



Speciation Study of Nitrite/Nitrate through Diazo-Coupling Reaction Using Silica-Perchloric Acid as a Novel Catalyst at Trace Level: Application to Environmental Samples

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A simple, sensitive and rapid spectrophotometric method has been developed based on the diazo coupling reaction in the quantification of nitrite/nitrate at trace level using silica perchloric acid (SPCA) as an acid catalyst. 5-Amino-2-mercapto benzimidazole has been used as a novel amine and coupled with *N*-(1-naphthyl)ethylenediamine dihydrochloride (NEDA) to produce an intense coloured azo dye. The proposed method showed wide linearity in the concentration range 0.1-1000 ppm of nitrite. The molar absorptivity and Sandell's sensitivity were found to be $1.6 \times 10^6 \text{ L mol}^{-1} \text{ cm}^{-1}$ and $0.0012 \mu\text{g cm}^{-2}$ respectively. Nitrate was measured after its reduction to nitrite using optimized copper-cadmium reductor column. The proposed method has been successfully applied to measure trace level nitrite/nitrate from a variety of sample matrices like water, soil, milk and radiator coolant.

Keywords: Silica perchloric acid, 5-Amino-2-mercaptobenzimidazole, Nitrate, Nitrite, Diazo-coupling reaction.

INTRODUCTION

Nitrites/nitrates are naturally occurring oxyanions of nitrogen present in the environment. The biodegradation of nitrates as well as ammonical nitrogen/nitrogenous organic matter is one of the sources of nitrite/nitrate in natural waters [1,2]. In water, the chemical reactions under anaerobic conditions may lead to the reduction of nitrates to nitrites to meet the oxygen deficiency [3]. Nitrates have been extensively used in the agricultural sector as fertilizers, in manufacturing of explosives and as an oxidizing agent in chemical industries [4,5]. Nitrites are widely used in radiator coolants, as well as packaged food industry as preservatives [6-9]. The excessive use of nitrates and nitrites in all these application domains may cause serious concern when their concentration exceeds certain limit. At low levels of concentration, it is nontoxic but in recent years nitrate sources have tremendously increased due to enhanced human activities, which are responsible for ecological imbalance in the concentration profile of nitrite/nitrate species [10,11]. It is primarily reacting with secondary or tertiary amines in the human body and living beings to form nitrosamines, which are known to be carcinogenic, teratogenic and mutagenic.

Even the presence of trace levels of nitrite/nitrate in water and milk powders may cause severe health risk in infants [12,13]. During the early stages of child development, nitrate is transformed to nitrite in the body, which reacts with hemoglobin, an oxygen carrier and turns into methemoglobinemia. This reduces the oxygen carrying capacity of heme species which causes blue baby syndrome. Even in adults, the accumulation of higher concentrations of nitrate/nitrite in various body fluids or biological systems may cause detrimental effects [14,15]. In order to check the nitrite/nitrate concentration a precise quantitative method has to be developed [16,17].

The threshold limit values (TLV) of these ionic species in drinking water are given by different controlling agencies like the World Health Organization (WHO) [18], United States Environmental Protection Agency (USEPA) [19], European Union (EU) [20], Central Pollution Control Board (CPCB), India [21], etc. Hence, throughout the world the prescribed limits of these two anions are very low; thus their quantification at trace level becomes very significant. Several methods have been reported for their quantification, including chromatography [22], capillary electrophoresis [23], flow injection spectrophotometry [24,25], amperometry [26], pulse polarography

[27] and UV-Vis spectrophotometry [28]. Among all these methods, UV-Vis spectrophotometric methods have been widely used due to their simplicity, high reproducibility and less expensive. In UV-Vis spectrophotometric method, the most significantly followed process is based on diazo-coupling, which is extensively known as Greiss-Ilosvey reaction [28]. In this reaction, a primary aromatic amine reacts with nitrite in acidic condition to form diazonium ion which subsequently couples with another reagent in neutral/acidic/alkaline medium to form a coloured azo dye. Several protocols have been reported based on this reaction due to its sensitivity and high reproducibility [29].

In recent years, several types of good catalytic acid materials are used in the synthesis of azo dyes, in which magnetic solid acid catalysts are the good substrate materials. The synthesis of these catalysts is very laborious and are coloured. Hence their use in UV-Vis spectrophotometric method was restricted [30]. Several colourless solid acids have been reported as catalytic agents to enhance the sensitivity in diazo-coupling reaction [31]. Silica acids exhibit higher activity and selectivity, due to the lower particle size and large surface area which have been used mainly in chemical transformations silica-periodic acid and silica-boron trifluoride have been reported as solid acid catalysts in recent years in the estimation of nitrite [29,32]. The method reported here involves the use of silica-perchloric acid (SPCA) as a substrate catalytic material for the first time in the quantification of nitrite at trace level.

