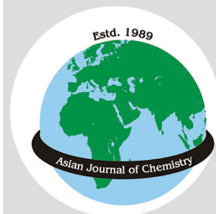

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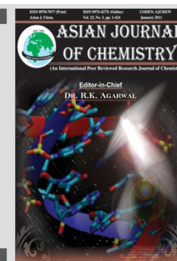

Kinetic Determination of Phenylhydrazine by its Catalytic Effect on the Reaction Between Tertrophene Blue and Bromate in Acidic and Micellar Medium

M. KEYVANFARD[1,*] and KH. ALIZAD[2]

[1]Department of Science, Majlesi Branch, Islamic Azad University, Isfahan, Iran
[2]Department of Science, Shahreza Branch, Islamic Azad University, Isfahan, Iran

*Corresponding author: Fax: +98 3355 452356; Tel: +98 3355 452360; E-mail: keyvan45638@yahoo.com

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A new, sensitive, simple, inexpensive and fast kinetic spectrophotometric method was developed for the determination of trace amounts of phenylhydrazine over the range of 1.5-15 nM. The method is based on the catalytic effect of phenylhydrazine on the reaction of tertrophene blue and bromate in acidic and micellar medium is reported. The reaction was monitored spectrophotometrically by measuring the decrease in absorbance of tertrophene blue at 632 nm with a fixed-time 0.5-2.0 min from initiation of the reaction. The detection limit is 0.6 nM and relative standard deviation of 3, 6 and 9 nM phenylhydrazine for 5 replicate measurements was 0.81, 0.40 and 1.04 %, respectively. The method was applied to the determination of phenylhydrazine in human serum and water samples.

Key Words: Phenylhydrazine, Tertrophene blue, Spectrophotometric, Micellar media.

INTRODUCTION

Phenylhydrazine induces a reactive oxygen species formation, peroxidation of lipids and oxidative degradation of spectrin in the membrane skeleton. Phenylhydrazine induced compound seems to be very useful in models studying mechanisms of hemolytic anemia. Phenylhydrazine induced hemolytic injury seems to be derived from oxidative alternations to red blood cell proteins. This compound can modulate immune reactions. Phenylhydrazine is toxic by single p.o. administration (LD50 80-188 mg/kg body weight) and is expected to be toxic by the inhalation and dermal routes. This chemical has potential for skin and eye irritation in humans. Exposure to phenylhydrazine may cause damage to red blood cells, potentially resulting in anemia and consequential secondary involvement of other tissues, such as the spleen, liver and kidney injury[1]. These, include titrimetry[2], spectrophotometry[3-6], gas chromatography[7], H-point standard addition method[8], kinetic methods[9-11] and electrochemical method[12]. These methods either lack sufficient sensitivity or are time consuming. In order to overcome these problems, we developed and validated a rapid, sensitive and selective kinetic spectrophotometric method for the determination of phenylhydrazine. Here, we report a kinetic method for ultra trace determination of phenylhydrazine, based on its catalytic effect on the reaction of tertrophene blue and $KBrO_3$ in acidic and micellar medium. Phenylhydrazine and tertrophene blue has the following structure (Fig. 1a-b).

