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Development and Validation of Kinetic and Atomic Absorption Spectrophotometric Methods for the Determination of Salbutamol Sulfate

R'afat Mahmoud Nejem[1*] , Mahmoud Mohamed Issa[2], Mozer Hosny AL-Kholy[2], Akila Amin Saleh[3], Alaa Abu Shanab[4] Raluca Ioana Stefan van Staden[5], Hassan Yousef Aboul-Eneien[6]

[1*]Analytical Chemistry, Department of Chemistry, Alaqsa University, P.O.Box 4051, Gaza, Palestine

[2]Pharmaceutical Analytical Chemistry, Department of Chemistry, Alaqsa University, P.O.Box 4051, Gaza, Palestine

[3] Inorganic Chemistry, Ain Shams University, Cairo, Egypt.

[4]Inorganic Analytical Chemistry, Department of Chemistry, Alaqsa University, P.O.Box 4051 Gaza, Palestine

[5]National Institute of Research for Electrochemistry and Condensed Matter, Bucharest, Romania.

[6]Pharmaceutical and Medicinal Chemistry Department, Pharmaceutical and Drug Industries Research Division, National Research Center, Dokki, Giza 12622, Egypt

Abstract

Two sensitive kinetic and atomic absorption spectrophotometric (AAS) methods where developed for the determination of salbutamol sulfate (SLS) in its dosage forms. The kinetic method was based on the bromination reaction of the drug by bromine generated in-situ by the reaction of bromate with bromide in acidic medium. The reaction was followed spectrophotometrically by measuring the decrease of bromine color at 380 nm. The reaction was carefully studied and optimized. Under

* Corresponding authors: Professor Hassan Y. Aboul-Enein ;Tel:+201003678948 ; Fax:+20233370931; E-mail: haboulenein @yahoo.com and Dr. R'afatt\M. Nejem ;Tel: +970599603459; Fax: +97282845488 ; E-mail: rafat_nejem@yahoo.com

optimum conditions, the stoichiometry and the order of the reaction were determined. The initial rate and fixed time methods were utilized for the determination of salbutamol sulfate concentrations. The AAS method was depended on the oxidation of iron (II) with excess bromine from the bromination reaction of the drug. A new method for separation iron (III) was used. Then iron (II) in aqueous layer was aspirated into air-acetylene flame and determined by AAS. The linear ranges for the proposed methods were 2.0-10.0 and 0.2-2.0 μgmL^{-1} with detection limit of 0.30 and 0.012 μgmL^{-1} for kinetic and AAS, respectively. The proposed methods were validated; the mean recovery ranges from 98.0 to 102.0% with RSD < 2.1%. Common excipients did not interfere the measurements of SLS. The methods were successfully applied to determined SLS in dosage forms; there was no significant difference between the proposed methods and official one.

Keywords: Salbutamol sulfate (SLS) – Atomic Absorption Spectroscopy – Kinetic method – Bromination – Initial rate method- fixed time method.

1. Introduction

Asthma is a chronic respiratory disease characterized by inflammation and narrowing of airways in the lungs, the bronchi. During an asthma attack, the smooth muscle surrounding the bronchi contracts and the lining of the bronchi swells; this swelling is life-threatening because the airways can become blocked. Salbutamol is one of the β-agonist bronchodilators, the largest group among the various classes of inhaled asthma drugs. Salbutamol sulfate, α-1-[[(1,1-Dimethylethyl) amino] methyl]-4-hydroxy-1,3-benzenedimethanol sulfate (2:1) (Scheme 1) is a direct-acting sympathomimetic agent with a relatively selective action on β_2-adrenoacceptors. The clinical use of salbutamol is the management of reversible airways observation such that occurs in asthma and delaying premature labour[1].

Literature survey shows that several spectrophotometric[2-12], spectrofluorimetric[13], conductometric[14], potentiometric[15], LC-MS[16] and HPLC[17-23] methods reported for the determination of salbutamol sulfate in various samples. So far, only one kinetic spectrophotometric method has been reported for determination of salbutamol sulfate[24]. No atomic absorption spectroscopic method has been reported for determination of salbutamol sulfate. Basavaiah and Prameela used the bleaching of methyl orange for the kinetic bromination of Salbutamol but nothing is mentioned in the paper about the different kinetic behavior for the simultaneous reaction of bromine with Salbutamol sulfate and methyl orange[24].

