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Research Article

SPECTROPHOTOMETRIC DETERMINATION OF NITRITE IN SOIL AND WATER USING CEFIXIME AND CENTRAL COMPOSITE DESIGN

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Abstract

The present paper seeks to develop a simple method for the spectrophotometric determination of nitrite in soil and water samples and also measure optimum reaction conditions along with other analytical parameters. The method is based on the diazotization-coupling reaction of nitrite with cefixime and 1-naphthylamine in an acidic solution (Griess reaction). The final product that is an azo dye has an orange color with maximum absorption at 360 nm which Beer's Law is obeyed over the concentration range 0.02-15.00 mg L⁻¹ of nitrite. Optimal conditions of the variables affecting the reaction were obtained by central composite design (CCD). A detection limit of 4.3×10⁻³ mg L⁻¹ was obtained for determination of nitrite by the proposed method. The proposed method was successfully applied to determine nitrite in soil and water samples. The molar absorptivity of the product of the reaction and RSD in determination of nitrite in real samples are 4.1×10³ (L mol⁻¹ cm⁻¹) and lower than 8%, respectively.

Keywords: Nitrite; Diazotization; spectrophotometric; cefixime; 1-naphthylamine.

Introduction

Public health and the environment have faced serious threats due to the increasing concentration of nitrite in soil, groundwater, rivers and lakes. Nitrite is an excellent indicator of the extent of pollution in environmental samples and the continuous ingestion of this ion can have serious implications for animal and human health. Nitrite acts as a precursor in the formation of many N-nitrosamines at physiological pH, which are notorious as potent carcinogens in a variety of animals and suspected of causing cancer in humans as well. Moreover, high concentration of nitrite in blood can react with iron (III) of the hemoglobin, turning it into methemoglobin which has no oxygen-carrying ability. This fatal disease is called methemoglobinemia (oxygen deficiency).

According to United States Environmental Protection Agency (USEPA), the maximum contaminant level that is allowed for nitrite in drinking water is 1 mg L⁻¹ [1]. Given that concentration of nitrite is widely recognized as a hazardous problem, there is a need to develop

methods for monitoring the nitrite ion levels in environmental matrices, which is desirable from the view point of environmental analytical chemistry.

Various methods have been already put forward for facilitating the determination of nitrite [2-4]. However, some of these methods such as ion chromatography [5-8], electrophoresis [12-15], chemiluminescence [16-17], spectrofluorimetry [18-21], electrochemical [22-24], voltametry [25], amperometry [26,27], potentiometry [28] are relatively expensive. Spectrophotometric methods are simple and cheaper methods for the analysis. Spectrophotometric methods for determination of nitrite [29-35] are usually based on Saltzman reaction [36], Jacobs-hochheiser reaction [37], Griess-liosvey reaction [38,39]. Kinetic spectrophotometric methods [9-11] have also been used for determination of nitrite.

Experimental design is a strategy to gather empirical knowledge based on the analysis of experimental data and not on theoretical models. The objectives of

experimental design are the following: efficiency (get more information from fewer experiments), and focusing (collect only the information you really need) [40]. An effective experimental design technique commonly used for process analysis and modeling is central composite design (CCD).

In this paper, we have developed a simple spectrophotometric method for determination of nitrite. The method is based on the reaction of nitrite with between 1-naphthylamine and cefixime. CCD was used to explore the factors affecting the reaction.

Experimental

Apparatus and software

An Agilent spectrophotometer model 8453 with diode array detector was used for recording spectra. Design and analysis of the central composite experiments were performed in MINITAB (Minitab Inc. Release 14.0) statistical package.

