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Spectrophotometric determination of nitrite and nitrate using phosphomolybdenum blue complex

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Abstract

A method for spectrophotometric determination of nitrite and nitrate is described. This method is based on the reduction of phosphomolybdic acid to phosphomolybdenum blue complex by sodium sulfide. The obtained phosphomolybdenum blue complex is oxidized by the addition of nitrite and this causes a reduction in intensity of the blue color. The absolute decrease in the absorbance of the blue color or the rate of its decrease is found to be directly proportional to the amount of nitrite added. The absorbance of the phosphomolybdenum blue complex is monitored spectrophotometrically at 814 nm and related to the concentration of nitrite present. The effect of different factors such as acidity, stability of the complex, time, temperature, phosphate concentration, molybdenum concentration, sodium sulfide concentration and the tolerance amount of other ions have been reported. Maximum absorbance is at 814 nm. The range of linearity using the conventional method is 0.5–2.0 ppm with molar absorptivity of 1.1×10^4 l mol^{-1} cm^{-1}. and a relative standard deviation of 2.6% for five measurements. The range of linearity using the reaction rate method is 0.2–3.6 ppm with a relative standard deviation of 2.4% for five measurements. The method is applied for determination of nitrite and nitrate in water, meat products and vegetables.

Keywords: Nitrate; Nitrite; Phosphomolybdenum blue; Spectrophotometry

1. Introduction

Nitrite is a characteristic pollutant [1]. It can react with secondary amines present in the body resulting in the formation of carcinogenic nitrosoamines [2–4]. On the other hand, when present at high concentration in blood nitrite can react with iron(III) of the hemoglobin, forming methemoglobin which has no oxygen-carrying ability. This fatal disease is called methemoglobinemia. Nitrate also at high concentrations can be considered as pollutant since it can be reduced to nitrite. Therefore, food and drinking water with high concentration of nitrate are also dangerous. The reduction of nitrate to nitrite is possible in the stomach of infants, where the low acidity allows the growth of nitrite-reducing microorganisms.

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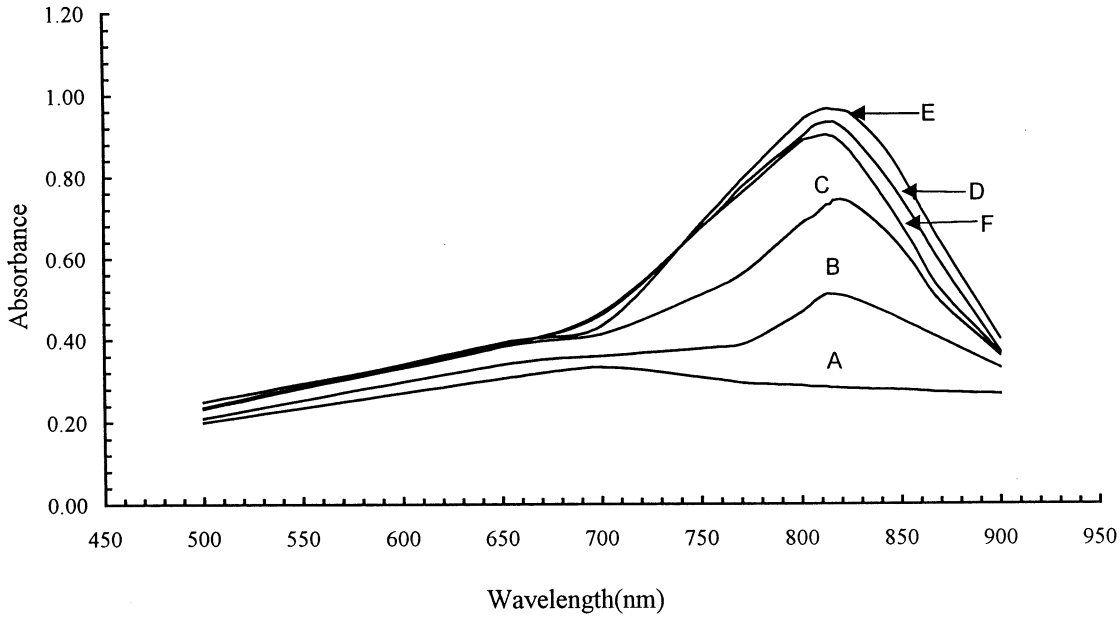

Fig. 1. Absorption spectra of phosphomolybdenum blue complex as a function of hydrochloric acid concentration: (A) 0.02 M, (B) 0.20 M, (C) 0.50 M, (D) 1.0 M, (E) 1.46 M, (F) 2.0 M. Conditions: [phosphate] = 3.0×10^{-3} M, [molybdenum(VI)] = 9.0×10^{-3} M, [sodium sulfide] = 3.0×10^{-4}%, temperature = 25°C, time = 30 min after mixing.