5-Amino-2-mercaptobenzimidazole has been used as a novel aromatic amine and *N*-(1-naphthyl)ethylenediamine dihydrochloride (NEDA) was employed as a coupling agent in the presence of silica-perchloric acid (SPCA) solid acid catalyst in the development of a new diazo-coupling protocol for nitrite sensing. The sensitivity, linearity and detection limit have been determined. The proposed method has been successfully applied to measure trace levels of nitrite/nitrate in water, soil, milk and radiator coolant sample matrices.

EXPERIMENTAL

All the chemicals used in this study were of AnalaR grade and silica gel 60 from Merck, 5-amino-2-mercaptobenzimidazole, *N*-(1-naphthyl)ethylenediamine dihydrochloride (NEDA) and sulphanilamide were purchased from Sigma-Aldrich and used without any further purification. Distilled water was used throughout the experiment. Standard nitrite stock solution (1000 µg/mL): prepared by dissolving 0.15 g of sodium nitrite (pre-dried at 105 ± 5 °C for 1 h) in distilled water containing about 1 mL of chloroform and diluted to 100 mL. Nitrate stock solution (1000 µg/mL): prepared by dissolving 0.16 g of pre-dried AnalaR grade potassium nitrate (at 105 ± 5 °C for 1 h) in distilled water and diluted to 100 mL in volumetric flask. Working standards were prepared by diluting the stock solution on the day of use. $\text{NH}_3\text{-NH}_4\text{Cl}$ buffer solution (pH = 8.5) was prepared by dissolving 0.531 g of NH_4Cl in 80 mL of water and adjusting the pH to 8.5 using 1:1 ammonia solution (v/v) and diluting it to 100 mL.

The absorbance measurements were carried out using UV-VIS spectrophotometer (Thermo scientific, Helios Zeta model:

361003, USA) with 1 cm quartz cuvettes. The synthesized solid acid, *i.e.* silica-perchloric acid (SPCA) and diazonium salts were characterized by using Fourier transform infrared spectrometer (FTIR) (Shimadzu model: 8400S, Japan) and powder XRD diffractometer (PAN analytical X pert PRO with graphite mono chromotized $\text{CuK}\alpha$ radiation source). Scanning electron microscopy images (SEM) of silica perchloric acid (SPCA) were recorded using scanning electron microscope (TESCAN Vega3 LMU, Netherland). The prepared azo compound was centrifuged using Remi centrifuge machine (FLOC 4502, India)

Synthesis of silica-perchloric acid (SPCA): Silica gel (150 mg) was taken in 250 mL beaker and 20 mL of perchloric acid (70% purity) was added and allowed for 1 h. The contents in the beaker were stirred for 30 min or until a gel type of mass was obtained. The gel type of mass was separated and washed thoroughly with methanol to remove excess of perchloric acid. The resulting mixture was homogenized by mechanical milling. The ground SPCA powder was stored in an airtight container for its analytical use [32].

Recommended procedure: 1 mL of amine, 300 mg of SPCA and 0.4 µg/L of nitrite solutions were taken in a mortar and mixed with pestle homogeneously. Then, 2 mL of NEDA was added and grinded for 5 min to form the blue coloured azo dye. The reaction mixture was quantitatively transferred into the glass tube and centrifuged. Then, the centrifugate was quantitatively transferred into 25 mL volumetric flask and diluted to the mark. Similarly, a reagent blank was also prepared without nitrite. The absorbance was measured at 576 nm against a reagent blank

Copperized-cadmium reductor column: Cadmium granules (25 g) were taken in a 100 mL beaker and washed with 6 N HCl and rinsed with distilled water. After washing, the granules were swirled in the presence of 50 mL of CuSO_4 (2% w/v) aqueous solution for 10 min until the blue colour fades completely. This procedure is repeated with 2% aqueous solution of copper sulfate in a beaker until the appearance of brown coloured colloidal precipitate in the beaker. Then, the granules were gently washed with distilled water several times to remove the unreacted copper sulfate solution. The granules were air dried and used in the construction of reductor column. A micro burette of the dimension (30 cm × 5 mm) was used as a column. Glass wool was used to plug the micro burette bottom and it was filled with water. The copperized cadmium granules were filled in the column without entrapment of air. The column was washed with 200 mL of NH_4Cl -EDTA buffer solution at a flow rate of 8-10 mL/min. The reductor column was optimized by using 1:3 volume ratio of standard nitrate and ammonium chloride-EDTA buffer solution (pH = 8.5) for its quantitative reduction to nitrite. All the parameters like column height and flow rate were optimized as per the ASTM protocol with known volumes of nitrate standards [33]. The concentration of the reduced nitrite was calculated with reference to the standard calibration plot constructed using standard nitrite through diazo-coupling reaction. This column can be used for 2-3 months as long as copperized cadmium granules are stored in NH_4Cl -EDTA buffer solution [33].

