Anthranilic Acid Schiff Base as a Fluorescent Probe for the Detection of Arsenite and Selenite: A Detailed Investigation of Analytical Parameters and Mechanism for Interaction

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The exploration of an anthranilic acid based Schiff base SB as a “Turn-ON” fluorescent probe for the detection of highly toxic selenite (Se IV) and arsenite (As III) species in an aqueous medium is described. The selectivity of SB towards Se IV and As III in the presence of other ions was investigated by some spectrofluorimetric and 1H NMR spectroscopic experiments. The studies revealed the interaction between SB and As III via the deprotonation of phenolic -OH, which enhanced the conjugation in phenolate ion and in turn enhanced the emission response. The SB has analytical prospects for the quantification of As III and Se IV with good sensitivity (LODs; 5.15 ppb for Se IV and 3.12 ppb for As III calculated by S/N = 3σ/ΔK). Furthermore, it can be used to evaluate real and synthetic samples for the presence of Se IV and As III species as well as the fabrication of on-spot recognition devices (in the form of silica gels SB@SiO2 and silica coated TLC aluminium strips SB@SiO2@Al).

Keywords Schiff base, arsenite, selenite, spectrofluorimetric method, Turn-ON switch

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Introduction

The chemistry of selenium and arsenic has gained immense interest due to their chemical properties, nutritional value and toxicological potential. Selenium is an important constituent in dietary supplements and industrial production of several electrical appliances, agrochemicals and domestic products.1–3 Similarly, arsenic is an important additive of animal feed, and its compounds are utilized in agriculture practices and electronic gadgets.4 Despite the biological importance of selenium and arsenic as micro-nutrients, excessive amounts in industrial effluents and agricultural drainage lead to their accumulation in plants, soil, and water, resulting in heavy toxicity. Selenium and arsenic exist in natural water in two toxic inorganic forms i.e. selenite (Se IV) and arsenite (As III) species in an aqueous medium, resulting in heavy toxicity.  Selenium and arsenic exist in natural water in two toxic inorganic forms i.e. selenite (Se IV) and arsenite (As III) species in an aqueous medium is described. The selectivity of SB towards Se IV and As III in the presence of other ions was investigated by some spectrofluorimetric and 1H NMR spectroscopic experiments. The studies revealed the interaction between SB and As III via the deprotonation of phenolic -OH, which enhanced the conjugation in phenolate ion and in turn enhanced the emission response. The SB has analytical prospects for the quantification of As III and Se IV with good sensitivity (LODs; 5.15 ppb for Se IV and 3.12 ppb for As III calculated by S/N = 3σ/ΔK). Furthermore, it can be used to evaluate real and synthetic samples for the presence of Se IV and As III species as well as the fabrication of on-spot recognition devices (in the form of silica gels SB@SiO2 and silica coated TLC aluminium strips SB@SiO2@Al).

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Experimental

Reagents and chemicals

All of the reagents, namely anthranilic acid (CDH), sodium thiosemicarbazide,23 cysteamine,24 oxime,25 and benzothiazoline26 have been reported as chemical probes for As III determination. It can be concluded from previous reports that the multidentate ligating systems provide better sites to interact with oxyanions, presumably due to H-bond formation (mostly in carboxylic acids and phenols). In our previous work, some multidonor systems i.e. dipodal and tripodal Schiff bases with HN(N-OH)2 and N(N-OH)3 skeleton were explored for the analysis of Fe II, Zn II, and Cd II ions.27–29 However, these molecules were spectrofluorimetrically insensitive towards As III and Se IV; therefore, we herein chose an anthranilic acid-based Schiff base (SB) with an HO-(N-COOH)2 skeleton for the recognition of oxyanionic species. This system possessed a highly acidic phenolic proton which was abstracted by Se IV and As III species, and a spectrofluorimetric response was displayed. Although SB responded both to Se IV/As III species and Zn II ions, however, it was found to be selective for Se IV and As III in the presence of competing ions up to 1/10th fold of their concentration. SB has analytical prospects, and thus it can be used for the quantification of As III and Se IV over a wide range of concentration. Moreover, it can be moulded into silica probes for routine analysis. To the best of our knowledge, a direct approach utilizing a Schiff-base probe for the analysis of both Se IV and As III has not been reported in the past (not even a single report on Se IV analysis by a Schiff base).

