Dual Chemosensor for the Rapid Detection of Mercury (II) Pollution and Biothiols

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Complete List of Authors:	Gholami, Mahnaz ; Queensland University of Technology - QUT Sonar, Prashant; Queensland University of Technology , School of Chemistry, Physics and Mechanical Engineering Manzhos, Sergei ; Institut national de la recherche scientifique Centre Énergie Matériaux Télécommunications Ayoko, Godwin; Queensland University of Technology, Chemistry, Physics & Mechanic Engineering Kiriakous, Emad; Queensland University of technology, Nanotechnology and molecular science Discipline

ARTICLE

Dual Chemosensor for the Rapid Detection of Mercury (II) Pollution and Biothiols

Mahnaz D.Gholami,^a Sergei Manzhos,^b Prashant Sonar,^a Godwin A. Ayoko^a and Emad L. Izake^{*a}

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A new benzothiazole azo dye [(E)-1-((6-methoxybenzo[d]thiazole-2-yl)diazanyl)naphthalene-2,6-diol] (also known as “BAN”), has been synthesised and used as a chemosensor for the rapid and selective detection of mercury (II) ions in water. The pink coloured chemosensor turns blue when reacted with mercury (II) ions due to the formation of a 2:1 coordination complex. The complex formation causes a bathochromic shift of the chemosensor’s UV absorption peak from 540 to 585 nm and turns on a highly selective fluorescence emission at 425 nm. The change in the optical property of BAN upon complexation with mercury (II) was confirmed by ab initio calculations. The new chemosensor was used to quantify mercury (II) ions in water by fluorescence spectroscopy down to 5×10^{-8} M (10 ppb). The limit of detection (LOD) of Hg^{2+} was 9.45 nM (1.8 ppb) which satisfies the maximum allowable Hg^{2+} concentration in drinking water that is set by the WHO organisation. The BAN- Hg (II) complex was used for the determination of cysteine (Cys) in aqueous solution by UV-Vis spectroscopy down to 1×10^{-7} M. The thiol- containing amino acid preferentially coordinates the mercury ions of the BAN- Hg (II) complex. This causes dissociation of the blue-coloured complex and the liberation of the pink-coloured BAN dye. The colour change of the BAN- Hg (II) complex from blue to pink was selective to the Cys biothiol while other non-thiol containing amino acids did not cause a colour change. For the in-field application, filter paper strips were loaded with BAN- Hg (II) complex and used as a disposable sensor for the detection of cysteine (Cys) by the naked eye. Therefore, BAN chemosensor offers a sensitive, and rapid tool for the detection of mercury (II) in water. In addition, the BAN- Hg (II) complex can be used as a simple and selective chemosensor of the screening of purified biothiols, such as cysteine, homocysteine and glutathione in biology research and pharmaceutical/ food industries.

1. Introduction

Azo dyes are a versatile class of synthetic organic compounds, which have many industrial applications in the textile, paper, and plastic industries. Benzothiazole azo dyes are heterocyclic compounds that have high stability against oxidation. They have strong colour shades that vary from red to blue according to the substituents attached in the dye’s molecular structure. The optical properties of benzothiazole azo dyes can be tuned by introducing appropriate functional groups at selected positions of their heterocyclic ring.¹ Additionally, benzothiazole azo dye derivatives have been successfully utilised in optoelectronic devices such as solar cells, nonlinear optics, liquid crystal displays, and optical data storage.² The electron- donating properties of benzothiazole derivatives can be used effectively to form coordination complexes with various ions.³ There are few reports of the use benzothiazole azo dyes as a chemosensor

to detect heavy metals and amino acids via colourimetric and fluorescence spectroscopy methods in the literatures.⁴⁻¹²

Mercury is used in various industries.¹³ The heavy metal bio-accumulates through the food chain and can easily be converted by bacteria into a highly toxic methyl mercury derivative. In addition, mercury can affect human body organs through binding to the thiol groups of amino acids and proteins, thereby causing serious damage to the central nervous system, brain, kidney, and respiratory system.¹⁴⁻¹⁹

The US Environmental Protection Agency (EPA) and the World Health Organization (WHO) have set the maximum allowed concentration of Hg^{2+} in drinking water at 2 ppb (10 nM).²⁰ The determination of Hg^{2+} pollution in environment is usually carried out by conventional advanced methods such as inductively coupled plasma mass spectroscopy (ICP-MS),²¹ atomic absorption spectroscopy (AAS),²² high performance liquid chromatography (HPLC),²³ and inductively coupled plasma-atomic emission spectrometry (ICP-AES).²⁴ However, these methods are time-consuming, and require expensive instrumentation and complex sample preparation protocols.^{25, 26} Therefore, there is a critical need for sensitive chemosensors for the rapid and selective detection of Hg^{2+} pollution in the environment.^{17, 25, 27}

Cys is a biothiol that plays a crucial role in biological processes. Cys deficiency can result in many diseases such as liver cirrhosis, delayed growth and muscle loss. In contrast, the high Cys

^a Queensland University of Technology (QUT), School of Chemistry, Physics and Mechanical Engineering, 2 George street QLD, 4000, Australia. E-mail: e.kiriakous@qut.edu.au
^b Centre Énergie Matériaux Télécommunications, Institut National de la Recherche Scientifique, 1650, boulevard Lionel-Boulet, Varennes QC J3X1S2 Canada.
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cellular concentrations may lead to cancer, Alzheimer's, and cardiovascular diseases.^{28, 29} In addition, Cys is used in nutritional supplements and drug formulations to improve human health.³⁰ Therefore, the determination of the concentration of Cys is important for the early diagnoses of diseases and human health.

