Kinetic Approach to the Mechanism of Reduction of (7-Amino-8-Methyl-Phenothiazin-3-Ylidene)-Dimethyl-Ammonium Chloride by Thiocyanate Ion in Acidic Medium

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ACIDIC MEDIUM

Strictly as per the compliance and regulations of :

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Abstract- The kinetics of reduction of (7-Amino-8-methyl-phenothiazin-3-ylidene)-Dimethyl-Ammonium Chloride (here after referred to as TB) by thiocyanate ion have been studied in acidic medium under the pseudo-first order condition of excess [SCN⁻] at 30 ± 1°C, [H⁺] = 1 x 10^{-3} mol dm^{-3} and ionic strength, I = 0.50 mol dm^{-3} (NaCl). The stoichiometry of the reaction was observed to be 1:1 mole ratio of TB to SCN ions. The redox reaction follows second order kinetics at constant hydrogen ion concentration and the rate also increases with increase in hydrogen ion concentration. The overall reaction conforms to the rate law

$$-\frac{d[TB^+]}{dt}= (a + b [H^+])[TB^+] [SCN^-]$$

where a = 2.8 x 10^{-2} dm^3 mol^{-1} s^{-1}, b = 1.20 x 10^{-1} dm^3 mol^{-1} s^{-1}

Variation of the ionic strength and dielectric constant of the medium altered the rate of the reaction, addition anions and cations(X = SO_4^{2-}, NO_3^-, Mg^{2+} and Ca^{2+}) to the reaction mixture decreased the rate of the reaction. Spectroscopic and kinetic investigation showed no sign of intermediate complex formation; free radical polymerisation test showed no free radicals. A plausible mechanism which accommodates all the experimental data was proposed.

Keywords: kinetics, mechanism, oxidation, thiocyanate, (7-amino-8-methyl-phenothiazin-3-ylidene)-dimethyl-ammonium chloride.

I. Introduction

Thiocyanate has reducing properties that include the ability to protect cells against oxidizing agents [1]. Thiocyanate ion is known to be an important part in the biosynthesis of hypothiocyanite by a lactoperoxidase [2, 3, 4]. Thus the complete absence of thiocyanate [5] or reduction in concentration of thiocyanate, in the human body, (cystic fibrosis) is damaging to the human host defense system [6, 7, 8].

(7-amino-8-methyl-phenothiazin-3-ylidene) - dimethyl- ammonium chloride is a dye belonging to the phenothiazine class same class as methylene blue and methylene green. Phenothiazines are important compounds in medicine and industry [9, 10]. The dye can be used to treat methaemoglobinaemia [11]. The use of toluidine blue in this respect has been attributed to its electron donor –acceptor property[12]. However despite these and numerous other uses of TB not much is known about the kinetic and mechanism information vital to the understanding of the chemical characteristics of this dye so that its full potentials can be harnessed for more informed use(s).

II. Experimental

There agents used were of analytical grade and were used as supplied. Stock solutions of TB, potassium thiocyanate and sodium chloride (BDH) was used to maintain ionic strength constant at 0.5 mol dm^{-3} and they were prepared by dissolving known quantities in distilled water; hydrochloric acid which was used to investigate the effect of [H⁺] on the rate of reaction. This was standardized titrimetrically using standard solution of sodium bicarbonate. The λ_{max} = 600 nm was determined by measuring the absorbance of the solution of TB in the wavelength range 500 – 650nm.

III. Stoichiometry

The stoichiometry of the reaction was determined by spectrophotometric titration using the mole ratio method by keeping the concentration of the dye constant at 1.0 x 10^{-5}mol dm^{-3}, [H⁺] = 1 x 10^{-3} moldm^{-3}, I = 0.50 mol dm^{-3}, λ_{max} = 600 nm, T = 30 ±1°C while [SCN⁻] was varied from 1.0– 6.0 x 10^{-5} mol dm^{-3} . The absorbance of the reacting mixture was measured after the reaction had gone to completion over a period of 24 hours when the absorbance attained a steady absorbance value. A point of inflexion on the curve of absorbance versus [SCN⁻] plot indicates the mole ratio of the reactants [13].