Reagents and Solutions

All chemicals used were of analytical reagent grade and doubly distilled water was used for preparation of the solutions. Sodium nitrite, ethanol and hydrochloric acid were supplied by Merck (Darmstadt, Germany). Drug cefixime was supplied by Sigma Chemical Company and 1-naphthylamine supplied by BDH Chemicals (LTD poole England). A stock 100 mg L⁻¹

standard solution of nitrite was prepared in doubly distilled water. Working solutions were prepared by diluting the standard stock solution to appropriate volumes with doubly distilled water whenever required. Stock 1000 mg L⁻¹ standard solutions of cefixime and 1-naphthylamine were prepared in doubly distilled water. Acidic solution (pH 1) was prepared by addition of the concentrated hydrochloric acid and sodium hydroxide to doubly distilled water.

Calibration curve

One calibration curve was obtained over the concentration range of 0.02 – 15.00 mg L⁻¹ of nitrite. Calibration points were obtained by adding different volumes of the concentrated stock solution of nitrite to the mixture of 1850 µL of stock solution of cefixime, 140 µL of 1-naphthylamine and 3010 µL of acidic solution with the optimal pH (pH 1). Then the mixture was shaken well and maintained at room temperature for 30 min for completion of the reaction. After 30 min, the spectra of the solutions were recorded against the reagent blank which is prepared in the same manner but in the absence of nitrite. Absorbance at 360 nm was used to obtain calibration curve. It must be mentioned that the maximum absorption was reached at 30 min after mixing. In Fig. 1, spectra of the calibration samples in optimal conditions have been shown. The proposed mechanism for the reaction of nitrite with cefixime and 1-naphthylamine has been shown in Scheme 1.

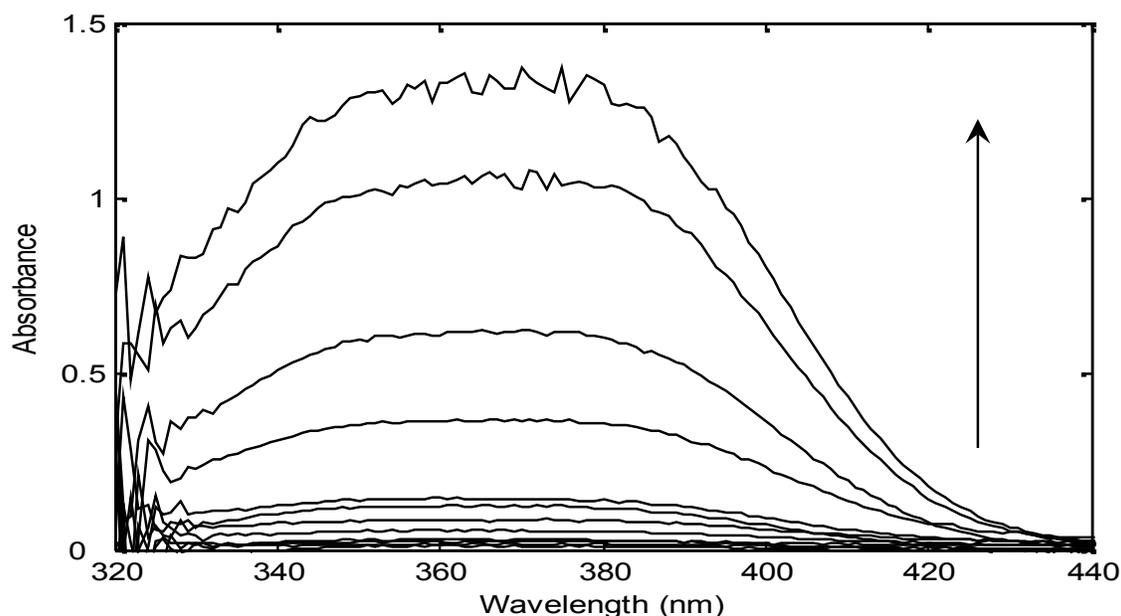
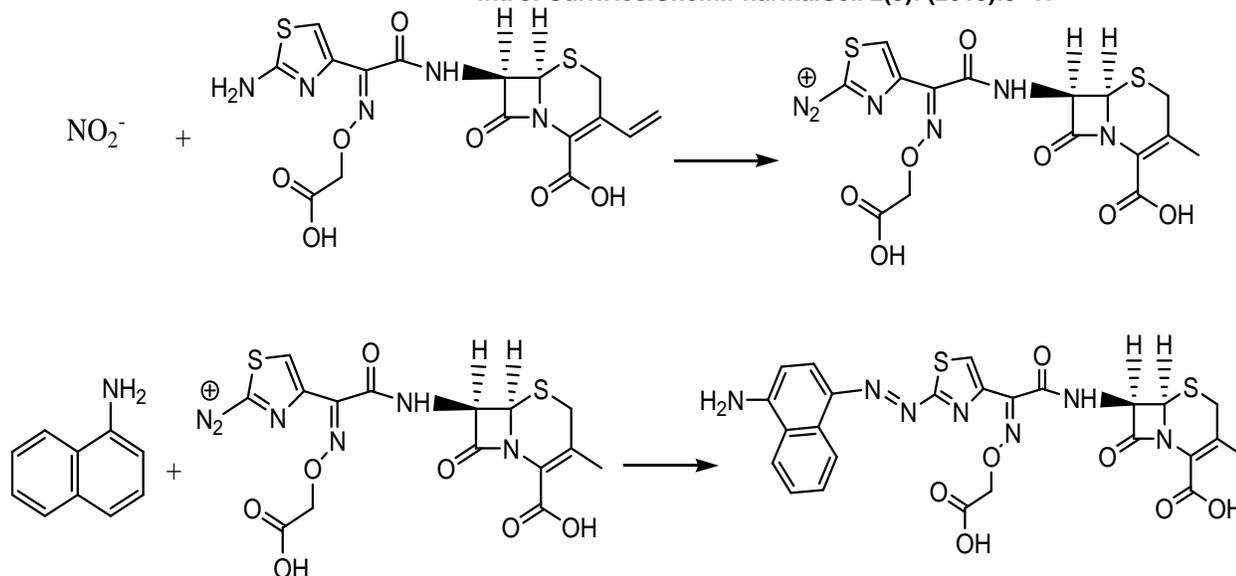