Herein, we report the synthesis and characterisation of (E)-1-((6-methoxybenzo[d]thiazole-2-yl)diazenyl)naphthalene-2,6-diol (BAN) as a new chemosensor for the detection Hg^{2+} ions in water by UV-Vis and fluorescence spectroscopy. The complex formation between BAN and Hg^{2+} ions causes a spontaneous colour change from pink to blue that is used to quantify mercury pollution down to 2.5 μM by UV-Vis. In addition, at pH 8, the complex formation between BAN and Hg^{2+} ions turns on a fluorescence emission at 425 nm after excitation at 252 nm. The fluorescence emission was highly selective towards the Hg^{2+} ion and allowed for its sensitive detection down to 1.8 ppb. The BAN- Hg^{2+} complex was utilised for the detection of Cys where the thiol-containing amino acid binds preferentially to Hg^{2+} ions and, therefore, cause the dissociation of the BAN- Hg^{2+} complex. This leads to a colour change from blue to pink due to the liberation of the free pink-coloured BAN dye.

2. Experimental section

2.1. Materials

2,6-Dihydroxynaphthalen, 6-methoxy-2-aminobenzothiazol, sulphuric acid, hydrochloric acid, sodium nitrite, methanol, ethanol, acetonitrile, potassium carbonate, sodium hydroxide, $\text{Hg}(\text{NO}_3)_2$, H_2O , NaCl , $\text{Ca}(\text{NO}_3)_2$, $4\text{H}_2\text{O}$, FeSO_4 , $7\text{H}_2\text{O}$, $\text{Al}(\text{NO}_3)_3$, $9\text{H}_2\text{O}$, $\text{Ni}(\text{NO}_3)_2$, $6\text{H}_2\text{O}$, $(\text{CH}_3\text{COO})_2\text{Mn} \cdot 4\text{H}_2\text{O}$, CuCl_2 , ZnSO_4 , $7\text{H}_2\text{O}$, $\text{Cd}(\text{NO}_3)_2$, $4\text{H}_2\text{O}$, $\text{Pb}(\text{NO}_3)_2$, CrO_3 , KCl , MgCl_2 , cysteine (Cys), phenylalanine (Phe), tyrosine (Tyr), and glycine (Gln) were purchased from Sigma Aldrich and used without further purification. Deionized water was used for the preparation of aqueous solutions of various metal ions and amino acids.

2.2. Instrumentation

FT-IR spectra were recorded using alpha-p FTIR spectrometer with ATR accessory ($500\text{--}4000\text{ cm}^{-1}$). ^1H and ^{13}C NMR were performed in DMSO-d_6 using a Bruker 600 MHz NMR spectrometer and tetramethylsilane (TMS) as the internal standard. Mass spectroscopy was carried out on a LTQ XL Ion Trap instrument. Varian Cary 60 and Eclipse spectrophotometers recorded UV-Vis absorption and fluorescence emission spectra. Differential scanning calorimetry (DSC) was carried out under nitrogen using a Chimaera instrument Q100. Thermal gravimetric analysis (TGA) was carried out using Pegasus Q500TGA analyser under nitrogen atmosphere and a heating rate of $10\text{ }^\circ\text{C}$ per min.

2.3. Synthesis and characterization of BAN

6-Methoxy-2-aminobenzothiazole (0.322 g, 2 mmol) was dissolved in H_2SO_4 (50%, 10 mL) and placed in an ice bath (-5 to $0\text{ }^\circ\text{C}$). An aqueous solution of NaNO_2 (0.296 g, 4.3 mmol in 3 mL

H_2O) was added dropwise with continuous stirring for 15 min. The resulting yellow diazonium salt was stirred between -5 to $0\text{ }^\circ\text{C}$ for 1 hour. 2,6-Dihydroxynaphthalene (0.36 g, 2 mmol) was dissolved in NaOH (10%wt, 10 mL) at temperature between -5 to $0\text{ }^\circ\text{C}$. The 2, 6-dihydroxynaphthalene solution was then added dropwise to the diazonium salt solution with continuous stirring in an ice bath (-5 to $0\text{ }^\circ\text{C}$) for 1 hour until the formation of a dark purple precipitate. Finally, the reaction mixture was neutralised to pH 6 by adding a saturated solution of potassium carbonate (K_2CO_3). The purple product was filtered and washed several times with distilled water and methanol and recrystallised in absolute ethanol. The weight of the purified dry purple powder was 652 mg (1.8 mmol) and the reaction yield 93%. (E)-1-((6-methoxybenzo[d]thiazole-2-yl)diazenyl)naphthalene-2,6-diol (BAN). ^1H NMR (600 MHz, DMSO-d_6) δ (ppm): 3.85 (s, 3H), 6.83 (d, $J=9.5\text{ Hz}$, 1H), 7.10 (dd, $J=8.9$, 2.6 Hz , 2H), 7.15 (dd, $J=8.8$, 2.6 Hz , 1H), 7.64 (d, $J=2.6\text{ Hz}$, 1H), 7.77 (d, $J=8.9\text{ Hz}$, 1H), 7.91 (d, $J=9.5\text{ Hz}$, 1H), 8.18 (d, $J=8.8\text{ Hz}$, 1H), 10.03 (s, 1H), 15.15 (s, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ (ppm): 55.17, 105.09, 112.89, 114.89, 118.77, 122.30, 123.19, 123.66, 129.52, 130.96, 133.14, 142.18, 145.33, 156.53, 156.81, 165.75, 171.56, 205.88. HRMS $[\text{M} + \text{H}]^+ \text{ m/z}$ calcd for ($\text{C}_{18}\text{H}_{13}\text{N}_3\text{O}_3\text{S}$) 352.07 found 352.07.

