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A novel method for the spectrophotometric determination of nitrite in water

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Abstract

A rapid, simple, selective and sensitive method for the spectrophotometric determination of nitrite in water has been developed and optimum reaction conditions along with other analytical parameters have been evaluated. Nitrite reacts with barbituric acid in acidic solution to give the nitroso derivative, violuric acid. At analytical wavelength of 310 nm, Beer's law is obeyed over the concentration range 0.00–3.22 ppm of nitrite. The molar absorptivity is 15330 ± 259.7 (95%) with pooled standard deviation of 355.57 and R.S.D. of 2.32%. As well as the method is sensitive (2.99×10^{-3} μg NO_2 cm^{-2}) and selective, it tolerates most of the potential interferents. It has been successfully applied to nitrite determination in natural waters by use of a calibration graph with determination limit of 1.66 μg NO_2 in 100 mL working solution corresponding to minimum 9.5 ppb NO_2–N in water samples. Lower concentrations of nitrite (3.0 μg NO_2/L sample) is precisely analyzed by using the method of dilution with sample, with R.S.D. of lower than 0.5%. The results were compared with standard *N*-(1-naphtyl)ethylenediamine dihydrochloride method and very good agreement between the data was observed. The method can easily be applied in the field.

Keywords: Nitrite determination; Barbituric acid; Nitrite in water; Spectrophotometry

1. Introduction

Nitrite ion formation is an important step in the nitrogen cycle. Nitrite is formed during the biodegradation of domestic or industrial nitrogenous wastes as well as some fertilizers. Air-borne nitrogen oxides are converted into nitrite ion, which is also a component of acid rains. Its activity on secondary amines causes the formation of *N*-nitrosamines, which are well-known potential carcinogens [1–4]. It causes the formation of methaemoglobin, for which its level in potable water and foods is restricted by regulations. Maximum permissible limit of nitrite is given by U.S. Public Health Association as 0.06 mg/L in potable water. Presence of nitrite and ammonia is an indication of the presence of bacterial activity and organic pollution [5].

Many analytical methods have been proposed for trace nitrite analysis. Most of the methods are based on the formation of strongly colored azo dyes. An amine is diazotized by means of nitrite under special conditions and the intermediate is let to react with a selected aromatic compound to couple. Several aromatic amines have been used for diazotization such as 4-aminosalicylic acid [6], 4-aminobenzoic acid [7,8], 4-amino-1-naphtalenesulfonic acid [9], 2-nitroaniline [10], 3-nitroaniline [11], 4-nitroaniline [12–18], *p*-rosaniline [19], 4-amino azobenzene [20], 4-aminophenylmercaptoacetic acid [21,22], 4-aminobenzotrifluoride [23], 4-aminoacetophenone [24] or sulfamate group compounds such as sulfanilamide [25,26], sulfapyridine [25] and sulfathiazole [25,27]. Aromatic partners for diazonium compounds are selected mostly from phenolic compounds such as *o*-methoxyphenol [10], 1-naphtol

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[6,9], 1-naphtol-4-sulfonate [25], 1-aminonaphtalene-2-sulfonic acid [10], phloroglucinol [13,28], chromotropic acid [14], 8-quinolinol [15], resorcinol [20] or from other aromatics such as *N*-(1-naphtyl)ethylenediamine (NEDA) [7,8,11,19,21,22,24,26,27,29] or citrazinic acid [30]. Active methylene compounds such as acetyl acetone [17], ethyl acetoacetate [18] or dibenzoylmethane [31] have also been used in coupling.

Azo dye formation is dependent on pH, diazotization temperature and coupling time. Toxicity of certain amines are also an important point for chemists. Coupling time is relatively long. Sulfanilic acid/NEDA method which is a reference method [32], color development is completed in 40 min. In some processes, extraction by a solvent [10,12,15,31] or concentration on a special solid support [25] of azo dye has been proposed to improve the sensitivity.

The methods suffer mainly from Cu(II) [6,12,33,34], Co(II), Fe(III) [6,12,24,33,34], Cr(III) and Al(III) interferences provided that masking agents are used. Sulphite [6,12,24,33,35], sulphite, thiosulfate and iodide are the most common reductant anionic interferences.

