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Standardization of Kinetic Determination of Nitrite Based on Its Catalytic Effect on an Indicator Reaction

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A catalytic kinetic procedure for nitrite determination based on its catalytic effect on the oxidation of brilliant cresyl blue (BCB) by bromate ion in acidic media is standardized and proposed. The reaction was monitored spectrophotometrically by measuring the decrease in absorbance of BCB at its λ_{max} 595nm. The calibration graph is linear from 1–80 ng.ml^{-1} of nitrite. The detection limit is 5.8 ng.ml^{-1} of nitrite. Nitrite in the range of 10–80 ng.mL^{-1} has been determined successfully where recoveries were 97.3 ~ 103.7% with the relative standard deviation 0.1 ~ 0.8%. The effect of many foreign ions upon determination of 70 ng.mL^{-1} of nitrite has been studied and shown that most common cations and anions do not interfere in the determination except Fe^{2+} which was masked. The procedure was successfully applied for the determination of nitrite in wastewater and seawater with satisfactory results. The typical feature of this procedure is that the determination could be carried at normal temperature of 25°C with analysis time of 4 min.

Key Words: Standardization, Kinetic determination, Nitrite, Brilliant cresyl blue.

INTRODUCTION

Nitrite is an active form of nitrogen cycle, resulting from incomplete oxidation of ammonium ions or reduction of nitrates in the environment by denitrification bacteria. It is a component of the salt mixtures, known as pickles, used for curing bacon and ham in the food industry[1]. Nitrite combines with myoglobin to form nitrosohaemoglobin, which is responsible for the characteristic red colour in meat production and hence sometimes used as meat preservative[2]. It is an important precursor in formation of N-nitrosamines, which can form at physiological pH, are potential carcinogens in a variety of animals and are suspected for causing cancer in humans as well.[3] Humans are exposed to nitrites through the ingestion of vegetables, water and cured meats. Nitrites at high levels can cause methemoglobinemia,[4] which causes reduction in the ability of heamoglobin in red blood

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cells to carry oxygen. Cattle have digestive systems capable of supporting nitrate reducing bacteria and they too can suffer from methemoglobinemia, if nitrate (nitrite) levels are high in the water they drink. Owing to its toxicity, nitrite is a characteristic pollutant[3]. Industrial effluents also add to contamination of the environment with nitrite. It is, therefore, extremely important in environmental studies to develop convenient, sensitive and cost-effective analytical methods for the determination of nitrite ion at ng mL^{-1} levels.

Many papers involving ion-chromatographic, potentiometric and spectrophotometric techniques have been published on determination of nitrite in atmosphere, natural water, meat and biological fluids[2,3,5–10]. Also higher sensitivities have been reported for some methods using large injection volumes in flow injection analysis (FIA) or the laborious extraction procedures[2,3]. In recent years, sequential injection analysis coupled with spectrophotometric monitoring has been used as an alternative to FIA for nitrite determination.[3] Many of the reported methods suffer from more or less time consuming procedures and complicated instrumentation. Among these, spectrophotometric methods based on the modified Griess-Ilosvay reaction, *i.e.*, the diazotization of an aromatic amine and subsequent coupling to form an azo dye are the most widely used[3,5,8,9]. These methods are generally sensitive but often have some drawbacks such as serious interferences, toxity of the reagents used and a relatively long diazo-coupling time with careful control of acidity for each step of process[5,10]. Catalytic kinetic methods (CKM) are the most attractive procedures for the trace determination of nitrite[11,12]. Few CKM for nitrite determination with good sensitivity have been published[2,5–8,10]. However, some are characterized by non-linear calibration graphs whereas others lack the high sensitivity as expected from kinetic techniques[5]. Development of CKM for determination of nitrite has still received little attention. Ensafi *et al.* have attempted to develop few CKM for determination of nitrite based on its catalytic effect on the oxidation reaction of pyrogallol red[13], nile blue[10,14] and brilliant cresyl blue[15] (BCB) with bromate ion in acidic media. However, the experimental data on BCB-bromate indicator reaction are confined to a rather narrow range of reactant concentrations while the dynamic range of determination reported by these authors is quite wider[15]. Based on our experience and notable contribution to the development of CKM for determination of various species it appears to us to be unlikely. These considerations raised doubt about the reported dependence study for the optimization of the indicator reaction[15]. This prompted us to investigate the detailed dependence studies on the reactant concentrations to clarify the optimum conditions and discover the feasible dynamic range for nitrite determination based on its catalytic effect on indicator reaction between BCB and bromate ion and the same is reported in the present paper.

