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A New Analytical Method for the Kinetic Spectrophotometric Determination of Trace Amounts of Nitrite in Some Environmental Water Samples

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Abstract

Nitrite is a characteristic pollutant ion, which is ubiquitous within environmental and physiological systems, and occurs in water as an intermediate during the nitrogen cycle. Therefore, the detection and determination of its concentration at trace levels is extremely important in biological and environmental studies. A simple, sensitive and rapid kinetic spectrophotometric method for the determination of nitrite in different natural and wastewater samples is presented. The reaction is initiated by adding the known volumes of a nitrite ion solution of identified concentration to acid solutions of Cresyl violet perchlorate, which the color of indicator reagent changes to yellow with time. Then, the absorbance changes are monitored spectrophotometrically at λ_{max}= 585 nm for the concentration range of 0.01-2 µg mL^{-1} and at λ_{max}= 409 nm for the concentration range of 0.5-5 µg mL^{-1} of nitrite ion at 40°C. The detection limit and quantification limit of the proposed kinetic method were 0.00627 and 0.0188 µg mL^{-1} respectively. The RSDs for the determination of 0.2 and 1.5 µg ml^{-1} of nitrite were 4.98% and 2.08% for five replicate measurements, respectively. The effect of possible interfering ions on the determination of nitrite is described. Many of cations and anions have no interfering effect but Fe^{3+}, Fe^{2+}, Cd^{2+}, I^-, N_3^-, WO_4^{2-}, S^{2-}, SO_3^{2-} and $S_2O_3^{2-}$ ions do interfere. The accuracy and validity of the proposed kinetic method was established by studying recovery of spiked nitrite ion and by parallel determination using a standard method based on diazo-coupling reaction. The method was successfully applied to the determination of nitrite in various natural and wastewater samples.

Keywords:

Kinetic spectrophotometry, UV-Visible region, nitrite ion, Cresyl violet perchlorate

1. Introduction

Nitrite is ubiquitous within environmental and physiological systems, and occurs in water as an intermediate during the nitrogen cycle. Traces of nitrite in environmental samples give an excellent indication of the extent of pollution and eutrophication. It is known that nitrite can interfere with the oxygen-transport system in the body, and may result in a condition known as methaemoglobinaemia, in which the ability of haemoglobin to exchange oxygen is seriously reduced [1]. Furthermore, nitrite can produce nitrosoamine, a carcinogenic material within the acidic conditions of the stomach, and then have subsequent implications in the pathology of gastric cancer [2]. Infants under three months are thought to be more susceptible than adults [3]. The oral lethal dose for humans was estimated to range

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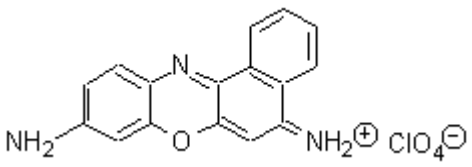
from 33 to 250 mg of nitrite per kg of body weight, the lower doses applying to children and elderly people. Toxic doses giving rise to methaemoglobinaemia ranged from 0.4 to 200 mg of nitrite per kg body weight [4]. Due to these toxic effects, it is important that sensitive and accurate methods are available for the determination of nitrite ions.

Until now, many approaches for nitrite determination have been put forward such as spectrophotometry [5-8], ion chromatography [9], voltammetry [10], spectrofluorometry [11, 12], chemiluminescence [13], and kinetic methods [14, 15]. These methods have been reviewed by Moorcroft et.al. [16]. Among them, spectrophotometric and fluorometric methods are widely used.

Various fluorometric methods have been reported for nitrite based on its effects on the fluorescence properties such as development [17, 18], inhibition [19,20] and enhancement [21], either in a direct or an indirect way. However, some of those methods are time consuming, heating is necessary, solvent extraction is necessary or the procedures are rather complicated [22]. A number of different fluorescent reagents have been developed. For example, Zhang et.al. [23-26] synthesized several fluorescent probes for the determination of nitrite in real samples with good sensitivity. Whereas, the methods require incubation, and the preparation of the reagents is inconvenient.

Spectrophotometry can be considered the classical detection principle for the determination of nitrite. The most widely used methods for the determination of nitrite are based on the well-known Griess reaction [27]. Nitrite reacts with a primary aromatic amine to form a diazonium salt. This is then coupled with another aromatic compound to form an azo dye of which the absorbance is spectrophotometrically measured [28]. Although the Griess reaction offers the advantage of sensitivity, it also has several limitations. For example, control of the pH, temperature and concentration of reagents are critical, and also the method causes a carcinogenic effect [29]. Increased precautions are necessary in the case of this kind of method based on the use of azo dyes. Griess reagents deteriorate during storage, even when refrigerated. The color-developing reaction has to be timed due to instability of the dye and to numerous side reactions. The kinetic spectrophotometric method is one of the most attractive approaches for the trace determination of some species. Several kinetic methods have been proposed for the determination of nitrite at trace amounts in literature [14, 15, 30-32].

