Spectrophotometric Determination of Trace Amount of Nitrite in Water and Soil

Sanjeela Shakya, Deepa D.C., Rajib Chaudhary and Surendra K. Gautam*

Department of Chemistry, Tri-Chandra Campus, TU, Kathmandu, Nepal
***Corresponding E-mail:** surendra.gautam@trc.tu.edu.np*

Abstract

A simple, sensitive, rapid and economical spectrophotometric method for the determination of trace amount of nitrite present in the water and soil samples is investigated. This method is based on the diazo-coupling reaction where nitrite reacts with p-nitroaniline in presence of hydrochloric acid to form diazonium cation which is subsequently coupled with 1-naphthol in presence of sodium hydroxide to form a stable azo-dye. The absorption maximum (λ_{max}) for azo-dye is found to be 610 nm and the method obeys Beer-Lambert's law at this analytical wavelength over the concentration range 0.02-0.12 μg/mL of nitrite. The molar absorptivity is found to be 3.29×10^4 liter.mol^{-1}cm^{-1}.

Keywords: *1-aphthol, Molar Absorptivity, Nitrite, p-nitroaniline, λ_{max}*

Introduction

Nitrite plays a crucial role as an intermediate in the biological nitrogen cycle, arising from the biodegradation of nitrogenous wastes from domestic or industrial sources and some fertilizers (Aydin et al., 2005; Govindasamy et al., 2022). It is commonly found as a pollutant in the atmosphere, natural water bodies, and soil (Pourreza et al., 2012). Due to its instability, nitrite can transform into nitrates or ammonia depending on environmental conditions (Mayouf, 2013; Moravsky et al., 2020). The excessive accumulation of nitrite contributes significantly to water and air pollution and poses various health risks. High levels of nitrite can lead to eutrophication and a reduction in dissolved oxygen in water (Nagaraj et al., 2016; El Hani et al., 2022).

Monitoring nitrite levels in environmental samples is crucial due to its potential adverse effects on human health (Thomas et al., 2015; Siegel et al., 2019). The US Health Association specifies a maximum allowable concentration (MAC) of nitrite at 0.1 mg/L, with a maximum permissible limit in drinking water set at 0.06 ppm (Khadka, 2014). Nitrite levels exceeding these limits can be harmful, particularly to infants and pregnant women (Khadka and Bhattarai, 2014; Khadka and Duwadi, 2014). High nitrite levels lead to the conversion of iron in hemoglobin to methemoglobin, which impairs oxygen transport in the blood, resulting in methemoglobinemia (Khaniki et al., 2007; Rajasulochana et al., 2022). Additionally, nitrite in the digestive system may contribute to cancer development by forming nitrosamines, potent carcinogens, when reacting with amines or amides. Prolonged exposure

to nitrite can also cause excessive urine discharge, increased starch deposits, and spleen hemorrhaging (Liu et al., 2015).

Nitrite can be detected using a variety of methods, including chemiluminescence, fluorometry, polarography, Raman spectroscopy, spectrophotometry, amperometry, potentiometry, flow injection, and kinetic techniques (Liu et al., 2015). Although many of these methods are sensitive, they can be time-consuming and require complex equipment. This study employs a spectrophotometric method due to its cost-effectiveness and efficiency. This approach relies on a diazo-coupling reaction for detection.

Experimental Methods

A standard nitrite solution with a concentration of 1000 ppm was prepared by dissolving 0.375 g of dried sodium nitrite in distilled water and adjusting the volume to 250 mL. To prevent the release of nitrous acid and inhibit bacterial growth, a sodium hydroxide pellet and approximately 1 mL of chloroform were added. The stock solution was then standardized by titration with a standard potassium permanganate solution. Dilutions of the stock solution were made to prepare nitrite solutions with concentrations of 0.02, 0.04, 0.06, 0.08, 0.10, and 0.12 ppm.