2.4. Stock solutions

A stock solution of the synthesised BAN (0.1 mM), was prepared by dissolving the appropriate amount of the dye in acetonitrile: water mixture (ACN: H_2O , 1:1, v/v). HCl (1 mM) was used to adjust the pH of the dye solution between 2 and 6. NaOH solution (1 mM), was used to adjust the pH of the dye solution between 6 and 12. Stock solutions of $\text{Hg}(\text{NO}_3)_2$, H_2O , NaCl , $\text{Ca}(\text{NO}_3)_2$, $4\text{H}_2\text{O}$, FeSO_4 , $7\text{H}_2\text{O}$, $\text{Al}(\text{NO}_3)_3$, $9\text{H}_2\text{O}$, $\text{Ni}(\text{NO}_3)_2$, $6\text{H}_2\text{O}$, $(\text{CH}_3\text{COO})_2\text{Mn} \cdot 4\text{H}_2\text{O}$, CuCl_2 , ZnSO_4 , $7\text{H}_2\text{O}$, $\text{Cd}(\text{NO}_3)_2$, $4\text{H}_2\text{O}$, $\text{Pb}(\text{NO}_3)_2$, CrO_3 , KCl , MgCl_2 (1 mM), Cys, phenylalanine, tyrosine and glycine (0.1 mM) were prepared by dissolving appropriate masses of the compounds in 10 mL of deionised water.

2.5. Complex formation, UV-Vis and fluorescence measurements

To study the complex formation between the BAN and Hg^{2+} , aliquots of stock Hg^{2+} solution were added to 250 μL of BAN (0.1 mM, in ACN: H_2O , 1:1 v/v, pH=8). ACN: H_2O solvent (1:1 v/v) was used to complete the volume to 5 mL. The final concentration of BAN in the mixture was 5 μM and the final concentration of the Hg^{2+} ions ranged from 0.1 μM to 10 μM . The colour change from pink to blue was observed by the naked eye, and UV absorption spectroscopy. UV-Vis spectra were recorded in the wavelength range 200–800 nm. The complex formation of between BAN and Hg^{2+} ions was also studied by fluorescence spectroscopy where the emission spectrum of the blue BAN- Hg^{2+} complex was measured between 400 and 600 nm using an excitation wavelength of 252 nm.

2.6. Study of pH effect and stoichiometry of the BAN- Hg^{2+} complex

To study the effect of pH on the complex formation between the BAN chemosensor and Hg^{2+} ions, equal volumes of Hg^{2+} ($5 \times 10^{-6}\text{ M}$) and BAN ($5 \times 10^{-6}\text{ M}$) (in ACN: H_2O , 1:1 v/v) were mixed and the pH of the solution changed from 6 to 12 by adding NaOH (1 mM) dropwise. The change in the colour of BAN from pink to blue after the addition of Hg^{2+} ions was monitored by

the naked eye and UV-Vis absorption spectra were acquired at different pH values. To determine the stoichiometry of the BAN-Hg²⁺ complex, UV-Vis spectroscopic titration of the BAN (5 × 10⁻⁶ M) was carried by adding different concentrations of Hg²⁺ ions (0.002 to 2 molar equivalents), and adjusting the pH of the solution to 8. The absorption maximum of the complex at 585 nm was acquired and the relationship between the absorption maximum and the dye-Hg²⁺ molar ratio was plotted.

2.7. Interference study of BAN chemosensor towards Hg²⁺ ions

For interference studies, stock solutions of various (10⁻³ M) of Na⁺, K⁺, Mg²⁺, Mn²⁺, Fe²⁺, Zn²⁺, Cu²⁺, Pb²⁺, Al³⁺, Cd²⁺, Ca²⁺, Ni²⁺ and Cr⁶⁺ salts were prepared in deionised water. 500 μL of the BAN (10⁻⁴ M in ACN: H₂O, 1:1 v/v, pH = 8) was mixed with five molar equivalents of the metal ion solution to the total volume of 5 mL. The UV-Vis adsorption and fluorescence emission spectra of the dye/metal ion mixtures were recorded in the wavelength range 200–800 nm and 400–600 nm respectively.

