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# KINETIC-SPECTROPHOTOMETRIC DETERMINATION OF NITRITE BY ITS ACCELERATING EFFECT ON THE OXIDATION OF TOLUIDINE BLUE BY BROMATE

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A kinetic-spectrophotometric method is proposed for the determination of trace amounts of nitrite. The method is based on its accelerating effect on the oxidation of toluidine blue by potassium bromate in acidic solution. The reaction was monitored spectrophotometrically, measuring the absorbance decrease of toluidine blue, at $\lambda = 631$ nm, for the first 15-45 s from the start of the reaction. The method permits determination of nitrite over the range 0.02–1 μg $mL^{-1}$, with a detection limit of 0.006 μg $mL^{-1}$. The method was applied to the determination of nitrite in spiked drinking water samples.

## INTRODUCTION

Nitrite ion is naturally occurring form of nitrogen that can be present in ground and surface water. In these media, nitrite is not ordinarily found in high concentration, but it can be present as an intermediate product in the microbial oxidation of ammonia or in the reduction of nitrate. It may also be excreted by phytoplankton as a result of excess assimilatory reduction. Nitrite is of particular health concern in the body because it causes the hemoglobin in the blood to change to methemoglobin. Methemoglobin reduces the amount of oxygen that can be carried in the blood. This results in cells throughout the body being deprived of sufficient oxygen to function properly. This condition is called methemoglobinemia which can lead to cyanosis, headache, nausea and dizziness. Nitrite may also react in stomach with nitrosable compounds (e.g. secondary and tertiary amines or amides in food) to form N-nitroso compounds which are well known potential carcinogens.[1,2] The determination of nitrite in natural waters is important, being harmful to human health. Maximum permissible limit of nitrite concentration as fixed by US Public Health Association is 0.06 μg $mL^{-1}$ in potable water. [3] Several reports have been published on the determination of nitrite including ion chromatography[4-6], capillary zone electrophoresis [7-9], amperometry[10-12], polarography[13], potentiometry[14], voltammetry[15], flow injection analysis.[16-18] In some of these methods, selectivity is poor; some demand expensive and complicated instruments or reagents and the others need to do difficult and time consuming separation procedures. Spectrophotometric methods are by far the most widely used for nitrite determination due to the high sensitivity, low detection limits, good selectivity, rapid analysis, inexpensive instrumentation such as a spectrophotometer and facile assay-type protocols. An important number of such studies reported kinetic-spectrophotometric methods for nitrite analysis. These methods are based on its accelerating effect action on the oxidation of some organic reagents with suitable oxidizing agents.[19-36] The nitrite is analyzed by photometrically recording the oxidation rate of the organic compounds.

The current paper describes another kinetic spectrophotometric method for determination of nitrite based on the toluidine blue (noted TB) –

bromate redox reaction. The optimum working conditions were established and the advantages of the proposed method in comparison with other reported contributions were pointed out. The method was applied for nitrite determination in spiked drinking water samples.

## RESULTS AND DISCUSSION

Reaction between toluidine blue (3-amino-7-(dimethylamino)-2-methylphenothiazin-5-ium chloride) and bromate in acid medium takes place very slowly which is confirmed by the slow decrease of absorbance (Fig. 1(-●-)). When the nitrite ion is added, the oxidation of TB is much faster, resulting in a considerable discoloration of mixture (Fig. 1(-■-)). The wavelength of maximum absorbance attributed to TB was found to be $\lambda = 631$ nm. Preliminary experiments showed that the $A_{631\ nm}$-[TB] graph was linear up to $5\times10^{-5}$ mol $L^{-1}$ and the position of the TB characteristic band does not change with varying the acidity and reagent's concentrations. The main parameters that can influence the performance of the proposed method were studied to arrive at the optimum working configuration. The discoloration rate values were calculated as difference between the rates obtained in presence and in absence of nitrite ions, performed in the same experimental conditions.