To determine the λ_{max} of the resulting azo dye, 0.6 mL of a 1 ppm sodium nitrite solution was placed in a 10 mL volumetric flask. Next, 1.5 mL of a PNA solution (1×10^{-3} M) in 20% aqueous ethanol was added. The mixture was shaken and allowed to react for 10 minutes for diazotization. Following this, 1 mL of 10% (w/v) EDTA and 1 mL of 0.1% 1-naphthol solution were added, and the solution was shaken for 1 minute. The final volume was adjusted to 10 mL with distilled water. The absorbance of the violet dye was measured using an ELICO SL 177 scanning mini spectrophotometer across a wavelength range of 550-680 nm, and the peak absorbance indicated the λ_{max}.

For the calibration curve, varying volumes of the 1 ppm nitrite standard solution (0.0, 0.2, 0.4, 0.6, 0.8, 1.0, and 1.2 mL) were transferred to separate 10 mL volumetric flasks. Each flask received 1.5 mL of the PNA solution (1×10^{-3} M) in 20% aqueous ethanol and was allowed to stand for 10 minutes to complete the diazotization reaction. Subsequently, 1 mL of 10% (w/v) EDTA and 1 mL of 0.1% 1-naphthol were added, and the contents were shaken for 2 minutes. The flasks were then filled to the mark with distilled water. The absorbance of each violet azo dye solution was measured at 610 nm using the ELICO SL177 scanning mini spectrophotometer, with measurements taken against a reagent blank. The λ_{max} (610 nm) was used for determining nitrite concentrations in water and soil samples.

Results and Discussion

Figure 1 illustrates the absorption spectra of the azo dye used to identify the λ_{max}, with 1 μg/mL of nitrite processed according to the specified method using 1-naphthol as the coupling reagent. The peak absorption (λ_{max}) is observed at 610 nm. Consequently, all subsequent absorbance measurements were conducted at this wavelength.
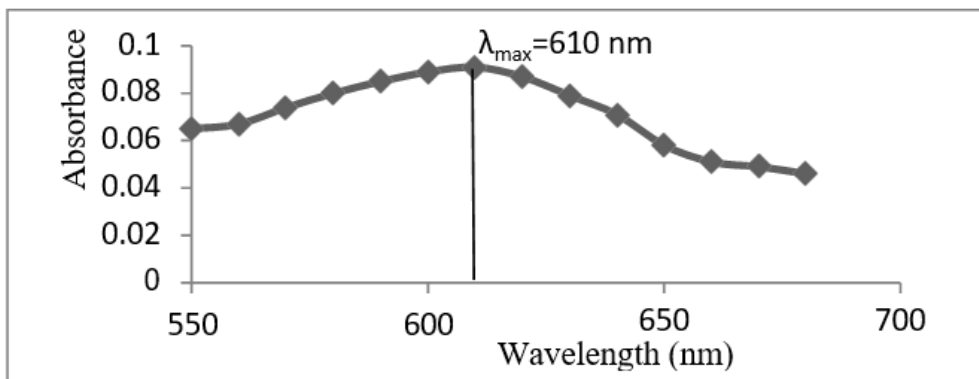
Fig. 1: Absorption spectra of azodye measured for λ_{max}

Adherence to Beer-Lambert's Law and Sensitivity

The compliance with Beer-Lambert's law was evaluated by reacting aliquots of a standard solution containing nitrite ions at concentrations of 0.02, 0.04, 0.06, 0.08, 0.10, and 0.12 ppm, with a final volume of 10 mL, according to the specified procedure. The absorbance of the resulting azo dye was measured at 610 nm. Figure 2 displays a graph of absorbance versus nitrite concentration in ppm, showing a linear relationship starting from the origin. This indicates that the method adheres to Beer-Lambert's law for nitrite concentrations ranging from 0.02 to 0.12 ppm in a 10 mL final volume. The molar absorptivity in the region with minimal photometric error was calculated to be 3.29×10^4 lit. $mol^{-1}cm^{-1}$.
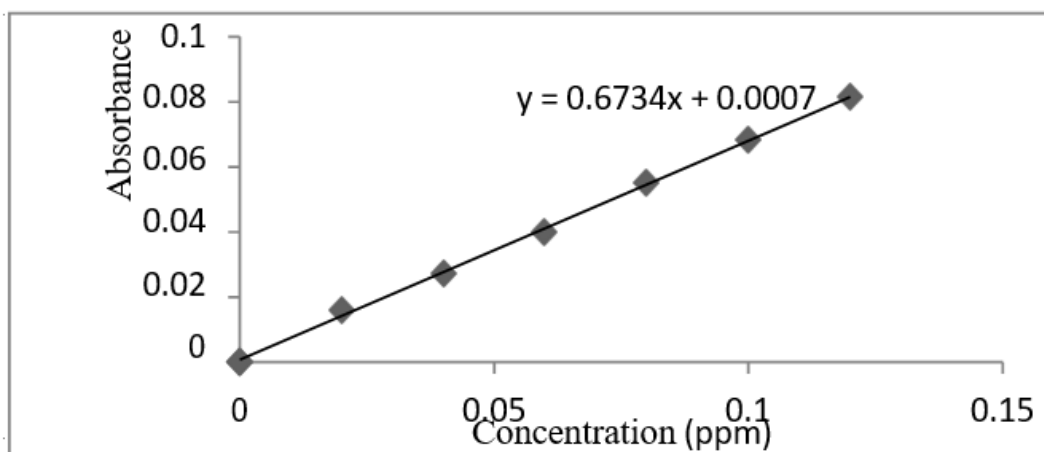

