KINETIC-SPECTROPHOTOMETRIC DETERMINATION OF THIOCYANATE BASED ON ITS INHIBITORY EFFECT ON THE OXIDATION OF PYROGALLOL RED BY BROMATE

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ABSTRACT : *A kinetic spectrophotometric method for rapid measurement of thiocyanate in serum, urine and sewage water with no need for removed of interfering substances is described. The method is based on the inhibitory effect of thiocyanate on the oxidation of pyrogallol red by bromate and nitrite which is monitored at 467nm.The variables affecting the rate of the reaction were investigated and the optimum conditions were established.Thiocyanate can be measured in the range of 0.010-0.500 μg/mL with a limit of detection of 0.001μg/mL. The relative standard deviation for ten replicate determination of 0.025μg/mL of thiocyanate is 1.5%.*

KEY WORDS : *Kinetic, Thiocyanate, Pyrogallol Red, Bromate, Oxidation, Inhibitory Effect.*

INTRODUCTION :

Thiocyanate is a detoxication product of cyanide, usually present at low concentrations in biological fluids and determination of thiocyanate in serum or urine or saliva have consequently been used for monitoring exposure to hydrogen cyanide from tobacco smoke [1-3], fire atmosphere [4], and certain vegetables that contain cyanogenic glycosides [5]. Moreover, thiocyanate is known to accentuate the anomalies of iodine deficiency, and the antiheroic properties of thiocyanate have been well documented [6,7]. The harmfulness of

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tobacco smoke containing thiocyanate is well known, and its concentration in the biological fluids of smokers is found to be several times higher than those of non-smokers.

Many methods have been reported for the determination of thiocyanate in biological samples, namely the spectrophotometric method based on the reaction with iron(III) [8,9], conga reaction [10-12], pyridin pyrazolone, pyridin benzidine [14], and methylene blue [15], but these reagents and products each have their drawbacks regarding preparation and stability and carcinogenic properties. Methylene blue method [15] is a complicated method which needs organic solvents.

Other methods such as inhibitory effect of thiocyanate on the reaction systems [16-19], or induction effect on the reaction [20-22] have also been used for the determination of thiocyanate. These methods usually are time consuming, and/or have high limits of detection [2.0μg/mL], or suffer from many interferences [19-22] or require expensive instrumentation.

Thiocyanate can be reacted with bromate [23] and nitrite [23] in trace levels. In our previous report [24], it was mentioned that thiocyanate acts as an inhibitory effect on the oxidation of pyrogallol red (PGR)by bromate in the presence of nitrite. In this work, we attempted to used such a system for the determination of thiocyanate. Its advantage is that only an ordinary spectrophotometer is required as the main instrumentation, has low limit of detection. The reaction is monitored spectrophotometrically using the maximum wavelength of the PGR (467nm) with measuring the change in the absorbance for a fixed time, namely 3.0min from the start of the reaction.

EXPERIMENTAL :

Apparatus

A *Perkin-Elmer* model 35- Spectrophotometer with 1.0cm glass cell was used for all of the absorbance measurements at 467nm. A thermostat bath (*Gallenkamp Griffin*, BJL-240-V) was used to keep the reaction temperature constant at 30°C.

Reagents

All solutions were prepared from doubly distilled water. The working solutions were all kept in a water- bath at 30°C, and analytical-reagent grade chemicals were used.

Sodium nitrite was dried at 110°C for 4hr. A 1000μg/mL nitrite solution was prepared by dissolving 0.3750g of $NaNO_2$ (*Merck*, Darmstadt, Germany) in water in a 250mL volumetric flask. A few milligrams (50-100) of sodium hydroxide were added to prevent its decomposition.

Sodium bromate solution (0.010M) was prepared by dissolving 0.3775g of $KBrO_3$ *(Merck)* in doubly distilled water in a 250mL volumetric flask.

Pyrogallol red solution (2.5×10^{-4}M) was prepared by directly dissolving 0.0100g of pyrogallol red *(Merck)* in mixture of water/ethanol (50%) in a 100mL volumetric flask. The solution is stable for at least two month.

