Diaminobenzene as a novel reagent for nitrite assay in environmental samples: An evidence for its mechanistic aspects

4.1 Introduction
Oxides of nitrogen (NO\textsubscript{x}) involving including nitrogen dioxide (NO\textsubscript{2}) and nitric oxide NO have been considered as suspected carcinogenic species as well as environmental pollutants and a precursor in acid rain formation [1].

Nitrite and nitrate ions are the other two important oxy anions of nitrogen found in abundance in nature. Nitrite is widely found in water, soil, milk and food products and produces nitrosamines in human body through its reaction with amines or amides [2]. The significance of nitrate to human health is related to the fact that nitrate, after being metabolized or reduced to nitrite, can react with secondary or tertiary amines to form N-nitrasocompounds, which are potent carcinogens [3]. The toxicity of high nitrate concentrations arises from the capability of the human body to reduce it in the stomach or in the lower intestine to nitrite, which will lead to methemoglobinemia [4 - 6]. Nitrite and nitrate ions take part in several important environmental transformations involving nitrogen. Nitrate is sources of nitrogen for the synthesis of proteins by microorganisms. However, large amounts of this species may be toxic and cause eutrophication [7]. The toxicity of nitrite is primarily due to the fact that it can react with secondary or tertiary amines present in human body to form nitrosamines, which are known to be carcinogens, teratogenic and mutagenic[8 - 11]. Nitrate was reduced to nitrite by a copperised cadmium reductor column and the nitrite and nitrate content can therefore be determined by measuring the difference between the responses with and without reduction [12]. Therefore the simultaneous determination of nitrite and nitrate is of great importance in the field of biological and environmental chemistry. A number of methods for the measurement of NO\textsubscript{x} have been developed in the past several decades. NO\textsubscript{2} is usually absorbed in a suitable absorber solution and converted into nitrite, followed by the determination of nitrite [13 - 16], in which a cyclization reaction is often employed [17 - 18]. Most of the methods for simultaneous determination of nitrite and nitrate are based on the reduction of nitrate to nitrite and subsequent colorimetric determination of the nitrite with a diazocoupling reaction.
[19 - 20]. However these methods have the disadvantages of Toxicity of the reagents used and insufficient sensitivity. Therefore the spectrophotometric methods are attractive alternatives in terms of sensitivity and selectivity [21 - 22]. The nitrite monitoring in environmental samples is most important. At present the maximum contamination level in drinking water is 1 μgM L⁻¹[23]. Excessive concentration of nitrite in drinking water could be hazardous to health especially for infants and pregnant women. The proposed work describes a new reagent for the determination of nitrogen dioxide in ambient air after fixing it as nitrite ion in sodium arsenate and nitrite/nitrate in a variety of sample matrices. The fixed nitrite is cyclized with Diaminobenzene (DAB) in aqueous medium to form an azo dye with an absorption maximum at 450 nm and the extraction procedure gives a very low detection limit.

4.2 Experimental Section

4.2.1 Apparatus and Reagents

The apparatus used for the characterization of synthesized and commercially procured benzotriazole samples were HPLC, FTIR spectrophotometer (Shimadzu, model FTIR 8400S), NMR (Bruker spectrophotometer at amx 400 MHz), Mass spectrometer (Bruker Daltonics Data Analysis 3.1 model) and CHN Analyzer (Eager 300 for EA 1112) and all absorbance measurements were made using the apparatus and reagents as described in the chapter 3 of section 3.2.2

**Diaminobenzene (0.05 %):** Prepared by dissolving 0.05 g of DAB in 5 mL of 2N HCl and diluted to 100 mL with distilled water.