By considering the important role of nitrite ion in human life, it still needs to new, sensitive, reliable, simple and fast analytical methods for the determination of trace amount of nitrite ion in real samples such as natural waters (hot- and cold-spring water), wastewater and foodstuffs by kinetic spectrophotometric method based on reduction of Cresyl violet perchlorate in acidic media.



The open molecular structure of Cresyl Violet Perchlorate (HIn^+)

This study describes a kinetic spectrophotometric method for the determination of nitrite using Cresyl violet perchlorate as a chromogenic indicator reagent. This new approach is based on the reaction of nitrite with Cresyl violet perchlorate, which is chemically known as 5-imino-5H-benzo[a] phenoxazin-9-amine monoperchlorate or oxazine 9 perchlorate, to give a new yellow-colored decay intermediate showing also an absorbance increasing with increasing nitrite concentration at 409 nm in approximately 0.2-0.3 mol L^{-1} H_2SO_4 media. The

method was successfully applied to the determination of nitrite in various natural and wastewater samples.

2. Experimental

2.1. Apparatus

In this study, a Shimadzu Model UV-Visible 1601 PC spectrophotometer equipped with a 1 cm quartz cell was used for absorbance measurements. This spectrophotometer has a wavelength accuracy of ±0.2 nm and a bandwidth of 2 nm in the wavelength range of 190–1100 nm. A TCC-140A mark temperature-controlled cell holder to this instrument was attached for absorbance measurements at a fixed wavelength. In order to control the temperature of reaction medium with an accuracy of ±0.1 ^{o}C, a Grant LTG-6G model thermostatic water bath regulated at the desired temperature operating in the range of -20 and 100 ^{o}C was used. A stopwatch was used for recording the reaction time. A pH meter consisting of a glass-calomel electrode double was used to determine pH values of solutions. Two standard buffer solutions of pH 7±0.01 and pH 4±0.01 (Sigma) were used for the calibration of pH meter. All solutions were preheated to an optimal temperature of 40±0.1^{o}C shortly before the initiation of indicator reaction with and without nitrite ion. The absorbance measurements were made at two separate working wavelengths of 585 nm and 409 nm.

2.2. Reagents and solutions

All chemicals used were of analytical reagent grade (Merck) or chemically pure grade and double distilled water was used for the dilution of reagents and samples. A Stock nitrite solution (1000 μg mL^{-1}) was prepared by dissolving 0.150 g sodium nitrite in water and diluting to 100 mL, preserved with 2 mL chloroform. The working solutions were prepared by appropriate dilution of each stock solution with doubly distilled water. The indicator dye reagent was prepared by dissolving 0.1 g Cresyl violet perchlorate and diluting to the mark in 100 mL in a volumetric flask with doubly distilled water. The reagent dye solution was stored in a brown bottle in a refrigerator. Sulfuric acid (2 M) was prepared by introducing 55.5 mL concentrated H_2SO_4 (95-97%, d= 1.84 g mL^{-1}) into a 500 mL volumetric flask and diluting to the mark with distilled water. Stock solutions of 1000 μg mL^{-1} of interfering ions were prepared by dissolving appropriate amounts of suitable salts of each ion in distilled water, HNO_3 or NaOH.

2.3. General procedures

An aliquot of the sample solution containing 0.01-2 μg mL^{-1} of nitrite or 0.5-5 μg mL^{-1} of nitrite ion was transferred to a series of 10 mL calibrated flasks. To this solution 1.5 mL of 3.11x10^{-3} mol L^{-1} Cresyl violet perchlorate, 1 mL of 2 mol L^{-1} H_2SO_4. The contents were diluted to 10 mL using doubly distilled water and mixed thoroughly for 5 minutes to allow the reduction reaction to go to completion. The reagent blank was prepared in the same way, excluding the analyte. After 5 minutes, the absorbance changes of the indicator dye and yellow colored-decay intermediate was monitored at 585 nm and 409 nm against the reagent blank at 40^{o}C. The absorbance change, ΔA corresponding to the nitrite ion concentration was obtained by subtracting the absorbance of the test solution from that of the blank. The amount of nitrite present in the unknown solution was computed from the calibration graphs.

