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The Chemical Behavior of Low Valence Sulfur Compounds. II.[*1] Thin-layer Chromatographic Separation and Photometric Determination of Low Valence Sulfur Compounds

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The quantitative determination of some low valence sulfur compounds, especially low polythionates, which appeared as intermediate products in the oxidation of elemental sulfur with compressed oxygen in aqueous ammonia was investigated by means of thin-layer chromatography and colorimetry with methylene blue to establish the mechanism of the oxidation of elemental sulfur. Thiosulfate and some low polythionates could be resolved clearly by thin-layer chromatography using silica gel as an absorbent. A suitable solvent for the development was a mixed solution of ethyl alcohol, *n*-butyl alcohol, aqueous ammonia, ammonium acetate and distilled water. The amount of sulfur in the developed spot of the thin-layer chromatogram was determined photometrically with precision of 10 to 20% using methylene blue as a color reagent. Sulfate, sulfite and thiosulfate could not be developed effectively by this method.

In a previous paper,[1] it was reported that ammonium sulfamate and ammonium sulfate were finally produced in the oxidation of elemental sulfur with compressed oxygen in aqueous ammonia solution. The reaction mechanism of the oxidation was not completely established, but its main course through some low polythionates has been disclosed. For the development of these serial studies, it is necessary to establish a rapid, reliable and simple analytical method for the mixture of polythionates in the presence of sulfite, sulfate and thiosulfate. Analytical methods have been proposed by many investigators. 1. A chemical method developed by Kurtenacker and Goldbach,[2] and Goehring[3] has generally been accepted for the detection and quantitative determination of polythionates. So far, this is the most reliable method. However, the skill and delicate operation required make it difficult to achieve reliable results. 2. A fractional precipitation method with benzidine was taken up by Spohn and Weitz[4] for the isolation of low polythionates, but the troublesome procedure prevents it from becoming popular. 3. A photometric method with ferric thiocyanate was proposed by Urban[5] and improved by Koh and Iwasaki.[6] As only a mean value of polythionate content is obtained, its application for the quantitative determination of polythionates is limited. 4. Recently, excellent works on the detection of polythionates using paper chromatography have been reported by Bighi and Trabanelli,[7] Scoffone and Carini,[8] and Pollard.[9] Two improved methods, in which radio-paper chromatography or thin-layer chromatography was used, were carried out by Blasius and his co-workers[10] and Seiler and Erlenmeyer,[11] respectively. These methods are superior in the effectiveness of qualitative separation of polythionates to any other

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[*1] Previous paper in series, M. C. Shieh, H. Otsubo and T. Okabe, This Bulletin, **38**, 1596 (1965).

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1) M. C. Shieh, H. Otsubo and T. Okabe, This Bulletin, **38**, 1596 (1965).

2) A. Kurtenacker and E. Goldbach, *Z. Anorg. Allg. Chem.*, **166**, 177 (1927); *ibid.*, **134**, 265 (1924).

3) M. Goehring, *Z. Anal. Chem.*, **128**, 6 (1948); *ibid.*, **129**, 346 (1949).

4) F. Weitz and K. Spohn, *Chem. Ber.*, **89**, 2332 (1956).

5) P. J. Urban, *Z. Anal. Chem.*, **179**, 415, 422 (1961); *ibid.*, **180**, 110, 116 (1961).

6) T. Koh and I. Iwasaki, This Bulletin, **39**, 352, 576, 703 (1966).

7) C. Bighi and G. Trabanelli, *Ann. Chim.* (Rome), **47**, 195 (1957); *Chem. Abstr.*, **51**, 9404a (1957); *Boll. Sci. Fac. Chim. Ind. Bologna*, **16**, 92 (1958); *Chem. Abstr.*, **53**, 4017g (1959).

8) E. Scoffone and E. Carini, *Ricerca Sci.*, **25**, 2109 (1956); *Chem. Abstr.*, **50**, 729e (1956).

9) F. H. Pollard, *J. Chromatogr.*, **15**, 393, 407, 518 (1964); *J. Chem. Soc.*, **1955**, 4377.