Fig. 2: Plot of absorbance verses concentration of nitrite ion in ppm.

Recommended Procedure for the Nitrite Determination in Water and Soil

Water and soil samples from different places of Kathmandu, Lalitpur and Bhaktapur district were collected and nitrite concentration was determined as described below.

17.5 mL of water sample was taken in 25 mL volumetric flask. 2 mL of PNA prepared in 0.05 M HCl was added to the above solution. The solution was shaken well and then kept aside for 15 minutes. 1.5 mL EDTA solution with 1.5 mL of 0.1% 1-naphthol solution was added. The solution was made up to the mark by adding distilled water. The absorbance of the resulting solution was measured at 610 nm using spectrophotometer. Finally, the amount of nitrite was calculated in ppm from the Calibration curve.

Soil samples were sieved to less than 2 mm. Dried soil was shaken with 2 M KCl solution (1:10 ratio). The resulting solution was filtered through Whatmann no 42. Then, the absorbance was measured at 610 nm using spectrophotometer. Finally, the amount of nitrite was calculated in ppm from the calibration curve.

Analysis of Water Samples

The proposed method was utilized to measure nitrite concentrations in water samples by analyzing 17.5 mL of water within a final volume of 25 mL, following the specified procedure. Table 1 presents the results of this analysis, which indicate that water samples from surface sources, like rivers, generally have higher nitrite concentrations compared to samples from deeper sources, such as wells, with some exceptions. This difference is attributed to the greater pollution and contamination at the Earth's surface. Figure 3 displays the nitrite concentrations (ppm) found in water samples from various locations.

Table 1: Analysis of various water samples

| S.N.	Volume of water (ml)	Sources of water	Location	Absorbance	Nitrite found (ppm)
1	17.5	Tap	Ghantaghar, Kathmandu	0.0165	0.024
2	17.5	Well	Kupondol, Lalitpur	0.0192	0.028
3	17.5	Well	Imadol, Lalitpur	0.0439	0.065
4	17.5	River	Imadol, Lalitpur	0.1153	0.171
5	17.5	Tap	Pepsicola, Bhaktapur	0.0149	0.022
6	17.5	River	Pepsicola, Bhaktapur	0.1090	0.16
7	17.5	Well	Samakhusi, Kathmandu	0.0206	0.031
8	17.5	Well	Chabel, Kathmandu	0.0144	0.021
9	17.5	Pond	Kirtipur. Kathmandu	0.0623	0.092
10	17.5	River (Bagmati)	Thapathali, Kathmandu	0.0854	0.127
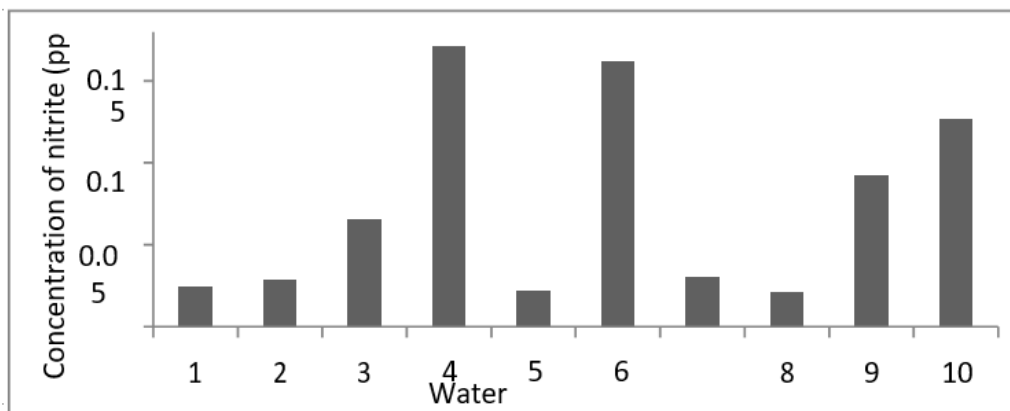
Fig. 3: The plot of concentration of nitrite verses water from different sources and locations