RESULTS AND DISCUSSION

XRD study: X-ray diffraction (XRD) study was carried out in order to know the adsorption of perchlorate anionic species on the silica substrate surface as well as the formation of diazonium salt on the silica surface. These materials were grinded uniformly for 3 min before the sample analysis. The X-ray diffraction peak patterns of the pristine silica, SPCA and SPCA + diazonium salt are shown in Fig. 1. The average particle size was calculated using Scherrer equation and it was found to be 9, 10 and 12 nm respectively for pristine silica, SPCA and SPCA + diazonium salt. The peaks height and full width at half maximum (FWHM) was smaller for SPCA than compared to silica. These confirm the adsorption of perchlorate anionic species on silica. The peaks height and FWHM of SPCA further become smaller for SPCA + diazonium salt. This reveals the formation of diazonium salt on SPCA substrate material (Fig. 1).

SEM study: The surface morphology of pristine silica and the synthesized SPCA substrate material was studied by

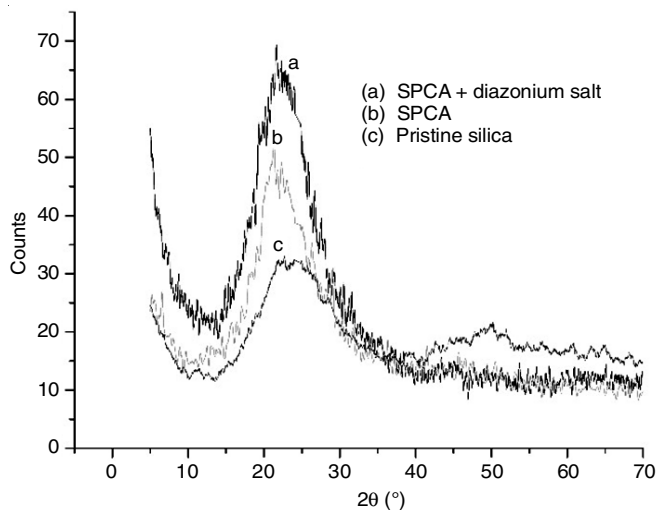


Fig. 1. Powder XRD diffraction patterns of (a) SPCA + diazonium salt, (b) SPCA and (c) pristine silica

recording scanning electron microscopy images as shown in Fig. 2a-b. The particle size of SPCA was higher when compared to pristine silica. These studies revealed the adsorption of perchloric acid species on the surface of silica in SPCA material (Fig. 2a-b).

FTIR study: The diazonium salt was synthesized by taking equal molar ratio of nitrite and amine with SPCA substrate material and grinded homogeneously to obtain the complex, it was filtered then finally dried with methanol. The FTIR spectrum of the synthesized diazonium-SPCA complex was recorded in the range 4000-400 cm^{-1} . Initially, the background correction was carried out and then the spectrum of diazonium-SPCA complex was recorded using FTIR instrument through ATR mode. A stretching vibration of $\text{N}\equiv\text{N}$ was observed at 1452 cm^{-1} indicating the existence of diazonium ion in the complex. The peak observed at 1495 cm^{-1} may be due to the stretching vibrations of C-N groups of the molecule. Then, C=N stretching bands were observed at 1627 cm^{-1} . However, the S-H and N-H stretching vibration bands were merged and appeared as a broad band at 3541 cm^{-1} . A strong and intense peak observed at 1071 cm^{-1} is due to the Si-O stretching vibrations indicating the presence of perchloric acid on the surface of silica substrate (Fig. 3). A strong absorption band at ~ 622 cm^{-1} corresponds to the stretching vibration of Si-OH. The peak at lower frequency 443 cm^{-1} is assigned due to out of plane bending of Si-O-Si stretching vibration [34].

