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Research Article

A New Rhodamine-Based Colorimetric and Fluorescence Chemosensor for the Real-Time Determination of Hg^{2+} in Aqueous Media

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Abstract

The selective and sensitive detection of trace mercury ion in aqueous solutions is vital because of their harmful effects for human health and living systems. In this study, a novel nitro-benzene thiosemicarbazide appended rhodamine derivative (the chemosensor **BODN**) was designed and synthesized. The chemosensor **BODN** is prepared as a fluorescence "turn on" and colorimetric probe for the spesific determination of Hg^{2+} ion without interference from other competitive metal cations in H_2O/ACN (v/v, 20/80) media. The chemosensor **BODN** was characterized by using the electrospray ionisation mass spectrometry (ESI–MS), NMR, and FT–IR spectroscopies. All fluorescence and absorbance spectral experiments were carried out at room temperature (25 °C). The chemosensor **BODN** showed an emission enhancing at 580 nm in the presence of Hg^{2+} ion because of "FRET on" (Fluorescence resonance energy transfer on) process based on the ring-opening mechanism of the rhodamine spirolactamin unit. This enhancement was investigated using fluorescence spectroscopy. A new absorbance band at 560 nm was formed and the drastic color change from colorless to pink upon the transferring of Hg^{2+} ion can act it a usable "naked eye" probe for the monitoring of Hg^{2+} ion. The binding stoichiometry between the chemosensor **BODN** and Hg^{2+} ion was found to be 2:1 by using Job's plot analysis and ESI-MS, respectively. The binding constant between the chemosensor **BODN** and Hg^{2+} was found to be 3.20×10^4 M^{-1}. In addition, the chemosensor **BODN** could detect Hg^{2+} ion at a concentration as low as 3.21 nM in H_2O/ACN (v/v, 20/80). Results of this study indicated that the fluorescence and colorimetric chemical sensor can be employed in aqueous media analysis to detect Hg^{2+} ion with high selectively and sensitively.

Keywords: Chemosensor, Colorimetric, Fluorescence, Hg^{2+}, Rhodamine.

Sulu Ortamda Gerçek Zamanlı Civa İyonunun Tayini için Yeni Bir Rodamin Bazlı Floresans ve Kolorimetrik Kimyasal Sensör

Öz

Sulu çözeltilerde eser miktardaki civa iyonunun seçici ve hassas tespiti, insan sağlığı ve canlı sistemler üzerindeki zararlı etkileri nedeniyle hayati önem taşımaktadır. Bu çalışmada, yeni bir nitro benzen tiyosemikarbazid modifiyeli rodamin türevi (kemosensör **BODN**) tasarlanıp, sentezlenmiştir. Kemosensör **BODN** diğer yarışmalı metal katyonların varlığında, H_2O/ACN (v/v, 20/80) çözelti ortamında Hg^{2+} iyonunun seçici olarak belirlenmesi amacıyla bir floresans "turn on" ve kolorimetrik kemosensör olarak hazırlanmıştır. Kemosensör **BODN** elektrosprey iyonizasyon kütle spektrometresi (ESI–MS), NMR ve FT–IR spektroskopileri kullanılarak karakterize işlemleri gerçekleştirilmiştir. Tüm floresans ve absorbans spektral ölçüm deneyleri, oda sıcaklığında gerçekleştirilmiştir (25 °C). Kemosensör **BODN**, yapısında bulunan spirolaktam ünitesinin halka açılma mekanizması prosesine dayanan "FRET on" (Floresans rezonans enerji transferi) mekanizması sebebiyle 580 nm dalga boyunda Hg^{2+} iyonları varlığında doğrusal artan bir emisyon şiddeti göstermiştir. Bu artış floresans spektroskopisi kullanılarak incelenmiştir. Ayrıca Hg^{2+} iyonu varlığında 560 nm'de yeni bir absorbans bandı oluşmuştur ve böylelikle kimyasal sensör çözeltisinin rengini renksizden pembeye değiştirmiştir. Bu durum kemosensör **BODN** ile Hg^{2+} iyonunun çıplak gözle tayini için bir kimyasal sensör olarak kullanılabilir