2.8. Density functional theory (DFT) simulations

Density functional theory (DFT) simulations were performed on the BAN molecules in protonated and deprotonated (single and double deprotonated, designated as BAN-H and BAN-2H, respectively) forms as well as their complexes with Hg²⁺.^{31, 32} The wb97xd functional³³ and Lanl2dz basis^{34, 35} set were used. The polarised continuum model (PCM)³⁶ of the water solvent was used. Spin-restricted calculations of appropriately charged singlet systems (BAN, BAN-H⁺, BAN-2H²⁺, Hg²⁺, Hg-BAN²⁺, [Hg-BAN-H]⁺, Hg-BAN-2H, Hg-BAN₂²⁺, Hg-(BAN-H)₂, [Hg-(BAN-2H)₂]²⁺ were performed. Optical absorption spectra are computed with time-dependent DFT (TD-DFT)³⁷ considering six lowest singlet excited states in complexes involving one BAN unit and twelve in complexes with two BAN units. The calculations were performed in Gaussian 09.³⁸

2.9. Detection of Cys by BAN-Hg²⁺ complex

To detect Cys by the BAN-Hg²⁺ complex, a 10⁻⁵ M stock solution of the complex was prepared by mixing 1000 μL of BAN (10⁻⁴ M in ACN: H₂O, 1:1 v/v) with 500 μL of Hg (II) ions (10⁻⁴ M) and adjusting the pH to 8 in the final volume of 5 mL. Aliquots of 10⁻⁴ M Cys aqueous solution were mixed with 250 μL aliquots of the BAN-Hg²⁺ complex (10⁻⁵ M) and the volume of the mixture was completed to 500 μL by adding ACN: H₂O solvent (1:1 v/v). The final concentration of the Cys amino acid in the prepared mixtures was in the range 0.5 μM to 20 μM while that of the BAN-Hg²⁺ complex was kept constant at 5 μM. The colour change from blue to pink was observed by the naked eye, and UV absorption spectroscopy. UV-Vis spectra were recorded in the wavelength range 200–800 nm.

2.10. Stoichiometry of cys detection by the BAN-Hg²⁺ complex

To study the stoichiometry of Cys detection by the BAN-Hg²⁺ complex, a UV-Vis spectroscopic titration of the BAN-Hg²⁺ complex (5 × 10⁻⁶ M) was carried out by adding different volumes of 10⁻⁴ M Cys stock solution. Therefore, the final concentration of the amino acid was in the concentration range of 0.1 to 4 molar equivalents of the BAN-Hg²⁺ complex. The absorption maximum of BAN at 540 nm was acquired and plotted against the molar ratio of Cys.

2.11. Interference study of the Cys detection by the BAN-Hg²⁺

Stock solutions of Cys, Phe and Tyr and Gln (10⁻⁴ M) were prepared by dissolving appropriate amount of the amino acid in 10 mL of deionised water. 250 μL aliquots of the BAN-Hg²⁺ complex (10⁻⁵ M) were mixed with 5 × 10⁻⁵ M of each amino acid solution (10⁻⁴ M) to the total volume of 500 μL. The UV-Vis adsorption spectra of the resulting mixtures were recorded in the wavelength range 200–800 nm.

2.12. Fabrication of disposable paper based sensor for Cys detection

A Paper based sensors for the detection of Cys was prepared by loading filter paper strips (1 × 5 cm) with 1 × 10⁻⁵ M BAN-Hg²⁺ complex and air dried. The paper sensors were stored at room temperature for future use.

3. Results and discussion

3.1. Synthesis and characterization

BAN was synthesised by the diazotation of 6-methoxy-2-aminobenzothiazole and coupling with 2,6-dihydroxynaphthalene (Scheme1). The structure of BAN was confirmed by ¹H NMR, ¹³C NMR, FT-IR and Mass spectroscopy.

The ¹H NMR of the synthesised dye is depicted in Fig. S1. The singlet at 3.85 pm is attributed to the three hydrogen protons of the methoxy group. The aromatic protons appear at 6.83 and 8.18 ppm. In addition, two singlet protons appear at 10.03 and 15.15 ppm and are attributed to the two-hydroxyl groups within the dye structure. The signal at 15.15 ppm may be attributed to hydrazone tautomer of the BAN dye (Fig. S2).³⁹

The ¹³C NMR of BAN is depicted in Fig. S3. The resonance at 55.17 ppm is attributed to the carbon atom of the methoxy group. The 17 aromatic carbons in the dye structure appeared between 105.09 ppm to 205.88 ppm. The signals at 171.56 ppm and 205.88 ppm may be attributed to the C-O bond of the hydroxyl group and the azo-hydrazone tautomer of the dye, respectively. All assigned proton and carbon peaks appeared in NMR data are matching with the chemical structure of BAN. The peak at 1464 cm⁻¹ is attributed to the N=N vibration in the azo form of the dye.⁴⁰

The C=N in the hydrazone form of BAN is also identified in the 1600 cm⁻¹ area. The peak at 1538 cm⁻¹ is assigned to C-N stretch at the benzothiazole ring in BAN.

Scheme. 1 Synthesis of (E)-1-((6-methoxybenzo[d]thiazole-2-yl)diazenyl)naphthalene-2,6-diol (BAN).

The mass fragmentation pattern of BAN is depicted in Fig. S5 and shows peaks at 352.07 and 350.07 that are assigned to the $[M+H]^+$ and $[M-H]^+$ fragments respectively. The thermal behaviour of BAN was investigated by thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC). The TGA analysis showed excellent thermal stability in nitrogen atmosphere. The decomposition temperature (T_d) of BAN was 242 °C (2.5% weight loss) (Fig. S6). The DSC analysis showed transition points at 219.7 °C and 233.2 °C that are attributed to the azo and hydrazone tautomers of the dye (Fig. S7).

3.2. UV-Vis spectra of BAN at different pH values

The UV-Vis absorption spectra of BAN at different pH values are depicted in Fig. 1. In the pH range 6-9, the pink-coloured dye shows an absorption maximum at 540 nm (molar absorption coefficient $= 1.3 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$) that is caused by $\pi - \pi^*$ electronic transitions. At pH values (pH= 10-12), the colour of the dye turns blue and the absorption maximum is shifted gradually from 540 nm to 630 nm due to the complete deprotonation of the dye (Fig. 1).