Thiocyanate solution (1000μg/mL) was prepared by dissolving 0.1932g of KSCN *(Merck)* in doubly distilled water in a 100mL volumetric flask.

Sulfuric, phosphoric and hydrochloric acids were all prepared from their concentrated acids *(Merck)*.

Recommended Procedure

The reaction was followed spectrophotometrically by monitoring the change in absorbance at 467nm by a fixed time method for the first 3.0min from initiation of the reaction.

A suitable aliquot of sample solution containing 0.01-10.00μg of SCN^- was transferred into a 10mL volumetric flask, then 1.0mL of 0.070M sulfuric acid, 1.0mL of 1.00μg/mL nitrite solution and 1.0mL of 2.5×10^{-4}M PGR solution were added. The solution was diluted to ca. 8mL with water and then 1.0mL of 0.010M bromate solution was added and the solution was diluted to the mark with water. Time was measured from just after the addition of bromate. The mixture was mixed and transferred into a 1.0cm

glass cell within 30sec from initiation of the reaction. The reaction was followed by recording the absorbance against water at 467nm, from 3.0min after initiation of the reaction. The decrease in absorbance of the sample and blank are designated as A_s and A_b respectively. The calibration graph was constructed by plotting (A_s - A_b) at a fixed time vs. thiocyanate concentration.

Determination of Thiocyanate In Urine, Sewage Water and Blood Serum

The sample should be free from suspended matter, which causes a noisy transmittance trace, and proteins, which are liable to precipitation. Urine samples are centrifuged for 5min, then 0.50mL of the sample was diluted to 10.0mL in 10mL volumetric flask.

Sewage water was initially filtered with a filter paper (Wattman No.1). Then 10mL of the sample was passed from a cation exchanger resin (strongly acidic, Na^+ form), to eliminate interfering ions (distilled water was used as the eluent). The eluent was collected to a 25mL volumetric flask, and the thiocyanate content was measured by the recommended procedure.

For serum samples, the following deproteinization procedure is used [25]: to 1.00mL of serum, 0.250mL of 0.8M $NaClO_4$ and 0.250mL of 20% trichloroacetic acid was added, then the sample was centrifuged for 20min at 2000rpm. Then 0.10mL of 1M NaOH was added to 1.00mL of the clear supernatant liquid and the mixture was diluted to 25mL in a 25mL volumetric flask. The thiocyanate content was measured by the recommended procedure.

Effect of Variables

The decrease in absorbance of PGR-bromate- nitrite system with and without thiocyanate was studied in the presence of sulfuric, hydrochloric and phosphoric acids to obtain the best sensitivity. The results show that sulfuric acid is the best (Table 1).Sulfuric acid is the best due to its acidity strength relative to hydrochloric, nitric and phosphoric acids at equimolar concentrations.

Table 1: Effect of various acids on the reaction rate (Conditions: PGR, 2.0×10^{-5}M; BrO_3^-, 0.0010M; NO_2^-, 1.00μg/mL; SCN^-, 0.100μg/mL; temperature 25°C, and measuring time of 3.0min).

Type of acid (0.005M)	A_{sample}-A_{blank}
H_2SO_4	0.029
HNO_3	0.015
HCl	0.010
H_3PO_4	0.008

The effect of sulfuric acid concentration on the rate of the reaction with and without thiocyanate was studied in the presence of 0.100 μg/mL thiocyanate.Fig.1 shows that the optimum concentration of sulfuric acid is 0.0070M. Thus 0.0070M H_2SO_4 was used throughout the study.