2.4. Determination of nitrite in water samples

A 5 mL of water sample containing not more than 10 μg mL^{-1} of nitrite was treated with 0.5 mL of 1 mol L^{-1} NaOH and 0.5 mL of 0.2 mol L^{-1} EDTA. The solution was

thoroughly mixed and centrifuged to remove any precipitate formed. The centrifugate was transferred to a 10 mL standard flask and directly used for the disappearance of the dye color at 585 nm or the appearance of the decay intermediate color at 409 nm in the presence of nitrite ion by following the procedure described above. The concentration of the nitrite was established by reference with a calibration graph prepared using 0.01-2 μg mL^{-1} of nitrite or 0.5-5 μg mL^{-1} in 10 mL standard flasks using distilled water [33].

3. Results and Discussion

Cresyl violet perchlorate is a cationic dye (HIn^{+}) belonging to the phenoxazine class [5-imino-5H-benzo[a] phenoxazin-9-amine monoperchlorate or oxazine 9 perchlorate] has a maximum absorption at 585 nm. It finds application in different areas wherein its redox chemistry plays an important role. The reaction can be followed spectrophotometrically at 585 nm for 0.01-2 μg mL^{-1} and 409 nm for 0.5-5 μg mL^{-1} of nitrite ion trace concentration. The reduction reaction was very slow in the absence of sulfuric acid. The absorbance of Cresyl violet has decreased and increased with time in the presence of nitrite ion at 585 nm and 409 nm respectively as shown in Fig.1.

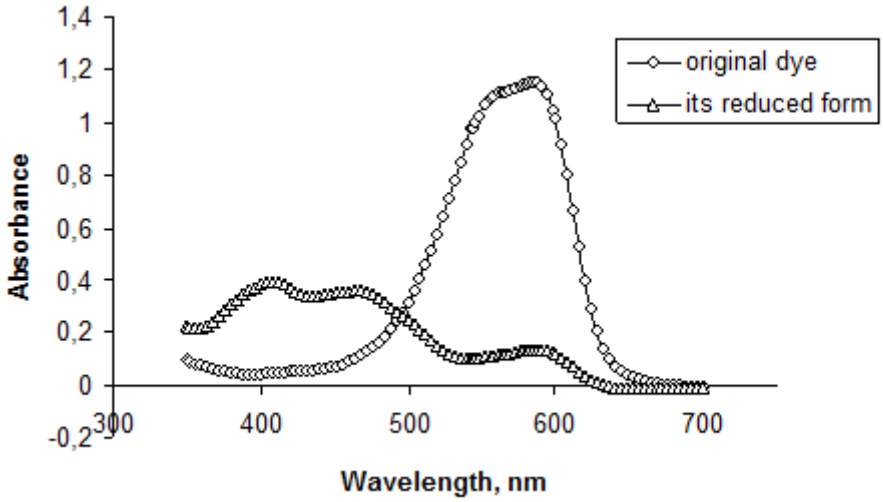


Fig.1. UV-Visible spectra of Cresyl violet and its redeuced form in the wavelength range of 350–800 nm. The optimum conditions: $5x10^{-5}$ M Cresyl violet, 0.3 mol L^{-1} H_2SO_4, 1 μg mL^{-1} NO_2^- and 5 min at 40°C.

3.1. The effect of acid concentration

To develop a quantitative analytical method based on this reduction reaction, preliminary studies were carried out to determine the most effective and optimum experimental conditions with of nitrite ion of 10 μg mL^{-1} in the final volume of 10 mL. The effect of acidity on the reaction rate was studied in the range 0.04- 0.44 mol L^{-1} sulfuric acid. Fig.2 shows the influence of sulfuric acid concentration on the rate of reaction between Cresyl violet and nitrite ion. The result show that the rate of reaction increased by increasing amount of sulfuric acid until 0.3 mol L^{-1}. At the higher acid concentrations the reaction rate decreased. This effect is due to the fact that in the presence of higher concentration of the acid, Cresyl violet is protonated, thus reducing the rate of reduction reaction in presence of nitrite ion. The optimum concentration of H_2SO_4 was chosen as 0.3 mol L^{-1} in final solution.