Analysis of Soil Samples

The proposed method was used to determine nitrite levels in soil samples by dissolving 1 g of dried soil in 10 mL of 2 M KCl solution, following the recommended procedure. The results, shown in Table 2, reveal that soil from riverbanks generally contains higher nitrite concentrations compared to roadside soil, with some exceptions. This is likely due to the discharge of industrial and municipal nitrogenous waste near rivers. Additionally, nitrite concentrations varied between locations, even among similar types of sources. Figure 4 illustrates the nitrite concentrations (ppm) found in soil samples from different locations.

Table 2: Analysis of soil samples

| S.N.	Amount of dried soil (g)	Sources of soil/15 cm depth from surface	Location	Absorbance	Nitrite found (ppm)
1	1	Farmland	Ghantaghar, Kathmandu	0.0212	0.031
2	1	Garden	Kupondol, Lalitpur	0.0022	0.003
3	1	Riverbank	Imadol, lalitpur	0.0892	0.132
4	1	Farmland	Imadol, lalitpur	0.0412	0.061
5	1	Riverbank	Pepsicola, Bhaktapur	0.1067	0.158
6	1	Roadside	Samakhusi, Kathmandu	0.0016	0.0024
7	1	Farmland	Shakhu, Kathmandu	0.0481	0.071
8	1	Riverbank (Bagmati River)	Thapathali, Kathmandu	0.0859	0.128
9	1	Roadside	Kalanki. Kathmandu	0.1067	0.158
10	1	Farmland	Bajrabarahi, Kathmandu	0.0315	0.047
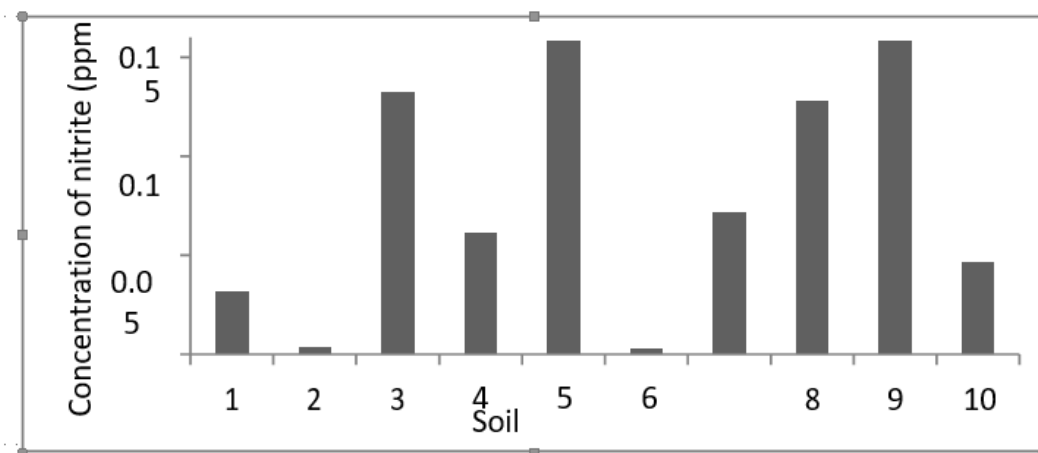
Fig. 4: The plot of concentration of nitrite verses soil from different sources and location

Comparison of the Proposed Method with Some Reported Methods

The suggested method for nitrite determination is sensitive, selective, straightforward, and cost-effective. It involves the reaction of p-nitroaniline with nitrite in an acidic environment to form a diazonium ion, which then couples with 1-naphthol to produce a violet azo dye. The evaluation and application of this method demonstrates its suitability for measuring nitrite levels in water and soil. Table 3 presents a comparison of the results, confirming the method's effectiveness.