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olduğunu göstermektedir. Kemosensör **BODN** ve Hg^{2+} iyonu arasındaki bağlanma stokiyometrisi hakkında bilgi sahibi olmak amacıyla Job's grafik analizi ve ESI-MS tekniği kullanılmıştır Kompleks oluşumunda bağlanmanın 2:1 oranında gerçekleştiği saptanmıştır. Kemosensör **BODN** ve Hg^{2+} iyonu arasındaki bağlanma sabit katsayısı ise $3.20x10^4$ M^{-1} olarak bulunmuştur. Ek olarak, kemosensör **BODN** H_2O/ACN (v/v, 20/80) çözelti karışımında 3.21 nM kadar düşük konsantrasyonda Hg^{2+} iyonunu tespit edebildiği hesaplanmıştır. Bu çalışmanın sonucu olarak, geliştirilen floresans ve kolorimetrik kimyasal sensörün (**BODN**) sulu ortamda yüksek seçicilik ve duyarlılıkla Hg^{2+} iyonunu saptamak amacıyla kullanılabileceğini göstermektedir.

Anahtar Kelimeler: Kemosensör, Kolorimetrik, Floresans, Hg^{2+}, Rodamin.

1. Introduction

Selective and sensitive determination of cations is of great attention, as they have important roles in the environment and living systems (D. Aydin, 2018; D. Aydin, 2020). Mercury is one of the most toxic heavy metal, has an immensely toxic effect on living organisms and ecosystem because of its accumulated characteristics in several natural and anthropogenic sources. Mercury pollution is a universal issue, bringing vital environmental and health problems, for examples kidney, stomach and neurological diseases due to the high affinity of Hg^{2+} for -SH groups in proteins and enzymes. The United States Environmental Protection Agency notified a maximum mercury concentration level in drinkable water at 0.002 $mg.L^{-1}$ (10 $nmol.L^{-1}$). Therefore, it is significant to determine the concentrations of mercury ion at trace concentrations in several biological and environmental samples (C. Camaschella, 2017; G. Chen, Guo, Zeng, & Tang, 2015; L. Chen et al., 2011; Han, Yuan, & Wang, 2009; Karuk Elmas & Yilmaz, 2018).

There have been several methods for the sensing of Hg^{2+} ion in the past decades for examples electroanalytical methods, atomic absorption inductively coupled plasma mass spectrometry, etc. Compared to those techniques, fluorescence sensing strategies have received deep interest for sensing of trace–level of Hg^{2+} because of the advantage of operational simplicity, high selectivity, rapidity, low–cost of instrument, selective and sensitive determination, real-time monitoring behavior and applications in vivo/vitro bio-imaging (Liu et al., 2015; Ş.N. Karuk Elmas et al., 2017).

Among of fluorophores, rhodamine-based fluorescence chemosensors have been remarkable attention due to their excellent photophysical behaviors such as high quantum yields and a broad range of emission and absorption wavelengths, and also they can be simply modified procedures. Rhodamine derivatives are not fluorescence molecules due to their spirolactam rings; however, the opening of the spirolactam structure causes strong emissions. Thus, rhodamine derivatives are regarded as excellent structures for the turn on/off types of fluorescence chemosensors.

Herein, a new rhodamine based fluorescence and colorimetric chemosensor (**BODN**), which was synthesized by the condensation of ethylenediamine appended rhodamine and 4-nitrophenyl isothiocyanate in methanol. The chemosensor was designed for the monitoring of Hg^{2+} ion in such a way to have binding sites like –C=O and -S group. The sensing behavior of the chemosensor **BODN** was evaluated in H_2O/ACN (v/v, 20/80) solution using UV-Vis and fluorescence spectroscopies. The chemosensor **BODN** determines Hg^{2+} ions selectively and sensitively with 'turn on' fluorescence response. Also, the chemosensor **BODN** can behave as a naked eye probe for the determination of Hg^{2+} ion in H_2O/ACN (v/v, 20/80) with the color changed from colorless to pink color.