Some methods use the reactions of nitrite to give the nitroso compounds with 4-iodo-*N*,*N*-dimethylaniline [36] and active aromatic compounds such as *N*,*N*-dimethylaniline, indole [37] or alkylresorcinols [38]. As to fluoresence methods, reduction of nitroso compounds into fluorescent amines is a method in nitrite detection [39]. Formation of fluorescent compounds such as diazotates [40,41] or Ga(III) complex of an azo dye [40] are used as well as chemiluminescence of uric acid-hexacyanoferrate-luminol system [42].

Decolorization of colored compounds such as acridine red [43] upon the nitrosation reaction have also been studied.

Catalysis of some reactions with nitrite also helps the nitrite analysis. Nitrite catalyses the hydrogen peroxide oxidation of chlorpromazine [44] and the bromate oxidations of brilliant cresyl blue [45] or bromocresol purple [46].

In some studies, preconcentration of nitrite has been needed [29].

Some instrumental methods, such as liquid chromatography [47,48], pulse polarography [49,50], flow injection [51–56], capillary zone electrophoresis [57–59] and membrane sensors [60–64] are also used in nitrite analysis. UV detection is preferred in chromatography.

In this paper, we have developed a simple and selective method for nitrite determination with low limits. The method is based on the reaction between nitrite and barbituric acid. The proposed method is sensitive, requires no control of temperature and do not suffer from most of the potential interferents.

2. Experimental

All chemicals were of analytical reagent grade and double distilled water was used in solutions.

A Shimadzu 240 double-beam spectrophotometer was used in spectral measurements with 1.00 cm quartz cuvettes. A NEL digital pH meter model 890 was used in pH adjustments:

- nitrite free water;
- stock sodium nitrite solution, 2.174×10^{-2} M (1 mg NO_2/mL)—prepared in a 1000 mL volumetric flask by dissolving 1.500 g dry sodium nitrite in acetate buffer solution of pH 5.0 containing 0.1 mL chloroform;
- standard sodium nitrite solution I, 2.174×10^{-5} M (1 μg NO_2/mL)—1 mL of $NaNO_2$ stock solution was diluted to 1000 mL with acetate buffer solution of pH 5.0;
- standard sodium nitrite solution II, 2.174×10^{-4} M (10 μg NO_2/mL): 10 mL of $NaNO_2$ stock solution was diluted to 1000 mL with acetate buffer solution of pH 5.00;
- barbituric acid solution 5×10^{-4} M is prepared in acetate buffer of pH 3.20;
- 5N and 10N sodium hydroxide solutions;
- 0.1N AcOH solution;
- acetate buffers of pH 3.20, pH 5.00 and pH 5.50.

2.1. Plotting of calibration graph

To a serie of nitrite aliquots containing 1.0–60.0 μg nitrite (NO_2) (1.00–60.00 mL) prepared from standard nitrite solution I is added 30 mL of 5×10^{-4} M barbituric acid solution in acetate buffer solution of pH 3.20 in 100 mL calibrated flasks. The pH of the solution should be between 3.25 and 3.50. The mixture is let to stand for half an hour at room temperature. 5.60 mL 5N sodium hydroxide solution is added and the mixture is diluted to the mark with acetate buffer of pH 5.50.

Absorbances at 310 nm are measured in 1.00 cm quartz cells against reagent blank which is prepared in the same manner but in the absence of nitrite. A calibration graph is plotted for nitrite. A straight line passing through the origin has been obtained. The amount of nitrite in the working solution can be extended up to 300 μg by use of appropriate standard solutions.

2.2. Nitrite analysis in water samples

In tap water samples, nitrite analysis is performed as follows: 90.0 mL of water sample in a 100 mL volumetric flask is acidified by 0.1N AcOH solution to get approximately pH of 5.0 and diluted to the mark with pH 5 buffer. An aliquot (maximum 60.0 mL) of that solution containing nitrite in the working range (1.7–300.0 μg NO_2) is used in nitrite analysis. Following the addition of 30 mL 5×10^{-4} M barbituric acid solution, the mixture is left to stand for half an hour. 5.6 mL of 5N NaOH solution is added and the mixture is diluted to the volume with buffer of pH 5.50. Absorbance is measured at 310 nm against reagent blank.

