Flow injection analysis of atmospheric nitrogen dioxide at ultra trace level through spirolactam ring opening process of Rhodamine B hydrazide

7.1 Introduction

Among the oxides of nitrogen, two oxides i.e. nitric oxide and nitrogen dioxide which are generally together represented as NO\textsubscript{x} and their presence in the atmosphere can cause a severe impact on the quality of ambient air. Nitric oxide even at 1 ppm concentration level in air is relatively harmless but nitrogen dioxide at higher concentration can cause respiratory distress \[1\]. It can also react with other compounds in the air to form inorganic and organic nitrates such as nitric acid and peroxyacetyl nitrate (PAN) respectively which are mainly responsible for the photochemical smog formation \[2\]. They are the precursors of acid rain and ozone formation which can damage human health, vegetation and aquatic ecosystems \[3 - 4\]. Several major reviews of the health effects of NO\textsubscript{x} have been reported in the literature in recent years \[5 - 10\]. Nitrogen dioxide damages lungs in three ways: (i) it is converted to nitric and nitrous acids in the distal airways which directly damages certain structural and functional lung cells (ii) it initiates free radical generation which results in protein oxidation, lipid peroxidation and cell membrane damage and (iii) it reduces resistance to infection by altering macrophage and immune function\[11\]. The immediate symptoms of exposure to nitrogen oxide vapors may include coughing, fatigue, nausea, choking, headache, abdominal pain and difficulty in breathing. A symptom free period of 3 - 30 hours may then be followed by the onset of pulmonary edema with anxiety, mental confusion, lethargy and loss of consciousness. At higher concentration, nitrogen oxides are generally causes respiratory irritation. The primary site of toxicity is the lower respiratory tract. Low concentrations may initially cause mild shortness of breath and cough then after a period of hours to days, victims may suffer bronchospasm and pulmonary edema. Inhalation of ambient air containing very high concentration of nitrogen oxides can cause burns, spasms, tissue swelling in throat and upper air way obstruction. Children may be more vulnerable to corrosive agents than adults because of the relatively smaller diameter of their airways. Absorption of nitrogen oxides can lead to a weak rapid pulse, dilated heart, chest congestion and circulatory collapse. High dose exposure may convert Fe\textsuperscript{2+} in hemoglobin to Fe\textsuperscript{3+} by
virtue of the presence of nitric oxide (NO) causing methemoglobinemia and impaired oxygen transport [12].

Increased NO$_x$ inputs into terrestrial and wetland systems can lead to changes in plant species composition and diversity. Nitrogen inputs to aquatic ecosystems such as those found in estuarine and coastal waters can lead to eutrophication (a condition that promotes excessive algae growth which can lead to a severe depletion of dissolved oxygen and increased levels of toxins which are harmful to aquatic life). Nitrogen alone or in acid rain form can acidify soils and surface waters. Acidification of soils causes the loss of essential plant nutrients and increased levels of soluble aluminum that are toxic to plants. Acidification of surface waters creates conditions of low pH and high levels of aluminum that are toxic to fish and other aquatic organisms [13].

Among the oxides of nitrogen nitric oxide (NO), nitrogen dioxide (NO$_2$), nitrous oxide (N$_2$O), dinitrogen trioxide (N$_2$O$_3$) and dinitrogen pentoxide (N$_2$O$_5$) are most stable forms [14]. NO and NO$_2$ are the most significant oxides among them and their high stability, reactivity causes severe impact on human health at even low concentrations. Nitrogen dioxide is a reddish brown gas with a characteristic pungent odour, corrosive and highly oxidizing in nature [15]. It is formed naturally in the atmosphere in substantial quantities and is also a by-product of human activity. Natural sources include oxidation of atmospheric nitrogen by lightning, forest fires, grass fires, bushfires and oxidation of ammonia releases from the soil [16]. Automobiles and other mobile sources contribute about half of the NO$_x$ that is emitted into atmosphere. Electric power plant boilers produce about 40% of the NO$_x$ emissions from stationary sources [17]. Substantial emissions are also added by such anthropogenic sources such as industrial boilers, incinerators, gas turbines, reciprocating spark ignition and diesel engines [18]. Among stationary sources, iron and steel mills, cement manufacture, glass manufacture, petroleum refineries and nitric acid manufacture units [19]. However the major sources of nitrogen oxides in urban areas are mainly from human activities. In the atmosphere, nitric oxide oxidizes slowly to nitrogen dioxide and the process is enhanced in outdoors in the presence of ozone.