2. Material and Method

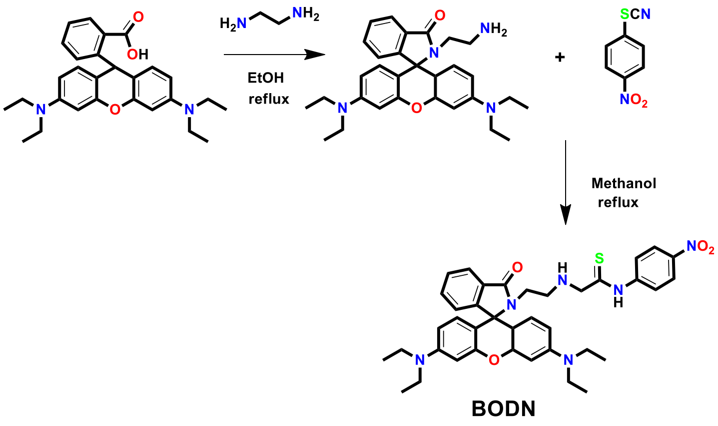
2.1. Reagents and Apparatus

All reagents were of spectroscopic grade and bought from commercial suppliers Sigma-Aldrich, VWR and Merck and utilized without further refinement.

^{1}H-NMR data were collected on a 600 MHz spectrometer (Bruker–DPX) by utilizing chloroform ($CDCl_3$). FT-IR spectra were measured using a Spectrum–100 infrared spectrometer (Perkin Elmer Inc.) and the mass spectra were obtained on a Daltonics – MicroflexTM spectrometer (Bruker). Cary Eclipse fluorescence and Cary 60 UV–Vis spectrophotometers (Agilent Tech., Inc.) were used to gain spectral data, respectively.

2.1. Synthesis of the Chemosensor BODN

As illustrated in Scheme 1, the ethylene diamine appended rhodamine (2) was synthesized according to previous literature procedures (H. Kilic & E. Bozkurt, 2018; S. Erdemir, 2019). For the chemosensor **BODN**, a solution of **2** (100 mg, 0.205 mmol) and 4-nitrophenyl isothiocyanate (38.7 mg, 0,215 mmol) in methanol (10 mL) was refluxed 24 hours in presence of molecular sieve. The mixture was cooled at room temperature and then the solvent was evaporated. The obtained solid was dissolved in CH_2Cl_2 (3 mL) then added to n-hexane (100 mL). Precipitated was occurred, filtered and washed with hexane, dried to give the chemosensor **BODN**. Yield: 20%. ^{1}H NMR (400 MHz, CH_3Cl-d_6) 8.34 (m, 1H), 8.18 (m, 1H) 8.01-7.70 (m, 2H), 7.68-7.37 (m, 2H), 7.21-6.95 (m, 2H), 6.37 (q, 6H), 3.82-2.89 (m, 12H), 1.17 (t, 12H). FTIR (ATR) cm-1: 3330 (N-H), 3265 (N-H), 3070 (Ar-H), 2969 (C-H), 2927 (C-H), 1665 (C=O), 1632 (C=O), 1511 (Ar-), 1327 (aromatic C-C), 1263 (aromatic C-O-C), 1116 (C-N), 1070 (C=S). MS–ESI (m/z) calculated for $C_{38}H_{44}N_6O_4S$ − 680.380. The spectral details were given in figures S1, S2 and S3 (in supporting information).



Scheme 1. Synthetic scheme of the chemosensor BODN

2.1. Synthesis of the Chemosensor BODN

The stock solution of the chemosensor **BODN** and metal perchlorate salts (Al^{3+}, Hg^{2+}, Ca^{2+}, Pb^{2+}, Cu^{2+}, Zn^{2+}, Fe^{2+}, Cd^{2+}, Ni^{2+}, Ag^{+}, Co^{2+} and Mg^{2+}) were prepared in ACN and then diluted to the required concentrations.