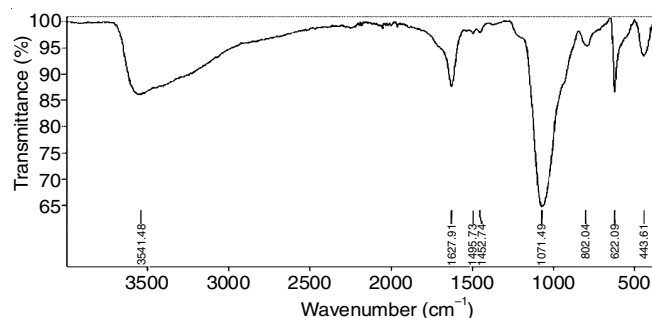


Fig. 3. FTIR spectrum of diazonium salt

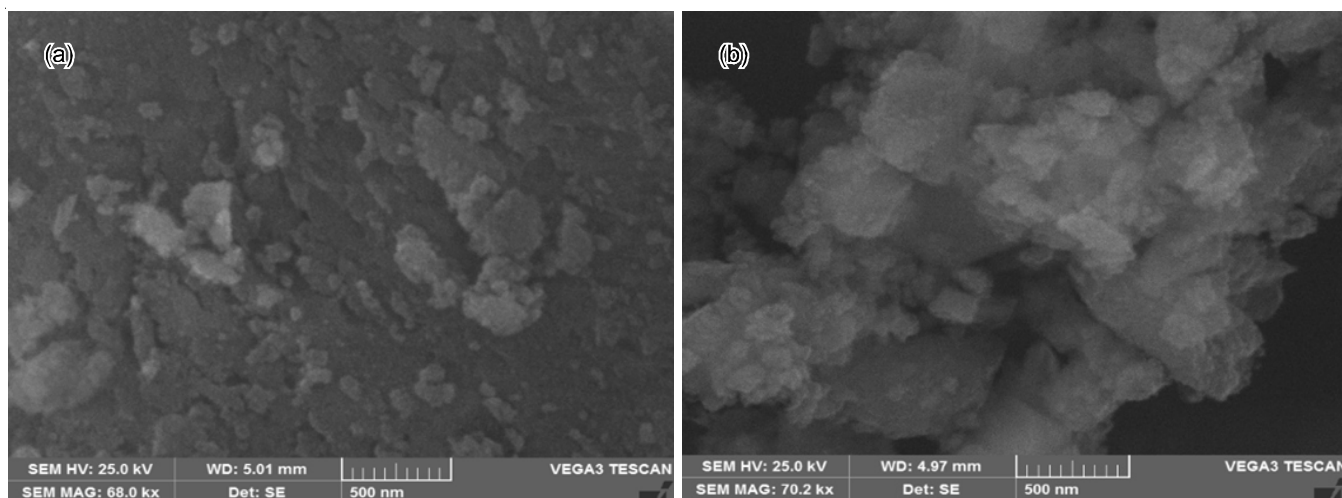


Fig. 2. SEM images of (a) pristine SiO_2 , (b) SPCA

Optimization study

Effect of amine: Initial studies were carried out using different aromatic primary amines (Table-1) in the diazotization process in presence of silica-perchloric acid (SPCA). Initially, 1 mL of primary aromatic amine (1 % w/v), 300 mg of SPCA and 2 ppm of nitrite was taken in a mortar and mixed thoroughly by grinding. Then 1 mL of NEDA (1 % w/v) was added and grinding was continued to form the azo dye. The reaction contents were quantitatively centrifuged for 5 min. The supernatant liquid was transferred into 25 mL volumetric flask quantitatively and diluted upto the mark with distilled water. Similarly, a reagent blank solution was also prepared. The measured λ_{\max} and absorbance values for the dye derived from different primary aromatic amines are shown in Table-1. The dye formed from 5-amino-2-mercaptobenzimidazole gave a better absorbance value than other amines. The concentration of selected amine (5-amino-2-mercaptobenzimidazole) was varied in the range of 0.2 to 3.5 mL in presence of 0.4 ppm of nitrite, 300 mg of substrate *i.e.* SPCA and 1 mL of NEDA. The absorbance values increased from 0.2 to 1 mL and remained constant after 1 mL

volume. Hence, an optimum value of 1 mL of amine was used in all further studies as an optimized concentration and its subsequent applications to real sample analysis (Fig. 4a).

Effect of acid on silica substrate: The acid adsorbed on silica is employed as a catalytic material for nitrite/nitrate detection through diazonium salt formation. In order to study the effect of acid adsorbed on silica substrate material has been studied using different mineral acids like hydrochloric acid, nitric acid, sulphuric acid and perchloric acid. The analysis was carried out in presence of 2 ppm of nitrite, 1 mL of amine and 1 mL of NEDA as coupling agent. The colour stability and absorbance values of azo dye for different acids adsorbed on silica substrate are listed in Table-2. In presence of hydrochloric acid, nitric acid and sulfuric acid, the silica causes degradation of formed azo dye. The presence of perchloric acid showed good colour stability due to the adsorption of perchloric acid species on silica surface. Hence, perchloric acid was used as an acid, which gave satisfactory results like colour stability and good absorbance value compared to other acids attributed to adsorption of perchlorate anionic species on silica surface. Thus, perchloric acid was used as a

TABLE-1
EFFECT OF AMINE

Amine	λ_{\max} (nm)	Absorbance
Sulfanilamide	540	0.795
<i>p</i> -Nitroaniline	560	0.749
2-Amino benzoic acid	530	0.473
4-Amino benzamide	548	0.519
5-Amino-2-mercaptobenzimidazole	576	0.807