In this paper, bromine is yielded in a slow reaction according to the following stoichiometry:

$$BrO_3^- + 5Br^- + 6H^+ \longrightarrow 3Br_2 + 3H_2O$$

and the rate law is[25]:

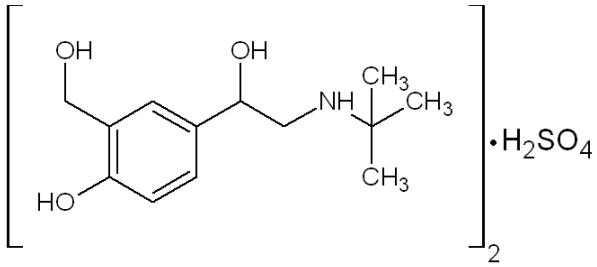
$$\frac{-d[Br_2]}{dt} = K_{Br}[BrO_3^-][Br^-][H^+]^2$$

The kinetic method depends on the bromination of the drug by bromine generated in-situ by the interaction of bromate with bromide in acidic medium. The absorbance of bromine color decreases with time and hence, a kinetically based method is elaborated. The use of bromine in kinetic spectrophotometric method is becoming of great interest[26-28] as it offered some advantages:

- Bromine can act in different reaction ways such as: substitution, addition and oxidation.
- Reaction between bromine and salbutamol sulfate is slow.
- Bromine can be detected in both UV and visible absorption bands.

The atomic absorption method is based on the oxidation of iron (II) with unreacted bromine from the bromination reaction of the drug. A new method for the separation of iron (III) with ammonium thiocyanate / n-propyl alcohol extraction system in the presence of sodium chloride has been used[29]. Then the remained iron (II) in aqueous layer is aspirated into air-acetylene flame and determined by atomic absorption spectrophotometer.

The proposed methods have the advantages of being simple, sensitive and free from interferences.



Scheme 1. Chemical structure of salbutamol sulfate

2. Results and discussion

In acidic medium, bromate oxidizes bromide to bromine and the in-situ generated bromine reacts with the drug. The absorbance of the unreacted bromine decreases with time and hence, a kinetically based method was elaborated (Figure 1). A study of optimum conditions was carried out and stoichiometry of the reaction of salbutamol with bromine was ascertained.

The pseudo-order rate constant (K') was determined by studying the reaction at different temperatures; 5, 10, 15, 20 and 25 ^{o}C using fixed concentration of SLS and Br_2 (Table 1). It was found that the rate of reaction affected by the change of temperature. The rate of the reaction at 10 ^{o}C was moderate, so that it was possible to estimate their reaction rate by fitting the kinetic data using a suitable regression method. Furthermore, the rates of reactions at 15, 20, 25 ^{o}C were so fast that was not possible to determine their rate constants.

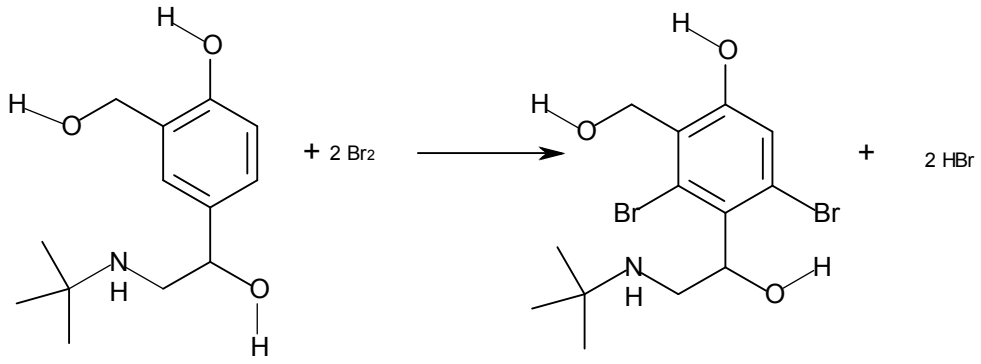
No change in the reaction stoichiometry was occurred when 0.5-2.0 mL of 5 molL^{-1} H_2SO_4 acid were used. So, a 1.0 mL of 5 molL^{-1} H_2SO_4 in a total volume of 10.0 mL was found suitable for bromination of SLS drug.

The bromide concentration was in large excess (0.1 molL^{-1}) in order to accelerate bromine generation. The amount of generated bromine was dictated by bromate.

An initial concentration of $3x10^{-4}$ molL^{-1} bromate generated $9.0x10^{-4}$ molL^{-1} bromine. This was a suitable concentration for experimental purpose. Figure 2 illustrates some reaction profiles of the bromine consumption, followed at 380 nm, for different initial bromine concentration when [SLS] > [Br_2]. Figure 3 explains the bromine consumption for different salbutamol sulfate concentrations when [Br_2] >[SLS].