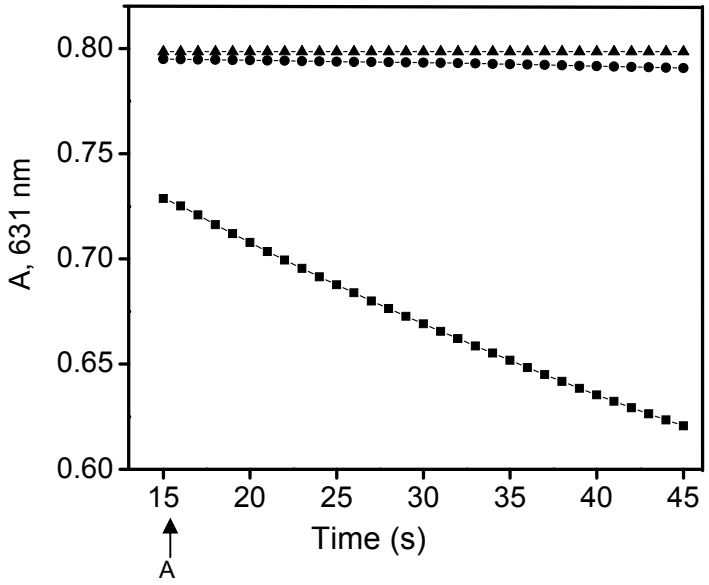


Fig. 1 – Variation of TB absorbance with time:
(●) TB + $H_2SO_4$ + bromate; (▲) Nitrite + TB + $H_2SO_4$; (■) Nitrite + TB + $H_2SO_4$ + bromate; [ $NO_2^-$ ] = 0.5 μg $mL^{-1}$; [TB] = $5 \times 10^{-5}$ mol $L^{-1}$; [$BrO_3^-$] = $5\times10^{-4}$ mol $L^{-1}$; point A: absorbance measurement was started.

Effect of acidity. The effect of $H_2SO_4$ on the reaction of TB oxidation with bromate was studied in the concentration range 0.18-0.58 mol $L^{-1}$. The acidity is a very important parameter, influencing the rate of the redox reaction. As can be seen in Fig. 2, the initial rate, in the presence of 0.5 μg $mL^{-1}$ [$NO_2^-$], increases with $H_2SO_4$ concentration up to 0.48 mol $L^{-1}$; then, a decrease was observed. This because, at concentration ≥ 0.48 mol $L^{-1}$ $H_2SO_4$, the rate of the reaction increases too much. As a consequence, the linearity of *A–t* graphs became poor and the linear parts of *A–t* graphs were shortened to values below 20 seconds, resulting in poor precision. The chosen concentration of $H_2SO_4$ was 0.48 mol $L^{-1}$.

Effect of TB concentration. The effect of TB concentration was studied in the concentration range of $1.5\times10^{-5}$–$7\times10^{-5}$ mol $L^{-1}$. The initial rates increases with TB concentration up to $5\times10^{-5}$ mol $L^{-1}$, then a slight decrease was observed (Fig. 3). This behavior is in accordance to the linearity of $A_{631\ nm}$-[TB] graph, valid up to $5\times10^{-5}$ mol $L^{-1}$. Therefore, a $5\times10^{-5}$ mol $L^{-1}$ TB was used in the recommended procedure.