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Selenite (Aldrich), sodium selenate (Aldrich), sodium arsenite (Loba Chemie), sodium arsenate (Loba Chemie), copper(II) nitrate trihydrate (HPLC Lab Reagents), nickel(II) nitrate (Merck), iron(III) nitrite anhydrous (Thomas Baker), iron(II) sulphate (Loba Chemie), manganese(II) acetate (Sigma-Aldrich), chromium(III) nitrate (Loba Chemie), cobalt(II) nitrate (Loba Chemie), zinc(II) nitrate hexahydrate (HPLC Lab Reagents), lead(II) nitrate (Fischer Scientific), mercuriic(II) chloride (Merck), cadmium(II) nitrate tetrahydrate (CDH), sodium acetate (Qualigens), sodium formate (Loba Chemie), sodium periodate (Nice), sodium bromate (Loba Chemie), sodium thiosulphate (Nice), ammonium thiocyanate (Loba Chemie), tetraethylorthosilicate (Acros), dipotassium hydrogen phosphate (Merck), oxalic acid (Avra), TLC silica gel 60 F254 (Merck), were purchased and used as such.

2,6-Diformyl-4-methylphenol (0.50 g, 3.05 mmol) and anthranilic acid (0.84 g, 7.14 mmol) were dissolved in 0.75 mL of DMSO-methanol mixture (0.5 mL dimethylsulfoxide (DMSO) and 4.5 mL methanol). It was further diluted to 50 μM (or the desired concentration) and used as stock solution for optimization experiments. A stock solution (10 mM) of AsIII/SeIV was prepared by dissolving 20 mg of SeIV in 5 mL stock solution of anion (SCN –, IO 42–, HPO 42–, BrO 3–, AsO 43–, SeO 42–, S 2O32–, CH 3COO–, Se IV, AsIII) and the emission response was recorded after each addition. Similarly, interference studies due to anionic species (SCN –, IO 42–, HPO 42–, BrO 3–, AsO 43–, SeO 42–, S 2O32–, CH 3COO–, CN –, SeIV/AsIII) were investigated by adding an anion species (SCN –, IO 42–, HPO 42–, BrO 3–, AsO 43–, SeO 42–, S 2O32–, CH 3COO–, CN –) prepared in DMSO –methanol; 10:90 v/v). The procedure was repeated for all of the above-mentioned species.

**Interference studies**

Briefly, 2 folds of AsIII/SeIV was added to 2 mL of stock solution of an SB solution followed by the incremental addition of various metal ion solutions (viz. Hg2+, Zn2+, Cd2+, Cu2+, Fe3+, Ni2+, Co2+, Mn2+, Fe2+, Cr3+ ions, 10 mM in water) up to 1/10 folds and the spectrofluorimetric response was recorded at an excitation wavelength of 427 nm. Similarly, interference studies due to anionic species (SCN –, IO 42–, HPO 42–, BrO 3–, AsO 43–, SeO 42–, S 2O32–, CH 3COO–, CN –) were investigated by adding an anion species (SCN –, IO 42–, HPO 42–, BrO 3–, AsO 43–, SeO 42–, S 2O32–, CH 3COO–, CN –) prepared in DMSO –methanol; 10:90 v/v). The procedure was repeated for all of the above-mentioned species.

The SB system was found to be sensitive towards Zn2+ ions; therefore, to check the effect of Zn2+ ions on the spectrofluorimetric response displayed by AsIII and SeIV, two separate experiments were performed.

In the first experiment, a mixture containing equimolar amounts (i.e. 1:1:1) of Zn2+ ions, SeIV, and AsIII was prepared. For this, solutions of 100 mM concentration of zinc nitrate (297 mg), sodium arsenite (130 mg) and sodium selenite (173 mg) were prepared by mixing in 10 mL of distilled water. These solutions were mixed to form one equimolar solution (33.3 mM each). Then, 1 μL of this mixture was added to 2 mL of SB (50 μM) and its emission profile was recorded. Next, the incremental addition of 1 μL of the same mixture was done up to ten additions and the spectrofluorimetric response was recorded after each addition.