10) E. Blasius and W. Burmeister, *Z. Anal. Chem.*, **168**, 1 (1959); *Z. Anorg. Allg. Chem.*, **313**, 248 (1959); E. Blasius, W. Burmeister and G. Wermbter, *ibid.*, **313**, 254 (1959); E. Blasius and G. Werbmter, *ibid.*, **316**, 251 (1962); E. Blasius and R. Kramer, *ibid.*, **318**, 113 (1962); *ibid.*, **319**, 1 (1962).

11) H. Seiler and H. Erlenmeyer, *Helv. Chim. Acta*, **47**, 264 (1964).

method proposed previously.

In the present study, chromatographic separation and photometric determination of polythionates and some sulfur-oxy acids are examined by thin-layer chromatography and the colorimetric technique with methylene blue as a color reagent.

Experimental

Sample Compounds of Sulfur. Compounds of sulfur used in the present study were potassium salts of thiosulfate, sulfate, sulfite and tri-, tetra-, penta- and hexathionates. Potassium salts of thiosulfate, sulfate and sulfite were of E.C.P. grade from Kanto Chemical Co., Inc. Potassium tri- and tetrathoinates were prepared from potassium thiosulfate and sulfur dioxide gas and from potassium thiosulfate and ethyl alcoholic solution of iodine by Martin's method,[12] respectively. Potassium penta- and hexathionates were prepared from sodium thiosulfate, sulfur dichloride and alcoholic potassium hydroxide solution and from sodium thiosulfate, sulfur monochloride and alcoholic potassium hydroxide solution by Goehring's method,[13] respectively. Each polythionate was recrystallized from aqueous solution. Its purity is as follows: trithionate 98%, tetrathionate 99%, pentathionate 80% and hexathionate 96%.

Preparation of Thin-layer Plate. Before the application on a glass plate, silica gel, Wako gel B–O of Wako Pure Chemical Industries, Ltd., was washed with distilled water to be free from chloride ion, and soluble starch was also washed with ethyl alcohol for several hours. Washing soluble starch with ethyl alcohol prevents the back-ground of the thin-layer from being colored, and clearly colored spots were visualized when silver nitrate solution was sprayed. Developing time is shortened to one-half or one-third by adding a small quantity of aqueous ammonia to the paste before spreading it on a glass plate. Development takes more than five hours on the thin-layer without adding aqueous ammonia. Thin-layer plates were prepared as follows: Two grams of soluble starch was kneaded with 20 m*l* of about 1.6 mol/*l* solution of aqueous ammonia in a mortar. Twenty grams of silica gel and 20 m*l* of distilled water were added little by little to the mortar to obtain a uniform paste. The paste was spread in about 330 μ thickness on a 200×200×3 mm of glass plate with an applicator. The plate was dried for 12 hr in air, and then activated by heating at 110 to 120°C for 2 to 3 hr and stored in a desiccator.

Development and Visualization. Two to five μl of the sample solution was taken accurately with a 0.05 m*l* micro-pipet and applied along one side of the thin-layer plate at intervals of 2 cm. Then the plate was developed in a closed vessel under saturated vapor of the solvent by the ascending technique. After development, the plate was taken out, dried and sprayed with 0.1 mol/*l* solution of silver nitrate. By this operation, polythionates and thiosulfate were visualized as dark-brown spots of silver sulfide formed by the following reactions:

$$S_2O_3^{2-} + 2Ag^+ + H_2O = Ag_2S + H_2SO_4 \qquad (1)$$

$$S_xO_6^{2-} + 2Ag^+ + 2H_2O = Ag_2S + 2H_2SO_4 + (x-3)S \qquad (2)$$

Though sulfate and sulfite generally appear in the reaction of sulfur compounds, they cannot be detected with silver nitrate differing from thiosulfate and polythionates. In this case, before the detection of polythionates and thiosulfate, the thin-layer plate was first sprayed with barium chloride solution acidified with acetic acid and with sodium rhodizonate solution. Sulfate and sulfite appeared then as pale-yellow spots on a pink colored background. Visualization of polythionates and thiosulfate was attained by spraying the spots with a silver nitrate solution.

Procedure of Colorimetric Determination. Sulfur amounts in the visualized spot was determined colorimetrically by the technique proposed by Kiba and his co-workers[14] as follows: The visualized spot was scratched as completely as possible and removed into a reduction quartz tube. Two or three m*l* of barium solution and bromine water were added to oxidize sulfur compounds, such as sulfide and elemental sulfur, to barium sulfate.