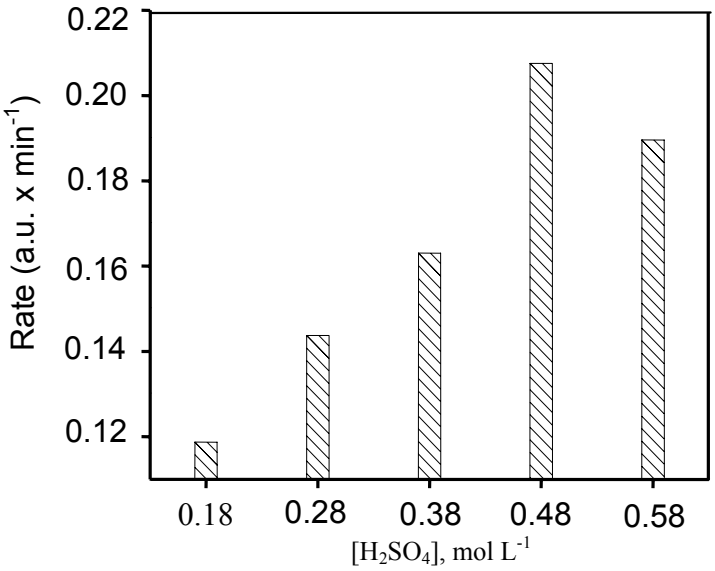


Fig. 2 – Discoloration rate dependence on sulfuric acid concentration.

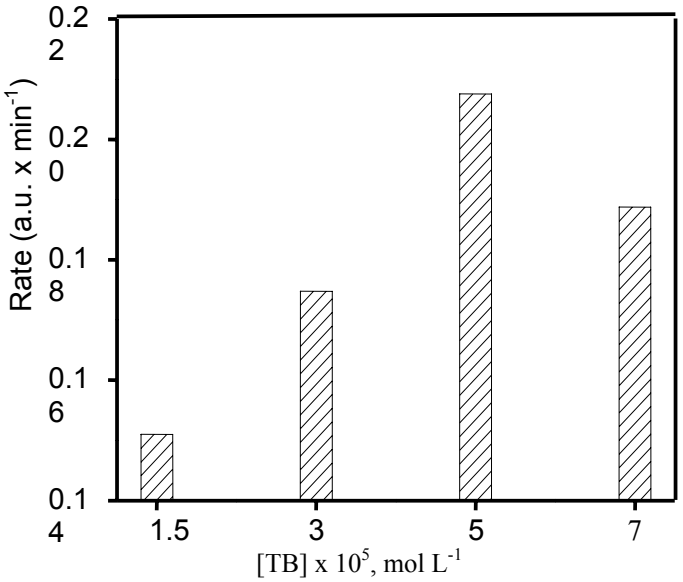


Fig. 3 – Discoloration rate dependence on TB concentration.

Effect of bromate concentration. The effect of bromate concentration was studied in the concentration range of $10^{-5}$–$10^{-3}$ mol $L^{-1}$. As can be deduced from Fig. 4, the initial rates increases with bromate concentration up to $5\times10^{-4}$ mol $L^{-1}$; over this value, a decrease of linearity of *A*–*t* graph was observed. This because, in the presence of $10^{-3}$ mol $L^{-1}$ bromate, a significant increase of the initial rate takes place. As a consequence, a quick decrease of the absorbance of TB takes place and the linear part of the graph is considerable shortened to a value which reaches 5 seconds when bromate of $10^{-3}$ mol $L^{-1}$ was used. So, $5\times10^{-4}$ mol $L^{-1}$ was chosen to be the optimum value of bromate concentration and adopted for further experiments.

Effect of the ionic strength. The effect of the ionic strength on the redox reaction accelerated by nitrite was studied in the presence of $NaNO_3$ solutions. As shown in Fig. 5, the discoloration rate of TB was independent of the ionic strength up to 0.05 mol $L^{-1}$ $NaNO_3$. The effect of $NaNO_3$ on the redox reaction between TB and bromate in presence of nitrite ions can be explained by considering the reaction on a microscopic scale. The solution in which equilibrium is established contains a variety of cations and anions $Na^+$, $H^+$, $BrO_3^-$, $NO_2^-$, $H_3O^+$ and $NO_3^-$. Thus, these species are surrounded by charged ionic atmospheres that partially screen the ions from each other. The redox reaction between TB and bromate in

presence of nitrite requires the disruption of the ionic atmospheres surrounding the mentioned ions. Increasing the concentrations of ions in solution, by adding $NaNO_3$, increases the size of these ionic atmospheres. Since more energy is now required to disrupt the ionic atmospheres, there is a decrease in the formation of reaction products, and an apparent increase in the equilibrium constant.[37]