The stoichiometry of the reaction was established from limiting logarithms plot in figure 4. The plots of log absorbance vs. log [Br_2] and [salbutamol] exhibit slope

values of 1.85 and 0.99, respectively. Hence, it is concluded that the reaction proceeds at the molar ratio of 2:1. The probable reaction is:



The initial-rate and fixed-time methods were adopted for constructing the calibration curves. The initial absorbance versus time is shown in figure 3. The rate expression can be written as:

$$rate = K\left[Br_2\right]^m\left[SLS\right]^n$$

where m and n are the orders of the reaction. Under optimized experimental conditions, using excess concentration of Br_2, the above equation is reduced to:

$$rate = K'\left[SLS\right]^n$$

and, $$Log\ rate = Log\ K' + n\ Log\ C$$

where K' is the pseudo-order rate constant , n is the order of the reaction and C is the concentration of salbutamol sulfate , in $molL^{-1}$.

The rate of the reaction may be estimated as $\frac{\Delta A}{\Delta t}$, where A is the absorbance and t is the time in seconds. By compensating the logarithm of rates and concentrations of SLS, as shown in Table 1, in the above equation, consequently:

$$Log\ rate = 1.665 + 0.983\,Log\ C \qquad (r = 0.9987)$$

Therefore, $K' = 46.23S^{-1}$ and the reaction is first order [n=0.983] with respect to salbutamol sulfate. The calibration graph showed a linear relationship over the concentration rang $5.97\text{x}10^{-6}$ – $2.99\text{x}10^{-5}$ $molL^{-1}$ (2-10 μgmL^{-1}). The low value of

detection limit 8.90×10^{-7} molL^{-1} (0.3 μgmL^{-1}) confirmed the good sensitivity of the proposed kinetic method.

Regression equation for salbutamol sulfate at different fixed time over the range of 5.97×10^{-6} – 2.99×10^{-5} molL^{-1} (2-10 μgmL^{-1}) at 380nm is shown in table 2. It is clear that the most acceptable values of the correlation coefficient and the intercept were obtained for a fixed time 10.0 min, which was therefore chosen as the most suitable time interval for measurement. After optimizing the reaction conditions , the fixed time methods was applied to the determination of salbutamol sulfate over the range 2-10 μgmL^{-1} using the following equation :

$$A_{10} = 0.002 + 0.0407C \qquad (r = 0.9990)$$

Where A_{10} is the absorbance at 380 nm and C is the concentration in μgmL^{-1} at a fixed time 10 min.

In atomic absorption spectrophotmetric method, the unreacted bromine is reduced by iron (II) and the resulting iron (III) complexed with thiocyanate. Then, the complex is extracted and iron (II) in aqueous layer determined by the proposed AAS. As a result there is a proportional increase in the absorbance of iron (II) with the increasing concentration of SLS. A 1.0 mL of 20 μgmL^{-1} iron (II) in a total volume of 10 mL resulted in convenient absorbance. This was found to be completely reduced by 10 μg bromate in the presence of excess bromide. Therefore, different amounts of SLS were reacted with 10μg bromate (each 1.0 μg SLS reacted with 0.333 μg bromate). An overall acidity of 0.5 molL^{-1} sulfuric acid used for the bromination between SLS and bromate was also maintained for reducing step. The bromination reaction was found to be complete in 10 min (fixed –time method) for the 0.2 -2.0 μgmL^{-1} ranges of SLS studied and a contact time of 10 min was found necessary for reducing iron (II). After

optimizing the conditions, the AAS method was applied to the determination of SLS over the range 0.2-2.0 μgmL^{-1} using the following equation:

$$A = -0.032 + 0.518C \qquad (r = 0.9982)$$

2.1 Analytical data

The analytical characteristics of the three proposed method are shown in tables 3-5. Initial rate method was found to be applicable over the range 5.97x10^{-6}–2.99x10^{-5} molL^{-1} (2.0-10.0 μgmL^{-1}) under the described experimental conditions. The limit of detection (LOD) was calculated and found to be 8.90x10^{-7} molL^{-1} (0.3 μgmL^{-1}) as shown in Table 3. In the fixed–time method, the absorbance of the reaction solution containing varying amounts of SLS was measured at a preselected fixed time. Beer's law was obeyed in the concentration range 2.0-10.0 μgmL^{-1}. The LOD value was 0.31 μgmL^{-1} as in table 4. The low value confirmed the good sensitivity of the fixed time method. The linear plot gave regression equation:

$$A_{10} = 0.002 + 0.0407C \qquad (r = 0.9990)$$

The calibration graph for AAS procedure was obtained by plotting the absorbance of iron (II) remain in aqueous layer with the concentration of SLS. Linearity was observed in the range 0.2-2.0 μgmL^{-1} and could be described by the recession equation:

$$A = -0.032 + 0.518C \qquad (r = 0.9982)$$

The LOD was calculated and found to be 0.026 μgmL^{-1} (Table 5). Based on the sensitivity results obtained, the AAS method has the superiority of over the other two methods.