**Solvent for extraction:** 1- butanol.

Procedure for the preparation of all other required solutions were explained in the chapter 3 of section 3.2.2

**4.2.2 Copperized cadmium reductor column:** The procedure for the preparation of copperized cadmium reductor column was explained in the chapter 3 of section 3.2.3
4.3 Results and Discussion

The present method involves the cyclization of DAB with fixed nitrite in acidic medium. The preliminary studies have been carried out by using various aromatic amines for cyclization process and for the determination of nitrite by cyclization reaction. Initial studies were carried out using 10 mL aliquots of sodium arsenite containing 50 μg of nitrite in aqueous medium. This solution was introduced into a 25 mL calibrated flask containing 3.5 mL of amine in acidic condition after allowing 2 min. for cyclization and the solutions were diluted up to the mark with distilled water and the reagent blanks were also prepared simultaneously for each combination. The absorption spectrum of the blank and sample was then recorded over the wavelength range 400 - 700 nm. Based on these observations the combination of diaminobenzene as amine and nitrite in acidic medium undergoes cyclization and to give $\lambda_{\text{max}}$ at 450 nm. The combination of these reagents gave high sample absorbance and very low blank absorbance in acidic medium for the determination of nitrite through the cyclization reaction.

4.3.1 Benzotriazole synthesis

Benzotriazole was synthesized on the large scale by the reaction of equimolar volumes of diaminobenzene and nitrite in acidic medium. The resulted product was isolated, filtered and dried. The dried product was purified by recrystallization method using alcohol and the recrystallized product was used for the following spectroscopic characterization.

4.3.2 Species responsible for color

1, 2 - diaminobenzene on treatment with nitrite undergoes cyclization in acidic medium and to form an orange colored azo dye which has $\lambda_{\text{max}}$ at 495 nm. The dye has been extracted quantitatively into organic solvent in alkaline condition to lower the detection limit. The dye has $\lambda_{\text{max}}$ at 495 nm in organic phase. The extracted organic phase was collected in 5 mL volumetric flasks and made up to the mark with methanolic hydrochloric acid to restore the original color as shown in the scheme 4.1.
4.3.3 Spectroscopic evidence

4.3.3.1 HPLC Analysis

The purity of the sample was confirmed by HPLC Analysis of the synthesized benzotriazole and commercially procured sample. The purity of the synthesized sample was found to be 98.18 % and commercially procured one was 99.69 %, with this it is evident that the purity of the synthesized product is almost equal to that of commercially procured sample (Fig. 4.1 and 4.2).

4.3.3.2 FTIR Study

An IR spectrometer (Shimadzu, model FTIR 8400S) with resolution of 1 cm\(^{-1}\) was used to scan the vibrational spectrum of synthesized solid benzotriazole from 4000 - 500 cm\(^{-1}\), first a background scan has been done and then the sample was scanned by making pellet with solid KBr salt. The spectral features of the sample as shown in the Fig. 4.3 that the –N==N– stretching frequencies fall in the region 1630 -1575 cm\(^{-1}\), where as the vibrational stretching frequencies of C–N exhibits strong absorption between 1340 - 1250 cm\(^{-1}\)and the spectral values of synthesized benzotriazole is almost comparable with commercially procured sample (Fig. 4.3 and 4.4).
Fig. 4.1 Chromatogram for benzotriazole (synthesized)

Fig. 4.2 Chromatogram for benzotriazole (commercial)
Fig. 4.3 FTIR spectra for benzotriazole (synthesized)

Fig. 4.4 FTIR spectra for benzotriazole (commercial)
4.3.3.3 NMR Study

The NMR spectrum of the benzotriazole was obtained with a Bruker spectrometer at amx 400 MHz by dissolving in CDCl\textsubscript{3} solvent (internal standard 1% tetramethylsilane solution). The spectrum shows that the signals at $\delta = 7.52$ ppm (doublet, $^a$H) and 7.95 - 8.16 ppm (doublet, $^b$H), indicative of the benzene group and at 7.26 ppm (wide singlet, $^c$H) indicative of the NH group as shown in the Fig.4.5 and Fig.4.6. The spectral values of synthesized benzotriazole are comparable with the commercially procured sample as shown in the Fig.4.7 and 4.8.

![NMR spectra for benzotriazole (synthesized) at low resolution](image)

**Fig. 4.5** NMR spectra for benzotriazole (synthesized) at low resolution
Fig. 4.6 NMR spectra for benzotriazole (synthesized) at high resolution

Fig. 4.7 NMR spectra for benzotriazole (commercial) at low resolution
4.3.3.4 Mass Spectral Study

Mass spectrometry has been used to study molecular mass of synthesized and commercially procured benzotriazole samples. Mass spectra were obtained on a (Bruker Daltonics Data Analysis 3.1 model) mass spectrometer. Electron ionization mass spectra were obtained at 40 eV which gives the positive mode of the mass for synthesized benzotriazole as [M+1] =119.9 (expected mass = 119), sodiated mass was 141.8 [M + Na] =141.8 (Fig.4.9). The result of synthesized benzotriazole is almost comparable with commercially procured sample (Fig.4.10).