TABLE-2
EFFECT OF ACID

Acid adsorbed on silica substrate	Colour stability	Absorbance
Hydrochloric acid	4 min	0.165
Sulfuric acid	6 min	0.345
Nitric acid	3 min	0.143
Perchloric acid	> 24 h	0.813

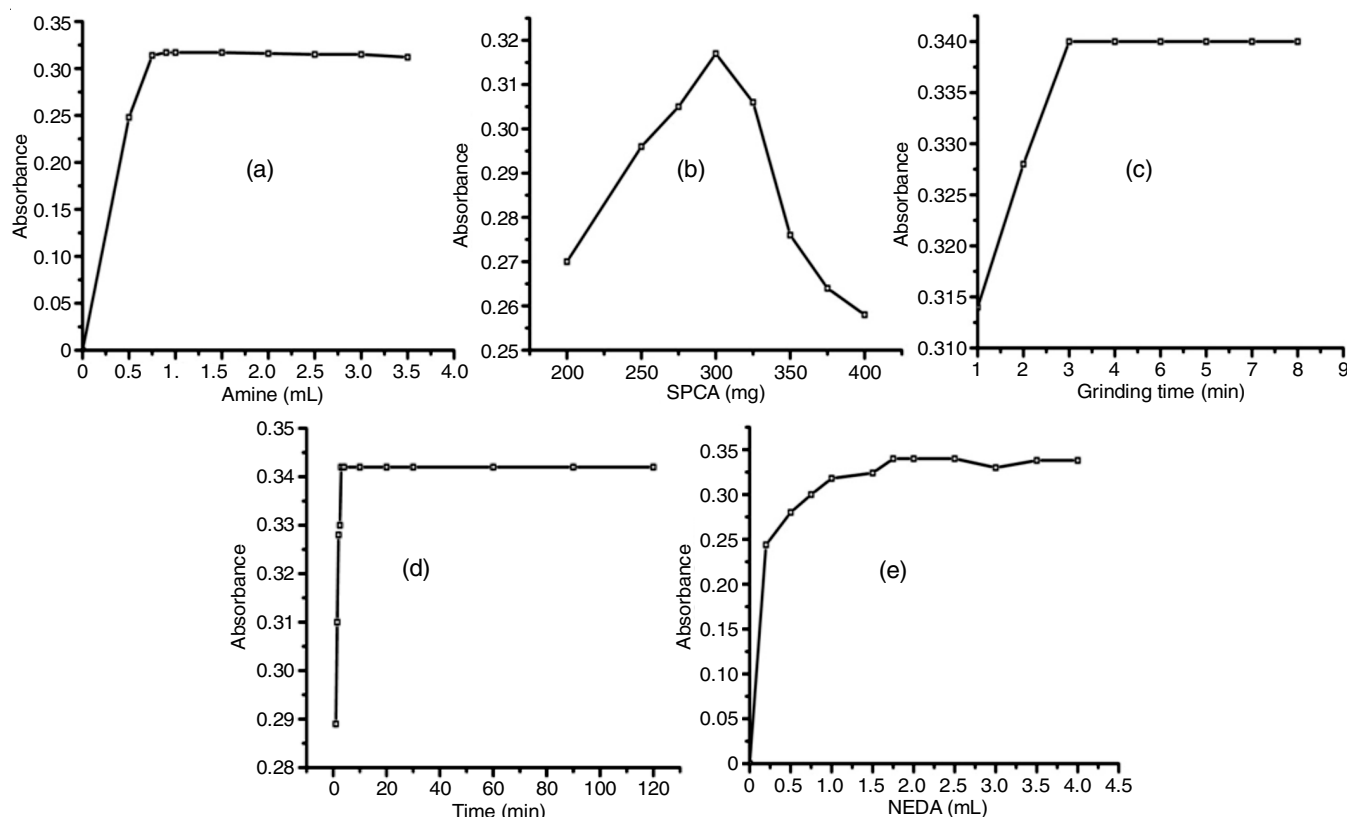


Fig. 4. Effect of (a) amine, (b) SPCA, (c) grinding time, (d) diazonium ion stability and (e) NEDA

substrate catalytic material in the present investigation to adsorb the acid on the surface of the silica substrate material to achieve the required colour sensitivity with high absorbance value for a fixed nitrite concentration.

The minimum quantity of silica perchloric acid substrate material required to get maximum absorbance in the present procedure at a fixed nitrite concentration has been studied by using 1 mL of amine, 0.4 ppm of nitrite, 1 mL of NEDA. Then, SPCA was varied in the range 200-400 mg. The absorbance value increases from 200 to 300 mg of SPCA and then suddenly decreases beyond 325 mg level. The SPCA (300 mg) was used as an optimized quantity in all further studies (Fig. 4b).