After evaporation to dryness, 5 to 10 m*l* of strong phosphoric acid solution of stannous chloride was poured into the tube and nitrogen gas was passed through the tube vigorously for first 5 min and then reduced to the flow rate of one bubble per second. After heating the quartz tube at 300 to 320°C for 30 to 60 min to reduce barium sulfate to hydrogen sulfide, nitrogen gas was passed again through the tube vigorously for 5 min. The absorption tube of hydrogen sulfide was taken off from the quartz tube and 1.5 m*l* of 1 : 1 sulfuric acid solution of *p*-amino dimethyl aniline and 0.5 m*l* of ferric ammonium sulfate solution were added under vigorous shaking to form methylene blue coloration. The colored solution of methylene blue was removed into a measuring flask and was diluted to 50 m*l* with distilled water. After standing for 20 to 30 min at room temperature, the absorbance was measured at 662.5 mμ through 0.03 mm width of slit in a glass cell of 10 mm thickness.

Results and Discussion

Selection of Developing Solvent. As developing solvents for sulfur-oxy acids, the following mixtures were used.

Bighi and Trabanelli:[7] isopropyl alcohol, pyridine and water (5 : 3 : 5)

Scoffone and Carini:[8] *n*-butyl alcohol, acetoacetic ester, acetic acid and water (10 : 2 : 1 : 7)

Pollard:[9] isopropyl alcohol, acetone and water (5 : 2 : 3)

Seiler and Erlenmeyer:[11] methyl alcohol, dioxane, aqueous ammonia and water (3 : 6 : 1 : 1)

The suitablity of mixed solvents of various alcohols given in literature was tested. From the results, it can be stated that no effective resolution of polythionates was obtained except for a case in

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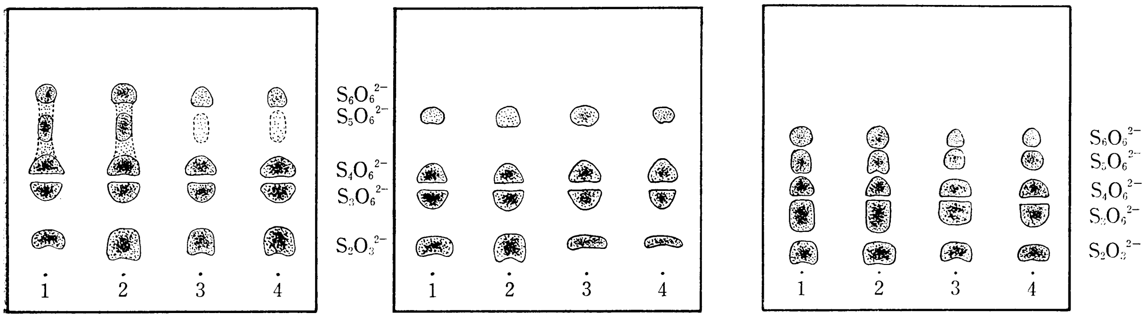
[12] F. Martin and L. Metz, *Z. Anorg. Allg. Chem.*, **127**, 83 (1923).

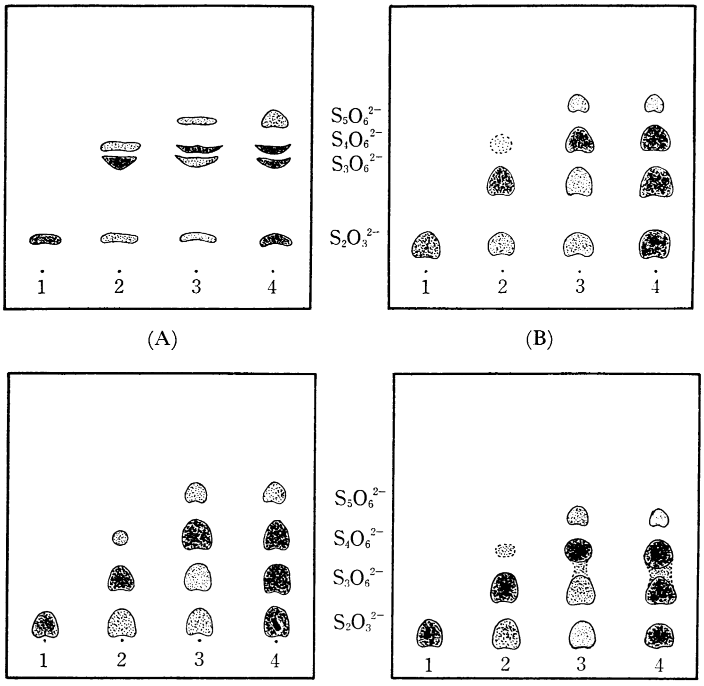
[13] M. Goehring and U. Feldmann, *ibid.*, **257**, 223 (1948).