EXPERIMENTAL

All chemicals used were of analytical reagent grade and were used without further purification. Double distilled water was used throughout the experiment. 250 mL standard stock nitrite solution of 1000 $\mu g\ mL^{-1}$, was prepared by dissolving

0.3750 g of sodium nitrite (BDH) in water containing a few drops of chloroform as a stabilizer to prevent bacterial growth. This stock solution was kept in a refrigerator at 4°C and was used within 2 weeks of preparation[16].

Working solutions were obtained daily by appropriate dilution with water. Potassium bromate stock solution of 2×10^{-2} M was prepared by dissolving 0.8350 g of $KBrO_3$ (APS) in appropriate amount of water and diluting to 250 mL. BCB solution (1.44×10^{-4} M) was prepared by directly dissolving 0.0500 g BCB in appropriate amount of water and diluted to 250 mL and was stored in dark at 4°C which was replaced every month.

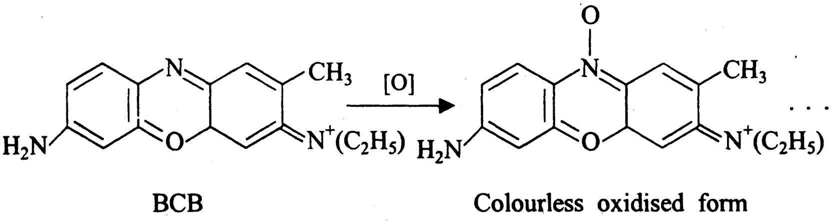
A Cintra double beam spectrophotometer, equipped with 10 mm silica cells and a recorder, was used for recording spectral changes as well as absorbance measurements. A thermostatic bath from Thermoline (Australia) BTC 9090, with circulating device and temperature measurement precision within ±0.1°C, was used to maintain the temperature of the reactants and reaction mixture. In order to eliminate any traces of metal ions coming from the glass surface, the glassware used for this work was cleaned scrupulously. Cuvettes were cleaned after use by immersion in nitric acid (1 : 2) for 15 min to remove traces of BCB absorbed on the walls. All the working solutions were kept at required temperature in the thermostatic bath for 30 min. In a 10 mL standard volumetric flask were placed, in sequence, appropriate volumes of solution containing 0.005–10 μg of nitrite, 0.88 mL of 4 M H_2SO_4, 2.6 mL of 1.44×10^{-4} M BCB and 3.5 mL of 2×10^{-2} M BrO_3. The stop clock was started immediately after the bromate solution was added and the solution was then diluted to the mark with double distilled water and mixed well. An aliquot of the reaction mixture was immediately transferred to a cuvette kept at temperature maintained at ± 0.1°C and the absorbance was recorded as a function of time. The catalyzed as well as uncatalysed reaction was followed spectrophotometrically by monitoring the decrease in absorbance at 595 nm. Absorbance measurement was started exactly after 0.5 min of initiation up to 4 min to use as *fixed time* measurement of *initial rate.* The *initial rate* of catalyzed reaction was calculated by subtracting the rate corresponding to a blank solution containing no nitrite, *i.e.*, uncatalyzed reaction.