Order of addition of reactants. It was observed that the sequence of addition of reactants can influence the rate of oxidation process, implicit of the discoloration of TB. Thus, the sequences (1) nitrite-TB-$H_2SO_4$-bromate; (2) bromate-TB-$H_2SO_4$-nitrite and (3) TB-$H_2SO_4$-nitrite-bromate gave higher values of descoloration rate. Sequences (4) bromate-nitrite-TB-$H_2SO_4$ and (5) bromate-$H_2SO_4$-nitrite-TB gave less values of descoloration rate. These results could be explained as follows: in the case of sequence (4), by adding bromate and nitrite before TB, a partial oxidation of nitrite could take place.[31] Moreover, this process is favored by the addition of nitrite to the acidified bromate solution (sequence (5)). On the basis of the oxidation rates illustrated in Fig. 6, the sequence (1) was chosen to be applied in the proposed method.

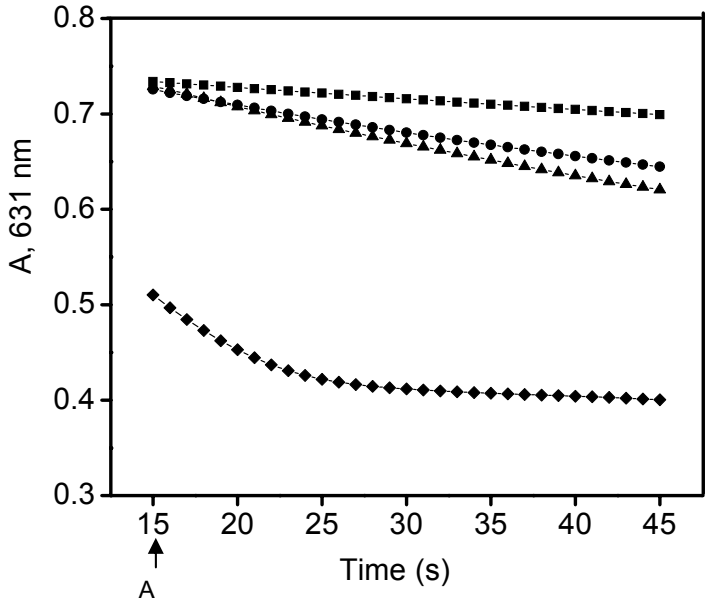


Fig. 4 – Variation of TB absorbance with time as a function of bromate concentration: $[BrO_3^-]$, mol $L^{-1}$: (■) $10^{-5}$; (●) $10^{-4}$; (▲) $5x10^{-4}$; (♦) $10^{-3}$; point A: absorbance measurement was started.

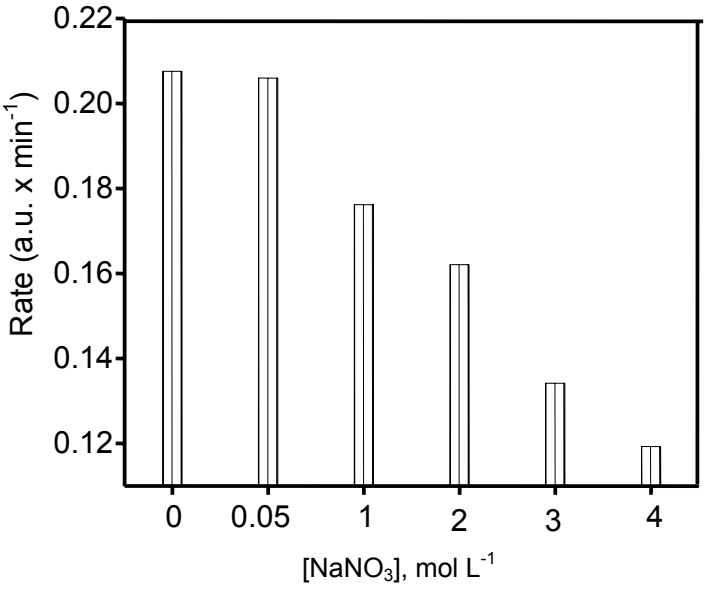


Fig. 5 – Discoloration rate dependence on sodium nitrate concentration.