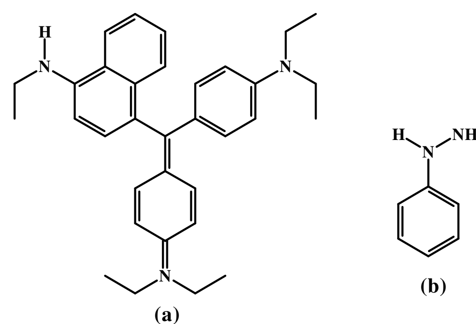


Fig. 1. Structure of (a) tertrophene blue and (b) phenylhydrazine

EXPERIMENTAL

Analytical-reagent grade and doubly distilled water were used. A 1.0×10^{-3} M stock standard solution of phenylhydrazine was prepared by dissolving 0.0147 g of phenylhydrazinium chloride (Merck, M = 144.6 g/mol) in distilled water and diluting it to 100 mL. Working solutions were prepared by appropriately diluting the stock standard solution. A 100 mL 0.001M potassium bromate solution was prepared by dissolving 0.0167 g of $KBrO_3$ (Merck, M = 167 g/mol) in distilled water and diluting it to mark in a 100 mL volumetric flask. A 4.0×10^{-4} M tertrophene blue solution was directly prepared by dissolving 0.02 g of tertrophene blue (Merck, M = 506.01 g/mol) in 50 mL ethanol and diluting it with distilled water in a 100 mL volumetric flask. Cetyl trimethyl ammonium

bromide (CTAB) solution (0.013 M) was prepared by dissolving 1.197 g CTAB (Merck) in distilled water and diluting to 250 mL in a 250 mL volumetric flask. The other surfactants tested, namely triton x-100, cetyl pyridinium chloride (CPC), sodium dodecyl sulphate (SDS), dodecyl trimethyl ammonium bromide (DTAB), hexa decyl pyridinium bromide (HDPB), tetra butyl ammonium bromide (TBAB) and hexa decyl pyridinium chloride (HCPC) were prepared in a similar way. Hydrochloric acid solution (1.0 M) was prepared by diluting a known volume of its concentrated solution (Merck).

Absorption spectra were recorded with a CECIL model 7500 spectrophotometer with a 1 cm quartz cell. A model 2501 CECIL spectrophotometer with 1 cm glass cuvettes was used to measure the absorbance at a fixed wavelength. A thermostat water bath (Gallen Kamp Griffin, BGL 240 V) was used to keep the reaction temperature at 30 ± 0.1 ºC. A stopwatch was used for recording the reaction times.

Determination of phenylhydrazine in human serum: Mineralization of 2 mL of the samples was carried out for 1 h at 100 ºC with the addition of 4 mL of concentrated nitric acid[13]. Then samples were analyzed directly after neutralization with sodium hydroxide solution and dilution with double distilled water to a suitable volume.

Recommended procedure: All the solutions and distilled water were kept in thermostated water-bath at 30 ± 0.1 ºC for 0.5 h before starting the experiment. An aliquot of the solution containing 1.5-15 nM phenylhydrazine was transferred into a 10 mL volumetric flask and then 2.2 mL of 1.0 M hydrochloric acid, 1 mL of 4.0×10^{-4} M tertrophene blue and 1.2 mL CTAB solution were added to the flask. The solution was diluted to 7 mL with water, then 2 mL 0.001 M potassium bromate solution was added and the solution was diluted to the mark with water. The solution was mixed and a portion of the solution was transferred to the spectrophotometic cell. The reaction was followed by measuring the decrease in absorbance of the solution against water at 632 nm for 0.5-2.0 min from initiation of the reaction. This signal (sample signal) was labeled as A_s. The same procedure was repeated without addition of phenylhydrazine solution and the signal (blank signal) was labeled as A_b. Time was measured just after the addition of last drop of bromate. The calibration graph was constructed by plotting of (A_s - A_b) *versus* phenylhydrazine concentration at a fixed-time of 0.5-2.0 min from initiation of the reaction.

RESULTS AND DISCUSSION

Optimization of variables: There are many methods, such as fixed-time, initial rate, rate constant and variable time methods for measuring the catalytic species. Among these, the fixed-time method is the most conventional and simplest, involving the measurement of A at 632 nm. Fig. 2 shows the relationship between A and reaction time for catalyzed reaction.

It was found that the rate of reaction is proportional to the phenylhydrazine concentration. Therefore, by measuring the decrease in absorbance of tertrophene blue for a fixed time of 0.5-2.0 min from initiation of the reaction, the phenylhydrazine contents in the sample can be measured.