Effect of grinding time: Grinding facilitates the adsorption of an amine on the surface of an acid catalyst. Hence, the effect of grinding time has been studied in the present study. After adding all the reagents in the mortar grinding was done for different time intervals from 1 to 9 min to get maximum absorbance for a given nitrite concentration. The absorbance values increase from 1 to 3 min and remained constant after 3 min of grinding time. Hence, 3 min of grinding time was optimized and used in all further studies (Fig. 4c).

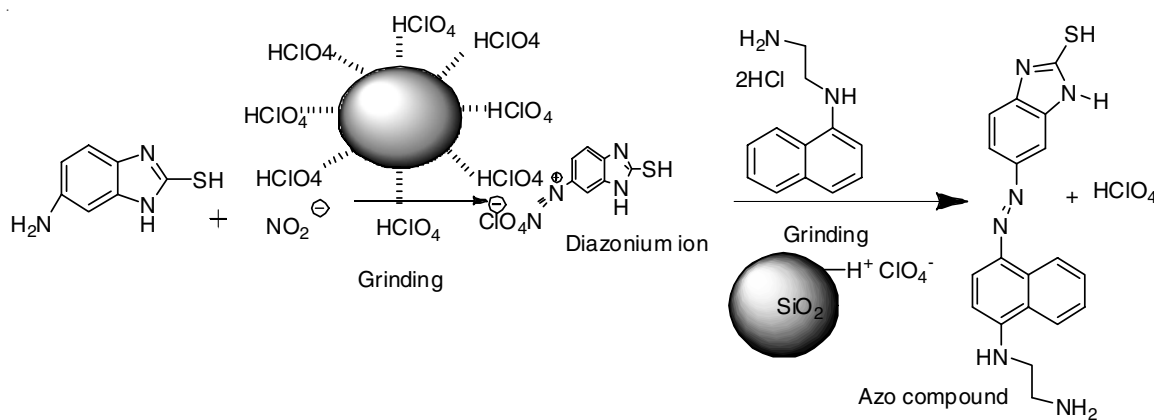
Effect of diazonium ion stability: In order to study the diazonium ion stability, different time intervals were allowed before adding NEDA as a coupling agent in the time range 1 to 120 min. The absorbance values increased from 1 min to 2 min. Then, it remained constant indicating that the ionic complex

is quite stable. Hence, in all further studies, 3 min time was allowed before adding coupling agent (Fig. 4d).

Effect of coupling agent: *N*-(1-Naphthyl)ethylenediamine dihydrochloride (NEDA) was used as a coupling agent in the volume range 0.2-4.5 mL and 2 mL was used as optimum volume in all further studies (Fig. 4e).

Species responsible for colour: The species responsible for colour is the amine *i.e.* 5-amino-2-mercaptobenzimidazole which gets adsorbed on the surface of acid catalyst and undergoes diazotization in presence of nitrite under acidic medium. Later, the diazonium ion couples with NEDA to form an azo compound, which gets desorbed from the SPCA surface. The contents were further quantitatively transferred into test tube and centrifuged. The absorbance value of the centrifugate was measured at λ_{\max} (576 nm) against a reagent blank after diluting to known volume. The nitrite detection process by azo dye formation and the species responsible for colour is shown in **Scheme-I**.

Absorption spectra: In order to find the λ_{\max} of the coloured species formed in the present diazotization reaction, the absorption spectrum was recorded in the wavelength range 400-800 nm (Fig. 5a). The absorption spectrum showed λ_{\max} at 576 nm wavelength. Hence, the absorption spectrum was recorded at 576 nm for different nitrite concentrations and measured absorbance values were plotted as calibration plot (Fig. 5b).