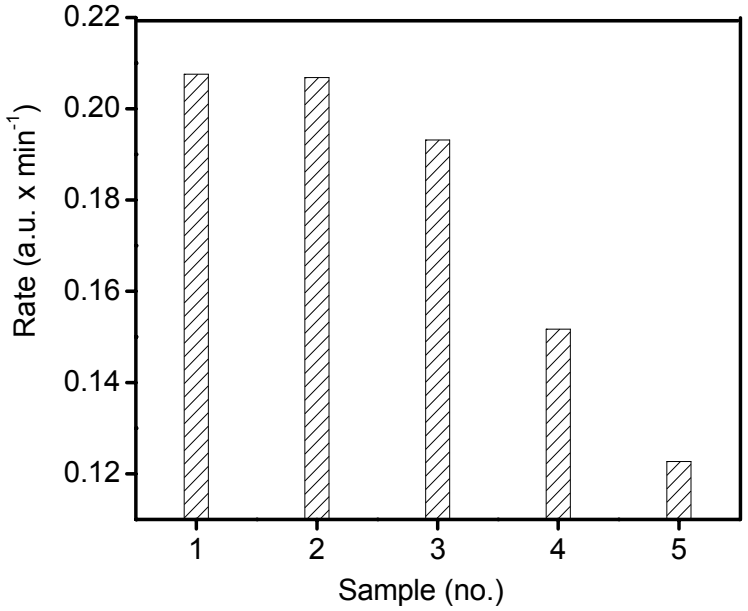


Fig. 6 – Discoloration rate variation with reactants addition order:
(1) nitrite-TB-$H_2SO_4$-bromate; (2) bromate-TB-$H_2SO_4$-nitrite; (3) TB-$H_2SO_4$-nitrite-bromate;
(4) bromate-nitrite-TB-$H_2SO_4$; (5) bromate-$H_2SO_4$-nitrite-TB.

The performances of the proposed method were verified on samples having the following composition and respecting this order of reagents addition: 1-mL sample solution containing a known amount of nitrite + 0.5 mL of $2\times10^{-4}$ mol $L^{-1}$ TB + 0.3 mL of 2.66 mol $L^{-1}$ $H_2SO_4$ + 0.2 mL of deionized distilled water + 20 μL of $5\times10^{-2}$ mol $L^{-1}$ bromate.

Analytical figures of merit. Once the optimum working conditions were established, the proposed spectrophotometric method was evaluated with respect to linearity, *LOD*, *LOQ*, accuracy, precision. The calibration graph was plotted by use of discoloration rate values obtained from five replicate samples of same nitrite content. The calibration graph was linear in the concentration range 0.02–1 μg $mL^{-1}$ of nitrite. The parameters of calibration graph were as follows: the linear regression's equation, $R = 0.3917\ [NO_2^-] + 0.0022$, where $[NO_2^-]$ = the nitrite concentration expressed in μg $mL^{-1}$; the squared correlation coefficient, $r^2 = 0.9987$; the detection limit, *LOD* (calculated as three times the standard deviation of the blank) = 0.006 μg $mL^{-1}$ $NO_2^-$ and *LOQ* (calculated as ten times the standard deviation of the blank) = 0.02 μg $mL^{-1}$ $NO_2^-$. In order to estimate the accuracy and precision of the proposed method, standard solutions of 0.10; 0.30 and 0.50 μg $mL^{-1}$ nitrite were analyzed according to the recommended procedure. For this purpose, six replicate determinations of each concentration were prepared. As it is seen in Table 1, relative standard deviations ranged from 1.40 % to 4.02 % and the percent recovery from 99.42 % to 101.12 %. The results in Table 1 were obtained by performing the experiments on samples with the following composition: 1 mL sample solution containing the nitrite ion at different concentration level + 0.5 mL of $2\times10^{-4}$ mol $L^{-1}$ TB + 0.3 mL of 2.66 mol $L^{-1}$ $H_2SO_4$ + 0.2 mL of doubly-distilled water + 20 μL of $5\times10^{-2}$ mol $L^{-1}$ bromate.

