

Spectrophotometric Determination of Nitrite using Diphenylamine as Complexing Agent

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A rapid, simple and sensitive method for determination of trace level of nitrite is described. Nitrite reacts with diphenylamine in sulfuric acid media with 1:2 stoichiometry and the blue coloured complex is determined at 588 nm. Beer's law is obeyed in the range of 38-1150 ng mL⁻¹. Detection limit is 38 ng mL⁻¹, analytical sensitivity is 6 ng mL⁻¹ and relative standard deviation is 5 %. The effect of different ions in determination of nitrite was also investigated.

Key Words: Nitrite, Diphenylamine, Spectrophotometry.

INTRODUCTION

The determination of nitrite ion in natural waters is important in environmental and marine chemistry. It is one of the nutrient species limiting the amount of biomass. Many methods have been proposed for the determination of nitrite¹⁻⁶. Spectroscopic methods are most widely used for nitrite determination due to its excellent limits of detection and facile assay-type protocols. A broad range of techniques have been evaluated, including UV-Vis⁷⁻¹³, chemiluminescence¹⁴, fluorimetric¹⁵⁻²⁰, IR²¹ and Raman²². The most common approach to the detection of nitrite²³ is the Griess Assay, first developed in 1897. Electrochemical methods such as voltametric techniques have also been used²⁴⁻²⁷. The present method describes a sensitive, rapid and simple method for determination of nitrite by spectrophotometry using diphenylamine as complexing agent.

EXPERIMENTAL

All spectrophotometric measurements were carried out using an Agilent UV-Vis spectrophotometer. The cells used for absorbance measurements were 1 × 1 glass cells.

All chemicals used were of analytical grade purchased from Merck, Germany. Doubly distilled water was used throughout the experiments.

The standard solution of nitrite (1000 µg mL⁻¹) was prepared by dissolving 0.1371 g of anhydrous sodium nitrite in doubly distilled water and diluting to 100 mL in a volumetric flask. A pellet of sodium hydroxide was added to prevent liberation of nitrous acid. Working standard solutions was prepared freshly by diluting the

stock solution with distilled water. Diphenylamine solution (0.5 % g/mL) was prepared by dissolving 0.0125 g of pure diphenylamine in 5 mL of doubly distilled water and diluting to 25 mL with concentrated sulfuric acid.

Sample preparation: A 2 mL portion of solution containing nitrite in the range of 38-1150 ng mL⁻¹ was transferred into the 10 mL volumetric flask and 3 mL of diphenylamine solution (0.05 % g/mL) was added and diluted to 10 mL with 9 M sulfuric acid.

RESULTS AND DISCUSSION

Optimization of parameters: The effects of different parameters affecting the sensitivity of the results were investigated.

Sulfuric acid concentration: Sulfuric acid in different concentrations was used for 0.6 µg mL⁻¹ nitrite solution. The absorbance increases until 9 M and then decreases with increase of concentration. The maximum absorbance was obtained in 9 M H₂SO₄.

Effect of time on the complex formation: A solution was prepared 0.05 % in diphenylamine and 0.06 µg mL⁻¹ in nitrite and 9 M sulfuric acid and the absorbance of the complex was measured per minute. After 15 min the blue colour was developed and maximum absorbance was achieved and kept constant.

Effect of diphenylamine concentration: Different concentrations of diphenylamine in the range of 0.01-0.1 % mg/mL were used for producing the complex and the absorbance of sample solution increases as diphenylamine concentration increases up to 0.03 % and levels off at higher values. Diphenylamine at 0.05 % concentration were used for further studies. Optimum condition are given in Table 2.

Effect of ionic strength: As ionic strength affect the activity of nitrite ion, its effects were investigated using KCl solution at different concentrations. The absorbance of the complex was decreased by increasing the KCl concentration and hence KCl was not added to maintain the ionic strength.