Fig. 1. Spectra of the calibration samples in conditions: 0.02-15.00 mg L⁻¹ of nitrite, 1850 µL of stock solution of cefixime, 140 µL of stock solution of 1-naphthylamine and 3010 µL of acidic solution with pH 1, 30 min after mixing.



Scheme 1. The proposed mechanism for the reaction of nitrite with cefixime and 1-naphthylamine.

Sample preparation for determination of nitrite in water and soil samples

Samples were analyzed within the same conditions as calibration samples. Firstly, water samples were filtered through a Whatman no. 41 filter paper. In order to prepare soil samples, we added 20 g of soil to 200 mL of doubly distilled water. The produced solution was then filtered through a Whatman no. 41 filter paper and centrifuged for 30 min, and again filtered through the filter paper. Then, the pH of the filtrates was adjusted to 1 by addition of concentrated hydrochloric acid. A volume equivalent to 3010 μL of the resulting sample without dilution was transferred to a 5 mL volumetric flask and reagents were added based on the procedure described in 2.3. After shaking well and standing for 30 min, the spectrum of the sample was recorded against the reagent blank.

Results and Discussion

Central composite design (CCD) and optimization of the factors

CCD was originally developed by Box and Wilson and later on improved upon by Box and Hunter [41]. The central point for each factor is assumed to be 0, around which the design is supposed to be symmetric. Factors and their considered levels of design are shown in Table 1. For a system with three factors, CCD can be represented by points on a cube, in which each axis corresponds to a factor and consists of 20 experiments. Table 2 shows the values of the factors in the experiments and corresponding measured responses. Response is the absorbance of the reaction mixture after 30 min at 360 nm. Tables 3 and 4 illustrate the analysis of variance (ANOVA) of the experiments in Table 2.

