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Kinetic Spectrophotometric Determination of Trace Amounts of Thiocyanate Based on its Catalytic Effect on the Bromate-Crystal Violet Reaction in Biological Samples

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Abstract

A new analytical method was developed for determination of the thiocyanate content of spiked water and biological samples. This method is based upon the catalytic effect of the ion on the reaction between sodium bromate and crystal violet (CV) in acidic media. The decolourisation of CV was used in order to monitor the reaction spectrophotometrically at 630 nm. The variables affecting the reaction rate were determined and optimized. The method is simple, rapid, relatively sensitive and precise. The limit of detection (3σ) was 6 ng mL^{-1} and the calibration range was linear over the thiocyanate concentration range of 10-1250 ng mL^{-1}. The relative standard deviations for ten replicate determinations of 0.250, 0.500 and 1.00 μg mL^{-1} were 4.36%, 3.04% and 1.11%, respectively. Influences of the potentially interfering substances were also studied. The method was successfully used to determine the thiocyanate content of spiked water and biological samples.

Keywords: Thiocyanate; crystal violet (CV); bromate; kinetic, spectrophotometry.

1. Introduction

Thiocyanate ion is the metabolite of cyanide and the product of detoxification of compounds containing cyanide through a reaction catalyzed by the enzyme rhodanase. Everyone has thiocyanate in their saliva, which means that we are exposed to cyanide sources throughout our daily lives. Clinical studies carried out on food and cigarette smoke have shown that thiocyanate concentration in urine and saliva is higher in smokers than in non-smokers, and thus it could be used to distinguish between smokers and non- smokers [1-4].

It has been reported that the presence of thiocyanate in the human body has some relations to local goiter.[5] Saliva thiocyanate may also have an antibacterial role in mouth, decreasing the corrosion potential of amalgams[6] or carries danger [7]. In addition, with increasing thiocyanate concentration, protein dialysis would be affected [8]. Although its toxicity is not comparable with cyanide, thiocyanate is harmful to aquatic life. Therefore, a simple

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reliable and accurate method for determination of the thiocyanate concentration in biological samples at low levels is of medical interest.

Several methods have been reported for determination of the thiocyanate concentration in samples, including the ion-selective electrode [9-13], spectrophotometric [14-17], fluorimetric [18,19], voltammetric [20,21] and chromatographic [4-5, 22-24] methods. Potentiometric methods based on ion-selective electrodes are complicated and laborious to perform. Many spectrophotometric methods based on the formation of the red Fe(III)-thiocyanate complex [14,15] have also been reported; these methods are sensitive but do not possess sufficient selectivity. Chromatographic methods such as electrophoresis [23,24], ion chromatography [22] and gas chromatography [4,5] have been used to determine the presence of thiocyanate in various samples. These methods have some drawbacks such as being time-consuming, having complicated operations and requiring expensive equipments.

Kinetic Spectrophotometric methods have been widely used to determine many analytes at trace levels. These methods have significant advantages such as high sensitivity, selectivity and the need for the use of a spectrophotometer as a cheap instrument for carrying out these investigations. A few number of indicator reactions have been reported for kinetic determination of the thiocyanate by spectrophotometric detection.[25-28]. Some of these methods have lower dynamic ranges [25,26] and higher limits of detection [26] and all of these methods have relatively low selectivity with respect to the proposed method.

In this work, the oxidation reaction of CV with bromate in acidic media was used as a new indicator reaction in determination of the thiocyanate ion concentration in the samples. This reaction is catalyzed by trace amounts of the ion. The reaction was followed spectrophotometrically by monitoring the decrease in absorbance of CV at 630 nm at a fixed time range of 15-265 s. The proposed method which is sensitive and selective was successfully applied for determination of the thiocyanate concentration in saliva and water samples.

2. Experimental

2.1. Reagents and chemicals

All the chemicals used were of analytical-reagent grade. Doubly distilled water was used throughout. A stock solution containing 1000 µg mL^{-1} of thiocyanate ion was prepared by dissolving 0.1676 g potassium thiocyanate (Merck) in distilled water and diluting it to 100 ml in a calibrated flask. Working solutions were prepared daily by appropriate dilutions of the stock solution. A 0.04 M stock solution of sodium bromate was prepared by dissolving 0.6036 g $NaBrO_3$ (Merck) in 100 mL standard flask. A 1.4 M HCl solution was prepared by diluting the

appropriate volume of concentrated hydrochloric acid (Merck), which was then standardized against sodium carbonate. A 4.9 $\times 10^{-4}$ M CV solution was prepared by dissolving 0.0500 g of CV (Merck) in distilled water and diluting it in a 250 ml calibrated flask. The mixtures of the CV and sodium bromate solutions with desired concentrations were prepared by diluting the appropriate volumes of their stock solutions.