Effect of diverse ions. To evaluate the selectivity of the proposed method, the effect of foreign ions on the determination of nitrite was studied by adding known quantities of each ion to a solution containing 1 μg nitrite (final volume = 2.02 mL) and determining the nitrite by the proposed method. The tolerance limits of foreign ions, taken as the concentrations (μg $mL^{-1}$) which cause errors less than 3 % are given in Table 2. These results clearly show that most of the ions which are normally associated with nitrite in water samples do not interfere. However, Hg(II), Ag(I), $Br^-$ and $S^{2-}$ have low tolerance limits. The majority of foreign ions mentioned in Table 2 are presented in drinking water at much lower concentration limits. Hence, the proposed method could be applied for the determination of nitrite in water samples for their quality control.

Application of the Method. The analytical potential of the method was tested by applying it to the determination of spiked amounts of nitrite in

drinking water samples. The spike is the addition of a known amount of analyte to a normal sample in the lab. Usually, matrix spike samples are analyzed by a proposed method to determine the effect of the sample matrix on the accuracy of the analytical results.[38] The difference between the sample and the spiked sample is determined and the percent recovery is calculated. Spikes help to determine if interferences are present. Generally, 85% to 115% recovery is acceptable.

For this experiment, the samples of potable water were collected from packaged water bottles. As it is seen in Table 3, very good recoveries of nitrite were obtained taking into account the presence of commonly constituents normally encountered in drinking water samples.

*Table 1*

Precision and accuracy of the proposed method

| Nitrite, μg mL$^{-1}$ | | *RSD*, % | *R*, % |
|---|---|---|---|
| Taken | *Found±$tSN^{-0.5}$ | | |
| 0.100 | 0.099±0.004 | 4.02 | 99.42 |
| 0.300 | 0.303±0.008 | 2.64 | 101.12 |
| 0.500 | 0.499±0.007 | 1.40 | 99.72 |

*Mean ± 95 % confidence limit, for $N = 6$; $t = 2.57$ = $t$-distribution for confidence level of 95 % with $N-1$ degrees of freedom; *RSD* = Relative standard deviation; *R*% = percent recovery.

*Table 2*

Influence of foreign ions on the determination of nitrite (0.5 μg mL$^{-1}$)

| Foreign ions | Tolerance limit, μg mL$^{-1}$ |
|---|---|
| $NH_4^+$, $Na^+$, $K^+$, Ca(II), Mg(II), Zn(II), Ni(II), Co(II) | 500 |
| $NO_3^-$, $PO_4^{3-}$, $CO_3^{2-}$, $SO_4^{2-}$ | > 500 |
| Mn(II), Al(III) | 150 |
| $CH_3COO^-$, $Cl^-$ | 200 |
| Fe(III), Cu(II) | 50 |
| Cr(III) | 30 |
| Pb(II), $C_4H_4O_6^{2-}$ | 20 |
| Fe(II), V(IV), $I^-$ | 5 |
| Ag(I) | 0.4 |
| Hg(II), $Br^-$ | 0.1 |
| $S^{2-}$ | 0.01 |