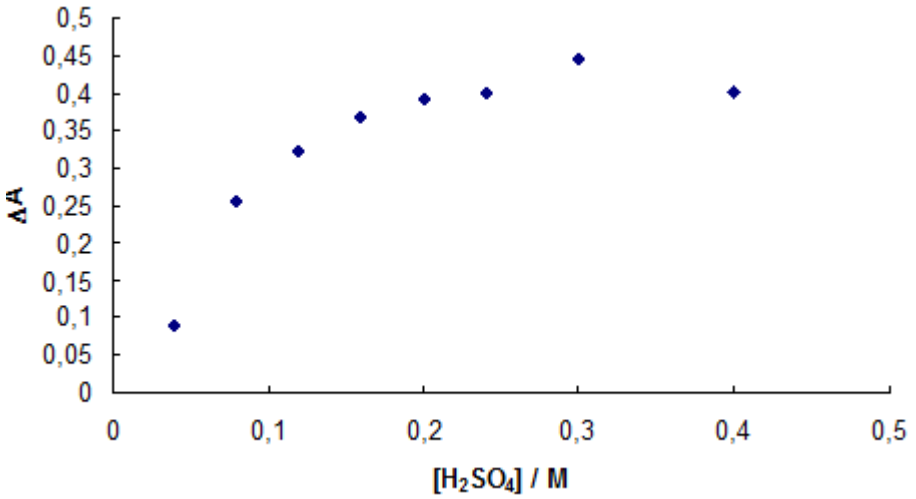


Fig.2. Effect of H_2SO_4 concentration on the reaction rate at λ_{max}= 585 nm. The optimum conditions: 2 mL $5x10^{-4}$ mol L^{-1} Cresyl violet, 1 µg mL^{-1} NO_2^- and 5 min at 40°C

3.2. The effect of indicator dye concentration

Figure.3 shows the influence of Cresyl violet concentration on the rate of reaction in the presence of sulfuric acid and nitrite. The effect of Cresyl violet concentration in the range of 0.75-7.5$x10^{-5}$ mol L^{-1} on the reaction rate was investigated. The data obtained were used for a plot of ΔA versus concentration of Cresyl violet as shown in Figure 2. The results show that a 2 mL 2.5$x10^{-4}$ mol L^{-1} Cresyl violet solution was sufficient to obtain a maximum reaction rate. Greater amounts of the Cresyl violet cause a decrease in the reaction rate. This is due to the fact that in higher concentrations of Cresyl violet in acidic media, Cresyl violet coagulated and precipitated in the solution. Thus, $5x10^{-5}$ mol L^{-1} of Cresyl violet was selected as optimal concentration for analytical purposes.

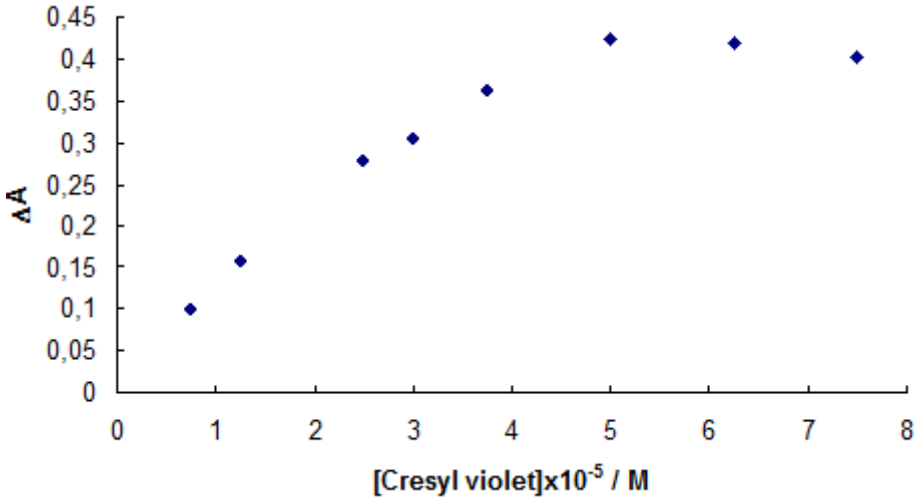


Fig.3. Effect of Cresyl violet concentration on the reaction rate at λ_{max}= 585 nm. The optimum conditions: 1.5 mL 2 mol L^{-1} H_2SO_4, 1 µg mL^{-1} NO_2^- and 5 min at 40°C

3.3. The effect of ionic strength

The ionic strength on the reaction rate of Cresyl violet and nitrite ion was considered by 2 M NaCl solution. In the whole of experiments the concentration of nitrite ion, Cresyl violet and sulfuric acid were 1 µg mL^{-1}, $5x10^{-5}$ mol L^{-1} and 0.3 mol L^{-1} respectively. The ionic

strength of solution was varied from 0.2 to 0.35 mol L^{-1} by using NaCl solution and no considerable effect on the reaction rate was observed.

3.4. The effect of the reaction time and temperature

The effect of time on reaction was shown in Figure.4. It is obvious that in the aqueous media the reaction is almost completed 10 minutes after mixing the reactants. With increasing time up to 5 min, the change in absorbance increased at a faster rate with the increasing slope in the plot of ΔA versus time. After that time, the change in absorbance increased at a relatively slower rate in the range of 5-13 min. Thus, a fixed time of 5 min was selected for optimum reaction time. Figure.5 shows the influence of temperature on the reaction rate. The effect of temperature was studied in the range of 15-60°C. With increasing temperature up to 40°C, the change in absorbance change increased for 0.5-5 minutes, whereas at higher temperature, the change in absorbance decreased. The temperature was fixed at 40°C due to give maximum absorbance change for subsequent experiments. The reaction rate increases by increasing temperature.