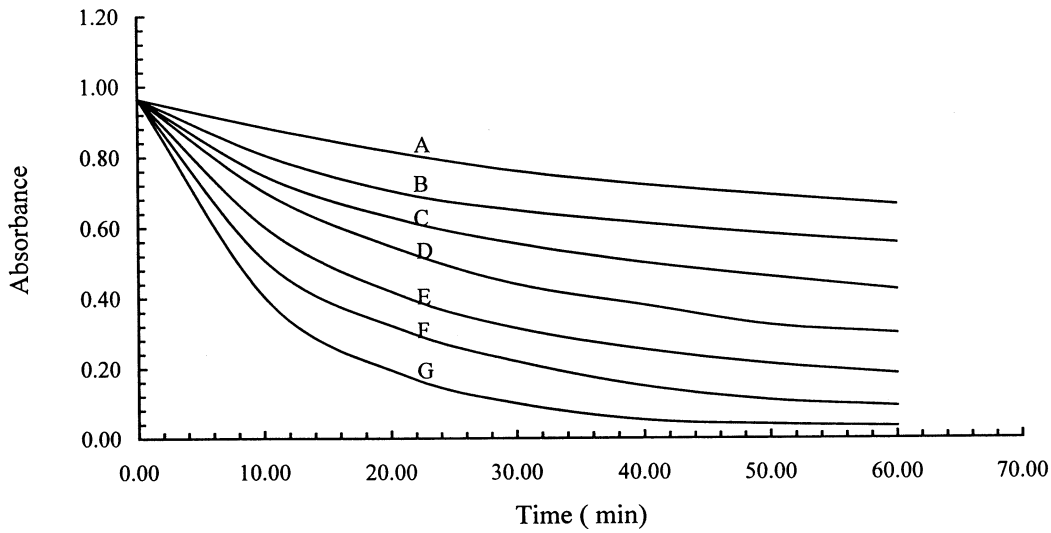

Fig. 2. Recorded absorbance–time curve for the reaction between nitrite and phosphomolybdenum blue complex. Nitrite concentration: (A) 1.1×10^{-5} M, (B) 2.0×10^{-5} M, (C) 3.0×10^{-5} M, (D) 4.0×10^{-5} M, (E) 5.0×10^{-5} M, (F) 6.0×10^{-5} M, (G) 7.0×10^{-5} M. Conditions: [phosphate] = 3.0×10^{-3} M, [molybdenum(VI)] = 9.0×10^{-3} M, [sodium sulfide] = 3.0×10^{-4}%, $\lambda = 814$ nm, temperature = 25°C.

Table 1
Calculated molar absorptivity and range of linearity as a function of time after mixing for absorbance measurement, using the conventional method

Time of measurement (min)	Molar absorptivity (l mol^{-1} cm^{-1})	Range of linearity (ppm)
10	0.70×10^4	0.50–4.20
20	1.00×10^4	0.50–3.70
30	1.10×10^4	0.50–3.00
40	1.20×10^4	0.50–2.90
50	1.24×10^4	0.50–2.50
60	1.25×10^4	0.50–2.40

Many methods have been reported for quantitative determination of nitrite and nitrate, including kinetic [5–8], chromatographic [9–11], potentiometric [12,13], amperometric [14], flow injection [15,16] and spectrophotometric [17–20] methods. Among the spectrophotometric methods that are adopted as an AOAC official method of analysis for nitrite and nitrate determination is its reaction with *N*-(1-naphthyl)ethylenediamine·2HCl and sulfanilamide [20]. This method requires careful control of acidity for each step of the process and causes a carcinogenic effect [21].