Scheme-I: Species responsible for colour

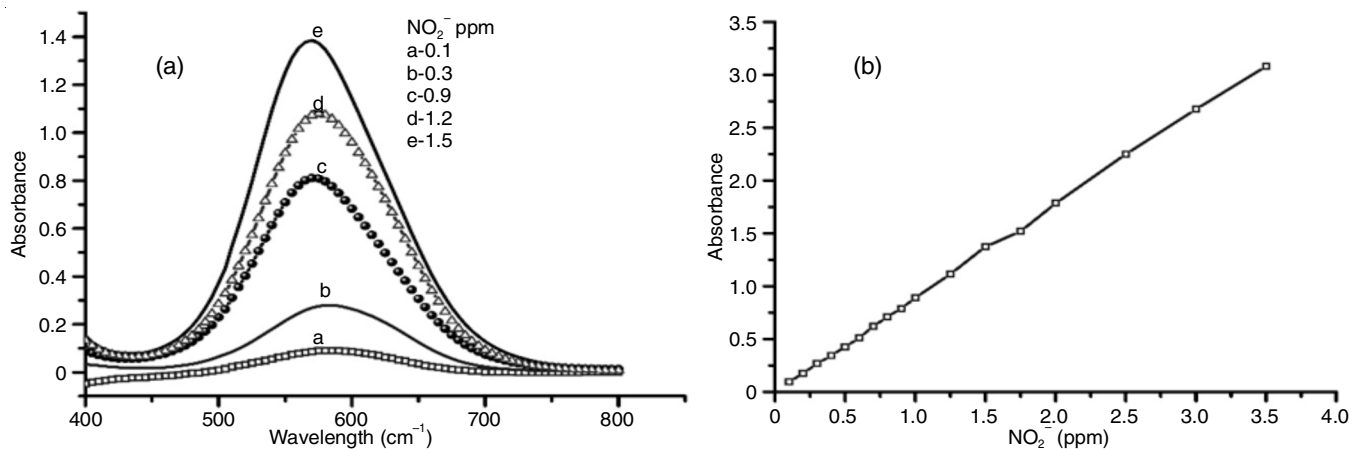


Fig. 5. (a) Absorption spectra and (b) calibration plot

Analytical merits of the present method: The proposed method for the nitrite detection at trace level has been validated by comparing with standard method [28]. The analytical parameters of the proposed method are shown in Table-3. The proposed method gave good linear working range, good sensitivity, short time diazonium ion formation and one of the major advantages is the formation of diazonium-SPCA complex with long time stability.

TABLE-3
ANALYTICAL PARAMETERS

Amine	5-Amino-2-mercapto benzimidazole	Sulphanilamide
Wavelength (λ_{max})	576 nm	540 nm
Time required for dye formation	30 s	2.0 min
Colour	Blue	Pink
Linearity (ppm)	0.1-1000	0.2-10
Molar absorptivity (ϵ) ($L\ mol^{-1}\ cm^{-1}$)	1.6×10^6	1.5×10^5
Correlation coefficient (R^2)	0.9996	0.9996
Colour stability	> 24 h	24 h

Interference study: The interference studies have been carried out in presence of 0.4 ppm of nitrite. The maximum tolerable concentrations are mentioned in Table-4. These tolerance limits did not cause more than $\pm 4\%$ error in the recovery of added nitrite/nitrate. In present method, formaldehyde did not interfere up to 30 ppm levels of nitrite and sulfate concentration level was 200 ppm beyond that a decrease in the absorbance value was observed. However, at higher concentration of sulfate, the tolerance level increases up to 450 ppm due to addition of 1 mL of 0.05% of formaldehyde solution which masks the sulfate, one of the most interfering ion. Another most interfering ion *i.e.* sulphite was not involved up to 5 ppm level, beyond that there was a decrease in the absorbance of the nitrite/nitrate, but addition of 1 mL of 0.01% (a) and (b) masking agents into the solution, the tolerance value has increased up to 10 and 25 ppm levels, respectively. The Fe^{2+} and Cu^{2+} effects were also determined by the addition of 1 mL masking agents (c) and (d), respectively. Most of the ions interference was checked for the estimation of the nitrite/nitrate in the water, soil, milk and radiator coolant samples. The ionic tolerance limit values are mentioned in Table-4.

Applications study

Determination of nitrite in real samples: The proposed diazotization method has been validated by applying to the nitrite present in various sample matrices like water, soil, milk and radiator coolants. The water samples were collected from bore well in Bangalore city rural areas. Known volumes of collected water samples were treated with 1 N NaOH solution and centrifuged. The centrifugate has been collected and then the residue was washed with water and again centrifuged. All the centrifugates were transferred quantitatively into 25 mL volumetric flask and then used for nitrite determinations based on above procedure. Same procedure was followed for soil samples matrices.

TABLE-4
INTERFERENCE STUDY

Interferent	Tolerance limit (ppm)
NH_4^+ , Fe^{2+} , Ca^{2+} , Mg^{2+} , Zn^{2+} , Hg^{2+} , Mn^{2+} , Cd^{2+} , Cr^{3+} , Pb^{2+} , K^+ , Na^+	10000
Ni^{2+} , Cu^{2+}	5000
Cl^- , CO_3^{2-} , HCO_3^- , PO_4^{3-} , ClO_4^- , CH_3COO^- , $C_2O_4^{2-}$	10000
HCHO	30
SO_3^{2-}	5
$SO_3^{2-(a)}$	10
$SO_3^{2-(b)}$	25
SO_4^{2-}	200
$SO_4^{2-(a)}$	450
Fe^{3+}	150
$Fe^{3+(c)}$	430
$Cu^{2+(d)}$	7000

^(a)1 mL of 0.05% formaldehyde (v/v) was added before the addition of coupling agent. ^(b)1 mL of 0.01% lead acetate (w/v) solution was added before the addition of coupling agent. ^(c)1 mL of 1 N NaOH (w/v) solution was added before the colour development. ^(d)1 mL of 0.05 M EDTA (w/v) solution was added before.