2.2 Precision and accuracy

The intraday and interday precisions for the three methods were found to be in the range 0.83–3.81 % and 0.9–3.5 %, respectively (Table 3-5). These data indicated that the proposed methods were reproducible within and between days.

The percent errors were found to vary from 1.0–2.0 %. The mean percentage recovery ranged from 98.0–102.5 % with relative standard deviation values < 4%. The percent errors and the RSD values indicate the high accuracy and precision of the methods.

2.3 Interferences

The selectivity of the proposed methods was preliminarily checked by determining of SLS in the presence of other compounds of the tablet. The results are summarized in table 6. It is evident that the excipients had no effect on the SLS estimation. Hence, the determination of the drug by the proposed methods is considered to be free from interference due to excipients.

2.4 Application to dosage forms

The proposed methods has been successful applied to the determination of SLS and the results were statistically compared with those obtained by use the official methods[31]. The results in Table 7 show that there is no significant difference between the performances of the methods compared.

3. Experimental

3.1 Apparatus

A shimadzu (Kyoto, Japan) UV-1650 PC, UV-Visible double-beam spectrophotometer with two matched 1cm path-length quartz cells was used for the measurements of molecular absorption. The subsequent statistical manipulation was performed by transferring the spectral data to Microsoft Excel 2007 program and processing them with the standard curve fit package and matrix calculation.

Flame atomic absorption measurements were performed using a Perkins-Elmer

AAS, Model A, Analyst 100 spectrophotometer equipped with an iron hollow-cathode lamp, under the following conditions: 302.1 nm wavelength, 30.0 mA lamp current, 0.2 nm slit width and 3.5:1.5 air: acetylene ratio.

3.2 Reagents and solutions

All reagents used were of analytical reagent grade and double-distilled water was used for the preparation of solutions. Pharmaceutical grade salbutamol sulfate was kindly provided by the Middle East pharmaceuticals and cosmetics laboratories, Palestine. Stock solution of $5.97x10^{-3}$ $molL^{-1}$ salbutamol sulfate was prepared daily by dissolving 0.20 g SLS in 100 mL of double-distilled water.

Ferrous ammonium sulfate of $3.57x10^{-2}$ $molL^{-1}$ was prepared by dissolving 1.4 g the solid salt in 100 mL of water. A standard solution of $9x10^{-3}$ $molL^{-1}$ potassium bromate was prepared by dissolving 0.15 g $KBrO_3$ in 100 mL of water. All required diluted working solutions were prepared by addition of double-distilled water.

A 10% potassium bromide, 5.0 $molL^{-1}$ sulfuric acid and 1.0 $molL^{-1}$ ammonium thiocyanate were also prepared.

3.3 Analytical procedures

3.3.1 Initial rate method

Aliquots of 0.1-5.0 mL of 20 μgmL^{-1} of SLS were transferred into a series of 10 mL volumetric flasks. To all flaks, 1.0 mL 10 μgmL^{-1} $KBrO_3$, 2.0 mL 10% KBr and 1.0 mL 5.0 $molL^{-1}$ H_2SO_4 were added and diluted to the mark. All solutions were immersed in an ice bath to keep temperature in the range 8-10 ^oC. After mixing, the reaction was monitored at 10 ^oC and the absorbance was recorded as a function of time for 10.0 minutes at 380 nm.

3.3.2 Fixed time method

All solutions were immersed in an ice bath to keep temperature in the range 8-10 ^{o}C. Aliquots of 1.0-5.0 mL of 20 μgmL^{-1} of SLS were transferred into a series of 10.0 mL volumetric flask. To this, 1.0 mL 10 μgmL^{-1} $KBrO_3$, 2.0 mL 10% KBr and 1.0 mL 5.0 molL^{-1} H_2SO_4 were added. The solution was diluted to volume with water and mixed thoroughly. The reaction was allowed to proceed for 10.0 minutes at 10 ^{o}C and the absorbance was measured at 380nm.