In the present work, a new method is proposed for the determination of nitrite and nitrate. It is based on the reduction of phosphomolybdic acid to phosphomolybdenum blue complex by sodium sulfide. The obtained phosphomolybdenum blue complex is oxidized by the addition of nitrite causing a reduction in intensity of the blue color. The decrease in the absorbance of the blue color is directly proportional to the amount of nitrite added. The absorbance of the phosphomolybdenum blue complex is monitored spectrophotometrically at 814 nm and related to the concentration of nitrite. The main advantage of the proposed method over the other methods is related to the short analysis time and the low detection limit.

2. Experimental

2.1. Chemicals

Unless otherwise stated, all chemicals and solvents used were of analytical reagent grade. Molybdenum(VI) solution 0.1 M was prepared by

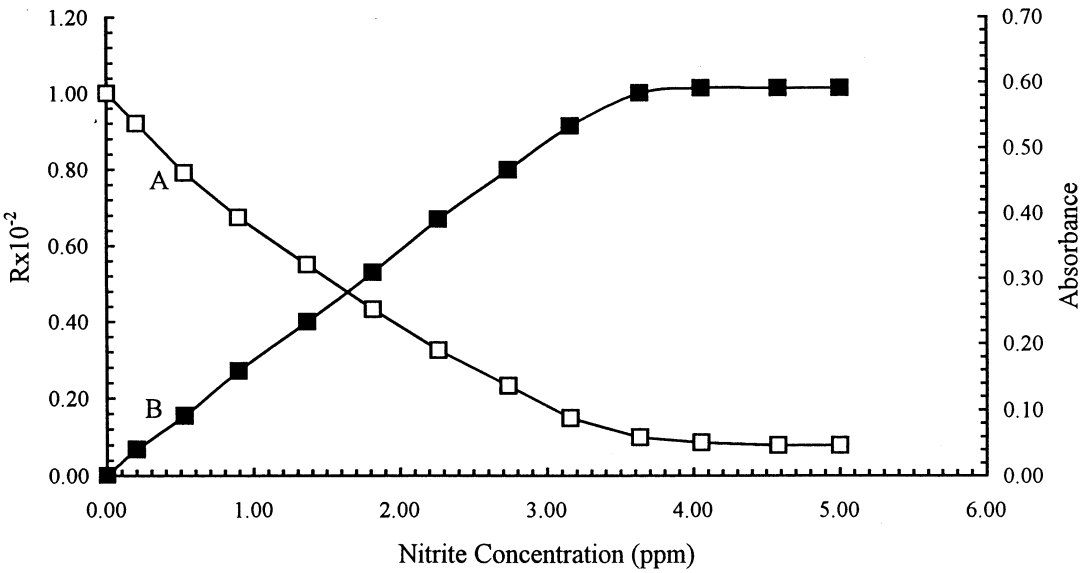

Fig. 3. Calibration curves for nitrite determination using (A) the conventional method and (B) the reaction rate method. Conditions: [phosphate] = 3.0×10^{-3} M, [molybdenum(VI)] = 9.0×10^{-3} M, [sodium sulfide] = 3.0×10^{-4} (w/v), $\lambda = 814$ nm, temperature = 25°C. Absorbance was measured at $t = 30$ min (conventional method) and $t = 5$ min (reaction rate method).

Table 2
Interference effect of other ions on the determination of 2.1 ppm of nitrite using the proposed method

Foreign ion	Nitrite:foreign ion mole ratio	Concentration of foreign ion[a] (ppm)	Error[b] (%)
Pb^{2+}	1:20	207.0	+20.0
Pb^{2+}	1:10	103.0	+9.0
Pb^{2+}	1:2	20.7	+5.0
Fe^{+3}	1:7	20.6	−8.0
Fe^{+3}	1:5	14.7	−1.0
Fe^{2+}	1:5	14.7	−12.0
Fe^{2+}	1:3	8.9	−5.5
Zn^{2+}	1:4	13.0	−11.0
Zn^{2+}	1:3	9.8	−6.0
Cu^{2+}	1:8	25.4	+10.0
Cu^{2+}	1:4	12.7	+6.0
Ni^{2+}	1:11	32.5	−9.0
Ni^{2+}	1:5	17.7	−4.0
Sn^{2+}	1:10	59.5	−11.0
Sn^{2+}	1:6	35.7	−6.0
Co^{2+}	1:11	32.0	−12.0
Co^{2+}	1:3	8.8	−5.0
Ag^{+}	1:9	48.0	−10.0
Ag^{+}	1:5	26.7	−6.0
I^{-}	1:7	44.5	−25.0
I^{-}	1:4	25.5	−7.0
I^{-}	1:1	6.3	−3.5
NO_3^{-}	1:20	62.0	+9.0
Cl^{-}	1:1000	1720.0	±3.0
Br^{-}	1:1000	4000.0	±3.0
CH_3COO^{-}	1:1000	2950.0	±3.0