IV. Kinetic Measurements

The kinetics of the reaction was monitored using a Corning colorimeter Model 252 at 30 ± 1°C, [H⁺] =1 x 10^{-3} mol dm^{-3} and I = 0.50 mol dm^{-3}. The progress of the reaction was monitored by following the decrease in absorbance of the dye at 600 nm. All kinetic

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runs were performed under pseudo-first order conditions with the concentration of the thiocyanate ions at least 20-fold greater than that of the dye. The pseudo-first order plots of the log $(A_t - A_\infty)$ versus time t were made (where A_t and A_∞ are the absorbance at time, t and the end of the reaction) and from the slope of the plots, the pseudo-first order rate constant (k_1) was determined. The second order rate constant (k_2) was obtained from the relation:

$$k_2 = \frac{k_1}{[SCN^-]}$$

a) Effect of [h+] on the Reaction Rate

The effect of $[H^+]$ on the rate of the reaction was studied in the range of $0.5 \times 10^{-3} \leq [H^+] \geq 2.5 \times 10^{-3}$ mol dm^{-3} while concentration of TB and SCN ion were kept constant at 1.0×10^{-5} and 20×10^{-5} mol dm^{-3} respectively at $30 \pm 1°C$ and $I = 0.50$ mol dm^{-3}.

b) Effect of Ionic Strength

The effect of ionic strength on the rate of the reaction was investigated in the range of $0.3 - 1.2$ mol dm^{-3}, while the concentrations of other reactants were kept constant at $30 \pm 1°C$. The results are presented in Table1.

c) Effect of Added Cation and Anion

The effect of added cation and anion were investigated for $[X] = 0.2 \times 10^{-5} - 1.0 \times 10^{-5}$ mol dm^{-3} (X $= Ca^{2+}$, Mg^{2+}, SO_4^{2-}, NO_3^-) and the concentration of all other reactants were kept constant at $30 \pm 1°C$ and the ionic strength was maintained constant at 0.50 mol dm^{-3}.

d) Test for Intermediate Complex

The electronic spectra of the reaction mixture were obtained after five minutes of the commencement of the reaction, over the wavelength range of $500 - 650$ nm. This was compared with the spectra of the dye alone within the same range. Michaelis-Mentens plot of $1/k_1$ versus $1/[SCN^-]$ was also made (Fig. 2).

e) Test for Free Radical

Acrylamidesolution was added to the partially oxidized reaction mixture of TB and the SCN ions in a large excess of methanol and to each of the reactants separately.

V. Results and Discussion

a) Stoichiometry and Product Analysis

A stoichiometric study showed that one mole of the dye was consumed by one mole of the thiocyanate ion. This conforms to the equation:

$$TB^+ + SCN^- \rightarrow Product \qquad\qquad -- 1$$

Product analysis was carried out by reacting equimolar amount of the dye and the thiocyanate at $[H^+] = 1.0 \times 10^{-3}$ mol dm^{-3} and $I = 0.5$ mol dm^{-3} (NaCl) After the completion of the reaction a colourless solution was obtained and UV visible spectra of the product showed no absorption peak at λ_{max}600 nm. This indicates the

destruction of the quinoid structure that gives the dye colour.