3.3.3 Atomic absorption method

Aliquots of 0.1-1.0 mL of 20 μgmL^{-1} of SLS were transferred into a series of 10.0 mL volumetric flasks. To this, 1.0 mL 10 μgmL^{-1} $KBrO_3$, 2.0 mL 10% KBr and 1.0 mL 5.0 molL^{-1} H_2SO_4 were added and the solution was left to stand for 10.0 minutes with occasional shaking. Then 1.0 mL 140.0 μgmL^{-1} ferrous ammonium sulfate was added and again the flask let stand for 10.0 minutes with occasional shaking followed by 1 mL 1.0 molL^{-1} ammonium thiocyanate and 0.2 g sodium chloride. The iron(III)-thiocyanate complex was extracted with three 10.0 mL portions of n-propyl alcohol and then the eliminated aqueous layer was aspirated into the air-acetylene flame to measure the absorbance of iron (II) at 302.1 nm and the drug concentration was determined from a calibration curve, previously constructed using standard SLS.

3.3.4 Analysis of dosage forms

Twenty tablets of SLS were accurately weighed and powdered. A portion equivalent to 2.0 mg SLS was dissolved in water filtered and analyzed by the recommended methods.

3.3.5 Analysis of synthetic mixture

Synthetic mixture containing SLS was prepared with excipients commonly used in solid dosage forms and analyzed to check the applicability of the proposed methods. The following excipients were used to prepare a synthetic mixture for solid dosage forms: SLS (2.0 mg), starch (30.0 mg), talk (2.0 mg), lactose (140.0 mg), magnesium

stearate (2.0 mg) and croscarmellose sodium (2.0 mg). A portion of the synthetic mixture was dissolved in water, filtered and analyzed by the proposed methods.

3.3.6 Reaction Stoichiometry

The stoichiometry of the reaction was studied adopting the limiting logarithmic method[30]. The disappearance of bromine color was alternatively measured in excess concentration of bromate-bromide mixture and the concentration of SLS was varied. Next, excess concentration of SLS was employed and concentration of bromate-bromide mixture was varied.

3.3.7 Validation of the method

For linearity evaluation, SLS was analyzed at five concentration levels. Each concentration was analyzed three times. The detection limit was evaluated as:

$$LOD = 3.3\frac{s}{b}$$

Where b is the slope and S is the standard deviation of the regression line. Three concentrations where selected and five solutions of each were used for intraday and interday analysis. Recovery experiments were performed using the standard addition method. The standard analytical error (SAE) and 95% confidence limits (CL) were calculated as:

$$SEA = \frac{S}{\sqrt{n}}$$

$$CL = \frac{St}{\sqrt{n}}$$

where S is the standard deviation of n measurements and t is the tabulated t-value.

4. Conclusion

The proposed methods described in this paper are simple, selective, sensitive and reproducible and don't require expensive reagents and sophisticated instruments. These methods are applicable for routine analysis of the studied drug in raw materials and pharmaceutical formulations over wide concentration range without interference from common excipients. The methods can use both spectrophotometric and (AAS) techniques for the final measurement step. The statistical parameters indicate the reproducibility and accuracy of the methods.

Sensitive kinetic and AAS methods are developed, to determine SLS in its dosage forms. The initial rate and fixed time methods are used for the determination of SLS in kinetic method and a new way for separation iron (III) was used in AAS methods. The AAS method is more sensitive than the kinetic method. It should be noted that this is the first AAS method for determination of the SLS. The kinetic method is comparable in accuracy and precision with the official method.

Acknowledgments

Authors thanks the Middle East pharmaceuticals and cosmetics laboratories, Palestine for supporting the salbutamol sulfate standard and drug formulation. As well thanking should be mentioned to Alaqsa University for offering all the required chemicals.