Sampling and Determination of Nitrite in Waste Water and Sea Water

Two water samples (**1** and **2**) from each the raw waste section (R), the primary section (P) and the final section (F) of Kinoya sewage treatment plant (KSTP) and seawater samples from Suva Harbour (SH) and Laucala Bay (LB) were collected using Nesken bottles. Seawater samples were collected approximately 20 m apart from each other stored to clean sample bottles in a cooler with ice cubes. 2 mL saturated potassium aluminium sulfate was added to 150 mL of water samples to precipitate the suspended particles, millipored using 0.45 μm millipore membrane and kept at 4°C to retard the bacterial growth[8]. 2 mL of the sample was used without any pre-concentration, for the determination of nitrite by the recommended procedure.

RESULTS AND DISCUSSION

Brilliant cresyl blue is a well known redox indicator and biological developer and is susceptible to oxidation irreversibly by bromate ion in acidic media leading to formation of colourless product as shown in eq. (1).



. . . (1)

The uncatalyzed reaction is quite slow. However, nitrite ion catalyzes the reaction at trace amounts and it proceeds much faster. The absorbtion spectra of uncatalyzed (curve 1) and catalyzed (curve 2–9) reactions at different time intervals in the presence of fixed concentration of nitrite are shown in Fig. 1. It can be seen that with increase in time the absorbance of catalyzed reaction decreases at 595 nm. The results suggest that the absorbance change ΔA, at 595 nm, is quite high where there is no absorption at all by other species involved in the reaction. The optimization of reaction variables and further determination were therefore carried out at 595 nm.

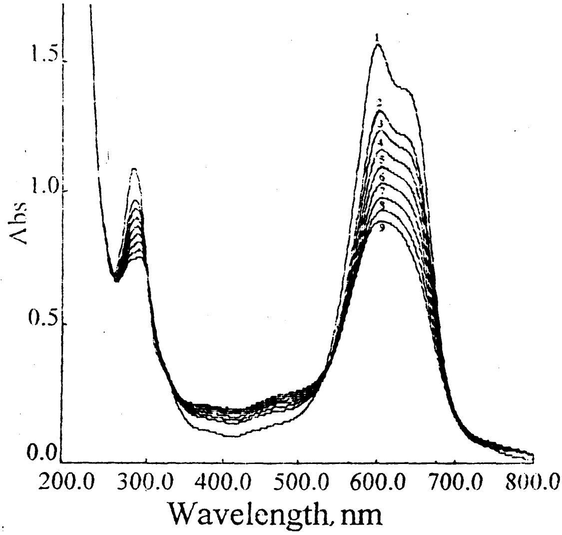


Fig. 1. Spectral scan of the uncatalysed and catalysed reaction at [H$_2$SO$_4$] = 7.4×10^2 M, [BCB] = 2.88×10^5 M, [BrO$_3^-$] = 1×10^3 M, temp. = 30°C. (1) Uncatalyzed after 1.5 min, (2) Catalyzed after 1.5 min, and (3) to (9) after intervals of 1 min each.

Reagents Concentration Optimization

In order to establish experimental conditions under which the catalytic effect of nitrite and therefore the sensitivity in its determination be at maximum, the dependence of rate on temperature and the concentration of H$_2$SO$_4$, BCB, and

BrO_3^- were studied. The change in absorbance after *fixed time* (ΔA_t) as measure of *initial rate,* were used to plot the graph for each variable and the optimum conditions were taken from the graphs for the subsequent study of the variables. The reagent concentration optimization was carried out on the uncatalyzed and catalyzed reactions for a constant time of 3 min at 30°C in the presence of 0.2 μg of nitrite.

Acidity: The effect of sulfuric acid on the uncatalyzed and catalyzed reactions was studied in the concentration range 0.1–0.45 M. The data obtained were used for a plot of ΔA_3 *vs.* $[H_2SO_4]$ as shown in Fig. 2. The reaction rate increases with increasing concentration of sulfuric acid up to 0.35 M. At still higher concentration, the rate decreases. This decrease in rate at higher acidic condition may be attributed to protonation of BCB, which might stop oxidation or make oxidation quite difficult to occur. Thus 0.35 M sulfuric acid was the optimum and was used for further study.