2.2. Apparatus

A Shimadzu UV-160 spectrophotometer with 1.0 cm quartz cell pairs was used to record the UV-VIS spectra and measure the absorbance at a fixed wavelength. A water bath thermostat (n-BIOTEK, INC., model NB-301) was used to control the reaction temperature. A stopwatch was used to record the reaction times.

2.3. Procedure

The reagent solutions and water were kept at 20.0 ºC in the thermostatic water bath for 30 min. 1.0 mL of 1.40 M hydrochloric acid and an appropriate volume of sample or standard solution containing 0.10-12.5 µg SCN^- were added to a 10 mL volumetric flask and diluted with water to nearly about 8 mL. Then 1.0 mL of the CV (1.96 $\times 10^{-5}$ M) and sodium bromate (2.06 $\times 10^{-3}$ M) mixture was added and the stopwatch was started. The solution was diluted to the mark with distilled water, and then a portion of the reaction mixture was transferred to the spectrophotometer cell. The absorbance change at 630 nm was measured at the fixed times 15 s and 265 s from the initiation time of the reaction ($\Delta A_s = A_{15} - A_{265}$). A blank solution (without thiocyanate) was prepared and measured in a similar way ($\Delta A_b = A_{15} - A_{265}$). Therefore, ΔA_s is the absorbance change of the sample solution or catalyzed reaction and ΔA_b is the absorbance change of the blank solution or uncatalyzed reaction. The difference between absorbance changes of the sample and blank reactions ($\Delta A = \Delta A_s - \Delta A_b$) was used as the analytical signal.

3. Results and discussion

CV is a dye that has been used as an indicator [29-30] and reagent [31-32] in analytical chemistry. The reaction of CV with sodium bromate in acidic media at room temperature is slow, but the reaction rate sharply increases by the addition of trace amounts of thiocyanate, and the absorption band of CV at 630 nm decreases with time (Fig. 1). Thus the absorbance changes at λ=630 nm was used for monitoring the reaction spectrophotometrically.

The effects of different variables on the reaction rate were studied in the presence and absence of thiocyanate in order to choose the optimum conditions. The system was optimized by one parameter at a time procedure. The difference between absorbance changes of the

sample and blank was measured at the times 15 s and 265 s after the initiation of reactions at 630 nm.