[a] Final concentration of foreign ion in the reaction solution.
[b] +, positive interference, causing decrease in absorbance at 814 nm (oxidation of PMBC to PMA); –, negative interference, causing increase in the absorbance at 814 nm (reduction of PMA to PMBC); ±, no interference.

weighing accurately 1.44 g of MoO_3 and dissolving it in 40 ml of 1 M NaOH; the volume was completed to 100 ml with water. Potassium dihydrogen phosphate solution 0.10 M was prepared by weighing accurately 1.33 g of KH_2PO_4 and dissolving it in water in a 100-ml volumetric flask. Sodium sulfide solution 0.01% (w/v) was prepared by weighing accurately 1.0 g of Na_2S and dissolving it in water in a 100-ml volumetric flask. Nitrite solution 0.10 M was prepared by dissolving exactly 0.69 g of $NaNO_2$ in water in a 100-ml volumetric flask; working solutions were prepared by diluting volumes of the stock solution to known volumes with water. Nitrate standard solution 0.10 M was prepared by dissolving exactly 0.85 g of $NaNO_3$ in water in a 100-ml volumetric flask; working solutions were prepared by diluting volumes of the stock solution to known volumes with water. Other solutions used for the interference study were prepared by dissolving the corresponding salt in water. Modified Jones reductor was prepared as described in the AOAC official methods of analysis [20].

2.2. Apparatus

All spectrophotometric measurements were carried out using a UV-2, Unicam UV–vis spectrophotometer. Constant temperature cell holder was used for absorbance measurements. The cells used for absorbance measurements were 1×1 cm glass cells. A Hanna 8521 model pH meter was used for pH measurements.

Table 3
Published methods for spectrophotometric determination of nitrite

Reaction system[a]	Range of linearity (ppm)	Reference
Sulfanilamide	10–1000	[20]
+ NED		
PCPH + BrO^{3-}	40–920	[5]
Thionine	0.3–55	[6]
+ BrO^{3-}		
Prochlorpenzine	0.8–70	[25]
+ BrO^{3-}		
Bindeschler's Green + Br_2	50–400	[26]
PCA + DAP	0.002–0.008	[27]
TAPP	0.00018–0.0018	[15]
Chlorpromazine	3–1500	[8]
+ H_2O_2		
PMBC	0.5–2.0	Proposed conventional method
PMBC	0.2–3.6	Proposed initial rate method

[a] NED, *N*-(1-naphthyl)ethylendiamine; PCA, *p*-chloroaniline; PCPH, pyridine-2-carbiadehyde-2-pyridylhydrazone; PMBC, phosphomolybdenum blue complex; TAPP, 5,10,15,20-tetrakis(4-aminophenyl)prophine.

3. Procedures

3.1. Preparation of phosphomolybdenum blue complex

A 30-ml volume of 0.1 M molybdenum(VI) solution is transferred into a 100-ml volumetric flask, then 10 ml of 0.10 M potassium dihydrogen phosphate solution is added, followed by 10 ml of 0.01% (w/v) sodium sulfide solution and 13 ml of 11.2 M HCl, in that order. The volume is completed with water. The absorbance of the solution is measured after 30 min at 814 nm against water as a blank in a thermostatted bath at 25 ± 0.2°C.

3.2. Spectrophotometric determination of nitrite

An aliquot of solution containing nitrite ions in the range 4.60–36.00 ppm is transferred into a 10-ml volumetric flask. Then 3.0 ml of phosphomolybdenum blue complex is added and the volume is completed with water. A portion of the solution was placed in the cell and the absorbance–time curve was recorded at 814 nm against water as a blank in a thermostatted bath at 25 ± 0.2°C. The concentration of nitrite can be calculated either by measuring the absorbance of the solution after exactly 30 min at 814 nm against water as a blank, or, in a different approach, by measuring the slope dA/dt of the reaction curve at 5 min after initiating the reaction.