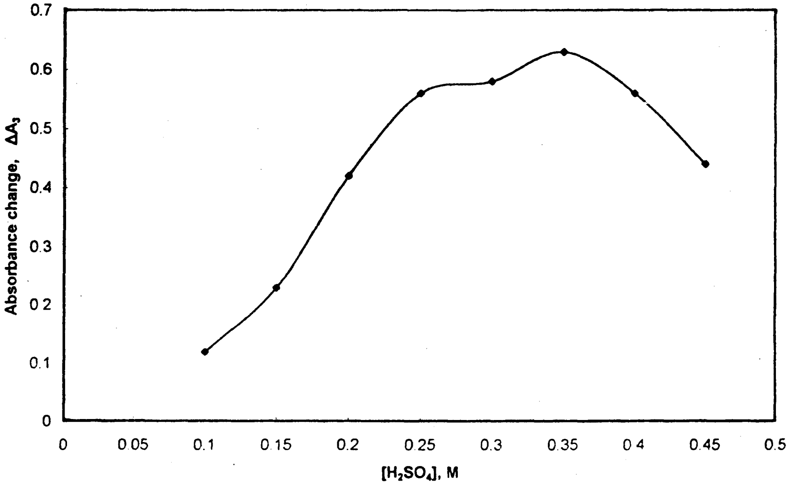


Fig. 2. Effect of H_2SO_4 concentration on reaction rate; $[BCB] = 2.88 \times 10^{-5}$ M, $[BrO_3^-] = 4 \times 10^{-3}$ M, $[NO_2^-] = 0.2$ μg ml^{-1}, temp. = 30°C.

The Reactant Concentrations: The experimental results on the study of the effect of BCB concentration (Fig. 3) in the range $3.6–57.6 \times 10^{-6}$ M indicate that the absorbance difference increases almost linearly with the concentrations of BCB at the level of $[BCB] < 36 \times 10^{-6}$ M. However, at $[BCB] > 43.2 \times 10^{-6}$ M the rate decreases. BCB concentration of 3.75×10^{-5} M was selected as the optimum. The dependence of bromate concentration on the rate of catalyzed oxidation of BCB was studied in the range of $5–90 \times 10^{-4}$ M $[BrO_3^-]$. Fig. 4 shows that with optimurn concentrations of H_2SO_4 and BCB the rate of catalyzed reaction increased up to 7×10^{-3} M of bromate. At still higher $[BrO_3^-]$ the reaction reaches the non-rate limiting stage. Thus 7×10^{-3} M was the optimum concentration selected for bromate.