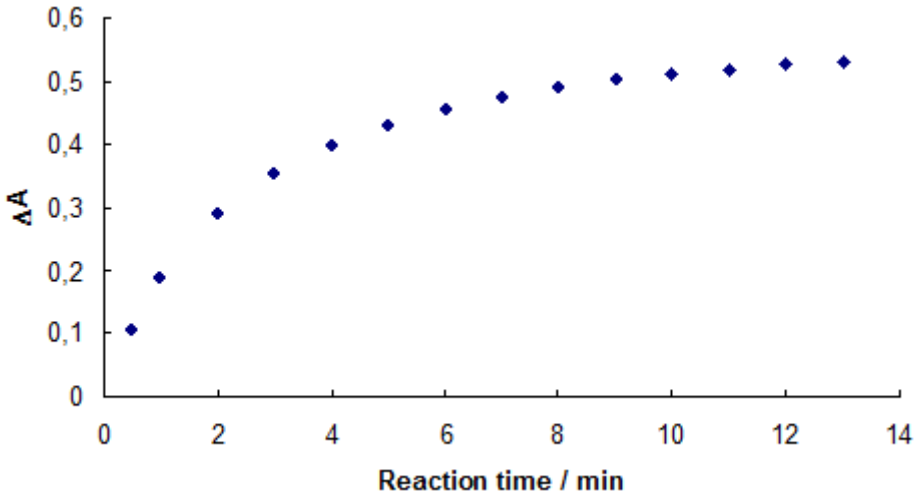


Fig. 4. Effect of reaction time on the reaction rate at λ_{max}= 585 nm. The optimum conditions: 1.5 mL 2 mol L^{-1} H_2SO_4, 2 mL 2.5x10^{-4} mol L^{-1} Cresyl violet and 1 µg mL^{-1} NO_2^- at 40°C

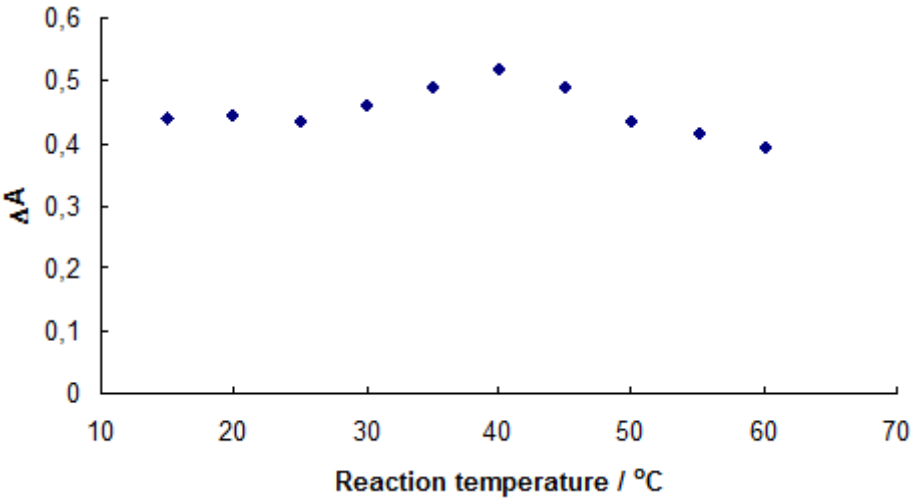


Fig.5. Effect of reaction temperature on the reaction rate for a reaction time of 5 min at λ_{max}= 585 nm. The optimum conditions: 1.5 mL 2 mol L^{-1} H_2SO_4, 2 mL 2.5x10^{-4} mol L^{-1} Cresyl violet and 1 µg mL^{-1} NO_2^-

3.5. Analytical Data

In the present study, it wasn't made an additional study about yellow colored-decay intermediate or by products after the reduction reaction and only final results have been taken into consideration. It was determined that nitrite ion can reduce the structures such as Cresyl violet as a redox function in sulfuric acid medium. It seems that H^+ ion with NO_2^- produce HNO_2. In 0.2-0.3 mol L^{-1} H_2SO_4 media, two moles of HNO_2 produce N_2O_3. The produced N_2O_3 takes part in an oxidation-reduction with $-NH_2$ function and second order amine function in the structure of Cresyl violet [34-39].

$$\left[HIn^{+}\right], violet \xleftrightarrow{NH_2^-,\ H^+} \left[H_2In^{2+}\right], yellow \qquad (1)$$

λ_{max}= 585 nm λ_{max}= 409 nm

Under the optimum conditions calibration curves were obtained by applying the fixed-time method. The calibration curves were linear in concentration range of nitrite for 0.01-2 µg mL^{-1} and 0.5-5 µg mL^{-1} in 585 and 409 nm, respectively.