In river water samples, nitrite analyses are done as given above, following the filtration of water samples through a filter of 0.45 μm pore size.

2.3. Application of the method of dilution with the sample

The pH of water sample is adjusted to 5.00 as mentioned above. Two or more working solutions are prepared in 100 mL volumetric flasks such as: (1) 5 mL (V_1) of standard nitrite solution II (2.174×10^{-4} M; 10 μg NO_2/mL) + 25 mL barbituric acid solution (5×10^{-4} M at pH 3.20); (2) 5 mL (V_1) of standard nitrite solution II + 25 mL barbituric acid solution (5×10^{-4} M at pH 3.20) + V_x mL (maximum 60 mL) water sample. After 30 min, into each flask 10N NaOH (approximately 3.6–4.7 mL) is added to get pH 5.50 and the mixtures are diluted to 100 mL with buffer 5.50. Absorbances are measured at 310 nm against reagent blank. By this way, 3 μg NO_2/L is analysed by R.S.D. of 0.5%.

3. Results and discussion

Reaction between barbituric acid and nitrite anion to give violuric acid occurs in acidic solutions. Completeness of the reaction depends on the pH of the solution.

Both barbituric acid and sodium nitrite give very weak absorption bands between 300 nm and 400 nm. 2×10^{-2} M Barbituric acid solution in nitrite-free water has pH 2.80 and gives a spectrum with two bands: 317 nm (sh) ($\varepsilon = 39.25$ L mol^{-1} cm^{-1}) and 328 nm ($\varepsilon = 42.0$ L mol^{-1} cm^{-1}) (Fig. 1). Molar extinction coefficient of barbituric acid solutions in the pH range of 2.80–6.50 are almost at same level, except a slight increase between pHs 3.50 and 4.00 and a decrease between pHs 4.00 and 5.00 (Fig. 2).

Nitrite solutions show several peaks with a maximum at 357 nm (Fig. 1), intensity of which decreases and slightly shifts to 355 nm by increase in the pH of solutions. Above pH 4.50, the spectrum becomes a broad peak of very low intensity.

Aqueous solutions of the reaction product, violuric acid, exhibit peak of 310 nm,which has been selected for analytical work and is independent from the pH change (Fig. 1). However, molar absorptivity is significantly changed by acidity of the solution below pH 5 (Fig. 3).

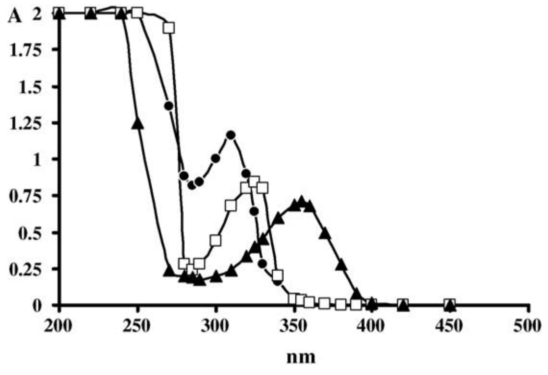

Fig. 1. Absorption spectra of aqueous (▲.) 3×10^{-2} M sodium nitrite (pH 5.58); (☐) 2×10^{-2} M barbituric acid (pH 2.80) and (●) 2×10^{-4} M violuric acid (pH 3.89) solutions (reference water).

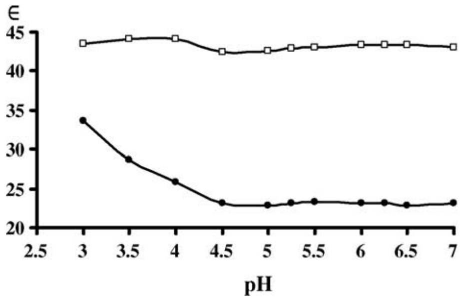

Fig. 2. pH dependent molar extinction coefficient changes of aqueous sodium nitrite (●) and barbituric acid (☐) solutions at maximum absorption wavelengths.