Table 1 Experimental factors and their investigated levels.

Factors	Levels		
	-1	0	1
X_1 (pH)	0.7	2.5	4.3
X_2 (Cefixime concentration)	70	250	430
X_3 (1-Naphthylamine concentration)	18	30	42

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

Run Order	1-Naphthylamine concentration	Cefixime concentration	pH	Response
1	40	100	4.0	0.1240
2	30	250	2.5	0.0517
3	20	400	1.0	0.0713
4	40	100	1.0	0.1510
5	30	250	2.5	0.1230
6	40	400	1.0	0.1050
7	42	250	2.5	0.0712
8	30	70	2.5	0.0289
9	30	250	2.5	0.0798
10	30	250	2.5	0.0517
11	18	250	2.5	0.0272
12	40	400	4.0	0.0936
13	20	100	4.0	0.1230
14	20	400	4.0	0.0959
15	30	250	2.5	0.0514
16	30	250	4.3	0.0588
17	20	100	1.0	0.0494
18	30	250	2.5	0.1110
19	30	430	2.5	0.0981
20	30	250	0.7	0.0599

Table 3 Analysis of variance for the experiments in Table 2.

Source	DF ^a	AdjMS ^b	F ^c	p ^d
Regression	9	0.001631	2.02	0.144
Linear	3	0.004226	5.24	0.020
Square	3	0.000463	57	0.645
Interaction	3	0.000205	0.25	0.857
Residual error	10	0.000807		
Lack-of-fit	5	0.001183	2.74	0.146
Pure error	5	0.000431		
Total	19			
R ²	64.53%			

^a Degree of freedom. ^b Adjusted mean of squares error. ^c F test.
^d Probability value.

Table 4 Analysis of variance (coded units)

Term	Coefficient	SE Coefficient	t^a	p^b
Constant	0.082546	0.011587	7.124	0.000
X1(1-NA)	0.004923	0.007688	0.64	0.536
X2(cefixime)	0.009432	0.007688	1.227	0.248
X3(pH)	-0.02855	0.007688	-3.714	0.004
X1X1	-0.000505	0.007484	-0.067	0.948
X2X2	-0.007222	0.007484	-0.965	0.357
X3X3	0.005895	0.007484	0.788	0.449
X1X2	0.000162	0.010045	0.016	0.987
X1X3	0.005387	0.010045	0.536	0.603
X2X3	-0.006912	0.010045	-0.688	0.507

^a t statistic. ^b Probability value.

As it can be inferred from data in Table 3, the overall effect of the linear terms is statistically significant at the 95% confidence level ($p < 0.05$). The interaction terms are not significant at the 95% confidence level. The coefficients of the nonlinear polynomial model and p values are reported in Table 4.

In order to gain insight about the effect of each factor and also analyze the variation of the response surfaces, the three dimensional (3D) graphs for the responses were plotted based on the nonlinear polynomial model as shown in Fig. 2. These figures show the relationship between two factors and response at the center level of other factors. It can be seen that in more acidic solutions, the response is higher (see Fig. 2b and Fig. 2c). Moreover, it is clear that in relatively higher concentrations of the 1-naphthylamine and moderate concentrations of cefixime, the response is higher. Moreover, curvatures in the direction of the change in the concentration of cefixime can be seen (see Fig. 2a and Fig. 2b). This

can also be inferred from the ANOVA table (Table 4) which the p value for the square of the concentration of cefixime is relatively low. On the other hand, variation in the response with concentration of 1-naphthylamine is highly linear (see Fig. 2a and Fig. 2c). Exploring the p values of the square terms shows that this term for 1-naphthylamine is relatively large. Amongst the linear terms, pH is highly significant since its calculated p value is very low (0.004).