In the second experiment, the displacement of Zn2+ ions by SeIV/AsIII was studied by commencing a spectrofluorimetric titration of the SB-Zn2+ conjugate with SeIV/AsIII ions. For this, two folds of Zn2+ ion solution i.e. 10 μL of 10 mM concentration were mixed with a 2 mL SB solution having a 25 μM concentration to form a SB-Zn2+ conjugate solution having a final concentration of Zn2+ ion and SB, 50 and 25 μM, respectively. Then, a spectrofluorimetric titration was commenced by a gradual addition of 1 μL of a 10 mM solution of SeIV/AsIII. The spectrofluorimetric response of each addition was recorded.

**Interaction studies**

The interaction between SB and SeIV was investigated by Job’s method and 1H NMR titration. For Job’s method, separate vials were charged with varying volumes of the SB solution i.e. 4.0, 3.5, 3.0, 2.5, 2.0, 1.5, 1.0, 0.5, 0.0 (mL of stock solution of SB). The volume of each solution was made to 4.0 mL by adding SeIV (50 μM in water) and the emission response was recorded at an excitation wavelength of 427 nm. A graph for the emission versus the mole fraction was plotted for respective species (Xs = [A]/[A] + [SB]), where Xs is the mole fraction of the anlyte (SeIV), [A] is the concentration of the analyte and [SB] is the concentration of the Schiff base. Similar studies were performed to determine the interaction between SB and AsIII.

For recoding 1H NMR, SB (20 mg, 0.05 mmol) was dissolved in 0.75 mL of DMSO-d6. Further, 1H NMR titration was done i) by adding 15 mg of AsIII (partially dissolved in DMSO-d6) to the SB solution and ii) Zn2+ ions (29 mg, 0.1 mmol) to the same solution and SB solution.
Quantification studies
In a 2-mL SB solution (50 μM), 2 μL of SeIV (10 mM) was added and mixed thoroughly. It was transferred to a quartz cuvette and excited at 427 nm to record the emission spectrum. The concentration of SeIV was gradually increased by the incremental addition of 2 μL of a 10 nM solution, and the respective emission spectral response was recorded until it attained a constant plateau. A calibration graph was plotted from the data and used to calculate the linear range, regression equation, and limit of detection.

Results and Discussion
Synthesis of SB probe
The Schiff base SB was obtained by a known method involving a condensation reaction of 2,6-diformyl-4-methylphenol and antranilic acid in a 1:2 molar ratio (Scheme 1).31 The formation of SB was confirmed by FT-IR and NMR spectroscopy. The appearance of vibrational bands at 3281, 1643, 1590 cm \(^{-1}\) in FT-IR spectrum of SB suggested the presence of –OH, –COOH, and C≡N, respectively. The formation of SB was further corroborated by the presence of characteristic signals in the \(^1\)H NMR spectrum of SB pertaining to aromatic rings (in the range 6.49 - 7.87 ppm), azomethinic NCH (at 10.23 ppm) and a carboxylic proton (as the broad signal at 8.57 ppm). The \(^1\)C NMR signals pertaining to the C≡N and -COOH were observed at 169.5 and 192.2, respectively. The literature reports and spectrophotometric studies suggested the formation of a centrosymmetric SB consisting of an HOOC-N-(OH)-N-COOH donor system embedded within a π-conjugated skeleton. The diverse donor sites are sufficiently powerful for coordinating metal ions as well as metal oxyanions via covalent coordinate and non-covalent interactions, respectively.