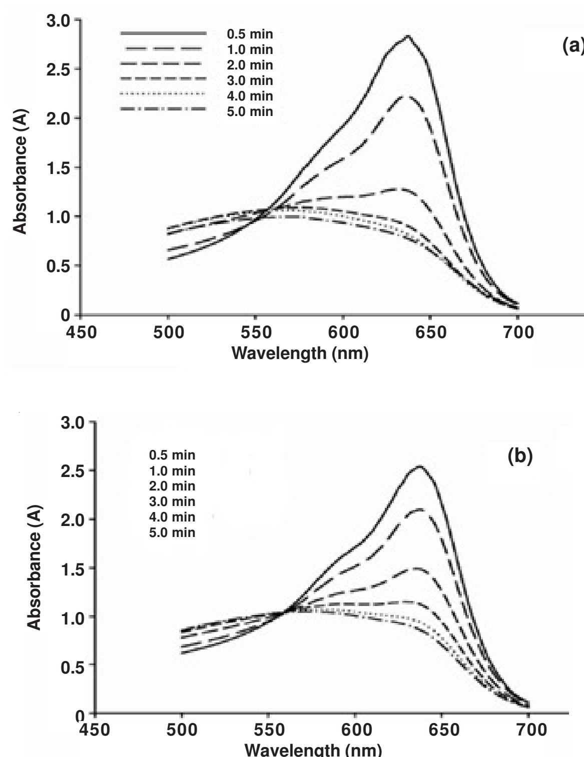


Fig. 2. Absorption spectrum for the phenylhydrazine - tertrophene blue - BrO_3^- system with time. Conditions: 0.22 M HCl; 1.6×10^{-3} M CTAB; 4.0×10^{-5} M tertrophene blue; 2.0×10^{-4} M BrO_3^-; 30 ± 0.1 ºC temperature; interval time for each scan, 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 min from initiation of the reaction. (a) in presence of 14 nM of phenylhydrazine and b) in absence of phenylhydrazine

The influence of HCl concentration, CTAB concentration, tertrophene blue concentration, bromate concentration and temperature on the analytical signal was studied to find the optimum conditions.

The effect of HCl concentration on the sensitivity was studied in the range of 0.10-0.24 M in the presence of 7.5 nM phenylhydrazine, 1.8×10^{-3} M CTAB, 3.2×10^{-5} M tertrophene blue and 2.6×10^{-4} M BrO_3^- at 30 ± 0.1 ºC. Fig. 3 shows that by increasing HCl values up to 0.22 M, the net reaction rate increases, whereas higher HCl values cause decreasing the analytical signal. This phenomenon is due to the fact that in acidic medium, tertrophene blue was protonated. Therefore, an HCl concentration of 0.22 M was selected for further study.

Fig. 4 shows the influence of tertrophene blue concentration on the analytical signal in the range of $(2.4\text{-}4.5) \times 10^{-5}$ M. The results show that by increasing tertrophene blue concentration up to 4.0×10^{-5} M, the net reaction rate increases, whereas greater amounts of the dye decrease the analytical signal. This may be due to the aggregation of the dye in higher concentration. Therefore, a tertrophene blue concentration of 4.0×10^{-5} M was selected for the study.

The effect of bromate on the reaction rate was studied in the range $(1.6\text{-}3.0) \times 10^{-4}$ M (Fig. 5). The results show that the net reaction rate increases with bromate up to 2.0×10^{-4} M, whereas the reaction rate decreases with increasing bromate concentration from 2.0×10^{-4} M to greater values. This means that the rate of uncatalyzed reaction increases with bromate concentration (> 2.0×10^{-4}) to a greater extent than the