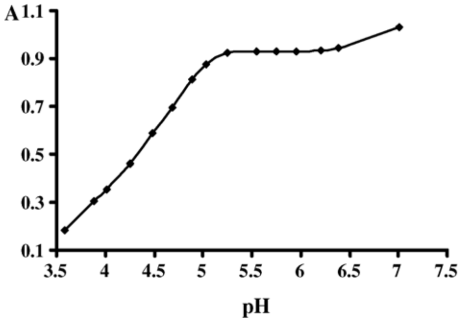

Fig. 3. Absorptions of violuric acid solutions at different pHs (λ 310 nm).

3.1. Formation of violuric acid

Barbituric acid reacts with nitrite anion in acidic medium (Fig. 4).

Nitrite determination procedure needs two pH adjustments. The first is applied for the violuric acid formation and the second is applied for the spectrophotometric evaluation of violuric acid content.

Formation of violuric acid has been followed by the measurements of absorbances at 310 nm in the pH range of 2–5.50 with 0.5 pH unit intervals for 0.25–8 h reaction times. The reaction is fast enough in acidic solutions. Both the highest absorbances at 310 nm and the lowest relative standard deviations (0.28–0.32%) have been obtained in the pH range of 3.25–3.50 for 15–45 min reaction time. Thus, the pH between 3.25 and 3.50 and the reaction time of 30 min have been selected as standardized conditions for the reaction between barbituric acid and nitrite (Fig. 5).

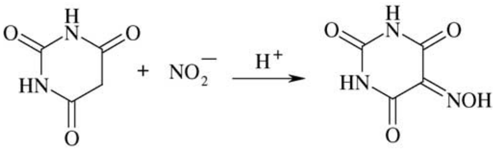

Fig. 4. Reaction between barbituric acid and nitrite ion.

Table 1
Determination of nitrite added to 20 mL tap water

Nitrite added (μg per 100 mL final solution)	Proposed method			Standard method		
	Nitrite found[a] ($\mu g \pm t\sigma N^{-0.5}$)	Recovery (%)	R.S.D. (%)	Nitrite found ($\mu g \pm t\sigma N^{-0.5}$)	Recovery (%)	R.S.D. (%)
1	1.08 ± 0.20	108.00	18.89	0.97 ± 0.16	96.6	13.05
2	2.10 ± 0.11	105.00	10.61	1.84 ± 0.16	92.0	6.52
3	3.06 ± 0.31	102.00	8.37	2.93 ± 0.16	97.72	4.44
4	4.02 ± 0.20	100.50	4.09	3.87 ± 0.16	96.67	3.38
5	4.98 ± 0.20	99.60	3.30	4.96 ± 0.16	99.16	2.63
10	10.14 ± 0.31	101.42	2.48	10.04 ± 0.21	100.42	1.77
16	16.14 ± 0.31	100.89	1.55	15.91 ± 0.19	99.41	0.98
21	20.94 ± 0.31	104.72	1.19	21.02 ± 0.16	100.09	0.62
25	24.85 ± 0.31	99.38	1.00	24.92 ± 0.16	99.67	0.52
50	50.18 ± 0.31	100.36	0.50	50.12 ± 0.22	100.23	0.36
100	100.24 ± 0.26	100.24	0.21	99.98 ± 0.16	99.98	0.13
200	199.71 ± 0.33	99.86	0.13	199.84 ± 0.22	99.92	0.09

[a] $N = 5$; $t = t$-distribution for confidence level of 95% with $N - 1$ degrees of freedom.

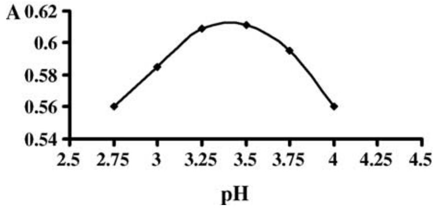

Fig. 5. pH dependence of violuric acid formation (reaction time 30 min).