All emission spectra were performed in the region of 540−700 nm (λ_{em}=580 nm, λ_{ex}=530 nm) and slit widths were 10.0 nm. Using the fluorescence titration study, the binding constant and limit of detection were calculated by the Benesi-Hildebrand equation and DL= 3xS/K, respectively. The absorbance spectra were obtained in the region of 200−800 nm at room temperature. The volume of the chemosensor **BODN** solution utilized in the UV-vis and fluorescence experiments was 3.0 mL.

3. Results and Discussion

3.1. Synthesis of the chemosensor BODN

As illustrated in Scheme 1, 4-nitrophenyl isothiocyanate was reacted with ethylenediamine appended rhodamine (2) in methanol. The characterization experiments of the chemosensor **BODN** were carried out by ^{1}H-NMR, IR and ESI-MS spectroscopies. The characterization details were given in supplementary data under section S1-S3.

3.2. Spectral experiments

The sensing behavior of the chemosensor **BODN** towards metal cations (Al^{3+}, Ca^{2+}, Cu^{2+}, Zn^{2+}, Fe^{2+}, Pb^{2+}, Cd^{2+}, Ni^{2+}, Ag^{+}, Co^{2+} and Mg^{2+}) was evaluated by using absorbance and emissions spectra. The emission properties of the chemosensor **BODN** were investigated in the presence of 20.0-fold excess of cations. As seen in figure 1, the fluorescence intensity at 580 nm was observed upon addition of the Hg^{2+} to the chemosensor solution while other metal cations caused small or no emission changes. The chemosensor **BODN** (5 µM) have almost non-fluorescence, 20-fold increment of fluorescence intensity at 580 nm upon the transferring of 10.0 equiv. of Hg^{2+} to the chemosensor solution was observed due to FRET mechanism. The fluorescence emission of the chemosensor **BODN** at 550 nm shifted at 580 nm during the complexation with Hg^{2+}. This situation could be defined by FRET (fluorescence resonance energy transfer) mechanism was supported the energy transfer from the isothiocyanate moiety (donor) to the rhodamine moiety (acceptor). The characteristic fluorescence of the rhodamine based structure in the spiro-ring closed form is inhibited, for this reason the donor moiety displays only the fluorescence. Subsequently, the FRET mechanism of the system is prohibited while after complexation between the chemosensor and metal ions, the spiro-ring of the rhodamine structure opens that causes a strong fluorescence (M. Maniyazagan et al., 2017). The overlap spectrum of the emission with absorbance clearly demonstrates the possibility of a FRET mechanism shown in figure S4 (in supporting information).

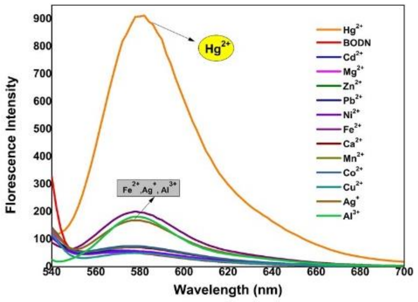


Figure 1 Spectra of the chemosensor BODN in the presence of metal ions (10.0 eq.) in H_2O/ACN media (v:v, 20:80) (5.0 µM) (λ_{ex} = 530 nm, λ_{em} = 580 nm)

The sensing behavior between the chemosensor **BODN** with Hg^{2+} was proved by the fluorescence titration experiment. The fluorescence at 580 nm reached a maximum intensity upon the transferring of 10.0 eq. of Hg^{2+}. Figure 2 displays the changes in emission spectra of the chemosensor **BODN** upon titration with Hg^{2+} (0.0–10.0 equiv). The emission intensity of the chemosensor **BODN** enhanced gradually when the amount of Hg^{2+} was increased stepwise.

Furthermore, Job's method for the evaluation of the binding behavior of the chemosensor **BODN** and Hg^{2+} was utilized and displayed in figure S5 (in supporting information). A Job's graph is a plot of the fluorescence intensities at 580 nm versus the corresponding values of the molar fraction of Hg^{2+} ion and a maximum value of the graph is about 0.33. The Job's plot graph promoted 2:1 complex formation between the chemosensor **BODN** and Hg^{2+}. The binding constant of **BODN**-Hg^{2+} complex was found 3.20×10^4 M^{-1} using the fluorescence titration experiment based on the Benesi-Hildebrand equation given in section S6 of the supplementary data. The limit of detection for Hg^{2+} was calculated to be 3.21 nM on the basis of 3*S*/*k*.