Qualitative test forcyanide ion was carried out. Cyanide ion was identified by precipitating with $AgNO_3$.

b) Kinetics

The pseudo-first orders of log $(A_t - A_\infty)$ versus time, t for these reactions were linear to about 90% of the reaction (Fig 1). The linearity of these plots indicates that these reactions are first order with respect to $[TB^+]$. A plot of log k_1 versus log $[SCN^-]$ was linear with a slope of 1.2 showing that the reaction is also first order with respect to $[SCN^-]$(Fig 2). This is also supported by the constancy of k_2 values. Thus the reaction is second order at constant $[H^+]$ and the rate equation for the reaction is:

$$-\frac{d[TB^+]}{dt} = k_2[TB^+][SCN^-] \qquad\qquad -- 2$$

where $k_2 = 9.96 \times 10^{-2}$ dm^3 mol^{-1} s^{-1}

c) Effect of Acid

The rate constants of the reaction were found to increase with increase in $[H^+]$ in the range of $(0.5 - 2.5) \times 10^{-3}$ mol dm^{-3}. Plot of k_2 versus $[H^+]$ was also linear with a positive intercept (Fig. 3) therefore the acid dependent rate constant is given by:

$$k_2 = a + b [H^+] \qquad\qquad --3$$

where $a = 2.8 \times 10^{-2}$ dm^3 mol^{-1} s^{-1}, $b = 1.20 \times 10^{-1}$ dm^3 mol^{-1} s^{-1}

From equation (3) it is evident that the reaction proceeds through acid dependent and acid independent reaction pathways. Similar acid dependence was observed in the reaction between toluidine blue and phenyl ascorbic acid [14].

d) Effect of Ionic Strength

The effect of ionic strength on the reaction was investigated by varying the ionic strength of the medium within the range $0.3 - 1.2$ mol dm^{-3}. The rate of reaction was found to decrease with increase in ionic strength of the reaction medium(figure 5). The result implies that positive and negatively charged species are participating in the rate determining step.[15]. The trend portrayed by the variation of ionic strength was verified by investigating the effect of the changes in dielectric constant of the reaction medium. Decrease in dielectric constant of the medium enhanced the rate of the reaction.

e) Effect of Added Species

Addition of Ca^{2+}, Mg^{2+} SO_4^{2-} and NO_3^- ions decreases the rate of the reaction. The inhibitive effect of these ions could be due to the columbic forces of attraction which tend to pull the reacting partners apart there by retarding the rate of the reaction and this

happens where there is no formal bond between the oxidant and the reductant prior to the rate determining step which is characteristic of outer sphere mechanism.
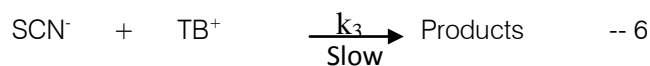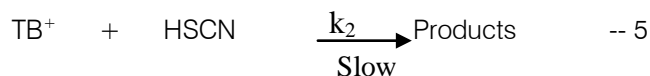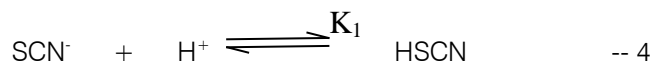
f) FreeRadical Test

The addition of acrylamide to the partially reacted mixture of the reactants followed by large excess of methanol did not lead to the formation of gelatinous precipitate. This suggests that free radicals might not likely be involved in this reaction[16].

g) Intermediate Complex Formation

The results of the spectroscopic studies indicated no shift from the absorption maxima of 600 nm characteristic of TB. This suggests the formation of an intermediate complex during the reactions is very unlikely. Michaelis-Mentens plots of $1/k_1$ versus $1/[SCN^-]$ gave a straight line which passed through the origin (Fig 5). This further suggests the absence of intermediate complex of significant stability thereby supporting the outer-sphere mechanism (Idris et al., 2005 and Benson, 1996) for this reaction.

