



Original paper

Quantitation of nitrite in soil and water samples based on central composite design and a new Griess reagent

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ABSTRACT

A simple and rapid spectrophotometric method for the determination of nitrite in water and soil preparation samples has been developed. Determination of nitrite is based on diazotization of p-nitro aniline with nitrite and subsequent coupling with 4-amino-3-hydroxynaphthalene-1-sulfonic acid in hydrochloric acid. The factors affecting the reaction i.e. concentration of p-nitro aniline, concentration of 4-amino-3-hydroxynaphthalene-1-sulfonic acid and concentration of the hydrochloric acid solution were studied and optimized using central composite design. The analysis of the results of experimental design showed that for obtaining higher signals the values of the above factors should be as 2.0×10^{-4} , 5.0×10^{-4} and 1.33 M, respectively. The reaction yields a product with an absorption maximum at 302 nm. Beer's law is obeyed in the range 0.10-2.00 mgL^{-1} of nitrite. The molar absorptivity of the product was found to be 6.74×10^4 . The method was applied successfully to the determination of nitrite in water and soil samples. Relative standard deviation of the method was below 2 %.

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1. Introduction

Nitrite is an important water pollutant. It is also an important intermediate formed during the biodegradation of nitrogenous organic matter (Black, 1977). During the biodegradation of nitrate and nitrogenous organic matter, nitrite is formed. Nitrites are regarded as hazardous compounds (Lijinsky et al., 1970). They react readily with secondary and tertiary amines and amides to form carcinogenic N-nitroso compounds (Lijinsky et al., 1976; Lijinsky, 1974). The maximum permissible limit, as fixed by the US Public Health Association, is 0.06 mgL^{-1} of nitrite in potable water (Standard Methods, 1976; Resource Agency, 1958). It affects the Biochemical Oxygen Demand (BOD) of water (Horn, 1978). It can react in the human body with dietary components to form toxic, carcinogenic and/or mutagenic nitrosamines (Glidewell, 1990). Furthermore, nitrite in the blood stream converts oxyhemoglobin to methemoglobin, thereby interfering with oxygen transport in the blood (Burden, 1961). Thus, the determination of nitrite is of great importance in environment and food analysis.

An excellent review on the detection and determination of nitrite has been reported by Moorcroft et al (Moorcroft et al., 2001). The most common technique for the determination of nitrite is spectrophotometry which is based on the diazotation of an aromatic amine and the formation of an azo dye. Electrometric (Issaq et al., 1983; Perez-Olmos et al., 1998; Gao et al., 1990; Muthukumar et al., 2014; Yildiz et al., 2014; Zhou et al., 2013), chromatographic (Wang et al., 2012; Iskandarani et al., 1982; Cortes, 1982), kinetic (Pettas et al., 1998; Kojlo et al., 1995) and spectrophotometric (Guo et al., 2013; Wang et al., 2011; Wang et al., 2000; Huang et al., 2006; Lapat, 1997) methods have also been developed. Other methods involve the use of ion chromatography (Wang et al., 2012; Crowther et al., 1956) and specific ion electrodes (Keeney, 1970).

Various spectrophotometric methods have been also used to determine nitrite in different samples (Zhang et al., 2014; Nagaraja et al., 2010; Filik et al., 2011; Pourreza et al., 2012; Abdul Galil et al., 2007). However, the most popular and sensitive spectrophotometric methods for determination of nitrite are based on the Griess reaction

(Greiss, 1879; Abbas et al., 2000; Kiso et al., 2006; Lopez Pasquali et al., 2007; López Pasquali et al., 2010; Nagaraja et al., 2010). In the present work, a new reagent for determination of nitrite based on the Griess reaction is used. For optimization of the reaction conditions, experimental design was employed.

2. Experimental

2.1. Apparatus and software

An Agilent UV-Vis spectrophotometer model 8453 with diode array detector with 1 cm path length quartz cells was used for recording spectra. Design and analysis of the central composite experiments were carried out by MINITAB (Minitab Inc. Release 14.0) statistical package.