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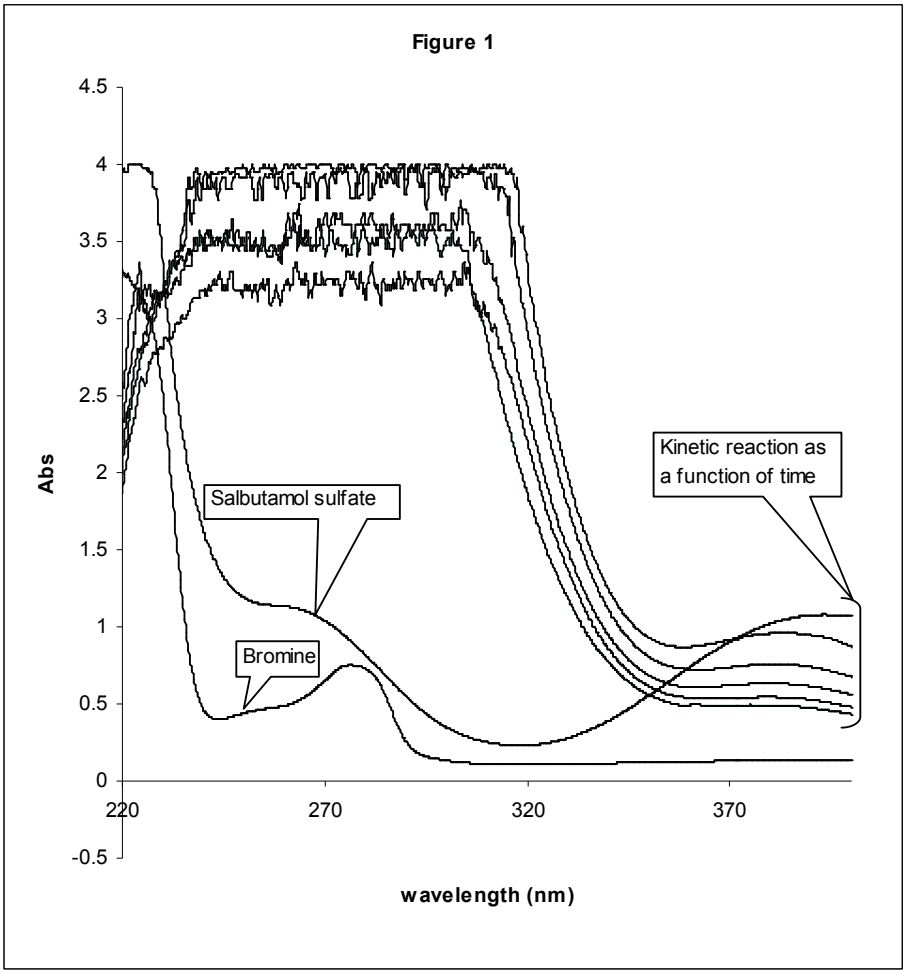
Figures captions

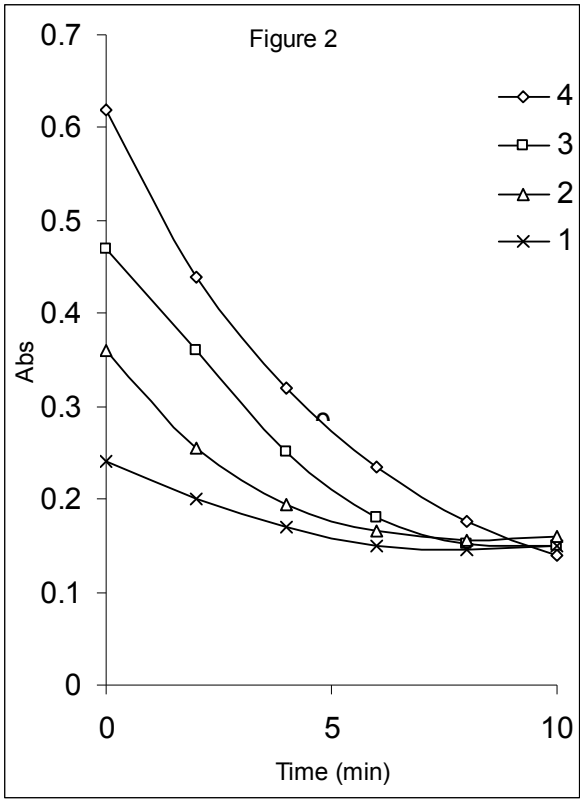
Figure 1. Absorption spectra of (1) Bromine, $1.8x10^{-3}$; (2) SLS, $3.0x10^{-5}$ $molL^{-1}$; (3) Kinetic reaction as a function of time at 10 ^{o}C.

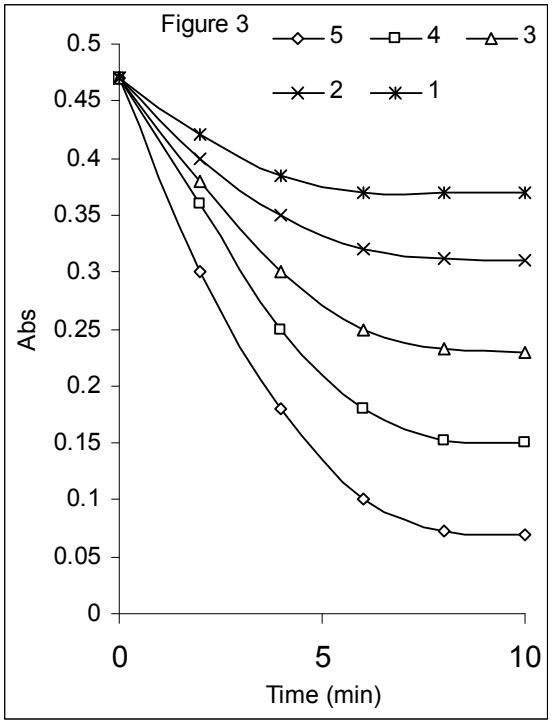
Figure 2. Bromine consumption in the SLS-Br_2 system for different initial bromine concentrations. [Br_2] (1) $4.5x10^{-4}$; (2) $6.75x10^{-4}$; (3) $9.0x10^{-4}$; (4) $1.13x10^{-3}$ $molL^{-1}$, [SLS] $3x10^{-3}$ $molL^{-1}$; H_2SO_4 0.5 $molL^{-1}$.