3.3. Spectrophotometric determination of nitrate

A 5-ml portion of solution containing an amount of nitrate in the range 10.00–100.00 ppm is transferred into the modified Jones reductor where the flow rate is adjusted to 3–5 ml/min. The reductor is then washed with 5 ml of water. The nitrite solution obtained from the reductor is treated as described in the above procedure for spectrophotometric determination of nitrite.

Table 4
Analytical results of nitrite and nitrate determination in water, meat and vegetables

Sample analyzed	Nitrite found (ppm, average ± S.D.)[a]		Nitrate found (ppm, average ± S.D.)[a]	
	Proposed method	AOAC official method [20]	Proposed method	AOAC official method [20]
Well water	None	None	9.9 ± 0.1	8.1 ± 0.2
Corned beef	24.2 ± 0.2	24.4 ± 0.1	13.1 ± 0.5	12.0 ± 0.7
Fresh tomato	None	None	10.2 ± 1.0	9.3 ± 0.5
Fresh cucumber	None	None	75.5 ± 2.0	80.5 ± 1.8

[a] Average of three separate measurements.

3.4. Preparation of real samples for analysis

For water samples, an appropriate volume of water is treated using the above procedure for the determination of nitrite and nitrate.

For meat samples, about 25 g of meat are weighed out accurately, minced and transferred into a 250-ml beaker. Then 50 ml of water is added and the mixture is heated to 80°C for 15 min and then transferred into a 250-ml volumetric flask. Enough hot water is added to bring the volume to about 200 ml. The flask is transferred to a steam bath for 2 h with occasionally shaking. The solution is cooled to room temperature. The volume is completed to 250 ml with water, filtered and centrifuged to clear. The concentration of nitrite and nitrate are determined following the procedures given above.

For vegetable samples, about 100 g of vegetable are weighed out accurately and blended into 400 ml of water for about 5 min. The solution is filtered and the above procedures are followed for the determination of nitrite and nitrate content.

4. Results and discussion

It was found that the reduction of phosphomolybdic acid to phosphomolybdenum blue by sodium sulfide and the oxidation of phosphomolybdenum blue by nitrite are affected by many factors. In the present work each of these factors is studied carefully in order to optimize the conditions for spectrophotometric determination of nitrite and nitrate.

4.1. Absorption spectra of phosphomolybdenum blue complex

Different workers [22–24] have reported different absorption spectra with a different wavelength of maximum absorbance for the phosphomolybdenum blue complex. In the present work it was found that when sodium sulfide is used as a reducing agent for phosphomolybdic acid and by the addition of hydrochloric acid an intense blue color is developed. The shape of the absorption spectra and the wavelength of maximum absorbance are found to vary by changing the concentration of hydrochloric acid in the solution. Fig. 1 shows the absorption spectra as a function of hydrochloric acid concentration in the solution. Comparison among these spectra show that maximum absorbance for the solution containing 0.02 M hydrochloric acid is obtained at 700 nm. A new absorption peak develops at 814 nm upon increasing the concentration of hydrochloric acid. On the other hand, increasing the acidity causes a gradual increase in the absorbance at 814 nm, while the absorbance at 700 nm remains almost fixed. The absorbance at 814 nm reached its maximum value at hydrochloric acid concentration of 1.46 M (Fig. 1) beyond which any further increase in the acidity caused a decrease in the absorbance. The results obtained suggested an absorbance wavelength of 814 nm and a hydrochloric acid concentration of 1.46 M as optimum for further work.

4.2. Effect of changing phosphate to molybdenum mole ratio on the absorbance of phosphomolybdenum blue complex

The effect is studied for solutions which contained 9.0×10^{-3} M molybdenum and various concentrations of phosphate. The solutions were prepared as described in the general procedure. The results obtained showed that increasing the concentration of phosphate leads to an increase in the absorbance, up to phosphate to molybdenum mole ratio of 1:3. Any further increase in the phosphate concentration affects a gradual decrease in the absorbance.

4.3. Effect of sodium sulfide concentration on the absorbance of phosphomolybdenum blue complex

The effect is studied for solutions containing fixed concentrations of molybdenum, phosphate and hydrochloric acid and various amounts of sodium sulfide. The solutions are prepared as described in the general procedure under the preparation of phosphomolybdenum blue complex. The results obtained showed that increasing the concentration of sodium sulfide results to an increase in the absorbance up to a concentration of 5.0×10^{-3}% (w/v), beyond which a brown precipitate is formed.