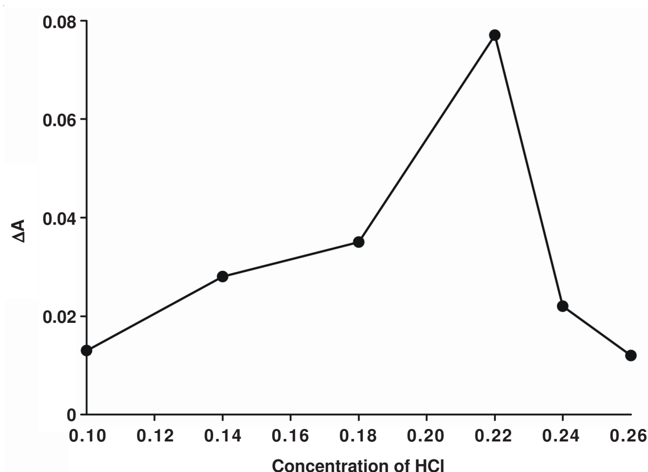


Fig. 3. Effect of HCl concentration on the sensitivity. Conditions: 7.5 nM phenylhydrazine; 1.8×10^{-3} M CTAB; 3.2×10^{-5} M tertrophene blue; 2.6×10^{-4} M BrO_3^-; 30 ± 0.1 ºC temperature and time of 2.0 min from initiation of the reaction

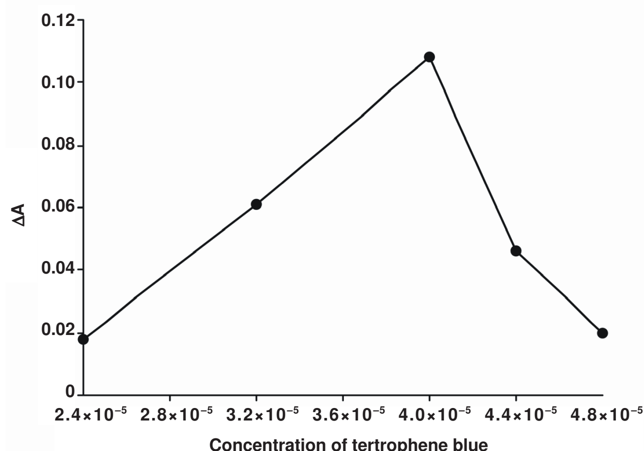


Fig. 4. Effect of tertrophene blue concentration on the sensitivity. Conditions: 0.22 M HCl; 7.5 nM phenylhydrazine; 1.8×10^{-3} M CTAB; 2.6×10^{-4} M BrO_3^-; 30 ± 0.1 ºC temperature and time of 2.0 min from initiation of the reaction

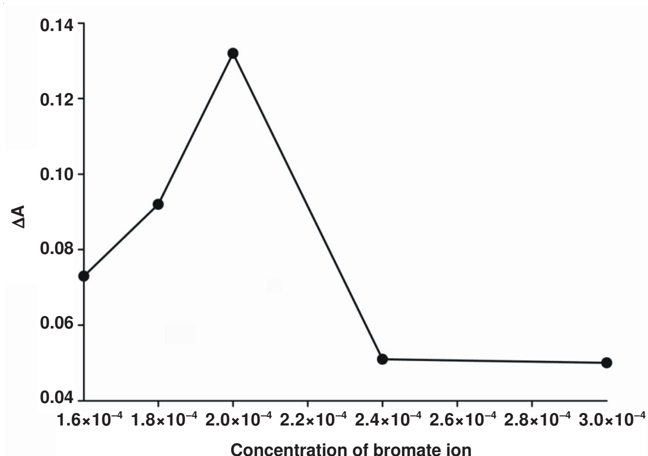


Fig. 5. Influence of BrO_3^- on the sensitivity. Conditions: 0.22 M HCl; 7.5 nM phenylhydrazine; 1.8×10^{-3} M CTAB; 4.0×10^{-5} M tertrophene blue; 30 ± 0.1 ºC temperature and time of 2.0 min from initiation of the reaction

catalyzed reaction and the difference between the rates of catalyzed and uncatalyzed reaction (A_s-A_b) diminishes at higher bromate concentration. Thus, a bromate concentration of 2.0×10^{-4} M was selected for further study.