Several methods for the measurement of NO$_x$ concentration from atmospheric air have been reported in literature [20]. Most of these methods are based on instrumental techniques like spectrophotometric, chromatography based as well as chemiluminescence are significant methods. Chemiluminescence methods are also widely applied, which are based either on the catalytic or photolytic reduction of NO$_2$ to NO and subsequent gas-
phase reaction with ozone [21 - 22]. In recent years flow injection based methods have been developed for the continuous measurement of nitrogen dioxide from ambient air and nitrite/nitrate levels from industrial effluents [23 - 24]. Simultaneous determination of nitrite and nitrate from various environmental samples is very significant speciation method to know the levels of these pollutants mainly from industrial samples. In the simultaneous determination of nitrite/nitrate, the methods are based on online nitrate reduction in a micro column containing copperised cadmium reductor column [25]. NO\textsubscript{x} is usually absorbed in a suitable absorber solution initially and fixed as nitrite ion followed by its determination by various methods [26 - 28]. In recent years flow injection analysis based on diazocoupling reaction and the color intensity measurement gained a lot of emphasis due to their simplicity, low cost and easy adoptability even under modest facility conditions. Rhodamine and its derivatives have been used as a chemosensors in the quantification of several anions and cations in recent years due to their excellent optical properties as well as high sensitivity. Rhodamine B hydrazide quantitatively reacts with nitrite at pH 3 to generate pink colored Rhodamine [29]. The intensity of the pink color produced has been correlated to nitrite concentration. In this chapter, this reaction has been explored to develop a simple flow injection analysis based on the reaction of NO\textsubscript{x} with Rhodamine B hydrazide and its catalytic activity to open the spirolactam ring in the molecule. The catalytic activity of NO\textsubscript{x} with Rhodamine B hydrazide has been used to monitor ambient nitrogen oxides (NO\textsubscript{x}) in the atmosphere. The method is based on the suction of atmospheric air through the sampling cuvette containing Rhodamine B hydrazide at pH 3 and its color intensity measurement at 563 nm. This chapter describes a sensitive flow injection analysis method developed using a colorimeter and a peristaltic pump for the continuous measurement of ambient nitrogen dioxide levels. The proposed method can be used in industrial locations to monitor ambient NO\textsubscript{x} levels continuously and it can be automated for measuring the variation of NO\textsubscript{x} concentrations thereby we can regulate the NO\textsubscript{x} emissions at source itself.

7.2 Experimental Section

7.2.1 Apparatus and Reagents

Absorbance measurements were made using Thermo Scientific UV-Vis Spectrophotometer (model Helios Zeta) with 1 cm quartz cuvettes, Peristaltic pump (Miclins, Chennai, model- PP 30) with suitable suction devices were used for sampling of
nitrogen dioxide from ambient air. Control Dynamics (Mumbai) digital pH meter (model APX 175 E/C) was used for all pH measurements. All reagents used were analar grade without further purification. Distilled water was used throughout the experiment.

**Standard sodium nitrite stock solution (1000 µgmL⁻¹):** Standard nitrite solution was prepared as described in the chapter 3 of section 3.2.

**Rhodamine B hydrazide (0.02M):** Prepared by dissolving 0.0913 g of RBH in small volume of acetonitrile and diluted to 100 mL with the same solution.

**Acetate buffer (pH: 3):** prepared by dissolving 6.8 g of sodium acetate in 3 mL of acetic acid and adjusting the pH to 3 using acetic acid. The solution is diluted to 100 mL with distilled water.