4.4. Effect of time on formation of phosphomolybdenum blue complex

The effect of time on the absorbance of phosphomolybdenum blue complex is studied for solution containing 9.0×10^{-3} M molybdenum, 3.0×10^{-3} M phosphate, 3.0×10^{-4} M sodium sulfide and 1.46 M hydrochloric acid. The solution is prepared as described in the general procedure and the results obtained showed that the absorbance increases gradually as a function of time and reached its maximum value after 30 min. The intensity of the color remained constant for at least 24 h after preparation of the sample.

4.5. Effect of time on the reaction between nitrite and phosphomolybdenum blue complex

The reaction between nitrite and phosphomolybdenum blue complex is studied as a function of time for solutions containing different amounts of nitrite and prepared as described in the general procedure. The results obtained (Fig. 2) showed that the absorbance of the solutions decreases gradually with time due to oxidation of phosphomolybdenum blue complex by nitrite. The reaction is slow and is attained after 60 min. It can be seen from the Fig. 2 that the sensitivity of the method is inversely proportional to time of reaction. In order to obtain maximum sensitivity the absorbance should be measured after 60 min. This can be considered as time-consuming. For the method to be more convenient for quantitative analysis the absorbance is measured after exactly 30 min.

4.6. Effect of temperature

The effect of temperature on the absorbance of two solutions prepared as described in the recommended procedure was studied. Solution A contained phosphomolybdenum blue complex and solution B contained nitrite and phosphomolybdenum blue complex. The results obtained showed that the absorbance for both solutions decreases gradually by increasing the temperature. Increasing the temperature above 70°C caused a color change from blue to light green. This change in color and the decrease in the absorbance of the solution could be due to the decomposition of the phosphomolybdenum blue complex. In the present work all measurements were carried out in a thermosatted bath at 25 ± 0.2°C.

4.7. Calibration curves and sensitivity

4.7.1. Using the conventional method

From the investigation of the variables that effect the absorbance, the conditions for the color development and the absorbance measurements were selected. Following the recommended procedure, reciprocal dependence was obtained between nitrite concentration and the corresponding absorbance. The range of linearity, the sensitivity and the calculated molar absorptivity were found to vary with the time of measurement (Table 1). It can be seen (Table 1) that the sensitivity and the molar absorptivity increase as the time of measurements increase while the linearity of the calibration curve is decreased. In order to simplify the method, the measurements were taken at 30 min, at which the linearity was in the range 0.5–2.0 ppm and the detection limit was 0.2 ppm, as shown in Fig. 3. The molar absorptivity was calculated to be 1.1×10^4 l mol^{-1} and the relative standard deviation (R.S.D.) was 2.6% for five measurements. Higher sensitivity can be achieved by measuring the absorbance at 60 min.

The reaction rate method has been applied for determination of nitrite by plotting ΔA $(A_0 - A_t)$ against concentration of nitrite. The results obtained (Fig. 3) show that the linearity of the calibration curve using the reaction rate method is in the range 0.2–3.6 ppm with a detection limit of 0.2 ppm. The R.S.D. was 2.4% for five measurements.

It can be concluded from the results that the linearity of the calibration curve and the time of analysis using the reaction rate method is much better than that using the conventional method. Also it can be seen from Fig. 3 that the absorbance of phosphomolybdenum blue complex decreases until the signal has stabilized with nitrite concentration, indicating that the reaction is stoichiometrically dependent on the nitrite concentration.

4.8. Interference studies

The effect of other ions on the determination of nitrite using the proposed method is studied for solutions containing 2.1 ppm nitrite and prepared as described in the general procedure. The results obtained are presented in Table 2. Passing the sample through a cation exchanger in the hydrogen form (such as Amberlite IR-120) before the general determination procedure is carried out can eliminate the interference from the cations.

4.9. Comparison with other published methods

Table 3 compares the results obtained for the determination of nitrite using the proposed method with that of published methods. The proposed method competes well from sensitivity, precision and interference of other ions with most of the published methods.

4.10. Applications

The proposed method is applied successfully to the determination of nitrite and nitrate in water samples, meat product and vegetables. The results obtained (Table 4) are compared with those obtained using the AOAC official method of analysis [20].

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