2.2. Reagents and solutions

All chemicals were of analytical reagent grade and doubly distilled water was used in the preparation of all solutions in the experiments. Sodium nitrite, hydrochloric acid and ethanol were supplied (Merck KGaA 64271 Darmstadt, Germany). 4-amino-3-hydroxynaphthalene-1-sulfonic acid (AHNSA) was supplied by BDH Chemicals Ltd Poole England and p-nitroaniline (PNA) was supplied by MERCK (E. Merck, Darmstadt). A 100.0 mgL^{-1} stock standard solution of nitrite was prepared in doubly distilled water. Stock $1.0 \times 10^{-3} \text{ M}$ solution of AHSA was prepared in doubly distilled water and ethanol (1:1) and stock 650 mgL^{-1} solution of PNA was prepared in doubly distilled water and ethanol (7:3). Hydrochloric acid solution with concentration of 1.33 M was prepared by dilution of concentrated hydrochloric acid with doubly distilled water. Working solutions were prepared by diluting appropriate volumes of the standard stock solutions with doubly distilled water in volumetric flasks whenever required.

2.3. Calibration curve

For constructing calibration curve, volumes equivalent to 0.8 mL of hydrochloric acid solution with concentration of 1.33 M, 1.5 mL of

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stock solution of AHNSA, 127.5 μL of stock solution of PNA and 572.5 μL were transferred to a 1 cm path-length quartz cell. To this mixture, 3 – 60 μL of stock 100.0 mgL^{-1} standard nitrite solutions were added. This mixture was maintained at room temperature for 65 min for equilibration. After this time, the spectrum of the solution was recorded against the reagent blank which is prepared in the same manner but in the absence of nitrite.

2.4. Sample preparation for determination of nitrite in water

Water sample (Drinking water) was collected without adding any preservative. By using the water sample, hydrochloric acid solution with concentration of 1.33 M was prepared. To this sample, volumes of the reagents based on the previous section (Recommended procedure) were added and mixed well. After 65 min the spectrum of the sample was recorded against the reagent blank.

2.5. Sample preparation for determination of nitrite in soil

Soil samples from manure garden farmland were collected. 5.0 g of the soil was dried at 55 $^{\circ}\text{C}$ in an oven for 5 hours. The dried sample was dissolved in 50.0 mL water shaken thoroughly and filtered. The filtrate was subjected to centrifugation. The supernatant liquid was taken and to it 1.0 mL of 1000.0 ppm EDTA solution was added to mask different interferent cations. The resulting solution was treated like a water sample as discussed in 2.4.

3. Results and discussion

3.1. Central composite experimental design and optimization of parameters

In the reaction of nitrite, three factors must be optimized. These are concentration of AHNSA, PNA and hydrochloric acid. The purpose of the experimental design is extraction of the maximum amount of information of the system in an economical way (Kincl et al., 2005). In experimental design, all variables are changed from one experiment to the next, simultaneously. The reason for this is that variables can influence each other and the optimum value for one of them might be

related to the values of the others (Gunaraj et al., 1999). Full factorial design requires many experiments at multilevel mode. Central composite design (CCD) provides almost the same information that multilevel full factorial design gives, but with fewer experiments (Box et al., 1978; NIST/SEMATECH e-Handbook of Statistical Methods). CCD was originally developed by Box and Wilson (Box et al., 1951) and improved by Box and Hunter (Box et al., 1957). The proposed method was compared with the reported spectrophotometric methods for determination of CAR.