In many reactions, suitable micelles can affect the rate of reactions[14-16]. A micelle usually can be formed by aggregation of charged organic molecules. These micelles have the same charge at the outer sphere. For those reactions which have charged species, these micelles can affect the rate of reaction by increasing the effective collisions. In order to choose an appropriate micellar system to enhance the rate of reaction, one should take into account the type of charge of the reactants, because the accelerating effect of micelles arises essentially due to electrostatic and hydrophobic interactions between the reaction and micellar surfaces[17]. Cationic (CTAB, CPC, DTAB, HDPB, TBAB, HCPC), anionic (SDS) and nonionic (Triton-X-100) micelles were tested at a concentration greater than the critical micelle concentration (CMC). The results are shown in Table-1. Phenylhydrazine and tertrophene blue are positively charged and bromate is negatively charged. Therefore, it seems logical to think that the cationic micelles can enhance the rate of phenylhydrazine-tertrophene blue-bromate reaction. In fact, both CTAB, CPC, DTAB, HDPB, TBAB and HCPC increased sensitivity, but CTAB increased sensitivity more than CPC, DTAB, HDPB, TBAB and HCPC; thus, CTAB was chosen for the study (Table-1). The effect of CTAB concentration on the rate of reaction was studied in the range of (1.3-2.1) × 10^{-3} M. The sensitivity increases with increasing CTAB concentration up to 1.6×10^{-3} M and decreases at higher concentrations. This is due to the high aggregation of the surfactant and change in the molar absorptivity of the tertrophene blue in the solution. Therefore, a final concentration of 1.6×10^{-3} M was selected as the optimum concentration of CTAB (Fig. 6).

TABLE-1
SURFACTANTS TESTED AS POTENTIAL MICELLAR CATALYSTS FOR THE ENHANCED RATE OF PHENYLHYDRAZINE-TERTROPHENE BLUE-BrO_3^- REACTION

Surfactant	Type	CMC (M)	Micellar catalysis
SDS	Anionic	8.1×10^{-3}	Negative
CPC	Cationic	1.2×10^{-4}	Positive
CTAB	Cationic	1.3×10^{-3}	Positive
DTAB	Cationic	1.5×10^{-2}	Positive
HDPB	Cationic	6.5×10^{-4}	Positive
TBAB	Cationic	7.5×10^{-5}	Positive
HDPC	Cationic	2.4×10^{-4}	Positive
Triton X-100	Nonionic	3.0×10^{-4}	Positive

The influence of reaction temperature on the analytical signal was studied in the range 25 ± 0.1 – 45 ± 0.1 ºC with the optimum other reagent concentration. The results showed that by increasing temperature up to 30 ± 0.1 ºC, the analytical signal increases, whereas higher temperature values because decreasing the analytical signal (A_s-A_b). This means that the rate of uncatalyzed reaction increases with temperature to a greater extent than the catalyzed reaction in higher temperature (> 30 ± 0.1 ºC). Thus, the difference between A_s and A_b diminishes at higher temperature. Therefore, a temperature of 30 ± 0.1 ºC was used in this study (Fig. 7).