Spectrophotometric studies
The UV-Vis spectrum of SB exhibited absorption bands centered at 227, 326, and 460 nm, (Fig. 1a) and a weak emission band centered at 545 nm (when excited at 427 or 460 nm). The emission band experienced selective enhancement in the presence of SeIV and AsIII species when excited at 427 nm (Fig. 1b); however, no significant change in the signal was observed when excited at 460 nm (Fig. 1c). In addition, the time-course curve of the fluorescence of SB after the addition of SeIV and AsIII species, over the time interval of 300 s, was investigated (see Fig. S1, Supporting Information). The reaction was quick, as observed from the spectrum. Hence, the fluorescence measurements were done immediately after the addition of analytes. Although, a noteworthy color change of SB was observed in the presence of other cationic and anionic species when illuminated under a UV lamp at 365 nm, however, these species caused a negligible change in the emission profile, except for Zn\(^{2+}\) ions. The presence of Zn\(^{2+}\) ions showed an enhancement of the emission signal, accompanied with a blue shift from 545 to 479 nm (Figs. 1d - e).

Due to the sensitivity of SB towards Zn\(^{2+}\) ions, the effect of Zn\(^{2+}\) ions on the spectrofluorimetric signal displayed by SeIV/AsIII ions was studied in detail by executing two separate experiments. The first experiment was carried out to know the selective interaction between SB and SeIV/AsIII ions in the presence of equimolar amounts of Zn\(^{2+}\) ions. Initially, 1 μL of an equimolar mixture containing 1:1:1 folds (33.3 mM) of SeIV, AsIII, and Zn\(^{2+}\) ions each was added to the 2 mL of an SB solution (50 μM). It displayed an asymmetric broad band centered at 479 nm due to the interaction of SB with all of the ions (Zn\(^{2+}\) ions as well as SeIV and AsIII). However, the consistent addition of the same solution (i.e. mixture containing SeIV:AsIII:Zn\(^{2+}\) ions in 1:1:1 folds) to Zn\(^{2+}\) ions resulted in a gradual suppression of the contributory maximum with a simultaneous enhancement at 545 nm (Fig. 2). This observation clearly revealed that SB interacted with SeIV and AsIII ions completely; however the free SB (as the concentration of AsIII and SeIV was less than the concentration of SB) was interacted with Zn\(^{2+}\) ions. Therefore, Zn\(^{2+}\), AsIII, and SeIV ions displayed a contributory response at 479 nm. When AsIII and SeIV ions were present above 1 fold (i.e. equivalent to the concentration of SB), SB preferred these ions over Zn\(^{2+}\) ions and displayed a response at 545 nm due to SeIV and AsIII species. This study suggested the displacement of Zn\(^{2+}\) ion from the SB-Zn\(^{2+}\) conjugate by SeIV/AsIII ions.

This observation was also validated by a parallel study. For this, two folds of Zn\(^{2+}\) ions were added to one fold of SB to make final concentrations of 30 and 25 μM of Zn\(^{2+}\) ions and SB, respectively, which showed emission signal at 479 nm due to the formation of SB-Zn\(^{2+}\) conjugate (see Fig. 3, i). The concentration of Zn\(^{2+}\) ions in the solution was 12.5 μM when the first fluorescence signal was obtained. The incremental addition of 1 μL of a 10 mM solution in 2 mL of SB-Zn\(^{2+}\) conjugate to make a final concentration of 5 μM of SeIV and AsIII species was done. However, by the continuous addition of a SeIV(or AsIII) solution from 0 fold to 1.2 fold to SB (on the addition of 6 μL of a 10 mM solution in a 2 mL SB-Zn\(^{2+}\) conjugate to make a 30 μM SeIV or AsIII solution), the signal was suppressed and reached to a minimum at 1.6 fold (upon the addition of 8 μL of 10 mM solution in a 2 mL SB-Zn\(^{2+}\) conjugate to make a 40 μM SeIV or AsIII solution) due to the displacement of Zn\(^{2+}\) ions from the conjugate (see Fig. 3, ii). Further addition of SeIV (or AsIII) species resulted in a simultaneous rise of the signal at 545 nm, suggesting an interaction between the SeIV (or AsIII) species and SB (see Fig. 3, iii). Overall, this study suggested that SB prefers SeIV and AsIII species when the molar ratio of SB to SeIV/AsIII species exceeds