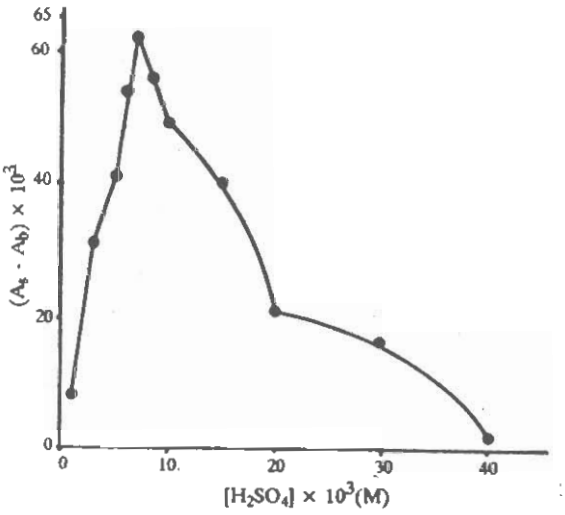

Fig.1 : Effect of sulfuric acid concentration on the reaction rate; Conditions: Nitrite, 1.00μg/mL; PGR, 2.5×10^{-5}M; Bromate, 0.0010M; SCN^-, 0.100μg/mL; temperature: 30°C, measuring time: 3.0min.

The effect of bromate concentration on the rate of blank and sample reactions in the range of 0.0001-0.003M bromate concentration was studied in the presence of 0.100μg/mL thiocyanate. Fig. 2 shows that sensitivity increases up

to 0.0010M Bro^{3-}. At higher values of bromate concentration, the sensitivity decreases, owing to the rate increase for A_s. Thus 0.0010M bromate was used for the study.

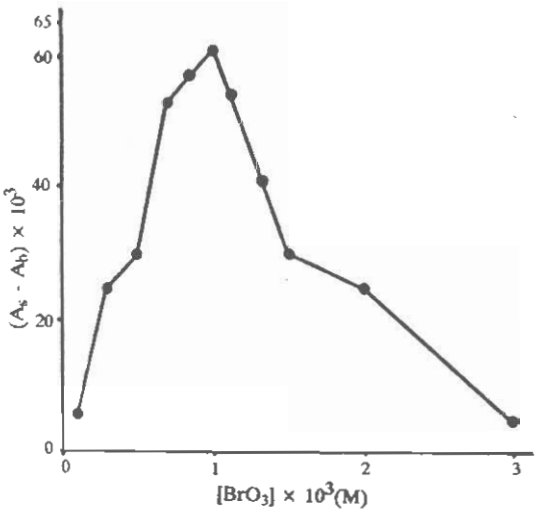

Fig.2 : Effect of bromate concentration on the reaction rate; Conditions: PGR, 2.5×10^{-5}M; NO_2^-, 1.00μg/mL; Sulfuric acid, 0.0070M; temperature, 30°C; measuring time: 3.0min.

The effect of nitrite concentration on the reaction rate with and without thiocyanate was studied in the range of 0.100-3.00μg/mL of nitrite(Fig.3). The results show that 1.00μg/mL of nitrite gives the best sensitivity. Thus 1.00μg/mL nitrite was adopted.

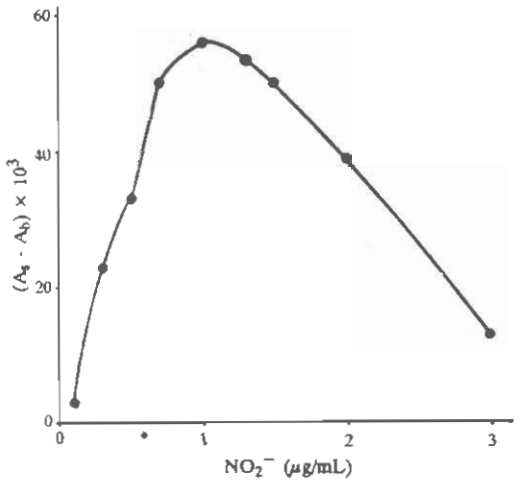

Fig.3 : Effect of nitrite concentration on the rate of reaction; Conditions: PGR, 2.5×10^{-5}M; SCN^-, 0.100μg/mL; Sulfuric acid, 0.0070M; BrO_3^-, 0.0010M, temperature: 30°C; measuring time: 3.0min.