In CCD, it is assumed that the central point for each factor is 0 and the design is symmetrical around it. Factors and their considered levels of design are shown in Table 1. For a system with three factors ($n = 3$), CCD consists of 18 experiments. Values of the factors in these 18 experiments and obtained responses are shown in Table 2. ANOVA results have been collected in Tables 3 and 4. As can be inferred from Table 3, values of statistics parameters F and p for the model show that the model is significant in 95% confidence level since calculated p is below 0.05. Moreover, linear portion of the model is also significant based on the calculated p value. Among the individual factors, X2 (concentration of PNA) is a significant factor at 95% confidence level (see Table 4) because its corresponding p value is very low and corresponding t value is high. None of the interaction terms are significant. However, the calculated p value for the interaction term X2X3 is low (see Table 4) which indicates that to some extent the effect of the concentration of PNA is influenced by concentration of hydrochloric acid.

Table 1. Experimental factors and their levels investigated.

| Factors | Levels | | |
|-----------------------------------------|----------------------|----------------------|----------------------|
| | -1 | 0 | 1 |
| Concentration of AHNSA (M) (X1) | 2.0×10^{-4} | 3.5×10^{-4} | 5.0×10^{-4} |
| Concentration of PNA (M) (X2) | 2.0×10^{-4} | 6.0×10^{-4} | 1.0×10^{-3} |
| Concentration of hydrochloric acid (X3) | 1.0 | 2.0 | 3.0 |

Table 2. Experiments based on central composite design with three factors.

| Exp # | Concentration of hydrochloric acid (M) | Concentration of PNA (M) | Concentration of AHNSA (M) | Experimental response |
|-------|----------------------------------------|--------------------------|----------------------------|-----------------------|
| 1 | 2.6 | 8.4×10^{-4} | 2.6×10^{-4} | 0.125 |
| 2 | 3.0 | 6.0×10^{-4} | 3.5×10^{-4} | 0.000 |
| 3 | 2.0 | 6.0×10^{-4} | 3.5×10^{-4} | 0.147 |
| 4 | 2.0 | 6.0×10^{-4} | 3.5×10^{-4} | 0.107 |
| 5 | 2.0 | 1.0×10^{-3} | 3.5×10^{-4} | 0.055 |
| 6 | 1.4 | 3.6×10^{-4} | 2.6×10^{-4} | 0.376 |
| 7 | 1.4 | 3.6×10^{-4} | 4.4×10^{-4} | 0.357 |
| 8 | 2.0 | 6.0×10^{-4} | 5.0×10^{-4} | 0.649 |
| 9 | 1.4 | 8.4×10^{-4} | 4.4×10^{-4} | 0.000 |
| 10 | 2.0 | 6.0×10^{-4} | 3.5×10^{-4} | 0.169 |
| 11 | 1.0 | 6.0×10^{-4} | 3.5×10^{-4} | 0.072 |
| 12 | 2.6 | 3.6×10^{-4} | 4.4×10^{-4} | 0.293 |
| 13 | 2.0 | 6.0×10^{-4} | 3.5×10^{-4} | 0.209 |
| 14 | 2.0 | 2.0×10^{-4} | 3.5×10^{-4} | 0.324 |
| 15 | 1.4 | 8.4×10^{-4} | 2.6×10^{-4} | 0.000 |
| 16 | 2.6 | 8.4×10^{-4} | 4.4×10^{-4} | 0.030 |
| 17 | 2.0 | 6.0×10^{-4} | 2.0×10^{-4} | 0.380 |
| 18 | 2.6 | 3.6×10^{-4} | 2.6×10^{-4} | 0.242 |

Fig. 1 shows the variation of response (absorbance of the product at 303 nm) versus the factors studied. In Fig. 1a can be seen that at intermediate concentrations of hydrochloric acid response can be higher. On the other hand, in the two extremes of the concentration range of AHNSA (see Fig. 1a), response may be optimum. For concentration of hydrochloric acid, Fig. 1b gave the same information as Fig. 1a gave. Moreover, the effect of concentration of PNA on the product formation is such that the lower its concentration the higher the response. This inverse effect can also be inferred from the sign of

the coefficient of X2 (-0.262, concentration of PNA). Furthermore, as can be observed from Fig. 1b, as concentrations of hydrochloric acid increases the effect of concentration of AHNSA on the response is inversed. This can be attributed to some interaction between these two factors which was inferred from the ANOVA results in Table 4 (p for the interaction term X2X3 is 0.063). In Fig. 1c, the suitability of the lower concentrations of PNA and intermediate concentrations of AHNSA for formation of the product is observed. Results of the optimization of experimental factors have been collected in Table 5.