Scheme 1 Synthetic route to obtain SB by 1:2 reaction of 2,6-diformyl-4-methylphenol and antranilic acid.
above 1 fold, even if it is conjugated with Zn$^{2+}$ ions. Moreover, if the Zn$^{2+}$ ions are present in very high concentrations, their interference can be minimized by the addition of dithio-carbaminoacetic acid (TCA) as a masking agent.32 Besides, the interference of various anionic interferents (SCN$^{–}$, IO$_4^{2–}$, HPO$_4^{2–}$, BrO$_3^{–}$, AsO$_4^{3–}$, SeO$_4^{2–}$, BrO$_3^{–}$, AsO$_4^{3–}$, SeO$_4^{2–}$, S$_2$O$_3^{2–}$, CN, CH$_3$COO$^{–}$, Se$^{IV}$/As$^{III}$) and metal ions (Hg$^{2+}$, Zn$^{2+}$, Cd$^{2+}$, Cu$^{2+}$, Fe$^{3+}$, Ni$^{2+}$, Co$^{2+}$, Mn$^{2+}$, Fe$^{3+}$, Cr$^{3+}$) was studied to investigate their impact on the recognition of As$^{III}$ and Se$^{IV}$ species. The SB was found to be selective towards As$^{III}$ and Se$^{IV}$ in the presence of coexisting anions (except for CN$^{–}$) up to 2 fold and common metal ions up to 1/10th fold concentrations (see Figs. S2 and S3, Supporting Information). The higher concentrations of these anionic and cationic species showed interference in the results. Similarly, the interference of CN$^{–}$ was observed even at lower concentrations, and all efforts to remove its interference failed, so the sample must be CN$^{–}$ free. A similar case concerning the recognition of CN$^{–}$ and As$^{III}$ was reported earlier, where CN$^{–}$ is reported to interact with the phenolic proton leading to deprotonation of the probe.23

Proposed interaction between As$^{III}$/Se$^{IV}$ and SB
Firstly, the stoichiometry of oxyanion: SB was studied by Job’s method, which revealed the formation of 1:1 conjugates (see Fig. S4, Supporting Information). The site for the interaction between oxyanions and SB was investigated by performing $^1$H NMR titration between SB and As$^{III}$ ions (in DMSO-$d_6$) in

Fig. 1 (a) Absorption spectrum of SB (50 μM in DMSO-methanol; 10:90 v/v) showing absorption bands at 227, 326, and 460 nm; (b) Emission spectra of SB (50 μM in DMSO-methanol; 10:90 v/v, at $\lambda_{ex} = 427$ nm) in the presence of different oxyanions/anions (10 mM each in water) showing enhancement in case of Se$^{IV}$ and As$^{III}$; (c) Emission spectra of SB (50 μM in DMSO-methanol; 10:90 v/v, at $\lambda_{ex} = 460$ nm) in the presence of different oxyanions/anions (10 mM each in water) (d) Emission spectra of SB (50 μM in DMSO-methanol; 10:90 v/v, $\lambda_{ex} = 427$ nm) in the presence of different metal ions (10 mM each in water) showing enhancement in the presence of Zn$^{2+}$ ions; (e) Color change observation of SB in the presence of various metal ions/oxyanions under UV light at 365 nm.