### 7.2.2 Laboratory setup used for nitrogen dioxide generation and its measurement

A portable colorimeter or spectrophotometer has been used to measure the absorbance values of the pink colored Rhodamine B formed from the catalytic reaction of NO₂ with Rhodamine B hydrazide. A glass carbyl containing known mass of pure metallic copper wire turnings has been used as NO₂ generator. On addition of 1:1 nitric acid into container which generates nitrogen dioxide. The generated nitrogen dioxide is purged into a sampling cuvette containing Rhodamine B hydrazide solution at pH 3 using peristaltic pump with an optimum flow rate. The gas flow rate has been adjusted in such a way that the bubble residential time in the reactant solution should be high enough so that the gaseous nitrogen dioxide catalyses the reagent solution to form pink colored Rhodamine. The color intensity of the compound has been measured at 563 nm and it is correlated to NO₂ concentration. All tubing connections were given using teflon tubes of different diameters. The setup used to generate nitrogen dioxide and its measurement has been shown in the Fig. 7.1.

### 7.2.3 Experimental setup used for the measurement of ambient nitrogen dioxide at traffic junction point

A portable colorimeter and a peristaltic pump combination have been used as a field sampling experimental setup for the measurement of ambient nitrogen oxides. The atmospheric air has been purged using peristaltic pump with suitable suction devices. Ambient air containing nitric oxides [NO + NO₂] has been purged through a colorimeter fitted with a sampling cuvette containing Rhodamine B hydrazide solution buffered at pH
3. The color intensity of the product i.e. Rhodamine B has been measured continuously and the absorbance values are correlated to the concentration of nitric oxides. The setup used to measure ambient nitrogen oxides has been shown in the Fig.7.2.

Fig.7.1 Schematic of laboratory setup used for nitrogen dioxide generation and its measurement using colorimeter or spectrophotometer

Fig.7.2 Experimental setup used for ambient nitrogen oxides [NO+NO\textsubscript{2}] measurement at traffic junction point within the city limits.
7.3 Results and Discussion

Nitrite quantitatively reacts with Rhodamine B hydrazide in acidic medium to give pink colored Rhodamine B [29]. Hence an attempt has been made to explore the catalytic reaction of nitrogen oxides \([\text{NO} + \text{NO}_2]\) with colorless leuco form of Rhodamine B hydrazide to produce pink colored Rhodamine B as a simple flow injection procedure in this chapter. The intensity of pink colored compound produced in the reaction has been correlated to the \(\text{NO}_x\) concentration and it has been applied to measure the ambient nitrogen dioxide from atmospheric air.

Initial studies have been carried out using standard nitrite and its catalytic reaction on Rhodamine B hydrazide to produce pink colored Rhodamine B compound. Known concentrations of nitrite samples were treated with colorless Rhodamine B hydrazide to generate pink colored Rhodamine B. The color intensity of the Rhodamine B was measured at 563 nm and correlated to nitrite concentration. The catalytic reaction of nitrite on Rhodamine B hydrazide in aqueous medium has inspired us to extend this method to measure the atmospheric nitrogen oxides concentration from ambient air. Hence a single reagent like RBH has been used to measure the ambient \(\text{NO}_2\) through its catalytic effect on Rhodamine B hydrazide solution through spirolactam ring opening process.

7.3.1 Species responsible for color

The catalytic reaction of nitrogen dioxide with colorless Rhodamine B hydrazide in acidic medium to form pink colored Rhodamine B. This transformation from colorless leuco form to colored Rhodamine B was induced by the catalytic effect of \(\text{NO}_2\) through spirolactam ring opening process and the absorbance values were measured at 563 nm. The species responsible for color in the reaction is given in the scheme 7.1 and Fig.7.3. as shown.
Scheme 7.1 Schematic representation of catalytic reaction of NO\(_2\) with Rhodamine B hydrazide to generate pink colored Rhodamine B.