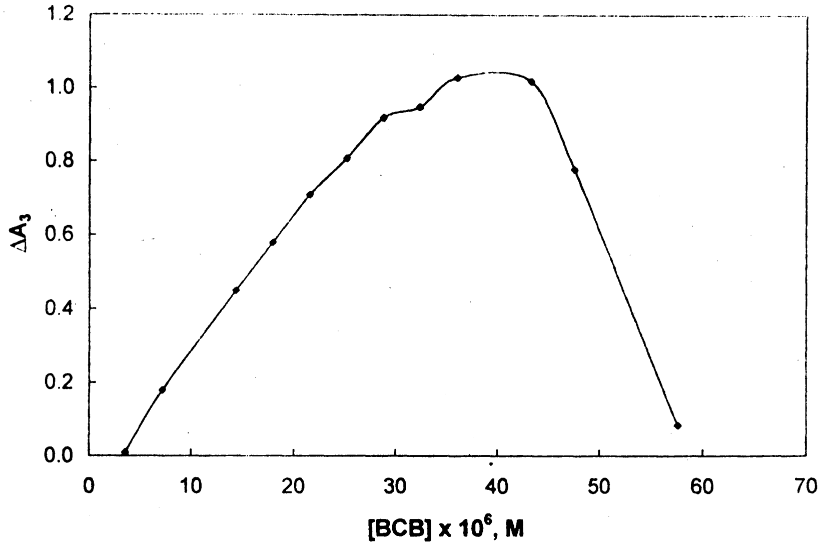


Fig. 3. Effect of BCB concentration on the rate of reaction; $[H_2SO_4] = 0.35$ M, $[BrO_3^-] = 4 \times 10^{-3}$ M, $[NO_2^-] = 0.2$ μg mL^{-1}, temp. = 30°C.

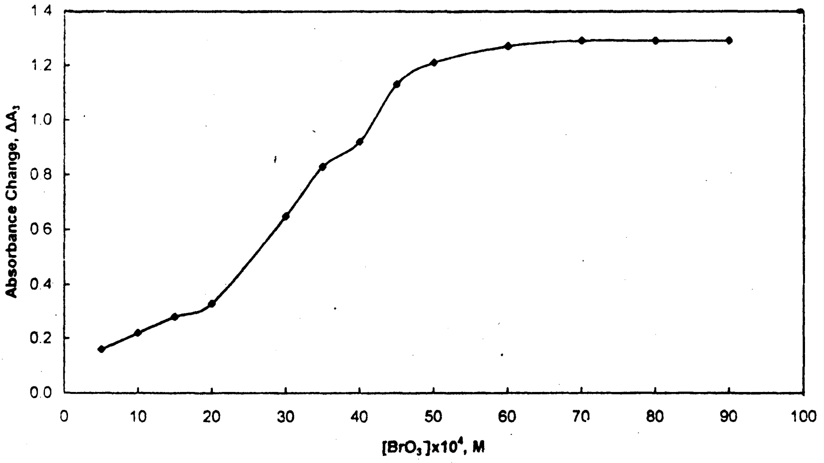


Fig. 4. Effect of BrO_3^- concentration on the rate of reaction; $[H_2SO_4] = 0.35$ M, $[BCB] = 3.75 \times 10^{-5}$ M, $[NO_2^-] = 0.2$ μg mL^{-1}, temp. = 30°C.

Effect of Temperature: The effect of temperature was studied in the range of 10–40°C under optimum reagent concentrations with 0.2 μg mL^{-1} of nitrite, where absorbance changes are remarkable (Fig. 5). With increasing the temperature up to 35°C the change in absorbance increased during the 0.5–4 min, whereas at higher temperatures the change in absorbance decreased which is contrary to the earlier report[15]. The decrease in the reaction rate at higher

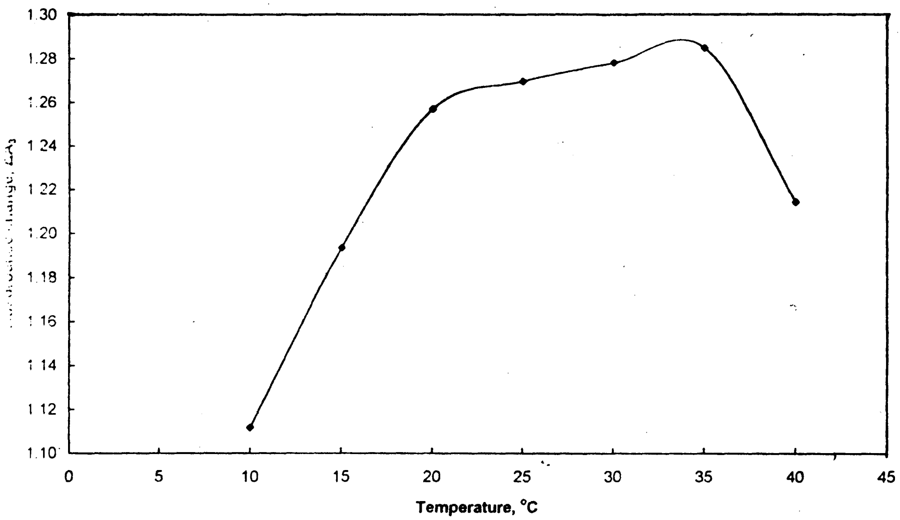


Fig. 5 Effect of temperature on the rate of reaction; $(H_2SO_4] = 0.35$ M, [BCB] = 3.75×10^{-5} M, $[BrO_3^-] = 7 \times 10^{-3}$ M, $[NO_2^-] = 0.2$ μg mL^{-1}, temp. = 30°C