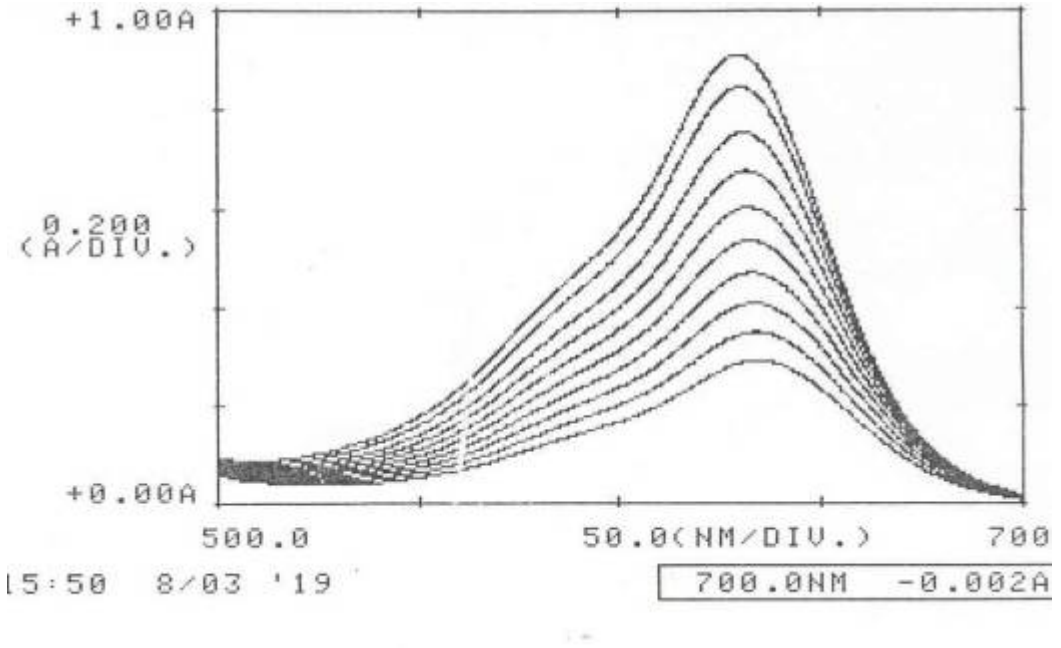

Fig.1. Absorption spectra of reaction system of CV-bromate-SCN^-.

Conditions: HCl, 0.14 M; $NaBrO_3$, 2.04×10^{-3} M; CV, 1.96×10^{-5} M; SCN^-, 0.25 µg mL^{-1} and temperature of 20.0 ºC with scan time intervals of 25 s.

Preliminary experiments were carried out for choosing the best type of acid as the reaction medium. Solutions with the same concentration of hydrochloric, nitric and sulfuric acid (0.10 M) were tested. The analytical signals for hydrochloric, nitric and sulfuric acid were obtained to be 0.061, 0.013 and 0.008, respectively. The results showed that hydrochloric acid had the best sensitivity, and thus it was selected as the best reaction medium.

The effect of hydrochloric acid on the uncatalyzed and catalyzed reactions was investigated in the 0.10-0.24 M concentration range of this acid. The results show that with increase in the hydrochloric acid concentration, ΔA_s and ΔA_b increase, due to increasing oxidation ability of bromate. Also the analytical signal (ΔA) increased and reached a maximum value at 0.14 M hydrochloric acid (Fig. 2). At higher concentrations, the analytical signal decreased. This decrease in the analytical signal at higher acidic concentrations is resulted from the increase in the blank reaction rate compared with the sample (catalyzed) reaction rate. Thus 0.14 M hydrochloric acid was concluded to be the optimum concentration and was used for further study.

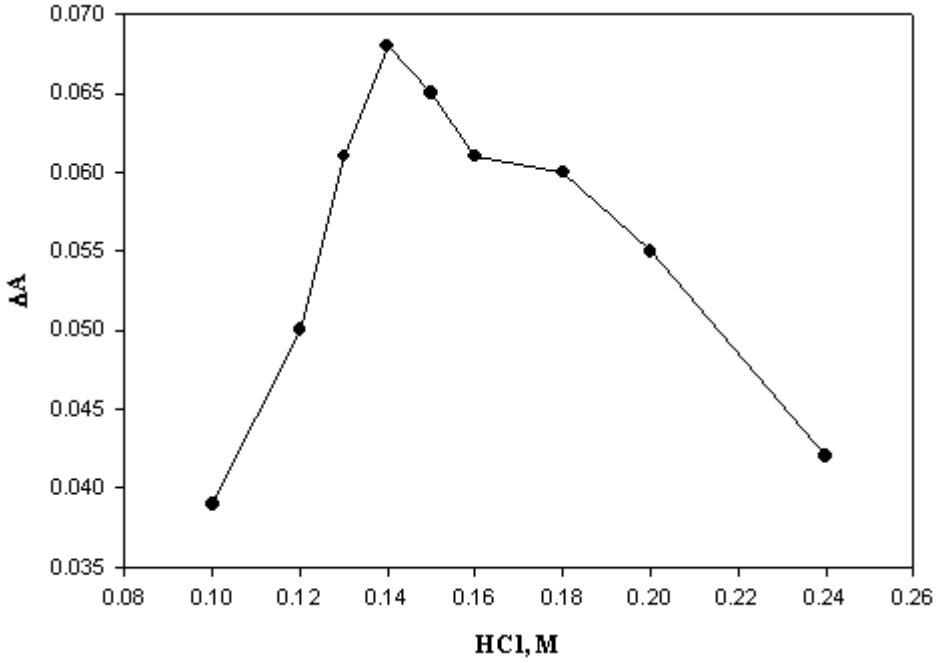

Fig 2. Influence of HCl concentration.
Condition: $NaBrO_3$, 2.0×10^{-3} M; CV, 1.96×10^{-5} M; SCN^-, 0.25 µg mL^{-1} at 20.0 ºC.