Fig.4.8 NMR spectra for benzotriazole (commercial) at high resolution
Fig. 4.9 Mass spectra for benzotriazole (synthesized)

Fig. 4.10 Mass spectra for benzotriazole (commercial)
4.3.3.5 CHN Analysis

It was used to compare the percentage of elements present in synthesized benzotriazole and commercially procured one. The percentages of $N = 35.2069$, $C = 60.7468$ and $H = 4.1443$ total of 100.0979 in synthesized sample, where as the percentages of $N = 35.1823$, $C = 60.6816$ and $H = 4.1401$ total of 100.004 in commercially procured sample are shown in the tables 4.1 and 4.2. It is evident from the tables that there is the total elementary percentage of the synthesized sample exactly matches with the commercial procured sample.

**Table 4.1** CHN Analysis of synthesized benzotriazole compound

<table>
<thead>
<tr>
<th>Element name</th>
<th>Ret. time</th>
<th>Area</th>
<th>BC Area ratio</th>
<th>K factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.0000</td>
<td>27</td>
<td>42507</td>
<td>0.0000</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>35.2069</td>
<td>37</td>
<td>816878</td>
<td>0.165745 + 07</td>
</tr>
<tr>
<td>Carbon</td>
<td>60.7468</td>
<td>60</td>
<td>2927175</td>
<td>0.391037 + 07</td>
</tr>
<tr>
<td>Hydrogen</td>
<td>4.1443</td>
<td>160</td>
<td>481836</td>
<td>0.948361 + 07</td>
</tr>
<tr>
<td>Totals</td>
<td>100.0979</td>
<td>4268396</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 4.2 CHN Analysis of commercially procured benzotriazole compound

<table>
<thead>
<tr>
<th>Element name</th>
<th>Ret. time</th>
<th>Area</th>
<th>BC</th>
<th>Area ratio</th>
<th>K factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.0000</td>
<td>27</td>
<td>42649</td>
<td>FU</td>
<td>0.0000</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>35.1823</td>
<td>37</td>
<td>815305</td>
<td>FU 3.564101</td>
<td>0.165745 + 07</td>
</tr>
<tr>
<td>Carbon</td>
<td>60.6816</td>
<td>60</td>
<td>2913145</td>
<td>FU 1.000000</td>
<td>0.390005 + 07</td>
</tr>
<tr>
<td>Hydrogen</td>
<td>4.1401</td>
<td>160</td>
<td>481613</td>
<td>FU 6.005120</td>
<td>0.945631 + 07</td>
</tr>
<tr>
<td>Totals</td>
<td>100.004</td>
<td></td>
<td>4252712</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
4.3.4 Optimization Study

4.3.4.1 Effect of amine concentration

The effect of amine concentration was varied in order to establish the optimum quantity of amine required for maximum absorbance by varying its concentration in the range 0.1-5 mL using 0.05 % DAB. Different volumes of amine were taken in a series of 25 mL volumetric flasks containing 2 mL of 2N hydrochloric acid and these solutions were treated with 10 mL aliquots of sodium arsenite containing 50 of nitrite. The solutions were allowed to stand for five minutes and diluted up to the mark with distilled water. These studies revealed that 3.0 mL of amine is sufficient enough to give maximum absorbance to the sample. Higher concentrations of amine did not enhance the sample absorbance values, hence 3.5 mL of 0.05 % of amine has been fixed as optimum concentration. In all further studies 3.5 mL of 0.05 % of amine has been used (Fig.4.11).