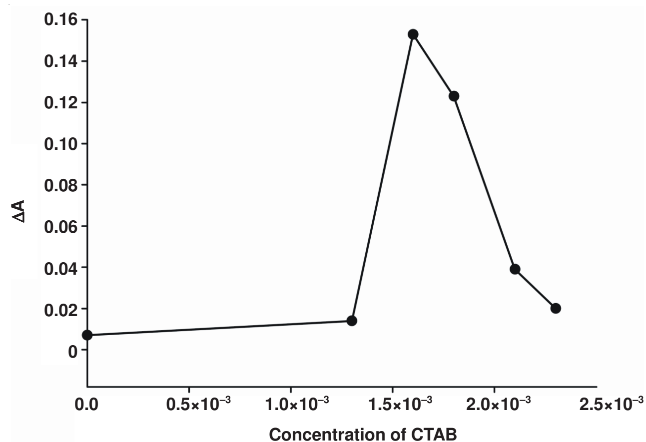


Fig. 6. Influence of CTAB on the sensitivity. Conditions: 0.22 M HCl; 7.5 nM phenylhydrazine; 4.0×10^{-5} M tertrophene blue; 2.0×10^{-4} M BrO_3^-; 30 ± 0.1 ºC temperature and time of 2.0 min from initiation of the reaction

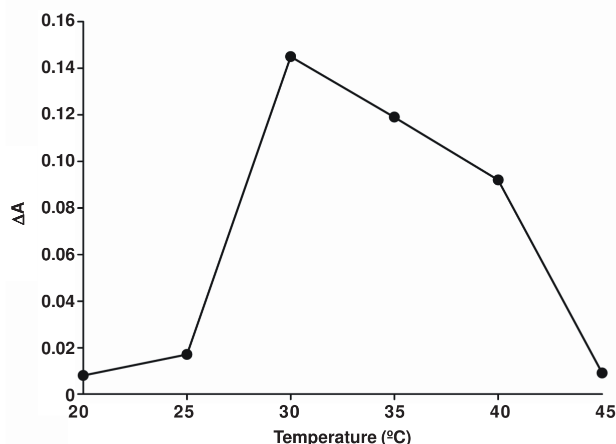


Fig. 7. Influence of temperature on the sensitivity. Conditions: 0.22 M HCl; 7.5 nM phenylhydrazine; 1.6×10^{-3} M CTAB; 4.0×10^{-5} M tertrophene blue; 2.0×10^{-4} M BrO_3^- and time of 2.0 min from initiation of the reaction

Under the optimum conditions described above the fixed-time method was applied to measure the change in absorbance over an interval times of 0.5-2.0 min from initiation of the reaction, because it gave better sensitivity and reproducibility.

Calibration graph, detection limit, reproducibility and accuracy: The calibration graph was linear for phenylhydrazine concentration in the range 1.5-15 nM with the regression equation of $\Delta A = 0.048C + 0.400$ with (r = 0.9998 n = 10), where ΔA is change in absorbance for the sample reaction for 0.5-2.0 min from initiation of the reaction (catalytic reaction) and C is the phenylhydrazine concentration in nM. The limit of detection (defined as $DL = 3S_b/m$, where DL, S_b and m are limit of detection, standard deviation of the blank signal and slope of the calibration graph, respectively) is equal to 0.6 nM phenylhydrazine. The relative standard deviations (RSD) for ten replicate determinations of 3, 6 and 9 nM phenylhydrazine are 0.81, 0.40 and 1.04 %, respectively.

Interference study: More than 30 foreign substances added in solution were studied for the possible influence on the determination of phenylhydrazine by the proposed method. The maximum amount of substance causing an error of more than 3 % in the determination of 7.5 nM phenylhydrazine was taken as the tolerance limit. The results are showed in Table-2. The results show that the method is relatively selective.

TABLE-2
EFFECT OF FOREIGN IONS ON THE DETERMINATION OF 7.5 nM PHENYLHYDRAZINE

Foreign species	Tolerated ratio $W_{species}/W_{phenylhydrazine}$
NO_3^-, SO_3^{2-}, Cl^-, BO_3^{3-}, ClO_3^-, Br^-, IO_4^-, SCN^-, I^-, CH_3COO^-, IO_3^-, Na^+, K^+, Ba^{2+}, Cr^{3+}, Mn^{2+}, Ni^{2+}, Pb^{2+}, Al^{3+}, Ag^+, Co^+, Mg^{2+}, Cd^{2+}, Mo^{6+}, Se^{4+}, Rh^{3+}, V^{5+}, Pd^{2+}, Glucose, saccharose	1000
CO_3^{2-}, histidine, aspirine, thiourea	400
NO_2^-, caffeine, dopamine, ascorbic acid, uric acid	200
$S_2O_8^{2-}$, Hg^{2+}	50
Cu^{2+}	30
N_2H_4	Inhibited