On the basis of the above experimental results, the following plausible mechanism is hereby proposed:

$$SCN^- \ + \ H^+ \ \underset{}{\overset{K_1}{\rightleftharpoons}} \ HSCN \qquad\qquad -- 4$$

$$TB^+ \ + \ HSCN \ \xrightarrow[\text{Slow}]{k_2} Products \qquad -- 5$$

$$SCN^- \ + \ TB^+ \ \xrightarrow[\text{Slow}]{k_3} \ Products \qquad -- 6$$

The equations 5 and 6 are the rate determining steps

Rate $= k_2\,[TB^+]\,[HSCN] + k_3\,[TB^+]\,[SCN^-]$ -- 7
From equation 4

$[HSCN] = K_1\,[SCN^-]\,[H^+]$ -- 8

Substitute equation 8 into equation 7

Rate $= k_2\,K_1\,[TB^+]\,[SCN^-]\,[H^+] + k_3\,[TB^+]\,[SCN^-]$ -- 9
Rearranging equation 9

Rate $= k_3 + k_2 K_1[H^+]\,[TB^+]\,[SCN^-]$ -- 10

Where $k_3 = a$
       $k_2\,K_1 = b$

Equation 10 becomes
Rate $= (a + b\,[H^+])\ [TB^+]\,[SCN^-]$ -- 11

Where $a = 2.8 \times 10^{-2}$ dm^3 mol^{-1} s^{-1} and $b = 1.20 \times 10^{-1}$ dm^3 mol^{-1} s^{-1}

VI. Conclusion

The reaction is found to be second order overall at constant $[H^+]$. And the proposed reaction steps point to an outer sphere electron transfer process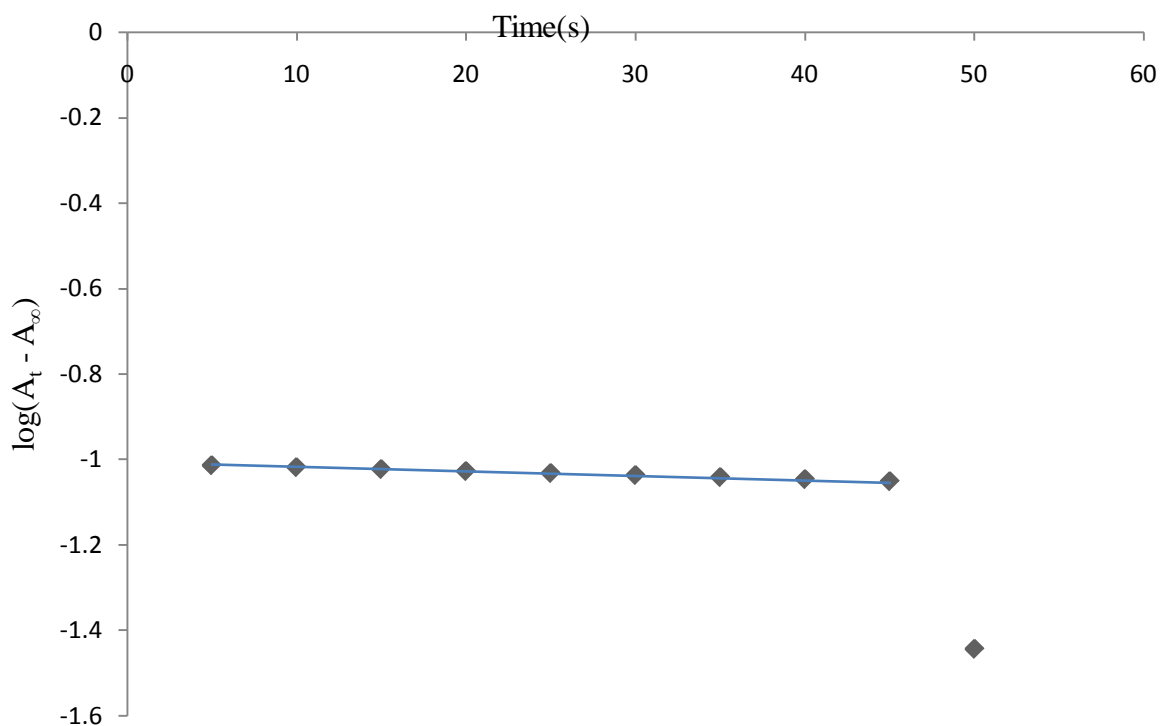, considering the fact that addition of foreign ions led to the inhibition of the reaction rates, when the absorbance of the reaction mixture were measured as the reaction progresses the λ_{max} of TB remains at 600nm, this is an indication of the absence of intermediate complex formation which would be as a result of a chemical bond between the reactants, thereby tempering with the electronic transition of the TB, and hence a shift in the λ_{max} of TB$^+$ or appearance of a new peak which was hitherto absent. Also Michaelis-Menten plot of $1/k_1$ against $1/[SCN^-]$ passed through the origin. These points greatly favour the outer sphere mechanistic pathway for this reaction.