Dependence of the rates of the catalyzed (ΔA_s) and uncatalyzed (ΔA_b) reactions and the analytical signal (ΔA) on the bromate ion concentration was studied in the concentration range of 1.6-2.4×10^{-3} M for sodium bromate at 20.0 ºC. The results show that both ΔA_s and ΔA_b increased with increasing bromate concentration and the analytical signal increased up to 2.04×10^{-3} M, then it remained constant up to the 2.08×10^{-3} M bromate concentration (Fig. 3). At higher concentrations, the rates of the catalyzed and uncatalyzed reactions increased. Therefore, 2.06×10^{-3} M sodium bromate was chosen for the subsequent studies.

Effect of the CV concentration on the sensitivity of the method was studied in the 1.47×10^{-5}- 2.21×10^{-5} M concentration range of CV at the optimum concentrations of the other reagents. The results obtained show that ΔA_s and ΔA_b increased with increasing CV concentration; the analytical signal increased up to 1.96×10^{-5} M and then decreased. Thus 1.96×10^{-5} M CV was used for the subsequent study.

Effect of temperature on the rates of the catalyzed and uncatalyzed reactions were studied between 5.0 and 40.0 ºC at the optimum concentrations of the reagents. As the temperature increased, both the sample and blank reaction rates increased. However, the effect was more pronounced for the latter. The results show that 20.0 ºC is the optimum temperature, since it provides a good reaction rate and could easily be maintained. Thus 20.0 ºC was used throughout the study.

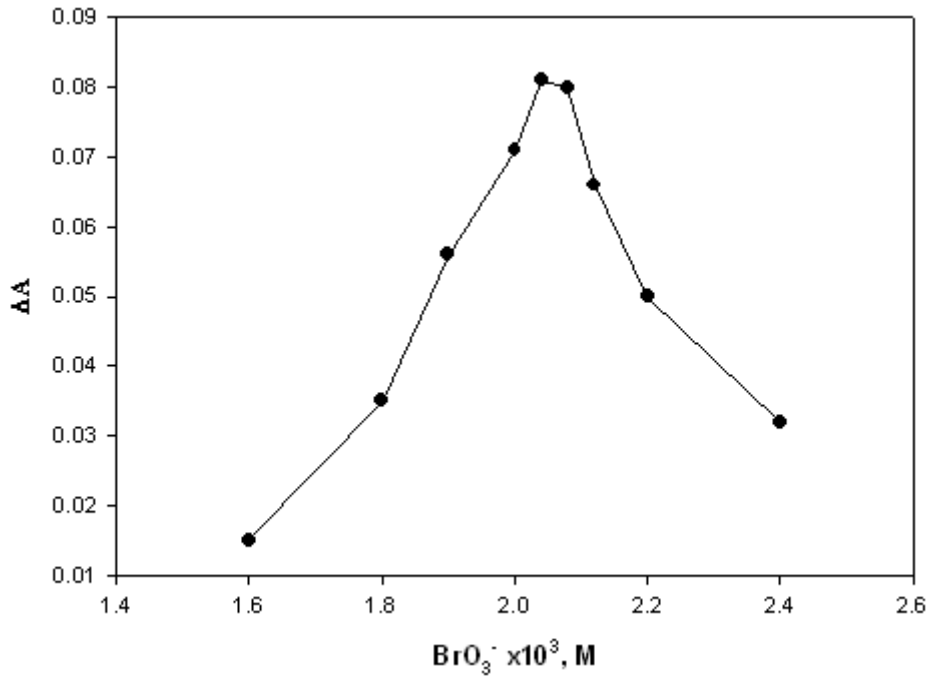

Fig 3. Effect of sodium bromate concentration.
Condition: HCl, 0.14 M; CV, 1.96×10^{-5} M; SCN^- 0.25 μg mL^{-1} at 20.0 ºC.

Effect of ionic strength on the analytical signal was studied using potassium nitrate (2.0 M). The results show that the sensitivity of the method is independent from the ionic strength until the potassium nitrate concentration is 0.4 M.