3.3. Complex formation between BAN and Hg^{2+} and effect of pH

The formation of coordination complexes between benzothiazole azo dyes and metal ions has been demonstrated in the literature.³⁻⁵ Therefore, BAN was utilised as a chemosensor for the detection of Hg^{2+} ions. The formation of a coordination complex between BAN and the Hg^{2+} ions in the pH range 2-12 was monitored by UV-Vis spectroscopy. At pH values less than 6.7 there was no change to the colour and the absorption band of BAN at 540 nm. When the pH of the solution was increased from 6.7 to 8.0 in the presence of Hg^{2+} ions, a colour change from pink to blue was observed by naked eye. Also the absorption band of the dye shifted from 540 nm to 585 nm (Fig. 2a). The shift in the absorption band of BAN is attributed to the formation of a coordination complex between the dye and the metal ions. At pH values above 10 and in the presence of Hg^{2+} ions, the absorption band of the dye shifted gradually to 630 nm and could not be distinguished from that of the deprotonated form of BAN (Fig. S8).

The complex formation between BAN and Hg^{2+} at pH 8 was confirmed by fluorescence spectroscopy. BAN did not show any appreciable fluorescence in the pH range 2-12 after excitation using a wavelength of 252 nm (Fig. 2b, blue spectrum). However, when Hg^{2+} ions were added to the dye solution and the pH adjusted to 8, a strong emission at 425 nm turned on using excitation wavelength of 252 nm (Fig. 2b, red spectrum). The emission quantum yield of the BAN chemosensor increased sharply from $\phi_f = 0.011$ to $\phi_f = 0.15$ after complexation with Hg^{2+} ions.

3.4. DFT simulation of BAN- Hg^{2+} complex formation

DFT simulation was carried out to identify the complex formation between BAN and Hg^{2+} ions. Based on the DFT calculations, the Hg^{2+} cations coordinate to the neighbouring oxygen and nitrogen atoms of deprotonated BAN. The

computed binding energy of Hg^{2+} ions to the protonated dye structure was found to be negligible. The binding of the metal ion was thermodynamically favoured to BAN as long as the hydroxyl group in the ortho position to the azo group is deprotonated. The binding energy of the metal ion to a deprotonated BAN molecule is computed to be -0.85 eV. The bond length between the Hg^{2+} cation and both the O and N atoms of deprotonated BAN is $\sim 2.40 \text{ \AA}$.

The DFT predicted no change in the 540 nm absorption band of protonated BAN when it interacts with Hg^{2+} ions (grey, light blue and deep blue spectra in Fig. S9). To the contrary, the computed spectra showed a red shift from 548 nm to 614 nm when the complex is formed between the Hg^{2+} ions and deprotonated BAN (orange and yellow spectra in Fig. S9). The predicted shift was in a good agreement with the experimental shift that was observed in the UV absorption of BAN from 540 nm to 585 nm upon complexation with Hg^{2+} ions at pH 8 (Fig. 2a).

To support the DFT simulation of the BAN- Hg^{2+} complex formation, FT-IR spectra of BAN and BAN- Hg^{2+} were acquired (Fig. S10). The FT-IR spectrum of the BAN- Hg^{2+} complex showed weak vibration modes between 3078 and 3376 cm^{-1} that are attributed to OH vibrations. In addition, the N=N vibration of the azo group in the BAN- Hg^{2+} showed at 1380 cm^{-1} and was shifted from its position at 1464 cm^{-1} in the free dye.⁴ This shift is attributed to the formation of a coordination complex between the azo group of the dye and the Hg^{2+} ions.

Fig. 1 UV-Vis spectra of BAN ($5 \times 10^{-6} \text{ M}$) in ACN:H₂O (1:1, v/v) at different pH values.

Fig. 2 (a) UV-Vis spectra of the BAN chemosensor ($5 \times 10^{-6} \text{ M}$) after the addition of different volumes (0-2 eq of $5 \times 10^{-6} \text{ M}$) of Hg^{2+} ions. (b) Fluorescence emission spectra of the BAN (10^{-6} M) before and after the addition of Hg^{2+} ions (10^{-6} M) and excitation at $\lambda_{\text{ex}} = 252 \text{ nm}$.

3.5. Stoichiometry of the BAN-Hg²⁺ complex

The concentration of BAN was kept at 5×10^{-6} M while the concentration of Hg²⁺ ion changed from 0 to 2 molar equivalents while maintaining the pH of the solution at pH 8. The UV-Vis spectra of the formed complexes showed an isosbestic point at 560 nm and an absorption band at 585 nm, which is attributed to the formation of a BAN-Hg coordination complex. As indicated by Fig. 3a, the stoichiometry of BAN in the complex was found to be 2:1 BAN: Hg²⁺. To rationalise this stoichiometry, we also performed DFT calculations of Hg complexes with two protonated or deprotonated BAN units. The binding energy to protonated BAN is insignificant. The binding energy to two deprotonated BAN units was -1.74 eV i.e. the 2:1 stoichiometry is indeed energetically preferred. The effects on the absorption spectra were found to be similar to the case of complexes with a single BAN unit. The energetically most stable BAN: Hg²⁺ complex is shown in Fig. 3b. In this complex, the Hg-O and Hg-N bond lengths are both around 2.41 Å.

