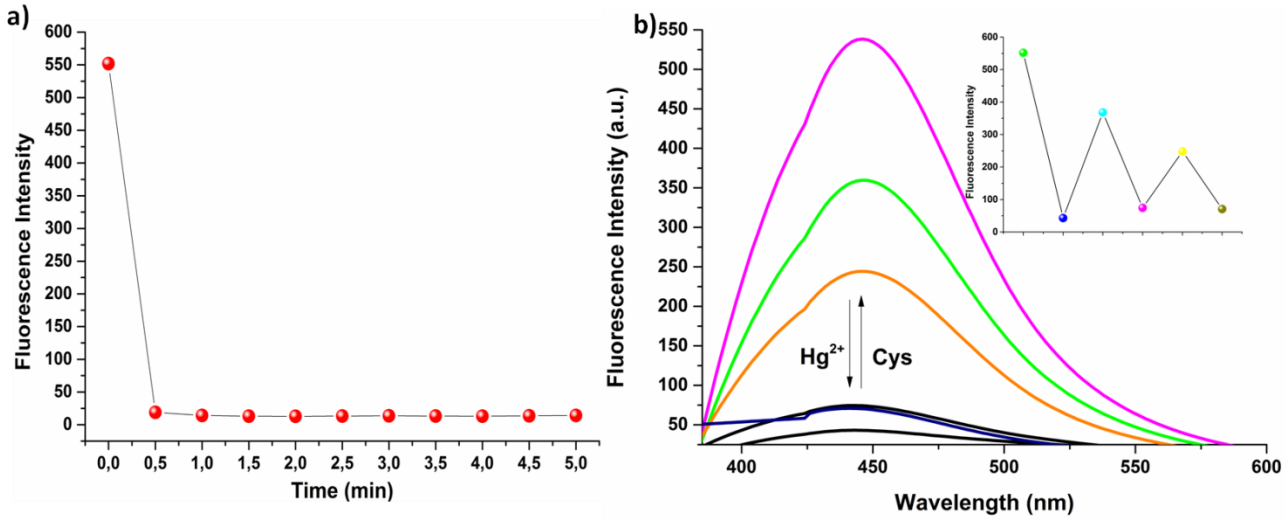


Figure 9. (a) Response time of **PENIM**-Hg^{2+} complex (b) the reversibility of **PENIM** with Hg^{2+} and Cys.

4. Conclusion

Herein, we reported the synthesis of a new reversible turn off-on fluorescence probe with high conversions. Fabricated **PENIM** showed high sensitivity and selectivity in determining of Hg^{2+} ions and reversible property for a mercapto containing biomolecule such as cysteine. The complexation between **PENIM** and Hg^{2+} was verified from fluorescence spectra, FT-IR spectroscopy and DFT calculations. The response time of the complexation is so fast and this quick respond time enables us an advantage to provide effective and selective determination of toxic Hg^{2+} in a low time. Also, prepared sensor could display reproducibility against Hg^{2+} and a mercapto containing biomolecule for example cysteine. These results indicate that the sensor could be utilized a promising sensor for biomedical applications.

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