Figure 1 : Pseudo-first Order Plot for the Reduction of TB by SCN

Table 1 : Pseudo – first order and second order rate constants for TBreduction by SCN⁻ in aqueous HCl medium at [TB⁺] = 1.0 x 10⁻⁵ mol dm⁻³, T = 30 ± 1˚C and , λ_{max} = 600 nm

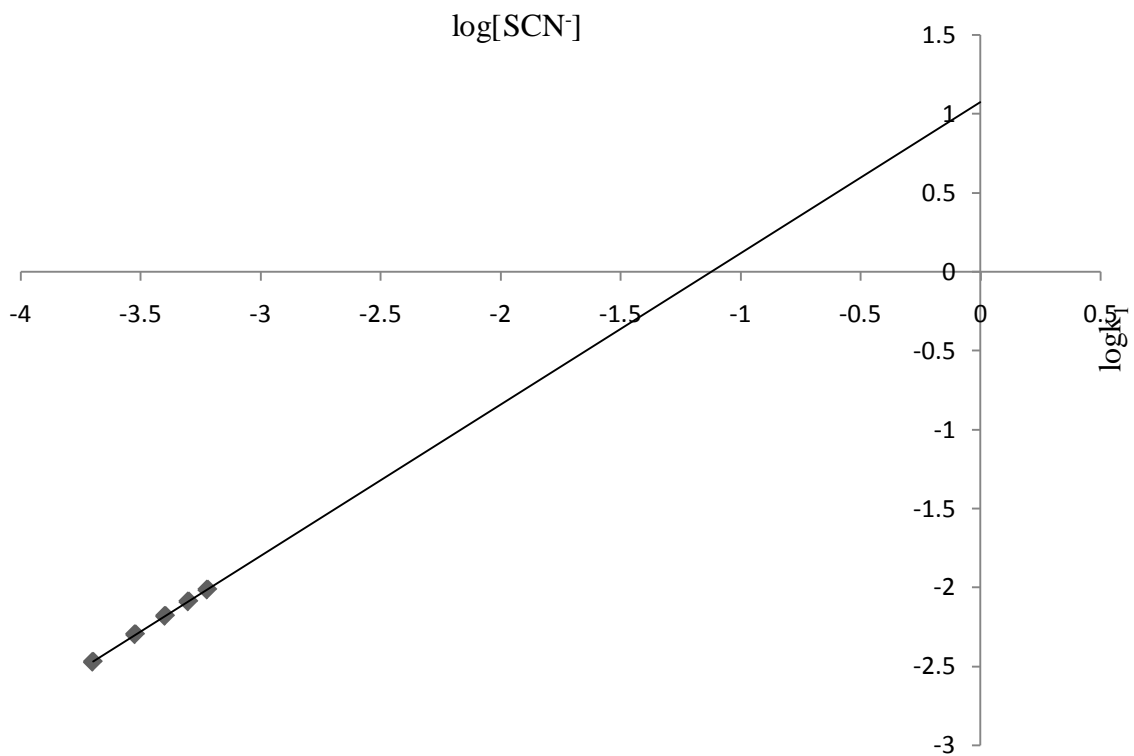| 10^5 [SCN⁻] mol dm⁻³	I (NaCl) mol dm⁻³	10^3 [H⁺] mol dm⁻³	$10^1 k_1$ s⁻¹	$10^3 k_2$ dm³ mol⁻¹ s⁻¹
20	0.5	1.0	2.03	1.02
30	0.5	1.0	3.04	1.01
40	0.5	1.0	3.98	1.00
50	0.5	1.0	4.91	0.98
60	0.5	1.0	5.84	0.97
20	0.5	0.5	1.81	0.91
20	0.5	1.0	1.90	0.95
20	0.5	1.5	2.02	1.01
20	0.5	2.0	2.35	1.17
20	0.5	2.5	2.60	1.30
20	0.3	1.0	2.21	1.10
20	0.5	1.0	1.92	0.96
20	0.7	1.0	1.70	0.85
20	1.0	1.0	1.55	0.77
20	1.2	1.0	1.44	0.67