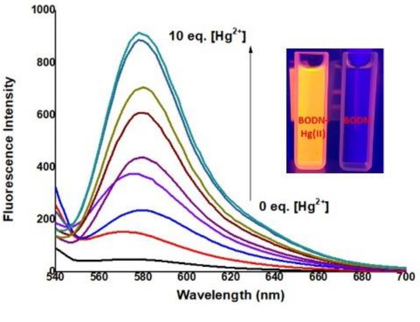


Figure 2 Spectral changes of the chemosensor BODN upon the addition of Hg^{2+} (0 to 10.0 equiv) in H_2O/ACN media (v:v, 20:80) (5.0 µM) (λ_{ex} = 530 nm, λ_{em} = 580 nm)

On the other hand, absorbance measurements were performed due to the investigation of the binding behavior of the chemosensor **BODN** towards metal ions (Figure 3). The absorbance of the chemosensor **BODN** displayed bands at 240, 270, 320 and 360 nm corresponding to π-π* and n-π* transitions. Upon transferring of enhancement amounts of Hg^{2+} (10.0 eq.), three absorbance bands at 240, 270 and 320 nm increased while the band at 360 nm was disappeared. Also, a new absorption band at 560 nm was observed due to the hydrolysis of the chemosensor **BODN** after the ring-opening process (Figure 4). The binding constant of **BODN**-Hg^{2+} complex and the limit of detection for Hg^{2+} were found 9.06×10^4 M^{-1} and 0.27 µM, respectively.

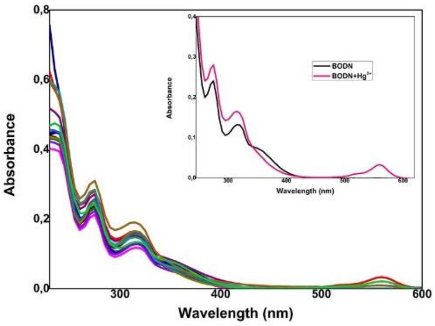


Figure 3 Absorbance of the chemosensor BODN in the presence of different cations (10.0 eq.) in H_2O/ACN media (v:v, 20:80) (5.0 µM)

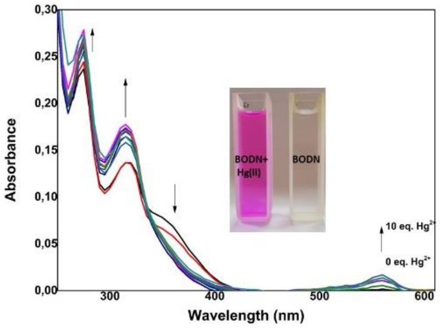


Figure 4 Absorbance changes of the chemosensor BODN upon the addition of Hg^{2+} (0 to 10.0 eq.) in H_2O/ACN media (v:v, 20:80) (5.0 µM)

To find out the fluorescence selectivity behavior of the chemosensor **BODN**, the interference measurements were performed in company with other metal cations (20 equiv.) with Hg^{2+} ion (20 equiv.) (Figure 5). The chemosensor **BODN** could detect Hg^{2+} without any influence of competing cations. Thus, Hg^{2+} was easily determined in the presence of competing metal ions with fluorescence 'turn-on' responses. The results displayed that the chemosensor **BODN** could be utilized as a Hg^{2+} sensor over the other competing metal cations.

It is commonly known that the parameter of pH would influence the response mechanisms of chemosensors. Thus, the fluorescence measurements were carried out in the presence and absence of the Hg^{2+} ion using buffer solutions (acetate buffer, phosphate buffer ammonium buffer) having a pH range of 4.0–11.0 (Fig. S8). When the obtained results were evaluated, it was determined that the the chemosensor **BODN** could determine Hg^{2+} ion at pH range of 4.0–11.0.