3.6. Selectivity of the BAN chemosensor towards Hg²⁺ ions

To explore the chemosensor selectivity toward Hg²⁺ ions, the UV-Vis and fluorescence spectra of BAN were monitored after the addition of 13 different metal ions at pH 8. As indicated by Fig. S11a, the UV-Vis spectra of the chemosensor did not change after the addition of the individual metal ions except for Ni²⁺, Hg²⁺ and Cu²⁺ ions which caused the absorbance of BAN dye to shift from 540 nm to 560 nm, 585 nm and 630 nm respectively. However, the fluorescence spectra of BAN after the addition of the metal ions did not show emission at 425 nm except in the presence of Hg²⁺ ions (Fig. S11b). Therefore, the detection of Hg²⁺ ions by fluorescence spectroscopy was highly selective.

3.7. Quantification Hg²⁺ by UV-Vis and fluorescence spectroscopy

For the determination of Hg²⁺ in water and UV-Vis spectroscopy using BAN, the absorption band of BAN: Hg²⁺ complex at 585 nm was monitored against the concentration of the metal ion. A linear relationship between the UV absorbance at 585 nm and metal ion concentration was found within the concentration range 5×10^{-5} M (50 μ M) to 1×10^{-6} M (1 μ M) of Hg²⁺ ions and represented by the equation $y = 0.0207x + 0.0264$ (correlation coefficient $R^2 = 0.996$). The limit of detection (LOD) was calculated using the equation $DL = 3 \sigma / K$ (σ = standard deviation and K is the slope of the calibration curve), and found to be 4.7×10^{-7} M.⁴¹⁻⁴³ The limit of quantification (LOQ) of the UV method was identified experimentally to be 2.5×10^{-6} M (Fig. 4a).

The quantification of Hg²⁺ by BAN was also carried out by fluorescence spectroscopy. The emission band of the BAN: Hg²⁺ complex at 425 nm was monitored against the concentration of the metal ion. A linear relationship that is represented by the equation $y = 0.2708x + 48.57$ ($R^2 = 0.993$) was found in the concentration range 1×10^{-6} M to 1×10^{-8} M (Fig. 4b). The LOD and LOQ of Hg²⁺ determination by the fluorescence spectroscopy method were 9.45×10^{-9} M (1.8 ppb) and 5×10^{-8} M (10 ppb) respectively. The LOD and LOQ of the Hg (II) detection by fluorescence spectroscopy was 2 orders of magnitude lower

than that by the UV-Vis method. Therefore, the determination of Hg (II) in water using the BAN dye was carried out by fluorescence spectroscopy.

3.8. Determination Hg²⁺ in water

To demonstrate the potential of the new chemosensor for the detection of Hg²⁺ pollution, tap water was collected and screened by ICP-OES for Hg²⁺ contamination. The ICP-OES measurement confirmed the absence of the heavy metal in the water sample.

The tap water sample was then spiked with Hg²⁺ ions to the final concentration of 1×10^{-7} M. One volume of the spiked water sample was mixed with one volume of BAN (2×10^{-7} M), the pH adjusted to 8. The final concentration of Hg²⁺ ions in the mixture was 5×10^{-8} M. The intensity of the fluorescence emission at 425 nm was recorded (Fig. S12). Using the regression equation in Fig. 4b, the concentration of Hg²⁺ was determined to be 4.46×10^{-8} M ($n = 3$) and the % recovery was 89.2 ± 3 . The detection of Hg²⁺ by the new chemosensor was compared to that carried out by other chemosensors (Table S1).

Fig. 3 (a) Change in the chemosensor UV absorbance at 585 nm with the concentration of Hg²⁺ ions. The inset shows the change in colour due to the addition of Hg²⁺ ions. (b) Complex between Hg²⁺ ions and deprotonated BAN molecules. Atom colour scheme here and elsewhere: C- brown, O- red, N- blue, S- yellow, H- pink, and Hg- turquoise.

Fig. 4 (a) Linear correlation plot of absorbance at 585 nm versus the concentration of Hg(II) and corresponding UV-Vis spectra from 1×10^{-6} M to 5×10^{-5} M. (b) A linear correlation plot of intensity of emission at 425 nm ($\lambda_{ex} = 252$ nm) versus the concentration of Hg(II) and the corresponding emission spectra from 1×10^{-8} M to 1×10^{-6} M.

As indicated by Table S1, the new chemosensor is very sensitive and can detect 1.8 ppb of Hg^{2+} ions in water. The maximum allowed concentration of Hg^{2+} in drinking water is set by the EPA and WHO authorities at 2 ppb (10 nM).²⁰ Therefore, the new chemosensor is satisfactory for the detection of Hg^{2+} pollution in drinking water.

3.9. Detection of Cys by the BAN- Hg^{2+} complex

It is known that mercury has high affinity towards thiol-containing amino acids.⁴⁴ Therefore, the BAN- Hg^{2+} complex was used to detect Cys. Upon the addition of aqueous Cys to the BAN- Hg^{2+} complex, a distinct colour change from blue to pink was observed by the naked eye. This was confirmed by observing the UV-Vis spectrum of the BAN- Hg^{2+} complex before and after the addition of Cys. As indicated by Fig. S13, the absorption band of the BAN- Hg^{2+} complex at 585 nm disappeared and that of the free BAN dye re-appeared at 540 nm upon the addition of Cys. The change in colour and UV-Vis absorption spectra is attributed to the formation of a Cys- Hg^{2+} complex and the dissociation of the BAN- Hg^{2+} complex, thus leading to the regeneration of the pink-coloured BAN dye.⁴⁵

The UV-Vis spectrum of the BAN- Hg^{2+} complex after the addition of Cys was monitored at 20 second time intervals after the addition of Cys (Fig. S14 a,b). As indicated by the figures, the 540 nm absorption band of BAN arrived to a maximum after 20 seconds. Therefore, a reaction time of 20 seconds was selected in subsequent experiments.