Fig. 2 Emission spectral response of SB (50 μM) towards Se$^{IV}$ and As$^{III}$ in the presence of Zn$^{2+}$ ions obtained from the successive addition of 1 μL of mixture of Se$^{IV}$, As$^{III}$, and Zn$^{2+}$ in an equimolar ratio (1:1:1).
both the presence and absence of Zn$^{2+}$ ions, respectively. The partial solubility of As$^{III}$ and Se$^{IV}$ salts in DMSO pose some difficulties in this study; therefore, the study was performed with As$^{III}$ only (folds mentioned below are approximate). The $^1$H NMR spectrum of SB exhibited a broad signal pertaining to –COOH at 8.57 ppm and a singlet due to –OH at 11.4 ppm (Fig. 4a). Both of the signals disappeared with the upfield shifting of signals for H$_7$ (CH$_3$, $\Delta$δ = 0.22 ppm), H$_{1,5}$ (phenolic ring protons, $\Delta$δ = 0.45 ppm) and azomethinic proton H$_{8,16}$ ($\Delta$δ = 0.10 ppm) in the presence of 2 folds of As$^{III}$ species (Fig. 4b). A negligible shift of remaining signals was observed with a slight decrease in the intensity of each signal. The plausible cause of this observation is the interaction of As$^{III}$ species with the phenolic proton, followed by the deprotonation of SB. The formation of the phenolate ion enhanced the conjugation in the phenyl ring, and disturbed the electronic patterns at 1, 5 and 7 positions, thus resulting in the upfield shifting of signals. The addition of Zn$^{2+}$ ions (2 folds) in the same solution could not show any change in the $^1$H NMR spectrum, suggesting selectivity of SB towards As$^{III}$ (Fig. 4c). In a parallel study, the addition of 2 folds of Zn$^{2+}$ ions in the SB solution caused a slight variation in the intensity of the signals because Zn$^{2+}$ interacted with SB via donor-acceptor interactions without deprotonation (see Fig. S5, Supporting Information). However, the addition of As$^{III}$ ions to the same solution featured a similar spectrum, as observed in the first study (i.e. disappearance of –OH and –COOH signals with an upfield shift of some signals) suggesting the displacement of Zn$^{2+}$ ions from
the donor system. The overall observation confirmed the interaction between SB and As$^{III}$ via phenolic -OH leading to deprotonation of phenolic -OH to form a phenolate which enhanced the conjugation and thus emission response. The disappearance of carboxylic protons occurred plausibly due to their acidic nature, which would have enforced them to be exchanged with the sodium ions available from the salt (sodium arsenite) during titration, and therefore did not participate in As$^{III}$ conjugation. Furthermore, to check the involvement of -COOH in the interaction, a supporting experiment was done using the SB analogue (SB’). In SB’, anthranilic acid (with -COOH) was replaced with the 2-aminophenol (with –OH) arm keeping the rest of the skeleton the same as SB. Its emission spectrum recorded in the presence of As$^{III}$ exhibited a similar band as SB, suggesting the main role of the central –OH (of the p-cresol part) for binding As$^{III}$ ions (see Fig. S6, Supporting Information). In addition, the 1H NMR titration of SB with As$^{III}$ was also carried out, which suggested that the SB’ must be reacted through all of the –OH groups with the arsenite ion because multiple signals were observed in the spectrum. All of the –OH groups disappeared and the interaction seemed to be non-uniform due to three –OH groups; therefore, it was difficult to assign a particular interaction site in the SB’ (see Table S1.1, Supporting Information).

The mass spectrometric analysis of all of the conjugates formed with SB were carried out, and peaks at $m/z$ 510, 555, 468 revealed the formation of SB-As$^{III}$, SB-Se$^{IV}$, SB-Zn$^{II}$ complexes, respectively. The $m/z$ peak at 467 and 510 in the case of SB-Zn$^{II}$-As$^{III}$ revealed complex formation with both of the ions i.e. Zn$^{2+}$ ions and As$^{III}$ ions. (see Figs. S7 – S10, Table S2, Supporting Information).

### Analytical prospects of study

#### Quantification of As$^{III}$ and Se$^{IV}$

Due to the selective spectrofluorimetric response of SB for As$^{III}$ and Se$^{IV}$, some analytical parameters were also investigated. In order to optimize the quantification parameters, a spectrofluorimetric titration was commenced between SB and the analytes (Se$^{IV}$, As$^{III}$). The incremental addition of analyte (Se$^{IV}$/As$^{III}$) solution in the SB solution resulted in the continuous enhancement of the emission band (observed at 545 nm) in the range 0 to 250 μM for Se$^{IV}$ species (Fig. 5a) and 1 to 130 μM for As$^{III}$ species.