For λ_{max}= 585 nm (The optimum conditions: 1.5 mL 2 mol L^{-1} H_2SO_4, 2 mL 2.5x10^{-4} mol L^{-1} Cresyl violet and 1 µg mL^{-1} NO_2^- for final volume of 10 mL at 40°C) the regression equation is:

$$\Delta A = 0.1642\ [NO_2^-]\ (\mu g\ mL^{-1}) - 0.0059,\ r^2 = 0.9959 \qquad (2)$$

For λ_{max}= 409 nm (The optimum conditions: 1 mL 2 mol L^{-1} H_2SO_4, 4 mL 2.5x10^{-4} mol L^{-1} Cresyl violet and 2 µg mL^{-1} NO_2^- for final volume of 10 mL at 40°C) the regression equation is:

$$\Delta A = 0.1483\ [NO_2^-]\ (\mu g\ mL^{-1}) - 0.0053,\ r^2 = 0.9954 \qquad (3)$$

The detection limit and quantification limit were obtained from $C_{DL}= 3S_{blank}/m$ and $C_{QL}= 10S_{blank}/m$. These values were 0.00627 and 0.0188µg mL^{-1} where S_{blank} is the standard deviation of blank signals for 10 replicate blank absorbance signals and m is the slope of the calibration curve [40]. The RSDs for the determination of 0.2 and 1.5 µg mL^{-1} of nitrite were 4.98% and 2.08% for five replicate measurements, respectively.

The maximum relative deviation between the result obtained by the proposed kinetic method and conventional standard method was less than 1.50% for nitrite ion and for water samples was near zero. The results showed no systematic error in the present method and thus indicate its reliability. A good agreement between this method and conventional standard method was obtained, slope or calibration sensitivity for 585 nm is 0.1642 and 409 nm is 0.1483.

3.6. Effect of interfering species

In order to assess the possible analytical applications of the proposed kinetic method, the effect of various interfering ions on the determination of 1.5 µg mL^{-1} of nitrite ion were studied. Variation in absorbance value of ±3% from that obtained for 15 µg of nitrite in the final volume of 10 mL in the absence of any interfering ions was taken into consideration as indication of interference. The tolerance limit of the various interfering ions studied is listed in Table 1.

Table 1. Tolerance levels different interfering ions on the determination of 1.5 μg mL^{-1} of nitrite ion in the optimal conditions

Interfering species	Tolerance levels (μg mL^{-1})
Formaldehyde, HCHO	5000
Nitrate, NO_3^-	3000
Zn(II), Mn(II) and SO_4^{2-}	2500
Ca(II), Cu(II), Co(II), Ni(II), Mg(II), Sr(II), Cr(III) andAl(III)	1000-1200
PO_4^{3-}, HCO_3^-, CO_3^{2-}, Cl^-, F^-, CH_3COO^-, $C_2O_4^{2-}$, As(V), Sb(V), tartarate and citrate	750-850
Ba(II)	350 (700[a])
Hg(II)	375
Bi(III), As(III) and Sb(III)	150-200
Fe(III), VO_3^-, IO_3^-, $Cr_2O_7^{2-}$	75-100
$S_2O_3^{2-}$, $S_2O_5^{2-}$ and $S_2O_8^{2-}$	75
MoO_4^{2-}, WO_4^{2-}, N_3^- and I^-	30-50
SCN^-, BrO_3^-	15
HSO_3^- and SO_3^{2-}	5 (25[b])
S^{2-}	10 (50[c])
Cd(II) and Fe(II)	15 (75[d])

[a]Treated with 1 mL of 2% K_2SO_4 and centrifuged. Centrifugate was used for the analysis.
[b]Treated with 1 mL of 1% HCHO solution prior to nitrite determination.
[c]Treated with 1 mL of 1000 μg mL^{-1} Zn^{2+} ion solution. Centrifuged and the centrifugate was used for the analysis.