3.1. Calibration graph

Under the optimum conditions of all the effective variables (hydrochloric acid, 0.14 M; BrO_3^- 2.06×10^{-3} M; CV, 1.96×10^{-5} M; temperature, 20.0 ºC) a calibration graph was obtained in the concentration range of 0.010-1.25 μg mL^{-1} for SCN^-. The regression equation $\Delta A= 0.0144+ 0.245\ C_{SCN^-}$ (n=10) with the correlation coefficient 0.9960 was obtained; C_{SCN^-} is the concentration of SCN^- expressed in μg mL^{-1}. The theoretical limit of detection was 6.0 ng mL^{-1} thiocyanate (with 3σ of the blank signal). In order to examine the precision and accuracy of the method, standard solutions of 0.250, 0.500 and 1.00 μg mL^{-1} of thiocyanate were analyzed using the recommended procedure. Ten repeated determinations of each concentration gave the relative standard deviations of 4.36%, 3.04% and 1.11%, respectively. The Student's t-test at the 95% confidence level showed that there was no systematic error in the proposed method and this indicates its reliability.

3.2. Interference study

Under the experimental conditions chosen, the effects of various cations and anions on the determination of the SCN^- concentration were studied by adding a known quantity of the desired ion to a solution containing 2.5 µg of SCN^-, which was determined as described in the given procedure. The tolerance limit was defined as the concentration at which the species caused an error of less than ±5%. The results obtained are summarized in Table 1. As it could be seen in this table, most of the cations and anions do not interfere with the method. In the presence of EDTA, the interfering effects of Hg(II), Cr^{3+}, Fe(III), and Fe(II)[29] decrease up to 400, 800, 160 and 8 fold, respectively. However, presence of the NO_2^- and I^- ions caused serious interferences.

Table 1. Influence of foreign ions on the determination of 0.25 µg mL^{-1} SCN^-.

Ion	Tolerated ratio(w_{ion}/w_{SCN^-})
EDTA	4000
Na^+, K^+, Ba^{2+}, Ca^{2+}, Mg^{2+}, Ni^{2+}, Mn^{2+}, Li^+, Co^{2+}, Cu^{2+}, Zn^{2+}, NO_3^-, SO_4^{2-}, CO_3^{2-}	1000[a]
F^-, C_2O_4	120
PO_4^{3-}	300
Hg^{2+}	50
Al^{3+}, Cr^{3+}, SO_3^{2-}	20
I^-, NO_2^-, WO_4^{2-}, Br^-, Fe^{3+}, Fe^{2+}	2

[a].Maximum ratio tested

3.3. Analysis of real samples

To examine the analytical applicability and validity of the proposed method for determination of the thiocyanate concentration, some saliva and spiked water samples were analyzed.

The method was first applied for determination of the thiocyanate concentration in saliva collected from a smoker and a non-smoker. Prior to collection, mouths of the donors were washed once with a 5.0 g L^{-1} citric acid solution and thrice with doubly distilled water. The saliva samples were centrifuged at 2000 rpm for 5 min. The portions of the resulting solutions were diluted 20 fold for non-smoker and 50 fold for smoker donors with doubly distilled water. Exactly 1.0 mL of this solution was analyzed using the proposed method by the standard addition approach. The thiocyanate contents of the saliva samples and the results of the recovery tests are shown in Table 2. The results for determination of the thiocyanate concentration in spiked

water samples are shown in Table 3. The validity of the proposed method for analysis of real samples is evident from the calculated recoveries.

Table 2. Determination of the thiocyanate concentration in saliva samples.

Sample	Added μg mL^{-1}	Found μg mL^{-1}	Recovery (%)	RSD% (n=5)	Content in sample (mol L^{-1})	
					This method	Reference method[33]
Smoker	---	0.275	---	2.8	2.37×10^{-3}	2.33×10^{-3}
	0.050	0.334	98.0	1.2		
	0.250	0.529	102	1.8		
Non-smoker	---	0.142	---	2.8	4.89×10^{-4}	4.53×10^{-4}
	0.250	0.393	100.4	1.2		

Table 3. Recovery test for determination of the SCN^- concentration in water samples.

Sample	Added (μg mL^{-1})	Found (μg mL^{-1})	Recovery (%)	RSD%(n=5)
Tap water	0.500	0.494	98.8	1.9
	0.750	0.734	97.9	1.9
Mineral water	0.400	0.410	102.5	2.1
	0.900	0.885	98.3	1.8

4. Conclusion

The kinetic spectrophotometric method suggested for determination of the thiocyanate concentration in samples is simple, inexpensive, allows rapid determination, uses readily available reagents, and shows sufficient selectivity and low detection limit. It could successfully be applied for determination of the thiocyanate concentration in biological and water samples.

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