4.3.4.2 Effect of acidity

Further the effect of acidity during cyclization process was examined in order to establish the optimum acidity for maximum color development. In these experiments 10 mL aliquots of alkaline sodium arsenite containing 50 µg of nitrite were added into series of 25 mL calibrated flasks containing 3.5 mL of 0.05 % amine and various volumes of 2N hydrochloric acid (0.1-5.0 mL). These solutions were allowed to stand for two minutes and diluted up to the mark with distilled water. The absorbance values were measured at 450 nm. It is evident from the graph that the overall acidity in the range 0.16 - 0.30 gave maximum absorbance. Hence the required acidity was provided by the addition of 3 mL of 2N hydrochloric acid during cyclization process (Fig.4.12).
Fig. 4.11 Effect of amine concentration

Fig. 4.12 Effect of acidity
4.3.4.3 Effect of cyclization time

The optimum time period required for cyclization of amine with nitrite was examined by treating 10 mL aliquots of alkaline sodium arsenite solution containing 50 μg of nitrite in a series of 25 mL calibrated flasks containing 3.5 mL of 0.05 % amine and 1 mL of 2N hydrochloric acid. These flasks were allowed to stand for different time intervals and diluted up to the mark with distilled water and the absorbance values were measured at 450 nm. It is evident from the graph that the time required for maximum absorbance is in the range of 90-120 sec. Hence two minutes time period has been allowed in all further studies for complete cyclization (Fig. 4.13).

Attempts have been made to extract the formed azo dye into organic solvent to lower the detection limits so that the developed method can be extended to measure the trace levels of nitrogen dioxide present in the atmospheric air as well as in industrial flue gases.

4.3.4.4 Effect of extraction pH

In order to establish the most suitable pH range for the quantitative extraction of the azo dye into organic solvent was next investigated. In these experiments, 10 mL aliquots of alkaline sodium arsenite solution containing 10 μg of nitrite were added to a series of 25 mL calibrated flasks containing 3.5 mL of 0.05 % amine and 2 mL of 2N hydrochloric acid. After standing time of 2 min. these solutions were transferred into 60 mL separating funnels and treated with 5 mL of various buffer solutions in the pH range 2 - 14. The solutions were extracted with 7.5 mL of 1-butanol and the organic extracts were collected into 5 mL calibrated flasks. These extracts have been diluted to the mark with methanolic hydrochloric acid to restore the original color and the absorbance values were measured. It has been found that $\lambda_{\text{max}}$ has been shifted from 450 nm in aqueous phase to 495 nm in organic phase. These studies have revealed that the extraction is quantitative in the pH range 8 - 12. Hence the required pH range during extraction was maintained by the addition of 5 mL of 1N NaOH into separating funnels before extracting the dye into organic solvent (Fig.4.14).
Fig. 4.13 Effect of cyclization time

Fig. 4.14 Effect of extraction pH
4.3.4.5 Effect of variation of solvents during extraction

Several polar solvents like 1-butanol, isoamylalcohol, isoamyl acetate and non polar solvents like benzene, toluene were used for extracting the azo dye. Among several solvents 1-butanol gave the lower blank absorbance and higher sample absorbance values. In these experiments 10 mL aliquots of sodium arsenite solution containing 0 - 10 µg nitrite were added to series of 25 mL standard flasks containing 3.5 mL of 0.05 % amine and 2 mL of 2N hydrochloric acid. The contents were mixed well and allowed to stand for 2 min. and diluted to 25 mL with distilled water and the absorbance values were measured at 495 nm (Table 4.3).

Table 4.3 The effect of solvents

<table>
<thead>
<tr>
<th>SL No</th>
<th>Solvent^a (mL)</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Blank vs. Solvent</td>
</tr>
<tr>
<td>1)</td>
<td>Isoamyl acetate (5)</td>
<td>0.0143</td>
</tr>
<tr>
<td>2)</td>
<td>Isoamyl alcohol (5)</td>
<td>0.0271</td>
</tr>
<tr>
<td>3)</td>
<td>Isoamyl alcohol + (5)*</td>
<td>0.0215</td>
</tr>
<tr>
<td></td>
<td>+ Isoamyl acetate</td>
<td></td>
</tr>
<tr>
<td>4)</td>
<td>1-Butanol (6.5)</td>
<td>0.0205</td>
</tr>
<tr>
<td>5)</td>
<td>MIBK (5)</td>
<td>0.0039</td>
</tr>
<tr>
<td>6)</td>
<td>MEK (7.5)</td>
<td>0.0026</td>
</tr>
</tbody>
</table>

^aBased on the solubility of solvent in aqueous phase, different volumes were used. In all cases the extract was collected in to 5 mL standard flask and made up to mark with methanolic HCl to restore the original color.