As the result shown in Table 1, a large number of anions and cations examined have no considerable effect on the determination of nitrite ion. Cd^{2+}, Fe^{2+} and Fe^{3+} ions abundantly found in natural water samples interfered seriously, but Fe^{2+} was masked with 1.0 mL of 1% sodium tartarate or 1.0 mL of 1% ammonium fluoride and Fe^{3+} with 1.0 mL of 1% ammonium fluoride or 1.0 mL of 2% triethanolamine. Also, Cd^{2+} up to 75 μg mL^{-1} was masked with 0.5 mL of 1% sodium tartarate. The WO_4^{2-} , N_3^-, I^-, S^{2-}, SO_3^{2-} and $S_2O_3^{2-}$ ions do a serious interference. However, the higher concentration of sulfite (25 μg mL^{-1}) can be tolerated by the addition of 1 mL of 1% HCHO to the sample solution prior to nitrite determination. The interference of sulfide (50 μg mL^{-1}) was overcome by addition of 1 mL of 1000 μg mL^{-1} Zn(II) solution to the sample solution. The precipitated ZnS was removed and the clear supernatant liquid was taken for the analysis. Also, the elimination of the interference effect of W(VI) is possible by addition of Hg_2^{2+} ion, and the interference of N_3^-, I^- and $S_2O_3^{2-}$ ions may be removed by introducing Hg^{2+} ion to the solution [41]. Hence the proposed method shows considerable selectivity and it was applied to the determination of nitrite in various water samples without any prior separation.

3.6. Analytical applications

To confirm the analytical usefulness of the proposed kinetic method, the method was applied to some natural and waste waters for the determination of nitrite. The concentrations of common interfering species in natural and waste waters are generally far below their tolerance levels. Therefore, the kinetic method was directly applied to the determination of

nitrite in natural lake, river, spring waters and synthetically prepared wastewaters as shown in Table 3. A comparison with the standard APHA method [42] could not be performed as the nitrite levels in the analyzed samples were far below the quantification limit of the standard method. The reliability of the proposed kinetic method to analyze real water samples was checked by recovery experiments based on standard addition method which gave quantitative results (97.3-102.6%) with convenient reproducibility (RSD=0.78-1.75%).

Table 2. Determination of NO_2^- ion in some natural water samples (results of recoveries of spiked samples) and synthetically prepared waste water samples using the proposed kinetic method and standard APHA method

Samples	NO_2^- added (μg mL^{-1})	Proposed kinetic method		Standard APHA method[d]			
		NO_2^- found±SD[a] (μg mL^{-1})	Recovery %	NO_2^- found±SD [a] (μg mL^{-1})	Recovery %	Student t-test[b]	*F-test*[c]
Lake water	.	0.180±0.002	-	0.185±0.002	-	1.87	2.24
	0.2	0.377±0.003	98.5	0.378±0.003	99.0	1.37	1.76
	1.0	1.183±0.003	100.3	1.183±0.002	99.8	0.76	0.65
Different marked spring water samples							
S_1	-	not detected	-	not detected	-	0.89	1.78
	0.5	0.485±0.003	97	0.490±0.003	98	0.77	2.78
	1.5	2.025±0.003	102.6	2.035±0.002	103	1.01	4.00
S_2	-	not detected	-	not detected	-	1.05	1.56
	0.5	0.480±0.003	96	0.485±0.003	99.8	1.56	1.96
	1.5	2.010±0.002	102	2.020±0.002	102.3	0.62	2.25
S_3	-	not detected	-	not detected	-	0.62	2.25
	0.5	0.485±0.003	97	0.495±0.003	99	1.24	2.25
	1.5	1.980±0.003	99.67	2.015±0.002	101.3	1.24	2.25
River water	-	0.120±0.004	-	0.123±0.003	-	0.74	2.30
	0.2	0.319±0.003	99.5	0.318±0.03	97.5	1.27	2.38
	1.0	1.130±0.003	101.0	1.124±0.002	100.1	1.05	3.35
Synthetically prepared wastewater*	-	2.95 μg mL^{-1}	-	3.10 μg mL^{-1}	-	0.76	2.34

*Wastewater that is synthetically prepared by adding 100 mL distilled water to contain: 3 μg mL^{-1} NO_2^-; 1000 μg mL^{-1} [HCO_3^-, Cl^-, SO_4^{2-}, Li^+, Na^+, K^+, NH_4^+, $C_2O_4^{2-}$, F^-, ClO_4^-, NO_3^-, Be^{2+}, Ca^{2+}, Mg^{2+}, Pb^{2+}, Zn^{2+}, Al^{3+}]; 100 μg mL^{-1} [EDTA, Cd^{2+}, Mn^{2+}, Ni^{2+}, Cu^{2+}, Co^{2+}, Fe^{3+}, Mo(VI), W(VI)]; 10 μg mL^{-1} [I^-, S^{2-}, $S_2O_5^{2-}$, Hg^{2+}]

[a]Mean±standard deviation for five replicate measurements

[b]Tabulated t-value for 8 degrees of freedom at confidence level of 95% or probability level of 0.95 is 2.31

[c]Tabulated F-value for (4,4) degrees of freedom at probability level of 0.95 is 6.39

[d]The values obtained by using standard APHA method.