3.10. Stoichiometry of the Cys- Hg^{2+} complex

To determine the stoichiometry of the formed Cys- Hg^{2+} complex, constant volumes of the BAN- Hg^{2+} complex solution (5×10^{-6} M) were mixed with 0.1–4 molar equivalents of 5×10^{-6} M Cys solution. The UV-Vis spectra of the formed complexes are depicted in Fig. 5a and show an isosbestic point at 560 nm as well as the reappearance of the BAN absorption band at 540 nm. Fig. 5b indicates that the absorbance of the 540 nm band arrives to a maximum when the Cys: BAN- Hg^{2+} molar ratio is 2:1, thus indicating the formation of a 2:1 Cys- Hg^{2+} complex.

Fig. 5 (a) UV-Vis spectra of the BAN- Hg^{2+} complex (5×10^{-6} M) after the addition of different volumes of Cys solution (5×10^{-6} M). (b) Change in the UV absorbance of the BAN- Hg^{2+} complex at 540 nm with the concentration of Cys. The inset depicts the change in colour due to the addition of Cys solution.

3.11. Selectivity of the BAN- Hg^{2+} complex towards Cys

Selectivity study of BAN- Hg chemosensor to Cys over other 3 various natural amino acids including Tyr, Phe and Gln were conducted.

To determine the selectivity of the BAN- Hg^{2+} complex towards biothiols over non-thiol-containing amino acids, it was interacted with Cys, Phe, Tyr and Gln respectively. As shown in Fig. 6a, the colour of the BAN- Hg^{2+} complex changed from blue to pink and its UV-Vis absorption shifted to 540 nm when Cys was added while no obvious changes took place with the addition of the Phe, Tyr and Gln amino acids. This result is attributed to the high affinity of the Hg (II) ions towards the sulfhydryl end group of Cys and the formation of a Cys- Hg^{2+} complex.⁴⁵

3.12. Quantification of Cys by BAN- Hg chemosensor

For the determination of Cys by UV-Vis spectroscopy using the BAN- Hg^{2+} complex, the absorption band of BAN: Hg^{2+} complex at 585 nm and BAN at 540 nm were monitored against the concentration of the amino acid. A linear relationship between the A_{540}/A_{585} absorbance ratio and Cys concentration was found within the range 1×10^{-5} M (10 μM) to 1×10^{-7} M (0.1 μM) of Cys and represented by the equation $y = 0.0248x + 1.3145$ (correlation coefficient $R^2 = 0.972$) (Fig. 6b), which is satisfactory for the detection of Cys in healthy subjects and patients with liver cirrhosis condition where the average concentration of the Cys amino acid in human blood plasma is in the range of $= 6.9 \pm 1.1 \mu\text{M}$ to $7.8 \pm 1.0 \mu\text{M}$ respectively.⁴⁶

3.13. Paper-based sensors for the rapid detection of Cys

The rapid, sensitive and cost-effective detection of Cys in vitro and in living cells is important for human health. To utilise the BAN- Hg^{2+} for the detection of Cys, it was loaded onto filter paper strips. The BAN- Hg^{2+} coated paper strips were used for the detection of the purified amino down to $5 \mu\text{M}$ where the blue coloured strips turned pink colour in presence of the amino acid (Fig. 7). Therefore, the BAN- Hg^{2+} coated paper strips can be used as an optical sensor for the rapid screening of Cys in pharmaceutical products, and nutritional supplements.

Fig. 6 (a) UV-Vis spectra of BAN- Hg (5×10^{-6} M) after the addition of 4 amino acids (2.5×10^{-5} M). (b) Linear correlation plot of ratio

absorbance at 540 nm and 580 nm versus the concentration of Cys and corresponding UV-Vis spectra from 1×10^{-5} to 1×10^{-7} M.

Fig. 7 Visual detection of Cys by paper-based sensor.

4. Conclusions

A new benzothiazole azo dye was synthesised and utilised as a chemosensor for the rapid detection of Hg^{2+} ions in water. The new chemosensor forms a spontaneous 2:1 coordination complex with Hg^{2+} ions at pH 8. The complex formation caused the colour of the chemosensor to change from pink to blue and turned on a strong fluorescence emission at 425 nm. The chemosensor showed high selectivity towards Hg^{2+} over 13 other metal ions. The new chemosensor was utilised for monitoring Hg^{2+} contamination in water by fluorescence spectroscopy. The limit of detection by the fluorescence method was found to be 9.45×10^{-9} M (1.8 ppb) which satisfies the detection limit recommended by EPA and WHO organizations. The blue-coloured BAN- Hg^{2+} complex was used for the rapid detection of the thiol-containing amino acid "Cys" where its colour turns back to pink due to the dissociation of the BAN- Hg^{2+} complex and the release of the free pink-colour BAN. The described chemosensor has strong potential as a cost-effective, sensitive and selective chemosensor for the detection of Hg^{2+} in water while its mercury complex has a strong potential as a disposable sensor for the detection of purified Cys, and other biothiols such as homocysteine and glutathione in biology research and pharmaceutical/food industries.