Application of the method: In order to evaluate the applicability of the proposed method, real and synthetic water samples were analyzed to determine phenylhydrazine contents. The results are presented in Table-3. Good recoveries and precise results show good reproducibility and accuracy of the method. The kinetic spectrophotometric method developed for the determination of phenylhydrazine is inexpensive, employs available reagents, allows rapid determination at low operating costs and provides simplicity, adequate selectivity, a low limit of detection compared to other kinetic procedures. Using this method, it is possible to determine phenylhydrazine at levels as low as 1.5 nM without the need for any preconcentration steps.

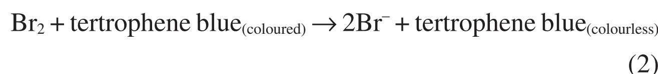
TABLE-3
DETERMINATION OF PHENYLHYDRAZINE IN REAL SAMPLES

Sample	Phenylhydrazine added (nM)	Phenylhydrazine found (nM)	Recovery (%)	RSD % n = 3
Well water Isfahan	2	1.9	95	0.43
	3	3.1	103	0.89
	4	3.8	95	0.87
	3	2.8	93	1.11
Human serum	7	7.5	107	1.05
	10	10.8	108	0.99

Mechanism of reaction: Bromate is reduced by the chloride ion in acidic media:

$$2BrO_3^- + 10Cl^- + 12H^+ \rightleftharpoons Br_2 + 5Cl_2 + 6H_2O \quad (1)$$

Tertrophene blue reacts with the products of the reaction and is decolourized.



$$Br_2 + \text{tertrophene blue}_{(coloured)} \rightarrow 2Br^- + \text{tertrophene blue}_{(colourless)} \quad (2)$$

The rate of this reaction is very slow. It is found that in the presence of cetyl trimethyl ammonium bromide (CTAB) as a micellar medium, this reaction rate is sharply increased by addition of trace amounts of phenylhydrazine. The rate equation of the catalyzed reaction is:

$$\text{Rate} = -d[\text{tertrophene blue}]/dt \quad (3)$$

$$\text{Rate} = k[\text{phenylhydrazine}][\text{tertrophene blue}]^m[BrO_3^-]_n \quad (4)$$

where k is the rate constant. Because $[BrO_3^-]$ > [tertrophene blue], BrO_3^- can be considered to be constant and m was found to be 1. By integration of eqn. 4 and by incorporating Beer's law, we obtain the final expression:

$$\Delta A = k[\text{phenylhydrazine}]t \quad (5)$$

where t is the reaction time.

Conclusion

The kinetic-spectrophotometric method developed for phenylhydrazine determination in water is inexpensive and readily available and allows rapid determination at low operating costs and shows simplicity, adequate selectivity, the detection limit of the proposed method was in the nanomolar range and in comparison with reported methods was better (Table-4). The method was found to possess good precision and accuracy, in relation to the other kinetic procedures. Therefore, the method could be proposed for environmental analyses.

TABLE-4
COMPARISON OF KINETIC SPECTROPHOTOMETRY METHODS FOR DETERMINATION OF PHENYLHYDRAZINE WITH PROPOSED METHOD

Method	DL (nM)	LDR (nM)	Ref. No.
H-point standard addition method	460	1841-92072	8
Kinetic spectrophotometry	185	460-74000	10
Kinetic spectrophotometry	74	994-9944	9
Kinetic spectrophotometry	20	1000-10000	11
Proposed method	0.6	1.5-15	–

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