* 1:1 ratio V/V.
4.3.4.6 Aqueous procedure

10 mL aliquots of sodium arsenite solution containing 0 - 250 µg nitrite were added to series of 25 mL standard flasks containing 3.5 mL of 0.05 % diaminobenzene and 1 mL of 2 N HCl. The contents were mixed well and allowed to stand for 2 min. Then diluted to 25 mL with distilled water and the absorbance values were measured at 450 nm using 1cm quartz cuvette (Fig. 4.15 and 4.16).

![Calibration plot (aqueous phase)](image)

**Fig. 4.15** Calibration plot (aqueous phase)
4.3.4.7 Extraction procedure

10 mL aliquots of sodium arsenite solution containing 0-50 µg nitrite were added to series of 25 mL standard flasks containing 3.5 mL of 0.05 % diaminobenzene and 1 mL of 2N hydrochloric acid. The contents were mixed well and allowed to stand for 2 min. The solutions were made up to the mark and transferred into 60 mL separating funnels. 3 mL of NH₃ - NH₄Cl buffer (pH = 8.5) and 7.5 mL of 1-butanol were added. The contents were equilibrated for one min. and the organic phase was collected into 5 mL volumetric flask. Then it is diluted up to the mark with methanolic hydrochloric acid and the absorbance values were measured at 495 nm against reagent blank (Fig. 4.17 and 4.18).

Fig. 4.16  Absorption spectra  (aqueous phase)
Fig. 4.17 Calibration plot (organic phase)

Fig. 4.18 Absorption spectra (organic phase)
4.3.4.8 Interference study

In order to evaluate the suitability of the proposed method for the determination of nitrogen dioxide in air and nitrite/nitrate in water, soil, milk and radiator coolant samples, the effect of interference of several ions in the determination was examined. Initially the effect of common atmospheric air pollutants like sulphur dioxide, hydrogen sulphide and formaldehyde in the determination of nitrogen dioxide was studied. These species were introduced in the form of their respective anions. Formaldehyde did not interfere up to 10 µg in the proposed method. While sulphite at concentrations above 50 µg interfered causing decrease in absorbance value. However higher concentrations (up to 100 µg) of sulphite can be overcome by the addition of 1 mL of 0.05 % formaldehyde solution which reacts with the sulphite to form a stable adduct prior to nitrite determination. Sulfide, up to 5 µg did not interfere but at higher concentrations it interfered by decreasing the absorbance values. Up to 50 µg of sulfide interference was overcome by precipitating as lead sulphide by the addition of 1 mL of 0.01 % lead acetate before nitrite estimation. The interference of other several anions and cations were evaluated to check the suitability of the method for the determination of nitrite and nitrate in water and soil samples. Anions like oxalate, carbonate, sulphate, citrate, phosphate, bicarbonate and nitrate did not interfere upto $1 \times 10^4$ µg levels. Cations like Fe$^{2+}$, Ni$^{2+}$, Hg$^{2+}$, Co$^{2+}$, Ba$^{2+}$, Mo$^{2+}$, Mg$^{2+}$ and Zn$^{2+}$ up to the $1.5 \times 10^4$ µg level did not interfere. However copper (II) interfered at $1 \times 10^3$ µg levels by decreasing the absorbance and this was overcome by adding 2 mL of 0.05 M EDTA solution. Iron (III) gives negative interference at $1 \times 10^2$ µg which was overcome by precipitating it as hydroxide and removing through centrifugation (Table 4.4).
Table 4.4 Interference studies