Table 3: Comparison of the proposed method with some reported methods (Liu *et. al,* 2015).

Method	Reagent	λmax (nm)	Molar absorptivity (lit.mol^{-1}cm^{-1})	Determination range (ppm)
1.	Sulfanilic acid + methyl anthranilate	493	1.03×10^4	0.2-8.0
2.	4-Aminophenylacetic acid + resorcinol	455	2.27×10^4	0.1-2.4
3.	*p*-Nitroaniline + acetylacetone	490	3.2×10^4	0.05-1.4
4.	4-Aminophenylacetic acid + 1-Naphthol	490	2.7×10^4	0.04-1.6
5.	*p*-Nitroaniline + Frusemide	680	3.314×10^3	0.4-2.0
12.	*p*-Nitroaniline + 1-Naphthol (proposed method)	610	3.29×10^4	0.02-0.12

Conclusion

Simple, sensitive and economical method has been proposed for the spectrophotometric determination of trace amount of nitrite present in water and soil. The proposed method involves diazotisation of *p*- nitroaniline followed by coupling with 1-naphthol to give stable violet colored azo-dye.

This method follows Beer-Lambert's law over the range 0.02-0.12 µg/mL of nitrite. The molar absorptivity was found to be 3.29×10^4 lit.mol^{-1}cm^{-1}. It was concluded that nitrite contents in water and soil can be higher than standard level in some areas and it can be a public health concern for people who consume such contaminated water and vegetables grown in such contaminated soil. Thus, it is needed to apply some actions to remove nitrite from water and soil.

Acknowledgements

References

Aydin A., Ercan O. and Tascioglu S., (2005) *Talanta*, **66,** 1181-1186.

El Hani O., Karrat A., Digua K. and Aziz A. (2022) *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy,* 267(2). 120574.

Govindasamy M., Wang S.F., Huang C.H., Alshgari R.A. and Ouladsmane, M. (2022) *Microchim. Acta,* **189,** 210.

Khadka D.B., (2014) *J. Institute of Science and Technology*, **19 (2),** 89-93.

Khadka D.B. and Bhattarai B., (2014) *Res. J. Chem. Sci*., **4(9),** 101-107.

Khadka D.B. and Duwadi A., (2014) *Int. Res. J. Environment Sci*., **3(4),** 64-69.

Khaniki G.R.J., Dehghani H., Mahvi A.H., Rafati L. and Tavanfar F.,(2007) *Department of Environmental Health Engineering*, 1-5.

Liu Y., Cai Q. and Ma W., (2015) *J. Applied Spectroscopy*, **82(3),** 470-473.

Mayouf J.A., (2013) *ARPN J. Science and Technology*, **3(10),** 1005-1008.

Moravský L., Troška P., Klas M., Masár M. and Matejčík, Š. (2020) *Contrib. Plasma Phys.,* **60,** e202000014.

Nagaraj S., Bhat N.G. and Chandrashekara K.G., (2016) *International J. Chem. Studies*, **4(3),** 101-105.

Pourreza N., Fait'hi M.R. and Hatami A., (2012) *Microchemical Journal*, **104,** 22-25.

Rajasulochana P., Ganesan Y., Kumar P.S., Mahalaxmi S., Tasneem F., Ponnuchamy M. and Kapoor, A. (2022) *Environ. Res.,* **208,** 112745

Siegel J.M., Schilly K.M., Wijesinghe M.B., Caruso G., Fresta C.G. and Lunte S.M. (2019) *Anal. Methods,* **11,** 148–156.

Thomas D., Aggarwal P. and Syed A.A., (2015) *Int. J. Recent Scientific Res.*, **6(11),** 7234-7239.