Milk samples were collected from local milk centre and extracted with acetic acid. The soil samples were collected in different areas in Hindupur city, India and these soil samples were treated with 0.5 % Na_2CO_3 solution. Radiator coolants are the most pollutants in the environments, the known volume of sample available in market has been taken and treated with charcoal and then used the further analysis. The results were compared with the standard method and shown in Table-5.

Determination of nitrate in real samples: A collected sample solution (10 mL) was treated with 5 mL of NH_3-NH_4Cl buffer solution (pH = 8.5) and then this solution passed through calibrated Cu-Cd redactor column at a flow of 1 mL/min and washed with water. By the end the converted (nitrite) solution was transferred into the 25 mL volumetric flask. The sample solution (1 mL) was taken for the estimation of reduced nitrite using the same procedure as described above. The nitrate content determined and the results were compared with the standard method (Table-6). The nitrate content can be determined by using eqn. 1:

$$\text{Nitrate (mg mL}^{-1}\text{)} = \frac{A - B}{C} \times \frac{62}{46} \quad (1)$$

where, A = Total NO_2^- ($\mu g\ L^{-1}$); B = NO_2^- originally present ($\mu g\ mL^{-1}$); C = sample volume.

Conclusion

A simple and rapid spectrophotometric protocol has been reported in the quantification of nitrite using silica-perchloric acid (SPCA) as a solid acid catalyst using 5-amino-2-mercapto-benzimidazole as a novel amine for the first time. The proposed method has been successfully applied to measure trace level nitrite/nitrate from a variety of sample matrices like water, soil, milk and radiator coolants. The protocol with wide linear range (0.1 to 100 ppm) with very low detection limit, limit of quantification can be used as an alternative method in nitrite assay. The results obtained by the proposed method are in good agreement with the results of the standard protocol.

TABLE-5
DETERMINATION OF NITRITE ION ($\mu\text{g L}^{-1}$)

	Sample	Nitrite originally present	Added nitrite	Total nitrite	Recovery of added nitrite (% RSD, n = 5)	
					Present method	Standard method
Water	Sample a	3.0	0.5	3.5	101.3 ± 2	102.1 ± 4
		2.9	1.0	4.1	102.5 ± 3	101.1 ± 1
	Sample b	2.8	0.5	3.4	101 ± 1	96.6 ± 3
		2.6	1.0	3.7	101.8 ± 2	99.8 ± 2
Soil	Sample a	2.8	0.5	3.3	100 ± 2	96.6 ± 2
		2.7	1.0	3.8	102.3 ± 2	96.5 ± 4
	Sample b	2.6	0.5	3.0	97.7 ± 3	100.6 ± 2
		2.7	1.0	3.6	99.3 ± 3	99.5 ± 5
Milk	Sample a	3.0	0.5	3.5	100 ± 2	100.2 ± 1
		3.2	1.0	4.1	97.6 ± 1	102.7 ± 2
	Sample b	3.3	0.5	3.9	102.5 ± 1	100.1 ± 4
		3.4	1.0	4.4	100.6 ± 2	102.4 ± 1
Radiator Coolant		12.1	0.5	12.5	99.3 ± 2	101.2 ± 3
		12.0	1.0	13.1	100 ± 2	103.8 ± 2

TABLE-6
DETERMINATION OF NITRATE ION (mg L^{-1})

	Sample	Nitrate originally present	Added nitrate	Total nitrate	Recovery of added nitrate (% RSD, n = 5)	
					Present method	Standard method
Water	Sample a	1.2	0.5	1.7	99.4 ± 2	100.2 ± 2
		1.2	1.0	2.2	99.9 ± 3	103.0 ± 2
	Sample b	1.5	0.5	2.1	100.1 ± 1	99.6 ± 3
		1.5	1.0	2.6	100.3 ± 2	101 ± 4
Soil	Sample a	12.0	0.5	12.5	103.6 ± 2	97.5 ± 4
		11.9	1.0	13.2	100.5 ± 1	99.4 ± 2
	Sample b	10.1	0.5	10.7	100.9 ± 2	100.8 ± 2
		10.6	1.0	11.5	96.1 ± 2	99.9 ± 3
Milk	Sample a	1.5	0.5	2.0	99.8 ± 3	97.4 ± 2
		1.6	1.0	2.6	99.6 ± 3	99.8 ± 1
	Sample b	1.5	0.5	2.1	105.0 ± 2	98.2 ± 5
		1.3	1.0	2.4	104.3 ± 3	97.0 ± 4
Radiator Coolant		83.2	0.5	83.6	99.8 ± 4	100.3 ± 2
		83.9	1.0	84.6	100.5 ± 2	98.6 ± 4

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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