The effect of PGR concentration on the reaction rate with and without thiocyanate was studied in the range of $(5\text{-}75)\times10^{-6}$M PGR at the optimum concentrations of other reagents. Fig.4 shows that the optimum concentration of PGR is 2.5×10^{-5}M. Thus 2.5×10^{-5}M of PGR was adopted.

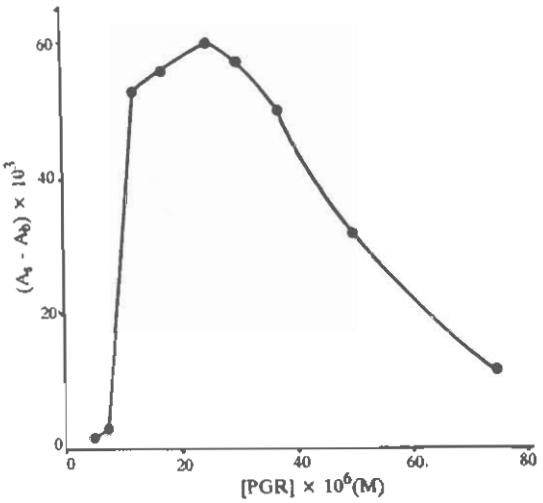

Fig.4 : Effect of PGR concentration on the reaction rate; Conditions: Nitrite, 1.00μg/mL; SCN^-, 0.100μg/mL; BrO_3^-, 0.0010M; temperature: 30°C; measuring time: 3.0min.

The effect of temperature on the rate of reaction was studied in the range of 5-60°C at the optimum reagents concentrations with 0.100μg/mL of SCN^- (Fig.5). The results show that 30°C is the best, since at higher temperatures, the inhibitory effect of thiocyanate is decreased causing a decrease in A_s; which means that $(A_s\text{-}A_b)$ diminishes at higher temperatures. Thus 30°C was used throughout the study.

The effect of measuring time for the decrease in absorbance with and without thiocyanate to give best sensitivity was studied in the optimum concentrations of the reagents at 30°C (Fig.6). The results show that 3.0min yields the best sensitivity. Thus 3.0min from initiation of the reaction was used as interval time for measuring the decrease in absorbance of the reaction system.

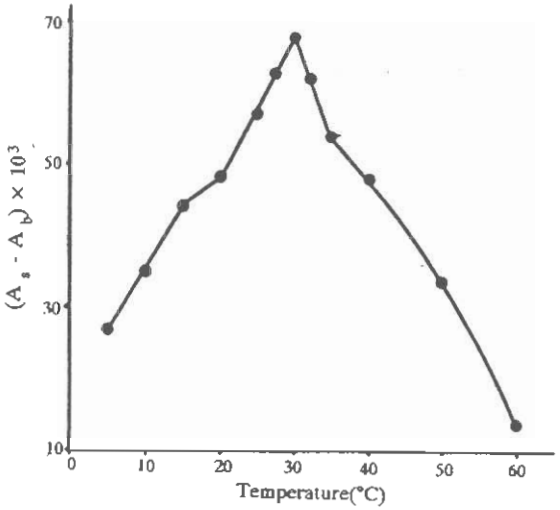

Fig. 5 : Effect of temperature on the reaction rate(under the optimum concentration of the reagents with 0.100 μg/mL).

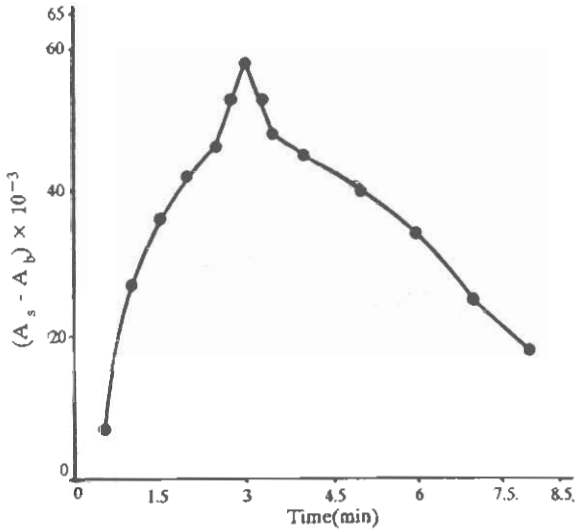

Fig. 6 : Effect of measuring time on the sensitivity; conditions as in Fig.5; temperature: 30°C.