Table 3. Analysis of variance for the results of CCD.

| Source | F ^a | p ^b |
|----------------|----------------|----------------|
| Regression | 3.47 | 0.047 |
| Linear | 5.27 | 0.027 |
| Square | 0.5 | 0.5 |
| Interaction | 1.58 | 0.27 |
| R ² | 79.61 | |

a. F test.
b. Probability value

Table 4. Analysis of variance (coded units).

| Term | Coefficient | t ^a | p ^b |
|-------------------------------|-------------|----------------|----------------|
| Constant | 0.167 | 2.22 | 0.057 |
| X ₁ | 0.05 | 0.738 | 0.481 |
| X ₂ | -0.262 | -3.84 | 0.005 |
| X ₃ | 0.048 | 0.708 | 0.5 |
| X ₁ X ₁ | 0.266 | 2.23 | 0.056 |
| X ₂ X ₂ | -0.059 | -0.495 | 0.634 |
| X ₃ X ₃ | -0.217 | -1.82 | 0.106 |
| X ₁ X ₂ | -0.036 | -0.246 | 0.812 |
| X ₁ X ₃ | -0.017 | -0.113 | 0.913 |
| X ₂ X ₃ | 0.320 | 2.162 | 0.063 |

a. t statistics. b. Probability value.

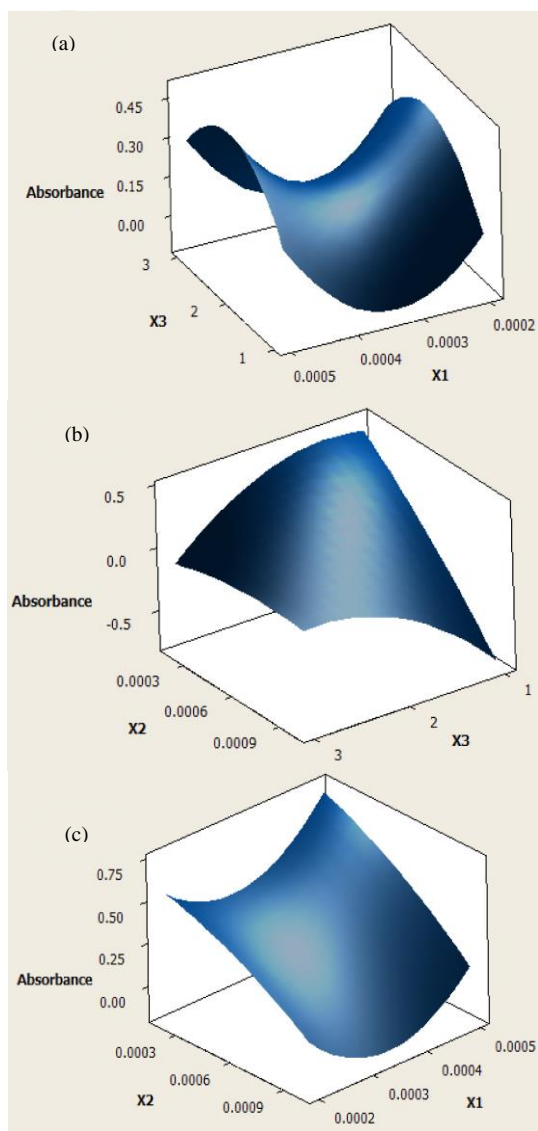


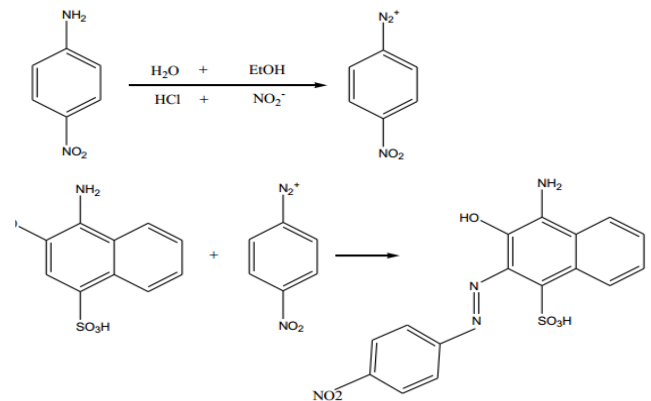
Fig. 1. Variation of the absorbance with (a) concentration of hydrochloric acid and concentration of AHNSA, (b) concentration of hydrochloric acid and concentration of PNA and (c) concentration of AHNSA and concentration of PNA.

Table 5. Optimized values of the factors for response during the determination of nitrite.

| Factors | Optimized values |
|-----------------------------------------|----------------------|
| Concentration of AHNSA (M) | 5.0×10 ⁻⁴ |
| Concentration of PNA (M) | 2.0×10 ⁻⁴ |