temperature may be due to partial dissociation of BCB at higher temperature. For simplicity and convenient application 25°C was selected as optimum.

Reagent concentrations, acidity and temperature selected in the indicator reaction are nearly of non-rate limiting stage so their effect could be considered to be constant during the determination except the effect of analyte, *i.e.*, nitrite. The nitrite dependence study showed that the *initial rate*, ΔA_t, is proportional to the nitrite concentration with pseudo first order in nitrite. Therefore, the kinetic equation for the oxidation of BCB can be given by equation (2), where k_1 and k_1' are composite rate constant.

$$d[BCB]/dt = k_1 + k_1'[NO_2^-] \qquad (2)$$

Calibration graph was obtained by applying the *fixed time* method under the optimum conditions. A plot of the absorbance changes after 4 min, *i.e.*, ΔA_4, *vs.* nitrite concentration in the reaction mixture are linear in the range of 1–80 ng mL^{-1} $[NO_2^-]$. The higher concentrations of NO_2^- were also tried but due to achievement of non-rate limiting stage the linear relation was not valid, which is completely contrary to the dynamic range reported earlier[15]. The linear regression equation relating *initial rate*, ΔA_4 to $[NO_2^-]$ up to 80 ng mL^{-1} is given in equation (3). It is in agreement with the proposed rate equation (2).

$$\Delta A_4 = 0.0159[NO_2^-] + 0.0145 \qquad (3)$$

To establish the reliability of the proposed method recovery experiments were carried out and the results are given in Table-1. The standard deviation and percentage errors corresponding to various determinations of nitrite concentration in the range of 10–80 ng mL^{-1} are also given in Table-1, where recoveries were in the range of 97.3–103.7 per cent.

TABLE-1
ACCURACY AND PRECISION OF NITRITE DETERMINATION IN SPIKED WATER SAMPLE BY THE PROPOSED KINETIC METHOD
$[H_2SO_4]$ = 0.35 M, [BCB] = 3.75×10^{-5} M, $[BrO_3^-]$ = 7×10^{-3} M, temp. = 25°C

$[NO_2^-]$ added (ng mL^{-1})	$[NO_2^-]$ ± s found* (ng mL^{-1})	Error (%)	Recovery (%)
10	9.8 ± 0.1	2.0	98.0
20	19.5 ± 0.8	2.5	97.5
30	30.8 ± 0.1	2.7	97.3
40	41.5 ± 0.5	3.8	103.7
50	48.9 ± 0.2	2.2	97.8
60	61.8 ± 0.3	3.0	97.0
70	70.6 ± 0.1	0.8	100.8
80	77.0 ± 0.3	3.8	103.7
		Av = 2.6	

*Mean of three determinations.
The ±s values represent per cent standard deviation of the mean for three determinations.

The statistical detection limit, $X_D = X_B + t\sqrt{2}\ SB$, defined by Tanaka[17] was used to calculate the detection limit for the proposed method, where X_B = av. blank value; SB = R/d_2 where R = $blank_{max} - blank_{min}$; $1/d_2$ = 0.5908 for use of 3 blank measurements and t = 3. By using the data for ΔA_4, the detection limit was calculated to be 5.8 ng mL^{-1} of nitrite.