log[SCN⁻]

Figure 2 : Plot of log k_1 vs log [SCN⁻] for the Reduction of TB by SCN⁻

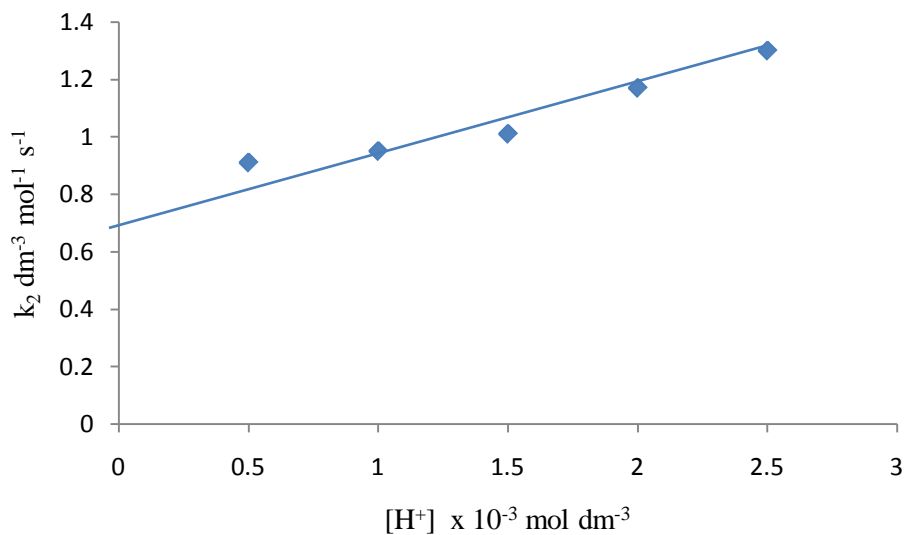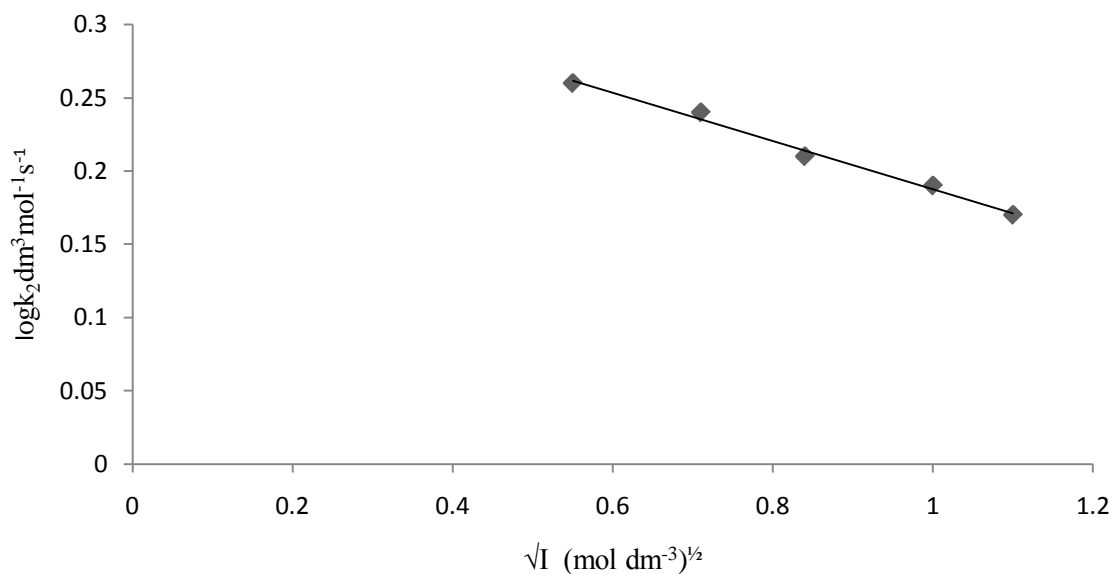*Figure 3 :* Plot of k_2 versus [H⁺] of reduction of TB by SCN⁻