| Concentrations of hydrochloric acid (M) | 1.33 |

3.2. Absorption spectra and the reaction

It must be mentioned that the maximum absorption was reached at 65 min after mixing. The reaction between nitrite, AHNSA and PNA in hydrochloric acid solution can be shown in Scheme 1. In the first step, nitrous acid from nitrite and hydrochloric acid can diazotize PNA. In the second step, this is coupled with AHNSA to form a dye.



Scheme 1. The reaction between the reagents PNA and AHNSA and nitrite.

The absorption spectrum of the colored dye shows maximum absorption at 302 nm in doubly distilled water and ethanol (7:3) mixture. In Fig. 2, the spectra of the calibration samples in optimum conditions are shown. The product has maximum absorbances at 303 and 347 nm. Moreover, a broad band in the region between 400-550 nm can be observed for the final product.

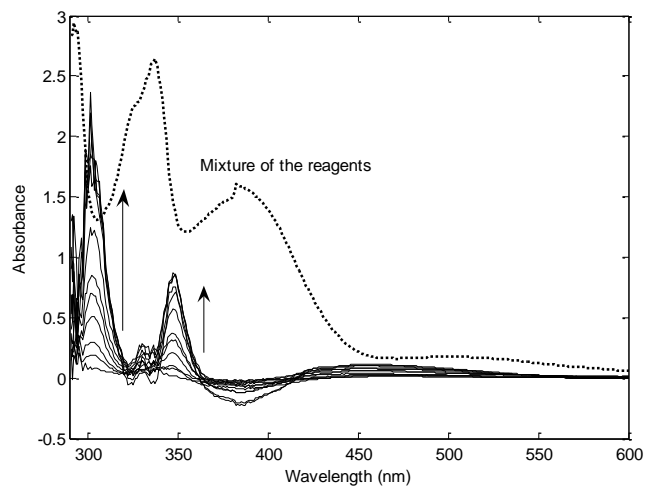


Fig. 2. Spectra of the mixture of the reagents and the product of the reaction between nitrite (0.1-2.0 mg.L⁻¹) with AHNSA (5.0×10⁻⁴ M) and PNA (2.0×10⁻⁴ M) in hydrochloric medium with concentration of 1.33 M. after 65 min.

3.3. Analytical data (linearity and sensitivity)

Under the optimized experimental conditions, one calibration curve was constructed covering a concentration range of 0.1-2.0 mg. L⁻¹ at the wavelength 302 nm. Adherence to Beer's law was studied by measuring the absorbance of the product in solutions with different amounts of nitrite.