The effect of ionic strength on the rate of reaction under the optimum conditions with and without thiocyanate was studied by using $NaNO_3$ (3.0M). The results show that reaction rate was independent of ionic stength up to 1.4M.

Calibration

Under the optimum conditions described above, in the concentration range 0.005-0.500μg/mL of thiocyanate, the following regression equation was obtained $A = 1.957 \times 10^{-4} + 0.6426C$ with a correlation coefficient of 0.9994 (n=5), where C is the μg/mL of thiocyanate and A is the difference in absorbance for the sample reaction and that of the blank reaction (A_s - A_b).

The theoretical limit of detection [26] is 0.001μg/mL of thiocyanate. The relative standard deviation for ten replicated determination of thiocyanate is 2.2% and 1.5% for 0.050 and 0.250μg/mL of thiocyanate.

Interferences

More than 30 other substances in solution were studied for their possible influence on the determination of 0.100μg/mL of thiocyanate by this method. The results show that large amounts of alkali and alkaline earth metal cations that generally accompany thiocyanate do not interfere; there were no effects from a 1000 fold amounts (weight/weight) of Zn(II), Ni(II), Cu(II), Cd(II), Cr(III), La(III), Ir(III), Co(II), Al(III), NO_3^-, NH_4^+, acetate, tartarate, citrate, ClO_4^-, SO_4^{2-}, PO_4^{3-}, $B_4O_7^{2-}$, and 200- fold amounts of CN^-, CO_3^{2-}, ClO_3^-, Mn(II), Ba(II), a 100- fold amounts of S^{2-}, SO_3^{2-}, $C_2O_4^{2-}$, $S_2O_5^{2-}$, Cl^-, and 50- fold Fe(III) and Pb(II) do not interfere, but more than 10- fold Br^-, F^-, Ag(I) and 5- fold Hg(II), V(III), Ce(IV) and Hg(I) interfere. The interference of ions may be due to the complex formation of PGR with metal ion or due to the catalytic effects of metal ion on the reaction or due to the reaction with bromate or with thiocyanate.

Determination of Thiocyanate in Real Samples

High concentration of thiocyanate, which is a major metabolite of cyanide, in biological fluids arises from tobacco smoke. Thiocyanate concentrations in the urine and serum of smokers are several times higher than those of the non-smokers (Table 2).

CONCLUSIONS :

The suitability of the PGR-BrO_3^--NO_2^- as a

Table 2: Determination of thiocyanate in real samples (at the optimum conditions).

Sample	Sex	Added SCN^- (μg/mL)	Smoker(S) or Non-smoker(N)	Found (μg/mL)	RSD% (n=5)
Sewage water	-	-	-	0.250	2.3
Sewage water	-	1.00	-	1.235	1.9
Sewage water	-	5.00	-	5.260	2.1
Urine	M	-	S	12.72	2.3
Urine	M	1.00	S	22.70	1.9
Urine	M	-	N	2.39	1.9
Urine	M	5.00	N	7.35	2.0
Urine	F	-	N	2.75	2.2
Urine	F	5.00	N	7.72	2.1
Serum	M	-	N	0.900	1.8
Serum	M	5.00	N	5.905	2.2
Serum	M	-	S	2.600	1.7
Serum	M	5.00	S	7.608	1.9
Serum	F	-	N	0.550	2.1
Serum	F	5.00	N	5.500	2.0

sensitive, precise, rapid and relatively selective system for the development of a kinetic- spectrophotometric method for determination of ultra trace amounts of thiocyanate down to 0.0005 μg/mL has been demonstrated. The applicability of the proposed method was demonstrated in the determination of thiocyanate in urine, serum and sewage water.

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