Interference studies

In order to investigate the analytical applicability, *i.e.*, selectivity of the method, the effect of several interfering ions that are usually present with nitrite was examined by carrying out the determination of 70 ng mL^{-1} nitrite. Table-2 shows the tolerance concentration ratio to nitrite. The tolerance limit was taken as the concentration, which caused an error of not more than ± 4% in nitrite determination. Fe(II) interfere seriously because nitrite converts the iron(II) to iron(III). The interference by Fe(II) was eliminated by addition of edta.

Determination of nitrite in real samples

To examine the applicability and validity of the proposed method for determination of nitrite, various samples collected, as in the procedure, were analyzed. Since the concentrations of other pollutants were far below the tolerance limit given in Table-2, the proposed method was applied directly, without any pre-concentration, for the determination of nitrite in collected samples. Table-3 shows the results obtained, which were confirmed by a standard method used by

KSTP authority and the results were in excellent agreement. It can be seen that the level of nitrite is lower in the wastewater samples from KSTP compared to the sea water samples. The results also show that the amount of nitrite in the sample decreases with delayed analysis time, may be due to bacterial action.

TABLE-2
EFFECT OF DIVERSE IONS ON THE DETERMINATION OF 70 ng mL^{-1} OF NITRATE
(Conditions are given in Table-1)

Ions added	Tolerance ratio[a] [Interfering ion]/ [NO_2^-]
Li^+, Cr^{3+}, CO_3^{2-}, CH_3COO^-, citrate ion, Na^+, SO_4^{2-}, ClO_4^-	2000
Cu^{2+}, Ni^{2+}, Ca^{2+}, Cd^{2+}, K^+, $C_2O_4^{2-}$, NH_4^+, NO_3^-, HCO_3^-, EDTA, NO_3^-, F^-, PO_4^{3-}	1000
Mg^{2+}, Mn^{2+}, Al^{3+}, Pb^{2+}	500
Fe^{3+}, Cl^-, Sr^{2+}	200
Zn^{2+}, Hg^{2+}, tartrate ion, Ag^+, I^-, Br^-	20
SCN^-, Co^{2+}, $S_2O_3^-$	10

[a]Based on mean of three analysis where error is within ± 4%.

TABLE-3
DETERMINATION OF NITRITE IN WASTE WATER AND SEA WATER SAMPLES

Type of samples	Delayed time (hours)	[NO_2^-] ± sd, found* (ng mL^{-1})
KSTPR-1	72	36.98 ± 0.04
KSTPP-1	72	23.83 ± 0.03
KSTPF-1	72	74.65 ± 0.04
KSTPR-1	96	36.26 ± 0.05
KSTPP-1	96	22.58 ± 0.05
KSTPF-1	96	71.16 ± 0.02
KSTPR-2	120	25.26 ± 0.03
KSTPP-2	120	17.83 ± 0.03
KSTPF-2	120	67.04 ± 0.04
KSTPR-2	144	20.66 ± 0.04
KSTPP-2	144	10.56 ± 0.05
KSTPF-2	144	67.04 ± 0.05
SH-1	02	421.68 ± 0.08
SH-2	02	421.06 ± 0.12
SH-1	24	368.30 ± 0.04
SH-2	24	363.08 ± 0.18
LB-1	02	371.16 ± 0.20
LB-2	02	368.24 ± 0.05
LB-1	06	369.28 ± 0.05
LB-2	06	365.44 ± 0.0

*Mean of three determinations.
The ± sd values represent standard deviation of the mean for three determinations.

Conclusion

The CKM described in the present paper shows that the BCB-bromate-acid system could be successfully used for the quantitative determination of trace amounts of nitrite in real samples. On the basis of optimization study we conclude the dynamic range of determination is to be 1–80 ng mL^{-1} and not 0.5–1000 ng mL^{-1} as reported in literature[15]. Also our optimization graphs obtained are quite different from those reported previously[15]. Among the various methods employed for environmental analytical work, the proposed CKM, involving spectrophotometric technique, is quite suitable for determination of nitrite in real samples.

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