<table>
<thead>
<tr>
<th>Interferent</th>
<th>Tolerance limit (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formaldehyde</td>
<td>10</td>
</tr>
<tr>
<td>Sulphide</td>
<td>5</td>
</tr>
<tr>
<td>^aSulphide</td>
<td>20</td>
</tr>
<tr>
<td>^bSulphide</td>
<td>50</td>
</tr>
<tr>
<td>Sulphite</td>
<td>50</td>
</tr>
<tr>
<td>^bSulphite</td>
<td>100</td>
</tr>
<tr>
<td>(\text{CO}_3^{2-}, \text{C}_2\text{O}_4^{2-}, \text{Citrate}, \text{NO}_3^-; \text{tartarate}, \text{Hg}^{2+}, \text{Ni}^{2+}, \text{Co}^{2+}, \text{Zn}^{2+}, \text{Ba}^{2+}, \text{Mg}^{2+}, \text{Fe}^{2+})</td>
<td>10000</td>
</tr>
<tr>
<td>(\text{Fe}^{3+})</td>
<td>100</td>
</tr>
<tr>
<td>^c(\text{Fe}^{3+})</td>
<td>500</td>
</tr>
<tr>
<td>(\text{Cu}^{2+})</td>
<td>1000</td>
</tr>
<tr>
<td>^d(\text{Cu}^{2+})</td>
<td>3000</td>
</tr>
</tbody>
</table>

^a treated with 1 mL of 0.01 % lead acetate solution centrifuged and washed the residue, the centrifugate and washings were mixed and used for color development.

^b treated with 2 mL of 0.05 % formaldehyde solution.

^c treated with 1 mL of 1M NaOH solution centrifuged and washed the residue, the centrifugate and washings were mixed and used for color development.

^d treated with 2 mL of 0.05M EDTA solution.
4.4 Application study

The proposed method has been applied to determine nitrogen dioxide, nitrite and nitrate in environmental samples like air, water, soil and radiator coolant and biological samples like milk. In order to check the validation of the proposed method, the samples were simultaneously determined by using Griess - Ilosvey reaction as standard method. The results obtained by the proposed method are in good agreement with those obtained by the standard method.

4.4.1 Determination of nitrogen dioxide in air

Air samples were drawn through 25 mL of sodium arsenite absorber solution taken in an impinger at a flow rate of 0.3 Lmin\(^{-1}\). The sampled solution was made up to 50 mL with sodium arsenite absorber solution. 10 mL of made up solution was taken into 25 mL calibrated flask containing 3.5 mL of 0.05 % DAB and 1 mL of 2N hydrochloric acid. The contents were mixed well and allowed to stand for 2 min. Then the solution was diluted to 25 mL with distilled water and the absorbance values were measured at 450 nm. The extraction procedure was adopted when the nitrite levels are well below the detection limit and the absorbance values were measured at 495 nm (Table 4.5).

Table 4.5 Determination of nitrogen dioxide in atmospheric air

Trapping solution: 25 mL of alkaline sodium arsenite.

<table>
<thead>
<tr>
<th>Sl.No.</th>
<th>Volume of air sampled(^{a}) (L)</th>
<th>proposed method</th>
<th>standard method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>NO(_2) (µg)</td>
<td>NO(_2) (ppb)*</td>
</tr>
<tr>
<td>1.</td>
<td>46</td>
<td>0.492</td>
<td>70.90</td>
</tr>
<tr>
<td>2.</td>
<td>42</td>
<td>0.364</td>
<td>64.68</td>
</tr>
</tbody>
</table>

\(^{a}\)Air was sampled on different days

\(* Concentration of NO\(_2\) (ppb) = \frac{NO\(_2\) (µg) \times 5 \times 532}{0.82 \times V}\)
Where V is the volume of air sampled, 0.82 is the factor of collection efficiency for sodium arsenite as trapping medium, 532 is the conversion factor to convert \( \mu \text{gL}^{-1} \) of NO\(_2\) to ppb of nitrogen dioxide at 298 K and 101.3 kpa.

4.4.2 Determination of nitrite/nitrate in water samples

10 mL of the water sample was treated with 1 mL of 1N sodium hydroxide and centrifuged. The centrifugate has been collected and the residue was washed with 5ml portions of water and centrifuged again. All the centrifugates were mixed well and made up to 25 mL in a calibrated flask.

**Nitrite determination** 10 mL of the made up solution was transferred to a 25 mL calibrated flask containing 3.5 mL of 0.05 % DAB and 1 mL of 2N hydrochloric acid. The contents were mixed well and allowed to stand for 2 min. Then the solution was diluted to 25 mL with distilled water. The absorbance was measured at 450 nm. If the color intensity is very low the extraction procedure can be followed and absorbance values can be measured at 495 nm against reagent blank.