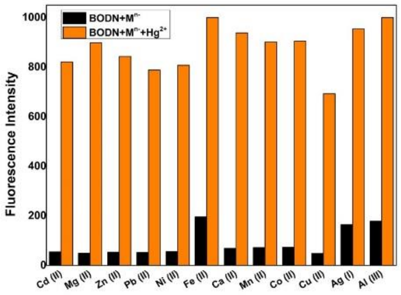


Figure 5 Competitive selectivity graph of the chemosensor BODN (5.0 µM) in the presence of different metal ions (20. Eq.) toward Hg^{2+} (20. Eq.) in H_2O/ACN media (v:v, 20:80) (λ_{ex} = 530 nm, λ_{em} = 580 nm)

The binding of the chemosensor **BODN** and Hg^{2+} was investigated by FT-IR experiments. The FT-IR spectra of the chemosensor **BODN** and BODN-Hg^{2+} were measured, and illustrated in figure 6a. The characteristic carbonyl (C=O) band of the chemosensor **BODN** at 1665 cm^{-1} disappeared after complexation between the chemosensor **BODN** and Hg^{2+}, due to the spirolactam ring-open process and a new C=N imine band was detected at 1645 cm^{-1} (A. Sikdar et al., 2013). The intensity of the band at 1090 cm^{-1} that is indicated to -C=S was increased and shift to 1070 cm^{-1} because of the formation of –C-S-Hg- bond (C. Kan et al., 2019). As seen in figure 6b, the peak at 1566.454 [m/z] could be supported [2·**BODN** + Hg^{2+}+ H_2O], which demonstrated that chemosensor **BODN** reacted with Hg^{2+} in a ratio of 2:1. Based on the obtained results, the proposed mechanism between the chemosensor **BODN** and Hg^{2+} ion was depicted in Scheme 2.

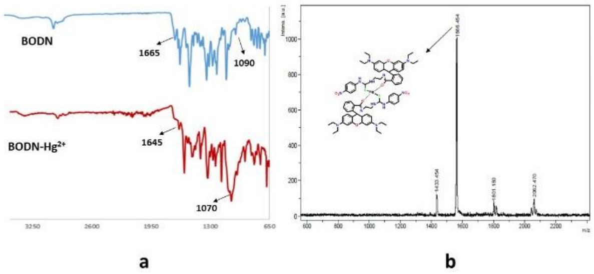
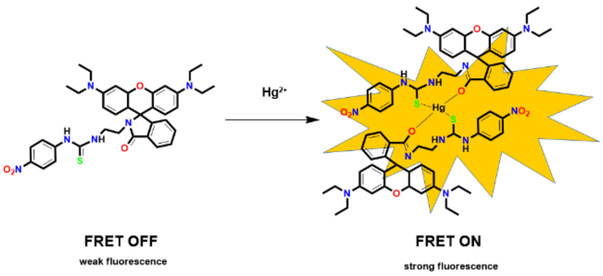


Figure 6 (a) Ir spectra of the chemosensor BODN and BODN-Hg^{2+} complex (b) ESI-MS spectrum of BODN-Hg^{2+} complex



Scheme 2. Possible binding mechanism of the chemosensor BODN upon the addition of Hg^{2+}

4. Conclusions and Recommendations

In a conclusion, a novel fluorescence chemosensor based rhodamine derivative (the chemosensor **BODN**) employed with FRET process was used for the detection of Hg^{2+}. The chemosensor **BODN** demonstrated excellent fluorescence "turn-on" response to Hg^{2+} in ACN/H_2O media (v:v, 80:20) at 580 nm with emission enhancement. Fluorescence measurements showed that Hg^{2+} binded to the chemosensor **BODN** with a 1:2 stoichiometry and a binding constant of 3.20×10^4 M^{-1}. The detection limit of the chemosensor was estimated to be 3.21 nM for Hg^{2+}. These promising results demonstrated that the chemosensor **BODN** has a high potential for the monitoring mercury ions.

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