### Table 1 Real sample analysis by utilizing samples from different sources

| S. N. | Source | Sample type | Analyte found (μM) | Accuracy, % |
|-------|--------|-------------|-------------------|-------------|
| 1     | Water Samples (Spiked) | Tap water | Se$^{IV}$ (15) | 14.5 | 96.6 |
|       |        |             | As$^{III}$ (15)  | 14.4 | 96.0 |
| 2     | Soil sample (Spiked) | Lake water | Se$^{IV}$ (15) | 13.5 | 90.0 |
|       |        |             | As$^{III}$ (15)  | 13.7 | 91.3 |
| 3     | Pharmaceuticals (Multi-vitamin tablet) | Methylcobalamin: | 1500 | 70.2 | 93.6 |
|       |        | Folic Acid IP: | 1.5 mg | 70.2 | 93.6 |
|       |        | Alpha Lipoic Acid: | 100 mg | 70.2 | 93.6 |
|       |        | Vitamin B6 IP: | 1.5 mg | 70.2 | 93.6 |
|       |        | Chromium Picolinate: | 500 μg | 70.2 | 93.6 |
|       |        | Sodium selenite: | 75 μg | 70.2 | 93.6 |
|       |        | ZnSO$\text{4}$$\cdot$H$_2$O IP | 7.5 mg | 70.2 | 93.6 |

**Fig. 5** Plot obtained by the spectrofluorimetric titration of SB (50 μM in DMSO-methanol: 10:90 v/v) with gradual increase of analyte (a) 2 μL of Se$^{IV}$ (10 mM in water) showing the enhancement of the emission band [inset: variation of emission intensity at 545 nm upon increasing Se$^{IV}$ concentration]; (b) 2 μL of As$^{III}$ (10 mM in water) showing enhancement of emission band [inset: variation of emission intensity at 545 nm upon increasing As$^{III}$ concentration].
(Fig. 5b), which resulted in a sigmoid-type curve ($\lambda_{em} = 545$ nm). The quantification parameters, such as the linear regression equation and the limit of detection (using 3$\sigma$/k), calculated from the linear points, were found to be 40.6 nM (5.15 ppb) ($R^2 = 0.9261$) for Se$^{IV}$ and, 29.1 nM (3.12 ppb) ($R^2 = 0.9451$) for As$^{III}$ species (see Fig. S1.11).

Further, the stability of the complexes is estimated by calculating the binding constant of SB with Se$^{IV}$ and As$^{III}$ as log $K_a = 3.09$ and 3.15, respectively, on the basis of the Benesi-Hildebrand equation,

$$1/I = 1/I_{max} + (1/K[C])(1/I_{max}).$$

In the equation, $I = I_0 - I_0$ and $I_{max} = I - I_0$, where $I_0$, $I$, and $I$ are the fluorescence emission intensities of SB measured in the absence of the analyte, at an intermediate analyte concentration, and at the concentration of its complete interaction, respectively. $K$ is the binding constant and [C] is the analyte concentration.

The present method was found to be better over some recently reported spectrofluorimetric probes due to the lower limit of detection, simplicity for handling, cost-effectiveness, and requirement of less-laborious protocols, as compared to sample preparation for sophisticated instrumental techniques and nanomaterials. The previous methods reported for the detection of As$^{III}$, like 2-((2-hydroxynaphthalen-1-yl)methylene)hydrazine-carbothioamide (7.06 ppb, DMF-water 9:1 v/v, Fluorescence), 23 2-hydroxy-5-methyl-benzene-1,3-dicarboxaldehyde di-oxime (24.61 ppb, Water, Fluorescence),25 NH$_2$-CMSs; (7 ppb, Electro-adsorptive X-ray fluorescence), work in higher detection ranges.33 Similarly, reports available on Se$^{IV}$ detection comprise (3-(3-(methoxycarbonyl) benzylidene)hydrazinyl) benzoic acid immobilized on mSiO$_2$, 2.02 ppb, ICP-AES),34 2-(2-Formyl-4-methyl-phenox)-N-phenyl-acetamide (1.20 ppb, Fluorescence),35 N,N-di(3-carboxysaliclydene)-3,4-diamino-5-hydroxyptpyrazole immobilised onto mSiO$_2$ monolith (1.14 ppb, ICP-AES),36 5-((2-hydroxy-1-naphthoyl)hydrazono)-methyl)benzoic acid immobilized on mSiO$_2$; (2.16 ppb, ICP-AES),37 2,3'-diamino-naphthalene (12 ppb, UV-Visible),38 bilogical fluorescent probe 1-Hydroxy-2-(benzothiazol-2-yl)naphthalene (HBTN-Se) (16.51 ppb), Fluorescence).39