Fig 7.3 Symbolic representation of transformation of Rhodamine B hydrazide solution (colorless) to pink colored Rhodamine B by nitrogen dioxide.
7.3.2 Optimization study

All the parameters influencing the color development were optimized and the optimized concentrations were incorporated in the recommended procedure. In all the optimization studies, standard nitrite solutions were added in place of nitrogen dioxide purging through the sampling cuvette containing Rhodamine B hydrazide solution. The variation of concentration of reagents is quite difficult in flow injection methods. Hence all the reagent concentrations have been optimized using stagnant solution in a 10 mL volumetric flask containing Rhodamine B hydrazide in presence of suitable buffer solution. Each time one reagent concentration has been fixed and the other reagent concentration varied till maximum absorbance to the sample was observed with minimum blank absorbance value

7.3.2.1 Effect of Rhodamine B hydrazide concentration

The effect of RBH concentration was varied in order to establish the optimum quantity of RBH required for maximum absorbance by varying its concentration in the range 0.5 - 5 mL using 0.02M RBH. Different volumes of RBH were taken in a series of 10 mL volumetric flasks containing 2 mL of acetate buffer (pH 3). To these solutions 2 µg of nitrite was added and they were allowed to stand for five minutes, then the absorbance values were measured at 563 nm. It is evident from the graph that the maximum absorbance value for the sample was obtained when the RBH concentration is in the range 2.5 - 4.5 mL of 0.02 M. Hence 3 mL of 0.02 M Rhodamine B hydrazide has been used as an optimum concentration in all further studies (Fig. 7.4).

![Effect of Rhodamine B hydrazide](image-url)
7.3.2.2 Effect of pH

The effect of pH for the catalytic reaction of nitrite on Rhodamine B hydrazide to produce pink colored Rhodamine B compound has been studied. In these experiments, various buffer solutions of 1 mL each in the pH range 0.5 - 7.0 were added to maintain the constant pH in 10 mL volumetric flasks containing 3 mL of 0.02M Rhodamine B hydrazide along with 2 µg of nitrite. The solutions were allowed to stand for five minutes and the absorbance values were measured at 563 nm. It has revealed that the maximum absorbance for sample was observed in the pH range 1 - 5. Hence the optimum pH for this reaction has been fixed 3 and it has been maintained by the addition of 1.5 mL of acetate buffer of pH 3 to get maximum sample absorbance. The absorbance values decreased above pH 5, hence pH 3 was maintained by the addition of buffer. In alkaline condition there is no catalytic effect by the nitrite on RBH.

![Graph showing effect of pH on absorbance](image)
7.3.2.3 Effect of reaction time

The optimum time period required for the quantitative catalytic reaction of nitrite on Rhodamine B hydrazide was next investigated by allowing the solutions to stand for different time intervals before absorbance measurement, the color development is instantaneous hence the effect of time on the catalytic reaction of nitrite on RBH is not required. The instantaneous reaction between RBH and nitrite to form pink colored dye has inspired us to develop a simple flow injection analysis for the quantification of nitrogen oxides from ambient air using RBH as a single reagent utilizing simple instrumental facilities like portable colorimeter for absorbance measurement and a peristaltic pump for air purging from atmosphere.

7.3.2.4 Interference study

In order to evaluate the suitability of the proposed method for the determination of NO\textsubscript{x} from atmospheric air, the effect of common atmospheric air pollutants like SO\textsubscript{2}, SO\textsubscript{3}, H\textsubscript{2}S, CO\textsubscript{2}, CO, Cl\textsubscript{2}, Br\textsubscript{2}, HCHO and NO in the determination of NO\textsubscript{x} was studied. These interfering gaseous species were introduced in the form of their respective anions. All these gaseous species did not interfere. However NO interfered even at smaller concentrations and gave a positive deviation. Nitric oxide reacts similarly with RBH and catalyses the spirolactam ring opening process similar to NO\textsubscript{2} gas to generate a pink colored Rhodamine B. The tolerance limits of the common interfering gaseous pollutants are given in the table 7.1. The reaction between RBH and NO\textsubscript{x} is quantitative and instantaneous; hence this reaction has been developed to monitor the trace level concentrations of nitrogen oxides from ambient air.
Table 7.1 Interference studies

<table>
<thead>
<tr>
<th>Interferent*</th>
<th>Tolerance limits (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formaldehyde</td>
<td>$2 \times 10^4$</td>
</tr>
<tr>
<td>Sulphur dioxide</td>
<td>$1 \times 10^3$</td>
</tr>
<tr>
<td>Hydrogen sulphide</td>
<td>$5 \times 10^2$</td>
</tr>
<tr>
<td>Carbon dioxide</td>
<td>$5 \times 10^2$</td>
</tr>
<tr>
<td>Carbon monoxide</td>
<td>$5 \times 10^2$</td>
</tr>
<tr>
<td>Chlorine</td>
<td>$1 \times 10^4$</td>
</tr>
<tr>
<td>Bromine</td>
<td>$1 \times 10^4$</td>
</tr>
</tbody>
</table>

7.4 Application study

In order to check the validation of the proposed method, the atmospheric nitrogen oxides were measured by the proposed method as well as by the Griess-Ilosvey reagent as a standard method.