In the next step, response surface optimization was used to find the optimal conditions of the factors to achieve higher responses. The results showed that response can be higher at pH 1, and concentration of cefixime and 1-naphthylamine of 370.0 and 28.0 mg L⁻¹, respectively. As was expected, in more acidic solutions, the response is higher. At these pHs, nitrite exists predominately as nitrous acid. It is worth mentioning that we chose room temperature as the temperature at which the reactions were conducted.

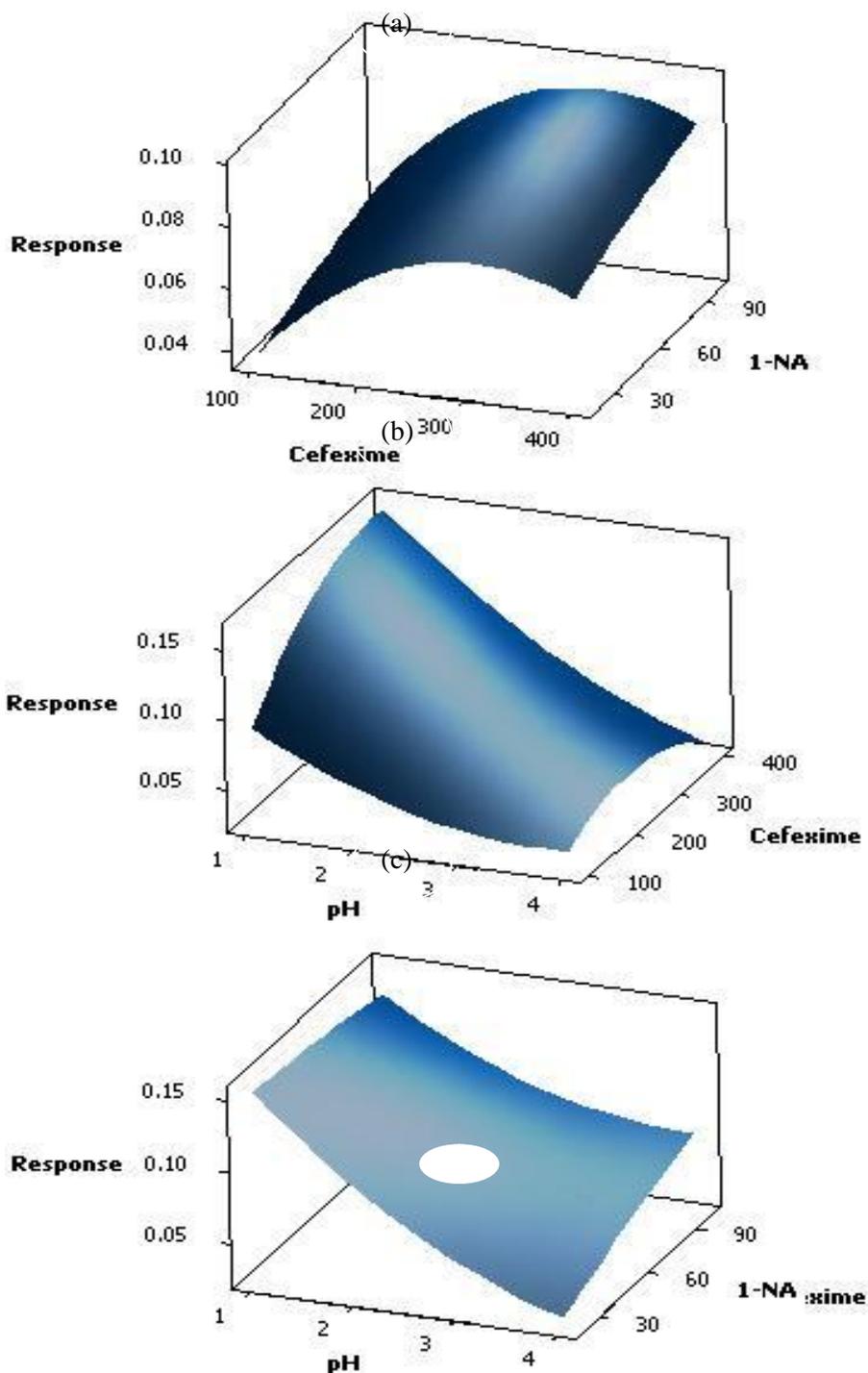


Fig. 2. Response surfaces obtained based on the model with the coefficients reported in Table 4 for pH, concentration of 1-naphthylamine and concentration of cefixime.