*Table 3*

Recovery data for drinking water samples spiked with nitrite

| Water mineral Sample, No. | Nitrite, μg mL$^{-1}$ | | *R*, % |
|---|---|---|---|
| | Added | *Found±$tSN^{-0.5}$ | |
| 1[a] | 0.300 | 0.290±0.008 | 96.67 |
| | 0.500 | 0.492±0.009 | 98.40 |
| 2[b] | 0.300 | 0.295±0.011 | 98.33 |
| | 0.500 | 0.493±0.010 | 98.60 |
| 3[c] | 0.300 | 0.291±0.007 | 97.00 |
| | 0.500 | 0.490±0.013 | 98.00 |

*Mean±95% confidence limit, for $N$=4; $t$=3.18=$t$-distribution for confidence level of 95% with $N$-1 degrees of freedom

[a]Dorna (source: Vatra Dornei-Suceava) – certified composition, μg mL$^{-1}$: $Na^+$, 22; $K^+$, 2.8; $Mg^{2+}$, 11.8; $Ca^{2+}$, 360; $Cl^-$, 10.2.

[b] Perla Harghitei (source: Sâncrăieni-Harghita) – certified composition, μg mL$^{-1}$: $Na^+$, 70.6; $K^+$, 9.72; $Mg^{2+}$, 43.8; $Ca^{2+}$, 107.12; $Cl^-$, 16; $SO_4^{2-}$, 3.3.

[c]Biborțeni (source: Biborțeni-Covasna) – certified composition, μg mL$^{-1}$: $Na^+$, 102; $K^+$, 7.2; $Mg^{2+}$, 84; $Ca^{2+}$, 270,7; $Cl^-$, 95.

## EXPERIMENTAL

**Chemicals and apparatus.** All chemicals were of analytical reagent grade and were purchased from Merck (Darmstadt, Germany). Doubly-distilled water was used throughout. A stock standard nitrite solution (1000 μg $mL^{-1}$) was prepared by dissolving 150 mg of pre-dried sodium nitrite in water containing a few milligrams of NaOH to prevent its decomposition. The resulting solution was diluted in a 100 mL volumetric flask after adding few drops of chloroform as a stabilizer, to prevent bacterial growth. This solution was stored in a brown bottle and kept at 4°C; it was used within two weeks of preparation. A stock solution of $5\times10^{-4}$ mol $L^{-1}$ TB was prepared by dissolving TB and then diluting to the mark with 0.32 mol $L^{-1}$ sulfuric acid in a 100 mL volumetric flask wrapped with an aluminum foil and kept at 4°C, when not in use. The solution was stable for at least 2-months. A 4 mol $L^{-1}$ solution of sulfuric acid was prepared by diluting concentrated sulfuric acid with water. Potassium bromate and sodium nitrate stock solutions were prepared in doubly-distilled water. Required working standard solutions were prepared by diluting the corresponding stock solutions.

Absorbance measurements were performed on a UV-VIS spectrophotometer (V-530 Jasco-Japan) equipped with cells holder thermostated by an external circulating water bath. Quartz cells of 1-cm path length were used.

The temperature was kept constant at 25±0.1°C by using the thermostated water bath, GFL 1003 type (Burgwedal, Germany), with an accuracy of ± 0.1°C. Eppendorf vary-pipettes (10-100; 100-1000 and 500-2500 μL) were used to deliver accurate volumes.