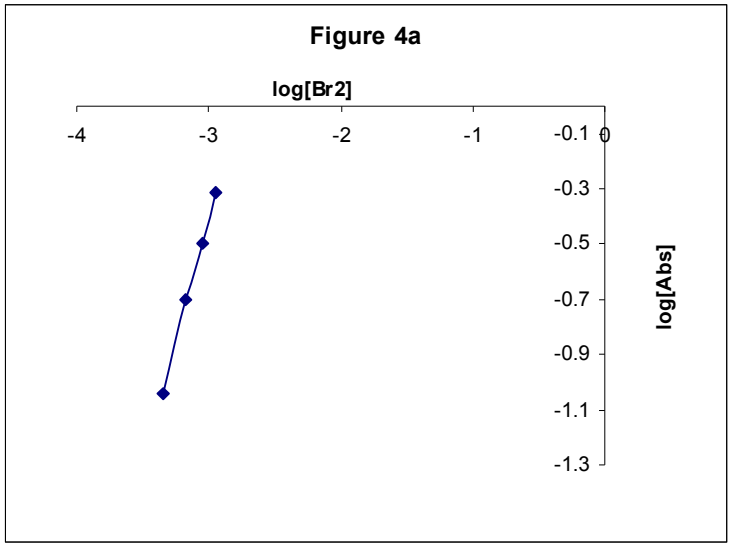
Figure 3. Bromine consumption in the SLS-Br_2 system for different SLS concentrations (1) $5.97x10^{-6}$; (2) $1.19x10^{-5}$; (3) $1.79x10^{-5}$; (4) $2.39x10^{-5}$; (5) $2.99x10^{-5}$ $molL^{-1}$, [Br_2] $9x10^{-4}$ $molL^{-1}$; H_2SO_4 0.5 $molL^{-1}$.

Figure 4. Limiting logarithmic plots for the molar ratio. (a) Log[Abs] vs. Log[Br_2] and (b) Log[Abs] vs. Log [SLS].









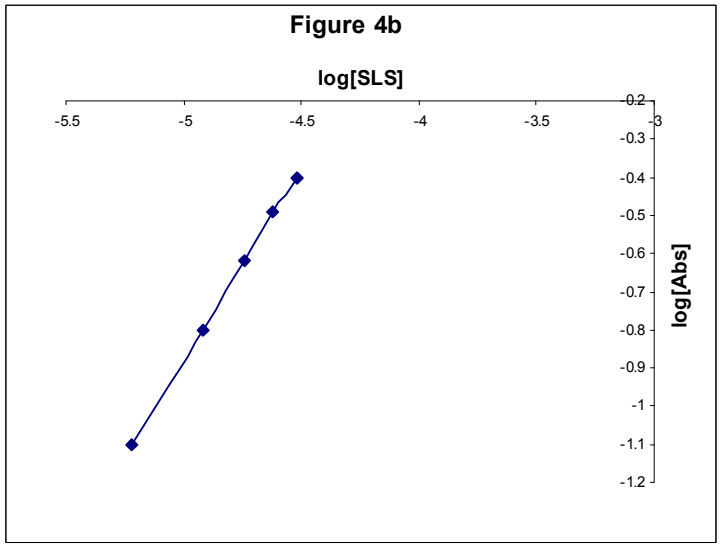


Table 1 Logarithms of rates for different concentrations ($molL^{-1}$) of SLS and at different temperatures.

T (^oC)	$Log\ \frac{\Delta A}{\Delta t}$	$Log\left[SLS\right]$	$K'S^{-1}$
10	-3.478	-5.22	46.23
10	-3.176	-4.92	46.23
10	-3.000	-4.74	46.23
10	-2.875	-4.62	46.23
10	-2.789	-4.52	46.23
5	-3.988	-5.22	15.50
15	ND	-5.22	ND
20	ND	-5.22	ND
25	ND	-5.22	ND

ND: not detected

Table 2 Calibration graphs of absorbance vs. initial concentration of SLS at fixed time.