Violuric acid solutions are stable at pH 3.50 ($\varepsilon = 2037\ \mathrm{L\ mol^{-1}\ cm^{-1}}$), which was followed within 5 days by absorbance measurements at 310 nm, with standard deviation of 0.0008 absorbance units with R.S.D. of 0.800%. Thus, although 30 min reaction time has been selected for proper conditions, the reaction solution of pH 3.50 can be let to stand more than 30 min before the pH adjustment, which is to be applied for spectrophotometric measurement. The high stability of violuric acid formed in the reaction medium gives us the chance to treat the water samples with the reagent in field and to analyse the treated samples in laboratory.

Violuric acid has tautomerism equilibrium between keto and enol forms, which is pH dependent. Enol form is predominant at lower pHs, by which the absorption of $n \rightarrow \pi^*$ is affected.

pH dependence of the 310 nm absorptions of violuric acid solution is given in Fig. 3, in which a plateau is observed between pHs 5.00 and 6.25. Since, the absorbance change is highly dependent on pH above pH 6.50 by taking the possible small pH adjustment failures into consideration, the pH 5.50 has been selected for spectrophotometric determination of violuric acid (Fig. 3). The absorbances of working solutions are stable at pH 5.50 within 24 h with standard deviations of ±0.0037 pooled units ($n = 21$ with 3 sets) in absorbances with relative standard deviation of 0.418%.

3.2. *Beer's law application*

Violuric acid solutions at pH 5.50 have linear response. Absorbances of violuric acid solutions at 310 nm are consistent and obey to Beer's law in the range of 0.00–3.22 ppm. The apparent molar absorptivity referred to nitrite in working solution is calculated as 15330.0 ± 259.7 (95%). Pooled standard deviation is 355.6 and R.S.D. is 2.32%.

The linear regression equation for the calibration graph plotted by use of mean values is as follows:

$$Y(\text{absorbance}) = 0.0033\,X + 0.0003$$

where X is the nitrite μg amount in 100 mL absorbance measured final solution and the correlation coefficient (r^2) is 0.9998. Mean absorbance values have been obtained from five replicate samples of same nitrite content.

The regression equation has been tried with observed absorbances of 115 samples consisted of 23 sets selected from nitrite concentration range of 1–25 μg NO_2^- per

Table 2
Determination of nitrite in river water

Sample volume[a] (mL)	Proposed method		Standard method	
	Nitrite found[b,c] ($\mu g \pm t\sigma N^{-0.5}$)	R.S.D. (%)	Nitrite found[b,d] ($\mu g \pm t\sigma N^{-0.5}$)	R.S.D. (%)
20	10.63 ± 0.20	1.55	10.80 ± 0.10	0.79
40	21.54 ± 0.20	0.76	21.5 ± 0.17	0.63
60	32.39 ± 0.20	0.51	32.3 ± 0.16	0.40

[a] Filtered water sample through 0.45 μm filter.

[b] $N = 5$; $t = t$-distribution for confidence level of 95% with $N - 1$ degrees of freedom.

[c] Calculated by regression equation $Y\,(\text{abs}) = 0.0033\,X\,(\mu g) + 0.0005$ ($R^2 = 0.9998$).

[d] Calculated by regression equation $Y\,(\text{abs}) = 0.0065\,X\,(\mu g) - 0.0008$ ($R^2 = 0.9998$).

100 mL analyte, with five replicate sampling from each set. The pooled standard deviation value of 0.252 μg has been obtained for this application. The regression equation can be used with the pooled R.S.D. of 2.1% for the least nitrite amount of 12 μg per 100 mL final solution.

3.3. Sensitivity

Detectable violuric acid concentration in solution calculated for the $\Delta A = 0.001$ unit is around 6.5×10^{-8} M which corresponds to sensitivity of 2.99×10^{-3} μg nitrite cm^{-2}. Detection limit calculated from $3 \times \sigma_{\text{blank}}/\text{m}$ is 0.500 μg $NO_2{}^-$ and determination limit calculated from $10 \times \sigma_{\text{blank}}/\text{m}$ is 1.660 μg $NO_2{}^-$, both in 100 mL final solution.