**General procedure.** The working solutions, sample solutions and pure water were kept at 25°C in the thermostated water-bath for at least 15 min to attain the equilibrium temperature. A 1-mL sample solution containing 0.001-1.2 μg $mL^{-1}$ $NO_2^-$ was transferred into a quartz cell. Then, 0.5 mL of $0.6\times10^{-4}$–$2.8\times10^{-4}$ mol $L^{-1}$ TB solution; 0.3 mL of 0.66–3.33 mol $L^{-1}$ $H_2SO_4$ and 0.2 mL of doubly-distilled water solution were added sequentially. Taking into account that the TB solutions were prepared in 0.32 mol $L^{-1}$ $H_2SO_4$, the final concentration of sulfuric acid in the prepared samples (after adding aliquots of 0.3 mL of 0.66–3.33 mol $L^{-1}$ $H_2SO_4$) were between 0.18 and 0.58 mol $L^{-1}$. The reaction was initiated by the injection of 20 μL of $10^{-3}$–$10^{-1}$ mol $L^{-1}$ bromate solution. Then, the cuvette was covered with cuvette lid after that the solution was quickly shaken and placed in the spectrophotometer cell holder using exactly time 15 s. The redox reaction was traced spectrophotometrically by monitoring the decrease in absorbance of TB with time from 15 to 45 s, at $\lambda$ = 631 nm (allowing a lag time of 1 s) against water as reference. A blank experiment was also performed by adding doubly-distilled water instead of the standard nitrite solutions. A calibration graph was constructed by plotting the difference between the discoloration rates of TB in the absence and in the presence of nitrite, as a function of nitrite concentration. The discoloration rate ($R$) was calculated from the slope of the linear part of the $A$ - $t$ graph, within 30 s, by applying the formula: $R$ [a.u. x $min^{-1}$]=$\Delta A_{15-45s}/\Delta t_{15-45s}=\Delta A_{15-45s}/0.5$, where a.u. =absorbance units. The nitrite content of the synthetic or real samples was determined from the calibration graph; the mentioned samples were prepared according to the general procedure.

## CONCLUSION

The oxidation of TB by bromate in a sulfuric acid medium and in presence of nitrite ion is an analytical reaction that can be applied in the kinetic-spectrophotometric determination of nitrite-containing water samples. The proposed method is inexpensive, fairly rapid and sensitive. The analytical parameters, especially sensitivity, recommend the proposed method as an alternative to other reported kinetic-spectrophotometric methods and as an instrument for quality control of drinking water samples. Under optimum working conditions the proposed method presents evident advantages in comparison with other reported contributions. As we observe in Table 4, some of the previous reported methods have higher limits of detection (see ref. no. 19; 21; 28; 33), lower dynamic range (see ref. no. 19; 21-23; 31-33; 35) or longer time needed for analysis (see ref. no. 19; 21; 28; 35) compared to the proposed method. Also, a comparison with previous reported results,[39,40] the present system of reagents permits the determination of nitrite up to 1000 ng $mL^{-1}$, making the method useful in the case of samples with high content of nitrite, without preliminary step of dilution.

*Table 4*

Comparison of dynamic ranges and detection limits of the present work with previously reported results

| Reaction system | Dynamic range, ng $mL^{-1}$ | Detection limit, ng $mL^{-1}$ | Time, s | Ref. |
|---|---|---|---|---|
| Methylthymol Blue -Bromate | 2-100 | 0.6 | 240 | 35 |
| Perphenasine - Bromate | up to 4.5 | 0.07 | 30 | 31 |
| Thymol Blue - Bromate | 5-80 | 4.5 | - | 23 |
| Bromcresol Purple - Bromate | 10-400 | 9 | 720 | 21 |
| Catechol Violet - Bromate | 20-350 | - | - | 22 |
| Brilliant Green - Bromate | 20-350 | - | - | 26 |
| Gallocyanine - Bromate | 20-500 | 10 | 120 | 28 |
| Chlorophosphonazo-pN – Bromate | 50-1000 | 18 | 30 | 33 |
| Methyl Red - Bromate | 50-1200 | 45 | 50 | 19 |
| Bromopyrogallol Red – Bromate | 200-1000 | - | - | 32 |
| Methylene Blue-Bromate | 5-500 | 1.5 | 30 | 39 |
| Tropaeolin 00-Bromate | 6-500 | 2 | 30 | 40 |
| Toluidine Blue - Bromate | 20-1000 | 6 | 30 | This work |

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