Analytical parameters of the calibration are given in Table 6. The Limit of detection (LOD) and quantitation (LOQ) were calculated according to ICH guide lines using the formulae: $LOD = 3.3 \times S/b$ and $LOQ = 10 \times S/b$, (where S is the standard deviation of the blank absorbance values, and b is the slope of the calibration plot) and have also been reported in Table 6. The linearity of the calibration curve is validated by the high value of correlation coefficient of the regression equation. The high values of molar absorptivity and low values of LOD indicate the high sensitivity of the proposed method.

Table 6. Statistical parameters of the calibration based on the proposed method for determination of nitrite.

| Parameters ^a | Results |
|-----------------------------------------------------------|--------------------|
| Beer's law limits (mg.L^{-1}) | 0.1-2.0 |
| λ_{max} (nm) | 302 |
| Molar absorptivity ($\text{L.mol}^{-1}.\text{cm}^{-1}$) | 6.74×10^4 |
| Limit of detection (mg.L^{-1}) | 0.001 |
| Limit of quantification (mg.L^{-1}) | 0.003 |
| Slope (b) | 0.9781 |
| Intercept (a) | -0.0032 |
| Correlation coefficient (r) | 0.9995 |
| Standard deviation of a (Sa) | 0.0912 |
| Standard deviation of b (Sb) | 0.0997 |
| Standard deviation of the regression | 0.1617 |

Statistical parameters were calculated based on the relations in reference (Miller and Miller, 2005).

3.4. Effect of foreign ions

As the system was developed for the analysis of water and soil samples, the interference from foreign ions commonly present in these samples were studied by adding known amounts of foreign ions to a solution containing 1.00 mgL^{-1} of nitrite. Several species that can occur in the water and soil samples together with nitrite were investigated. The tolerance limit of a potentially interfering ion was taken as its maximum amount causing an error of $\geq 5\%$ during determination of nitrite. The tolerance limits for the ions studied are given in Table 7.

Table 7. Effect of foreign ions on determination of nitrite with concentration of 1.00 mgL^{-1} .

| Foreign ion | Tolerance limit (mg.L^{-1}) |
|--------------------|----------------------------------------|
| SO_4^{2-} | 11.26 |
| NO_3^- | 8.20 |
| K^+ | 5.20 |
| Ca^{2+} | 2.73 |
| Mg^{2+} | 1.83 |
| Fe^{3+} | 1.72 |

3.5. Application

Under the optimized experimental conditions, validity of the proposed method was checked by determining nitrite in various water and soil samples.

The method provides a simple and sensitive procedure for the spectrophotometric determination of nitrite. The water sample was collected from different sources.

As the sample that was available contained no nitrite, samples were spiked with known amounts of nitrite, and then analyzed according to the proposed procedure. The proposed method was also used for the determination of nitrite in soil sample.

The soil sample was collected from different sources, and was filtered before analysis. Soil samples were also spiked by the known amount of nitrite.

Results of the analysis of the real samples have been collected in Table 8.

The percentage relative standard deviation (RSD %) for drinking water was 0.7% and for soil was 1.6% indicating high precision of the method.

Accuracy was evaluated as percentage relative error (RE %) between the measured mean concentrations and taken concentrations for nitrite. Percent relative error (RE %) for drinking water was 10% and for soil was 3%, these demonstrates the accuracy of the proposed method.

Table 8. Spectrophotometric determination of nitrite in water and soil samples based on the proposed method.

| Real sample | Added (mgL^{-1}) | Found ^a (mg L^{-1}) | RE % | RSD % |
|----------------|-----------------------------|-------------------------------------------|------|-------|
| Drinking water | 1.00 | 0.90 | 10 | 0.7 |
| Soil | 1.00 | 0.97 | - 3 | 1.6 |

a. Mean of three determinations.

4. Conclusions

The proposed method is a simple and rapid spectrophotometric method which can be used in routine determination of nitrite in soil and water samples. Most of the ions normally present in water and soil do not interfere. The proposed method was successfully applied in the determination of nitrite in soil and water samples.

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