Time (min)	regression equation	r	LOD (μgmL^{-1})
2.5	$A = -0.0020 + 0.0221C$	0.9820	1.23
5.0	$A = -0.0015 + 0.0294C$	0.9840	0.60
7.5	$A = 0.0010 + 0.0393C$	0.9953	0.44
10	$A = 0.0020 + 0.0407C$	0.9990	0.29

Table 3. Intraday and interday assay by the initial-rate method

Conc.(μg mL^{-1})	Intraday assay (LOD=0.30 μg mL^{-1})				
	Found±SD[a]	Recovery, %	SAE[b]	RSD %	CL[c]
2.0	1.98±0.02	99.00	0.009	1.010	1.98±0.024
6.0	6.10±0.06	101.7	0.026	0.980	6.10±0.072
10.0	9.80±0.09	98.00	0.040	0.920	9.80±0.111
Conc.(μg mL^{-1})	Interday assay (LOD=0.29 μg mL^{-1})				
2.0	2.06±0.02	103.0	0.009	0.971	2.06±0.025
6.0	5.90±0.07	98.00	0.031	1.180	5.90±0.087
10.0	10.3±0.12	103.0	0.053	1.170	10.3±0.149

[a] average of five determinations,
[b] SAE, standard analytical error,
[c] 95% confidence limits (n=5)

Table 4. Intraday and interday assay by the fixed-time method.

Conc.(μg mL^{-1})	Intraday assay (LOD=0.31 μg mL^{-1})				
	Found±SD[a]	Recovery, %	SAE[b]	RSD %	CL[c]
2.0	1.99±0.018	99.50	0.008	0.90	1.99±0.022
6.0	5.98±0.05	99.60	0.022	0.83	5.98±0.062
10.0	9.86±0.16	98.60	0.071	1.62	9.86±0.19
Conc.(μg mL^{-1})	Interday assay (LOD=0.30 μg mL^{-1})				
2.0	2.08±0.019	104.0	0.008	0.91	2.08±0.023
6.0	5.96±0.08	99.30	0.035	1.34	5.96±0.099
10.0	10.20±0.14	102.0	0.062	1.37	10.2±0.17

[a] average of five determinations,
[b] SAE, standard analytical error,
[c] 95% confidence limits (n=5)

Table 5. Intraday and interday assay by the AAS method

Conc.(μg mL^{-1})	Intraday assay (LOD=0.026 μg mL^{-1})				
	Found±SD[a]	Recovery, %	SAE[b]	RSD %	CL[c]
0.4	0.393±0.007	98.80	0.003	1.80	0.393±0.008
1.2	1.23±0.026	100.2	0.012	2.10	1.23±0.032
2.0	1.990±0.042	99.50	0.019	2.10	1.990±0.077
Conc.(μg mL^{-1})	Interday assay (LOD=0.028 μg mL^{-1})				
0.4	0.410±0.009	102.5	0.004	2.10	0.41±0.011
1.2	1.20±0.025	100.0	0.011	2.00	1.20±0.031
2.0	2.01±0.03	101.1	0.013	1.50	2.01±0.037

[a] average of five determinations,
[b] SAE, standard analytical error,
[c] 95% confidence limits (n=5)

Table 6. Preliminary selectivity data[a]

Initial-rate method			Fixed-time method			AAS method		
Taken (μg mL^{-1})	Found (μg mL^{-1})	Recovery[b], %	Taken (μg mL^{-1})	Found (μg mL^{-1})	Recovery, %	Taken (μg mL^{-1})	Found (μg mL^{-1})	Recovery, %
2.0	2.04	102.0	2.0	1.98	99.00	0.4	0.39	98.00
6.0	5.88	98.00	6.0	5.90	98.30	1.2	1.22	101.6
10.0	9.86	98.60	10.0	10.1	101.0	2.0	2.02	101.0

[a] Synthetic mixture containing excipients (starch (30mg), talc (2mg), lactose (140mg), magnesium stearate (2mg) and croscarmellose sodium (2mg)) in water
[b] Mean of five determination

Table 7. Comparison of the proposed methods with official method [31].

Formula	Parameters	Proposed methods of assay			Official method
		Initial-rate	Fixed-time	AAS	
Ventolin tablets	Recovery [a], %	102.4±	100.1±2.10	99.80±3.30	101.2±2.60
	t-test	0.96	1.10	1.30	
	F-test	2.20	3.90	2.40	

[a] Average of five determinations
t_{tab} (n=5) = 2.776
F_{tab} (5,5) = 6