Stoichiometry: In order to determine the stoichiometry of the complex, both Job and mole ratio methods were employed. Both methods show a stoichiometry of 2:1 confirming the formula of (diphenylamine)₂NO₂ for the complex. The results are given in Figs. 1 and 2.

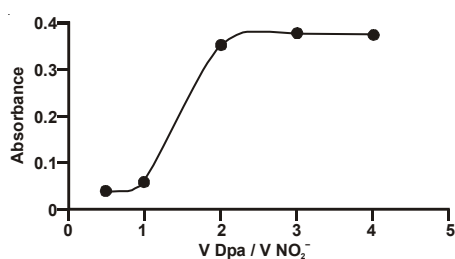


Fig. 1. Mole ratio method

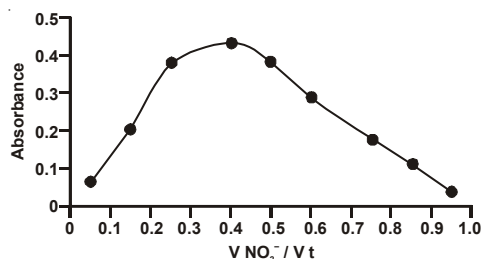


Fig. 2. Job method

Analytical performance: The calibration graph was obtained from the results for several nitrite standard solutions as shown in Fig. 3. The statistical limit of detection (based on 3s) was 38 ng mL^{-1} the relative standard deviation was 5 % ($n = 7$). The linear range of calibration graph was in the range of 38 to 1150 ng mL^{-1} .

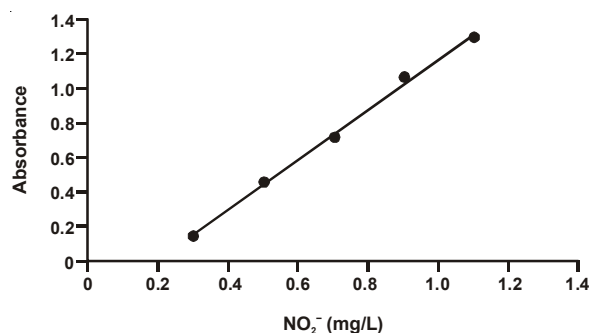


Fig. 3. Calibration curve

Interference studies: A $0.6 \mu\text{g mL}^{-1}$ nitrite solution containing 100 fold of foreign ions was analyzed as described in the procedure. The results are given in Table-1. As can be seen, most cations show considerable interference in determination of nitrite. In order to investigate the removal of the interference effect, a cation exchange resin (Amberlite IRC-718, Sigma) was used. The results show complete removal of interfering cations from nitrite and hence, its determination could be performed without the interference of metal ions. However nitrate causes severe interference effect on the determination of nitrite and its interference could not be removed even using different derivative analysis.

TABLE-1
EFFECT OF INTERFERING IONS ON DETERMINATION OF NITRITE

Interfering ion	RE (%)*	Interfering ion	RE ^a (%)
NO_2^-	—	NO_3^-	200.00
F^-	-29.50	K^+	-38.00
B^-	-19.02	Fe^{3+}	392.00
PO_4^{3-}	-22.70	Mg^{2+}	131.40
SO_4^{2-}	-30.17	NH_4^+	398.00
SCN^-	30.77	Ca^{2+}	-0.86
Cl^-	39.00	Al^{3+}	123.20

*Relative error in absorbance of $0.6 \mu\text{g mL}^{-1}$ nitrite with 100 fold interfering ion.

TABLE-2
OPTIMIZED CONDITION FOR DETERMINATION OF NITRITE

Sulfuric acid concentration (M)	Time (min)	Diphenylamine concentration (% mg/mL)
9	15	0.05

Conclusion

A rapid, simple and sensitive method for determination of nitrite is described. Nitrites react with diphenylamine in sulfuric acid forming a 2:1 complex which absorbs at 588 nm. The effects of different parameters affecting the sensitivity of the method were studied. The interferences of metal ions in determination of nitrite were removed using cation exchange resin.

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