7.4.1 Determination of nitrogen oxides [NO$_x$] from ambient air

Air samples were drawn directly from atmospheric air by using peristaltic pump at a flow rate of 0.3 Lmin$^{-1}$. The ambient air was purged into the sampling cuvette containing Rhodamine B hydrazide solution buffered at pH 3 using acetate buffer. The absorbance values were measured continuously at 563 nm for different time intervals on different days. The measured concentrations of NO$_x$ values are given in the table 7.2. The values of nitrogen oxides measured by the proposed method at a traffic junction point within the city limit has varied and are in agreement with the values measured by the standard method as well as reported by the Karnataka State Pollution Control Board, a statutory body of pollution prevention agency of the local area. The proposed method can be used
as a simple flow injection analytical procedure for the measurement of NO\textsubscript{x} in industrial atmospheres.

**Table 7.2** Determination of nitrogen oxides [NO\textsubscript{x}] from ambient air*

Flow rate: 0.3 L min\textsuperscript{-1}

<table>
<thead>
<tr>
<th>Sl.No.</th>
<th>volume of air sampled\textsuperscript{a} (L)</th>
<th>proposed method NO\textsubscript{x} (ppb)</th>
<th>standard method NO\textsubscript{x} (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1)</td>
<td>108</td>
<td>41.67</td>
<td>38.99</td>
</tr>
<tr>
<td>2)</td>
<td>90</td>
<td>42.01</td>
<td>38.76</td>
</tr>
<tr>
<td>3)</td>
<td>108</td>
<td>40.06</td>
<td>37.10</td>
</tr>
<tr>
<td>4)</td>
<td>90</td>
<td>38.13</td>
<td>34.04</td>
</tr>
<tr>
<td>5)</td>
<td>90</td>
<td>38.20</td>
<td>36.00</td>
</tr>
</tbody>
</table>

\*According to Karnataka State Environmental Pollution Control Board (KSEPCB), the NO\textsubscript{x} concentration measured by this agency on annual average basis is 40 ppb.

\textsuperscript{a}Air was sampled on different days

\textit{Concentration of NO\textsubscript{x} (ppb)} = \frac{NO_2^- (\mu g) \times 532}{V}

Where V = volume of air sampled in litres

### 7.5 Conclusion

The proposed method is based on the catalytic reaction of nitrogen dioxide with colorless Rhodamine B hydrazide to form pink colored Rhodamine B. A simple flow injection analysis for the quantification of oxides of nitrogen [NO\textsubscript{x}] from ambient air has been explored using simple instrumental setup which consists of colorimeter and peristaltic pump. The atmospheric oxides of nitrogen can be measured by purging the ambient air through the sampling cuvette containing Rhodamine B hydrazide buffered at pH 3 using peristaltic pump. The developed method has been applied for the continuous measurement of ambient nitrogen oxides present at ppb level at traffic junction points within the city limits. The results obtained are in good agreement with the standard method. The proposed method can be automated and used as a flow injection analytical procedure for continuous measurement of both NO and NO\textsubscript{2} which are present in the atmospheric air. This method serves as an alternative one in place of existing methods at ppb level.
concentrations of NO\textsubscript{x} in air or industrial atmospheres where oxides of nitrogen are the major flue gas emissions.

7.6 References


[16] Clapp L. J and Jenkin M. E, Analysis of the relationship between ambient levels of O\textsubscript{3}, NO\textsubscript{2} and NO as a function of NO\textsubscript{x} in the UK, *Atmospheric Environment*, **35**, 6391 - 6405 (2001).

[17] Selective catalytic reduction control of NO\textsubscript{x} emissions, *SCR Committee of Institute of Clean Air Companies* (1997).