3.4. Validation

The validity of the proposed method has been controlled by the comparison with the AOAC recommended sulfanilamide-NED (*N*-(1-napthyl)ethylenediamine hydrochloride) method in nitrite free tap water samples as well as the nitrite containing river water samples. The results are given in Tables 1 and 2. According to the results obtained from both kind of water samples, any appreciable difference in the results ($P < 0.001$) have not been observed, to indicate good agreement between the data obtained by both of the methods.

3.5. Interferents

The effect of possible interferents has been studied at a nitrite concentration of 0.5 μg/mL. Maximum tolerable concentrations are given in Table 3. The interferents given in Table 3 besides halogenides and ammonium have shown positive effects on absorbances. Interferences, arising from cations such as Cu(II), Fe(II), Co(II), Mn(II) and Pb(II), have been removed by filtration, following the treatment of water samples with 0.5N NaOH to get pH 9.00.

Interferents, arising from the suspended solids, can be removed by filtration through a filter with appropriate pore size.

The interferences from barbituric acid and nitrite were disregarded since their molar absorptivities at the analytic wavelength are too low (e.g., 7.3 and 14.14 L mol^{-1} cm^{-1} for nitrite and barbituric acid, respectively).

In the proposed method, the interferents are better tolerated than most of the former methods do [6,9,11,12,18,23].

3.6. Application of the method of dilution with sample for lower nitrite amounts

An adaptation of the standard addition method has been used to analyze the nitrite concentration down to 3 μg NO_2/L_{sample}. The method is applied by use of a concentrated standard nitrite solution, which gives the absorbance readings within the range of 0.1–0.5 with R.S.D.s of better than 0.5% (Table 1).

The working solutions for absorbance measurements have been prepared from both standard nitrite solution and standard nitrite solution plus aliquots of the unknown solution. The unknown concentration is calculated by:

$$C_x = \frac{(A_2 - A_1)V_1C_1}{A_1V_x}$$

where A_1 is the absorbance of the working solution prepared from a volume V_1 of standard nitrite solution with C_1 concentration and A_2 is the absorbance of the working solution prepared by the addition of V_x volume of unknown solution with concentration C_x onto the V_1 mL of standard nitrite solution.

This 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 mg NO_2–N/L) [65] or USEPA (1994) (1 mg NO_2–N/L) [66].

The method can also be used in quality evaluations of inland waters. The levels of foreign ions in inland waters do not affect the nitrite analyses. For instance, the Turkish regulations require 0.002 mg NO_2–N/L for first quality

Table 3
Effect of foreign ions on the determination of 0.5 μg mL^{-1} nitrite

Foreign ion	Maximum permissible concentration (μg/mL)[a]	Foreign ion	Maximum permissible concentration (μg/mL)[a]
Ammonium	1420[b]	Calcium	940
Nitrate	460	Zinc	1275
Phosphate	1210	Copper(II)	181(440[c])
Sulfate	1260	Cobalt(II)	141 (250[c])
Chloride	920[b]	Iron(II)	90 (240[c])
Carbonate	1100	Lead(II)	630 (835[c])
Bromide	990[b]	Manganese(II)	300 (555[c])
Fluoride	1160[b]	Magnesium	1245
Barium	1255	Strontium	1000

[a] Concentration causing an error of < ±2%. Acetate salts were used.

[b] Negative (−) effect.

[c] Analyses were performed following the filtration of hydroxides formed by pH 9.00 adjustment of water by 0.5N NaOH. The metal concentrations given are initial concentrations.

waters which can be easily analyzed by the proposed method.

4. Conclusions

The proposed method is simple, selective and reproducible for nitrite determinations of low levels in natural waters as well as the polluted river waters. The reaction product, violuric acid, is very stable under working conditions. The method can easily be applied in the field. During the field work, the water sample is let to react with the reagents to get violuric acid in solution and the treated samples can be kept in well-closed vessels for a long period of time, e.g., 5 days, without any need of temperature control. Final pH adjustments are done at laboratory before the absorbance measurements.

The method requires low cost equipment and the reagents are safe for health. It does not need an extraction as in the case of some coupling reactions. The method tolerates more to the foreign ion effects than other methods do. It is applicable to colored solutions provided that they do not absorb in mid-UV region. Application of the method of dilution with the sample makes it more favorable for lower nitrite concentrations.

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