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Supporting Information

Dual Chemosensor for the Rapid Detection of Mercury (II) Pollution and Biothiols

Mahnaz D.Gholami^a, Sergei Manzhos^b, Prashant Sonar^a, Godwin A. Ayoko^a, Emad L. Izake^{b*}

^a Queensland University of Technology(QUT), School of Chemistry, Physics and Mechanical Engineering, 2 George street QLD, 4000, Australia

^b Centre Énergie Matériaux Télécommunications, Institute National de la Recherche Scientifique, 1650, boulevard Lionel-Boulet, Varennes QC J3X1S2 Canada.

* Corresponding author e-mail: e.kiriakous@qut.edu.au

Fig. S1. ¹H NMR (600 MHz, DMSO-d₆) spectrum of BAN.

Fig. S2. Azo-hydrazone tautomerism of BAN.

Fig. S3. ¹³C NMR (151 MHz, DMSO-d₆) spectrum of BAN.

Fig. S4. FTIR spectrum of BAN.

Fig. S5. Mass spectrometry of BAN.

Fig. S6. TGA thermogram of BAN.

Fig. S7: DSC analysis of BAN.

Fig. S8. Colour change and UV-VIS spectra of chemosensor BAN in the absence and presence of Hg^{2+} ions at different values.

Fig. S9. Computed absorption spectra of BAN and deprotonated BAN (a Gaussian broadening of 0.25 eV HWHM is applied).

Fig. S10. FT-IR Spectra of BAN and BAN - Hg²⁺ complex.

Fig. S11. (a) UV-Vis spectra of BAN (1×10^{-5} M) after the addition of 13 metal ions (5×10^{-5} M), (b) Fluorescence emission spectra of BAN (1×10^{-5} M) after the addition 13 metal ions (5×10^{-5} M).

Fig. S12. Fluorescence emission spectra of BAN (1×10^{-7} M) before and after interaction with blank and spiked tap water (Hg^{2+} concentration = 5×10^{-8} M) at pH 8. The excitation wavelength was $\lambda_{\text{ex}} = 252$ nm.

Fig. S13. The UV-Vis spectra of a) BAN dye (5×10^{-6} M in ACN: H_2O , 1:1 v/v), b) BAN-Hg²⁺ complex and c) The BAN-Hg²⁺ complex after the addition of cysteine.

Fig. S14. (a) The UV-Vis spectra of BAN-Hg²⁺ complex (5×10^{-6} M in ACN: H_2O , 1:1 v/v) after addition of cysteine during 100 s, (b) Plotted absorbance at 540 nm against time (s).

Determination of Quantum yield

The luminescence quantum yield was measured using anthracene ($\Phi_f = 0.27$ in ethanol) as a reference dye. Both the BAN compound and the reference were excited at the same wavelength ($\lambda_{\text{ex}}=252$ nm), maintaining nearly equal absorbance (~ 0.1), and the emission spectra were obtained at 425 nm. The quantum yield is calculated according to the following equation:

$$\Phi_S = \Phi_R \left[\frac{A_S}{A_R} \right] \left[\frac{\text{Abs}_R}{\text{Abs}_S} \right] \left[\frac{n_S^2}{n_R^2} \right]$$

where A is the integrated intensity of the luminescence, Abs is absorbance at the excitation wavelength, n is the refractive index of the solvent and Φ is fluorescence quantum yield.

Table S1. Comparison of optical probes for Hg²⁺ ions.

Chemosensor	Detection mode	LOD	LOQ	Comments	Ref
Fluorescence	55nM	NA	Requires complex synthesis procedures. LOD is above the maximum allowable level of Hg ²⁺ in drinking water.	¹
Fluorescence	50 nM	NA	LOD is above the maximum allowable level of Hg ²⁺ in drinking water.	²
UV-vis	188 nM	NA	LOD is above the maximum allowable level of Hg ²⁺ in drinking water.	³
Fluorescence	27nM	NA	Suffers from interferes by Fe ³⁺ ions. LOD is above the maximum allowable level of Hg ²⁺ in drinking water.	⁴
Fluorescence	11 nM	NA	The LOD satisfies the maximum allowable level of Hg ²⁺ in drinking water. The screening time for Hg ²⁺ ions is 30 min.	⁵
Fluorescence	1.2 nM	NA	The LOD is below the maximum allowable level of Hg ²⁺ in drinking water. The screening time for Hg ²⁺ ions is 30 min.	⁶
Fluorescence	1.8 nM	NA	The LOD is below the maximum allowable level of Hg ²⁺ in drinking water. The screening time for Hg ²⁺ ions is 30 min.	⁷

Table S1. Continued.

Chemosensor	Detection mode	LOD	LOQ	Comments	Ref
UV-vis	150 nM	510 nM	The LOD is above the maximum allowable level of Hg ²⁺ in drinking water.	⁸
Fluorescence	27 nM	NA	The LOD is above the maximum allowable level of Hg ²⁺ in drinking water.	⁹
Fluorescence	9.45 nM	50 nM	Spontaneous detection. Can be used as a paper based sensor. The LOD satisfies the maximum allowable level of Hg ²⁺ in drinking water.	
	UV-vis	470 nM	2500 nM		

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