**Nitrate determination** 10 mL of made up solution was taken and treated with 5 mL of NH\(_3\)-NH\(_4\)Cl buffer solution (pH = 8.5) and passed through copperized cadmium reductor column at a flow rate of 1 mLmin\(^{-1}\). The column was washed with five 3 mL portions of water and the eluents were collected in a 25 mL standard flask and diluted to the mark with water, 5 mL of the made up solution was taken and analyzed for total nitrite content. The nitrate content can be calculated by the difference between total nitrite and original nitrite content (Table 4.6).
Table 4.6  Determination of nitrite/nitrate in water samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total nitrite found (µg)</th>
<th>nitrate (µgmL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>proposed method</td>
<td>standard method</td>
</tr>
<tr>
<td>Ground water (Bore well)a</td>
<td>17.60</td>
<td>18.01</td>
</tr>
<tr>
<td>Lake waterb</td>
<td>16.05</td>
<td>16.61</td>
</tr>
</tbody>
</table>

\[
Total \ nitrite(\mu g) = \left\{ \text{nitrite originally present (\mu g)} \right\} + \left\{ \text{nitrite formed by the reduction of nitrate (\mu g)} \right\}
\]

\[
NO_3^-(\mu g/mL) = \frac{Total \ NO_2^- (\mu g) - NO_2^- \ originally \ present (\mu g)}{10} \times \frac{62}{46}
\]

a, b Water samples were collected from different locations around Bangalore city

4.4.3 Determination of nitrite/nitrate in soil samples

A known weight (0.5 g) of soil sample was taken in a 50 mL beaker and extracted with three 5 mL portions of 0.5 % sodium carbonate solution and centrifuged. The clear centrifugate solutions were collected in 25 mL calibrated flask and diluted to the mark. The nitrite and nitrate contents can be measured by following the procedure described under water samples (Table 4.7).
Chapter 4

Table 4.7 Determination of nitrate in Soil samples

<table>
<thead>
<tr>
<th>Weight of soil(a) taken (g)</th>
<th>Total nitrite(b) found in 25 mL extract ((\mu g))</th>
<th>nitrate in soil sample(c) ((\mu g)g(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>proposed method</td>
<td>standard method</td>
</tr>
<tr>
<td>0.50(d)</td>
<td>4.66</td>
<td>4.75</td>
</tr>
<tr>
<td>0.50(e)</td>
<td>4.04</td>
<td>4.19</td>
</tr>
</tbody>
</table>

\(a\) nitrite was not detected in these soil samples

\(b\) \[
\text{Total nitrite}(\mu g) = \left\{\text{nitrite originally present (\(\mu g\)})\right\} + \left\{\text{nitrite formed by the reduction of nitrate (\(\mu g\)}\right\}
\]

\(c\) \[
\text{\(NO_3^-\) in soil (\(\mu g\)g\(^{-1}\))} = \frac{\text{total } NO_3^- (\mu g) - \text{\(NO_3^-\) originally present (\(\mu g\)})}{\text{weight of soil (g)}} \times \frac{62}{46}
\]

d, e Soil samples were collected from agricultural fields in the rural areas of Bangalore.

4.4.4 Determination of nitrite/nitrate in milk samples

A known volume of the milk sample was treated with 1 mL acetic acid and centrifuged to deproteinate the sample. The centrifugate has been collected and the residue was washed with 5 mL portions of water and centrifuged again. All the centrifugates were mixed well and made up to 25 mL in a calibrated flask.

Nitrite determination: 10 mL of the made up solution was transferred to a 25 mL calibrated flask containing 3.5 mL of 0.05 % DAB and 1 mL of 2N hydrochloric acid. The contents were mixed well and allowed to stand for 2 min. Then the solution was diluted to 25 mL with distilled water. The absorbance was measured at 450 nm. If the color intensity is very low the extraction procedure can be followed and the absorbance values can be measured at 495 nm against reagent blank.
Nitrate determination 10 mL of made up solution was taken and treated with 5 mL of NH₃-NH₄Cl buffer solution (pH = 8.5) and passed through copperized cadmium reductor column at a flow rate of 1 mLmin⁻¹. The column was washed with five 3 mL portions of water and the eluents were collected in a 25 mL standard flask and diluted to the mark with water, 10 mL of the made up solution was taken and analyzed for total nitrite content. The nitrate content can be calculated by the difference between total nitrite and original nitrite content (Table 4.8).