The method is rapid and shows advantages over the conventional calibration procedure in getting more precise results for lower nitrite concentrations. The proposed method is easily applied to the examination of drinking waters according to the Drinking Water Standards set by the WHO (1993) (0.91 μg mL^{-1} NO_2-N [43] or USEPA (1994) (1 μg mL^{-1} NO_2-N [44]. The present method can also be used in quality evaluations of inland waters. The levels of interfering ions in inland waters do not affect the nitrite analysis. For

instance, the Turkish regulations require 0.002 μg mL^{-1} NO_2-N for first quality waters, which can be easily analyzed by the proposed kinetic method.

4. Conclusions

The results obtained with the proposed kinetic method applied to monitoring of nitrite in natural and waste water samples show a good agreement with those obtained by the reference standard method. These results show that the analytical method could be used as an advantageous alternative to the conventional methods. The use of automatic or semi-automatic systems allow us to monitoring of different chemical species is widely recognized importance for the analysis of environmental samples. Small volume reagents and samples are important in chemical analysis. The proposed method was successfully applied to the determination of trace amounts of nitrite in natural and waste water samples. In conclusion as the results show, the present analytical method is simple, sensitive, economical, relatively selective and robust method as compared with conventional methods, and was proven to be useful in monitoring of nitrite ion in chemical and bioprocess effluents. The advantages of the present kinetic method over the previously reported methods [45-57] are summarized in Table 3.

Table 3. Comparison of the present analytical method with the previously reported analytical methods

No	Reagents	λ_{max} (nm)	The range of determination NO_2^- (μg mL^{-1})	Molar absorptivity (L mol^{-1} cm^{-1})	Remarks	Reference
1	Sulfanilamide + N-(1-naphtyl) ethylenediamine dihydrochloride	543	0.5-14	$4.0x10^4$	Sensitive to pH variation, requires 10 min for full color development and the product can be carcinogenic	42
2	p-nitroaniline + 8-quinolinol	550	0.08-1.12	$3.88x10^4$	Less sensitive, Cu^{2+}, Fe^{2+} and sulfide interfere. 8-quinolinol is acute toxic.	45
3	p-nitroaniline + Diphenylamine	500	0.05-0.8	$1.425x10^4$	Less sensitive, micellar media (Triton X-100) required, Fe^{3+} interfere.	46
4	p-nitroaniline + acetyl acetone	490	0.05-1.4	$3.2x10^4$	Less sensitive, Cu^{2+}, Fe^{3+}, Co^{2+} and Hg^{2+} interfere.	47
5	Neutral red	530	0-20	$2.5x10^4$	Less sensitive	48
6	Phenosafranine	520	0-12	$3.7x10^4$	Less sensitive	49
7	p-nitroaniline+1-naphthol	610	0.035-0.123	$5.24x10^4$	Sensitive, but it is extractive, Cu^{2+}, Fe^{3+} and SO_3^{2-} ions interfere.	50
8	p-aminobenzoic acid + 8-hydroxy quinoline	499	0.1-1.5	$3.2x10^4$	Less sensitive, sulfide interferes seriously.	51
9	Sulfanilic acid + 1-naphtylamine	520	1.4-35	$3.3x10^4$	Less sensitive, 1-naphtylamine is a potential carcinogenic.	52
10	5,10,15,20—tetrakis (4-aminophenyl) porphine	434	0.0-0.018	$2.65x10^5$	Highly sensitive, time consuming, requires 30 min heating, Fe^{3+} seriously interferes.	53
11	4-aminosalicylic acid + 1-naphtol	520	0.1-3.0	$1.47x10^4$	Less sensitive, Fe^{3+}, Cu^{2+} and sulfide interfere.	54

Table 3. (continued)

12	p-aminoaceto-phenone + Citrazinic acid	495	0.05-1.2	$2.9x10^4$	Less sensitive, Fe^{3+} and Cu^{2+} interfere.	33
13	p-nitroaniline + ethoxyethylene-maleic ester	439	0.5-16	$1.21x10^4$	Less sensitive, diazotization requires cooling (0-5^{o}C).	55
14	Thinonine	600	0.025-0.5	$4.1x10^4$	Simple, sensitive and rapid.	56
15	Cresyl Violet	555 405	0.0184-2.39 2.4-6.4	-	The method was applied to the determination of nitrite ion concentration in foodstuffs such as sausages.	57
16	Cresyl violet perchlorate	585 409	0.01-2.0 0.5-5.0	$7.55x10^4$	Simple, sensitive, rapid and relatively selective.	This method

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