**Application to real samples.** The standardized method was applied to real and synthetic samples, like water samples, soil samples, and multi-vitamin tablets. The soil and water samples were spiked with known concentrations of analytes and analyzed by the optimized method. However, the multi-vitamin samples were prepared by a known procedure (see Fig. S2.1). The outcomes showed that the concentrations of Se$^{IV}$ and As$^{III}$ found by the proposed method had a good consistency with the added amounts, and the recoveries of Se$^{IV}$ and As$^{III}$ were found to be in the range 91.3 - 96.6% (Table 1). Furthermore, a multivitamin tablet available in the market was analyzed for the presence of Se$^{IV}$. The method showed the good consistency of the experimental amount with the actual amount present in the tablet.

**Practical application:** Furthermore, an exploration of the SB probe for practical usage was achieved by the fabrication of silica coated aluminum TLC strips and silica gels (see Fig. S2.2 for experimental). The SB@SiO$_2$@Al strips were found to be greyish in sunlight and fluorescent blue under UV light. The strips showed significant interactions with Se$^{IV}$ and As$^{III}$ solutions and displayed a fluorescent green color in both the cases (Fig. 6).

Although the distinction between Se$^{IV}$ and As$^{III}$ was not possible, however, the presence of either of the species could be detected with usage of these strips. In another approach, silica gels were fabricated and utilized as portable solid gels for practical recognition of the targets (see Figs. S2.2, S1.12, and S1.13 for synthesis and characterization details). The fabricated gel displayed beautiful color variations like SB imparted a red color to the white silica gel which resulted in the formation of SB impregnated silica gel (SB@SiO$_2$). We found fluorescent

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![Fig. 6](image6.png)  
**Fig. 6** TLC plates coated with SB. (a) naked eye; (b) under UV light; (c) under UV light after dipping in the Se$^{IV}$ ion solution.

![Fig. 7](image7.png)  
**Fig. 7** Pictorial representation of silica gels for the recognition of Se$^{IV}$/As$^{III}$ ions.
purple and bluish green in both the absence and presence of either Se\textsuperscript{IV} or As\textsuperscript{III} under UV light (when illuminated at 365 nm), respectively. Silica gel impregnated with both SB and Se\textsuperscript{IV} (SB-Se\textsuperscript{IV}@SiO\textsubscript{2}) displayed blue color under UV light (at 365 nm) (Fig. 7). Therefore, SB@SiO\textsubscript{2} gel can be easily used for the on-spot detection of Se\textsuperscript{IV}/As\textsuperscript{III} simply by dipping the gel in the sample solution. The gel imparts a blue color to the solution when observed under UV light due to the interaction of SB released from the silica network with Se\textsuperscript{IV} or As\textsuperscript{III}.

Conclusions

In the present approach, anthranilic acid derived SB probe has been explored as a Turn-ON fluorescent switch for the Se\textsuperscript{IV} and As\textsuperscript{III} species. The heteroatomic SB system having the HOOC-N-(OH)N-COOH coordinating system was found to be highly selective and sensitive for Se\textsuperscript{IV} and As\textsuperscript{III} with wide detection ranges and low detection limits. Moreover, it can be used for the fabrication of SB modulated silica gels (SB@SiO\textsubscript{2}) and paper strips (SB@SiO\textsubscript{2}@Al) for the on-site detection of either of the species by the fluorescent green color formation.

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Supporting Information

This material is available free of charge on the Web at http://www.jsac.or.jp/analsci/.

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