Table 4.8 Determination of nitrite/nitrate in milk

<table>
<thead>
<tr>
<th>Sample (mL)</th>
<th>Total nitrite found (µg)</th>
<th>nitrate (µgL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>proposed method</td>
<td>standard method</td>
</tr>
<tr>
<td>Milk (5)</td>
<td>12.50</td>
<td>14.20</td>
</tr>
</tbody>
</table>

\[
\text{Total nitrite(µg)} = \left\{ \text{nitrite originally present (µg)} \right\} + \left\{ \text{nitrite formed by the reduction of nitrate (µg)} \right\}
\]

\[
\text{NO}_3^-(\text{µg mL}^{-1}) = \left[ \frac{\text{total NO}_2^-(µg) - \text{NO}_2^~- \text{originally present}(µg)}{10} \right] \times \frac{62}{46}
\]

*Milk sample was collected from milk dairy, Bangalore.*

4.4.5 Determination of nitrite and nitrate in radiator coolant

The proposed method was also applied for the determination of nitrite content in radiator coolants where nitrites are used as corrosion inhibitors. The known volume of coolant sample was diluted to 100 - 200 times with distilled water depending on the nitrite content. 10 mL of the diluted coolant was analyzed for the nitrite content following the procedure described under nitrite determination in water samples.
Nitrite determination: 10 mL of the made up solution was transferred to a 25 mL calibrated flask containing 3.5 mL of 0.05 % DAB and 1 mL of 2N hydrochloric acid. The contents were mixed well and allowed to stand for 2 min. Then the solution was diluted to 25 mL with distilled water. The absorbance was measured at 450 nm. If the color intensity is very low the extraction procedure can be followed and absorbance values can be measured at 495 nm against reagent blank.

Nitrate determination: 10 mL of made up solution was taken and treated with 5 mL of NH$_3$-NH$_4$Cl buffer solution (pH = 8.5) and passed through copperized cadmium reductor column at a flow rate of 1 mL min$^{-1}$. The column was washed with five 3 mL portions of water and the eluents were collected in a 25 mL standard flask and diluted to the mark with water, 10 mL of the made up solution was taken and analyzed for total nitrite content. The nitrate content can be calculated by the difference between total nitrite and original nitrite content (Table 4.9).

Table 4.9 Determination of nitrite/nitrate in radiator coolant

<table>
<thead>
<tr>
<th>Sample * (mL)</th>
<th>total nitrite found (µg)</th>
<th>nitrate (µgL$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>proposed method</td>
<td>standard method</td>
</tr>
<tr>
<td>Radiator coolant (5) *</td>
<td>13.55</td>
<td>13.67</td>
</tr>
</tbody>
</table>

*Nitrite was not found.

\[
NO_3^- (\mugmL^{-1}) = \left[\frac{\text{total } NO_2^- (\mu g) - \text{originally present } (\mu g)}{10}\right] \times \frac{62}{46}
\]

*aCoolant sample was taken from automobile garage.
4.5 Conclusion

The proposed method based on the cyclization reaction between diaminobenzene and nitrite in presence of acidic medium, is sensitive and simple for the estimation of nitrogen dioxide/nitrite/nitrate at trace level. The reaction conditions have been optimized and the method obeys Beer’s law over the concentration range 0 - 250 µg in aqueous phase and 0 - 10 µg in organic phase. The proposed method has been applied to determine nitrogen dioxide levels of ambient air after fixing it as nitrite using sodium arsenite trapping solution. The results obtained by this method are in good agreement with standard method [12]. It has been applied to measure nitrite and nitrate levels in bore well water, soil, milk and radiator coolant samples and the results are in quite agreement with the standard method. The application of this method for routine monitoring of nitrite/nitrate levels of industrial effluents at trace level will be a useful analytical procedure and it serves as an alternative to other existing procedures.

4.6 References