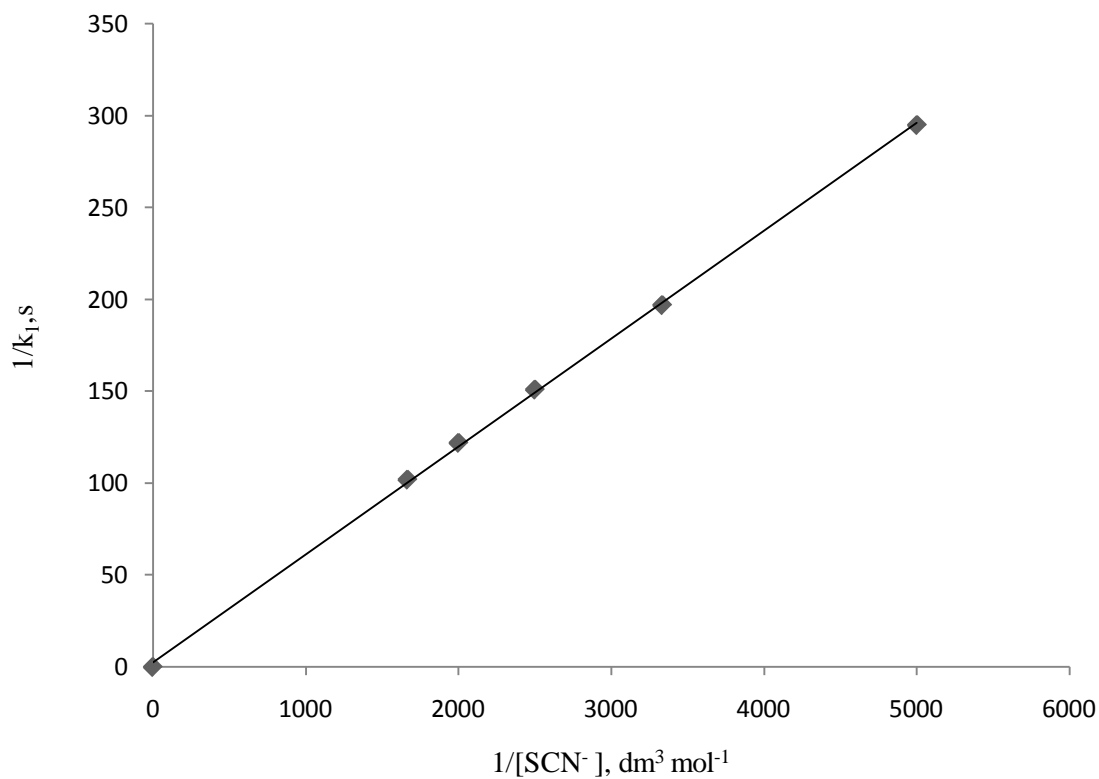*Figure 4 :* plot of log k_2 versus $\sqrt{I}$ for the reduction of TB$^+$ by [SCN$^-$]

Figure 5 : Michaelis-Menten's plot for the reduction of TB by SCN$^-$

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