Analytical data

In order to assess the efficiency of the proposed method for determination of nitrite, the absorbance of a series of solutions containing varying concentrations of nitrite in the optimal conditions were recorded

against the corresponding reagent blank at 360 nm. The univariate calibration curve was linear in the range of 0.02–15.00 mg L⁻¹ evaluated by linear regression. The statistical parameters of the constructed calibration curve have been summarized in Table 5.

Table 5 Statistical results of the calibration of nitrite using the proposed method.

Parameters	Characteristic
Color	Light Orange
wavelength max (nm)	360
Linear range (mg L ⁻¹)	0.02-15.00
Number of samples	18
Intercept of calibration curve	0.0273
Slope of calibration curve	0.0878
Correlation coefficient	0.9979
F statistic of the model	2308.46
Detection limit (mg L ⁻¹)	4.3×10 ⁻³
Quantification limit (mg L ⁻¹)	0.014

Effect of foreign ions

The effects of several species that can occur in the various samples together with nitrite were examined. This helped us to evaluate the validity of the method as the process of determination was progressing. The tolerance limit of a potentially interfering ion was taken as its maximum amount causing an error of $\pm 5\%$

during determination of nitrite in soil and water samples. The tolerance limits for the ions studied are given in Table 6. Metal ions such as NO₃⁻, Mg²⁺, HCO₃⁻, Fe²⁺ and Fe³⁺ were proved to interfere. The anions were used as sodium and potassium salts and the cations as chlorides salts.

Table 6 Tolerance limit of different ions in determination of nitrite with concentration of 0.1 mg L⁻¹

Foreign ion	Added as	Tolerance limit (mg L ⁻¹)
Ca ⁺²	CaCl ₂	15
CN ⁻	KCN	13.4
HCO ₃ ⁻	NaHCO ₃	7
Fe ⁺²	FeCl ₂ .4H ₂ O	3.3
Fe ⁺³	FeCl ₃ .6H ₂ O	less than 2
Mg ⁺²	MgCl ₂	6.7
NO ₃ ⁻	KNO ₃	less than 2

Application of the method

For examining the suitability of the recommended method for nitrite determination in real samples, it was

applied to soil, tap, rain and wastewater samples. The results of the analyses have been collected in Table 7. The results illustrates that the accuracy and the precision of the method are satisfactory (lower than 9%).

Table 7 Application of the recommended method to determination of nitrite in soil, tap, rain and wastewater

Sample	Added (mg L ⁻¹)	Found (mg L ⁻¹)	Relative standard deviation (RSD %)	% RE
Tap water	0.00	Not detected		
	1	1.08	7.57%	8.85%
Waste water	0.00	Not detected		
	0.50	0.47	7.14%	5.76%
Rain water	0.00	0.86	2.09%	
	2	2.16	7.83%	8.02%
Soil	0.00	0.39	2.35%	
	2	2.07	4.56%	3.92%

Conclusion

This paper proposes a simple spectrophotometric method for fast determination of nitrite in soil and water samples with no need for pretreatment or extraction. Applying the method to real samples resulted in satisfactory precision and accuracy. The low detection limit proved that the method in question was sensitive. The results of experimental design showed that an acidic pH is suitable for the studied modified Griess reaction. This method offers a simple, economical and rapid alternative to previous methods for nitrite determination.

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