[14] T. Kiba, Y. Yoshimura, T. Takagi and I. Kishi, This Bulletin, **28**, 641 (1955); T. Kiba, I. Akaza and N. Sugishita, *ibid.*, **30**, 972 (1957).



(A) (B) (C)

Fig. 1. Chromatograms obtained by varying the amount of aqueous ammonia.
Developing solvent: ethyl alcohol 75 m*l*, *n*-butyl alcohol 75 m*l* and aqueous ammonia
Developing time: 2 hr
Sample: a mixture of potassium thiosulfate, potassium tri- and tetrathionates
Amout of aqueous ammonia added
A: 4 m*l*, B: 8 m*l*, C: 12 m*l*



(A) (B)

(D) (C)

Fig. 2. Chromatograms obtained by addition of ammonium acetate.
Developing solvent: ethyl alcohol 75 m*l*, *n*-butyl alcohol 75 m*l*, aqueous ammonia 10 m*l*, ammonium acetate and distilled water
Developing time: 2.7 hr
Sample: 1. potassium thiosulfate 2. potassium trithionate
3. potassium tetrathionate 4. potassium pentathionate
Amount of 1 mol/*l* solution of ammonium acetate added
A: 0 m*l*, B: 10 ml*l*, C: 20 m*l*, D: 40 m*l*

which a mixture of ethyl alcohol, *n*-butyl alcohol, aqueous ammonia and water or ethyl alcohol, methyl isobutyl ketone, aqueous ammonia and water was used. Therefore, the solvent system consisting of ethyl alcohol, *n*-butyl alcohol, aqueous ammonia and water was studied by varying the amounts of aqueous ammonia. The results are shown in Fig. 1. The resolution of polythionates was almost independent of the amounts of aqueous ammonia.

Generally the addition of neutral salts affects activities of polythionates. It is expected that the resolution of polythionates might be improved. The addition of ammonium acetate to the solvent actually improved the resolution of the spots as shown in Fig. 2.

It is concluded that the following composition may be the most suitable as the developing solvent in the thin-layer chromatography of these sulfur compounds, *viz.* ethyl alcohol 75 m*l*, *n*-butyl alchol 75 m*l*, concentrated aqueous ammonia 8 to 10 m*l*, 1 mol/*l* solution of ammonium acetate 20 to 40 m*l* and distilled water 10 to 30 m*l*. Under these conditions, the R_s values of thiosulfate and some low polythionates obtained are shown in Table 1.

TABLE 1. THE R_s VALUES

Species	Present study	Pollard's results*
$S_2O_3^{2-}$	0.18—0.20	0.11
$S_3O_6^{2-}$	0.63—0.64	0.67
$S_4O_6^{2-}$	1.00	1.00
$S_5O_6^{2-}$	1.34—1.38	1.25

* Pollard's results were obtained by paper chromatography. R_s values: R_f values are normalized to that of tetrathionate.

The chromatographic separation of sulfate, sulfite and thiosulfate was studied by the same method using various mixed solvents, but a suitable solvent system for the resolution of these compounds was not found.

Colorimetric Determination of Sulfur in the Developed Spot. In the developed spot which was visualized by spraying silver nitrate solution, sulfur is contained as silver sulfide, sulfate and elemental sulfur. The amounts of sulfur can be roughly determined by comparing the color intensity of these spots with the standard color, but the accuracy is not satisfactory for quantitative determination of sulfur. Thus, the colorimetric technique proposed by Kiba and his co-workers[14] was chosen.

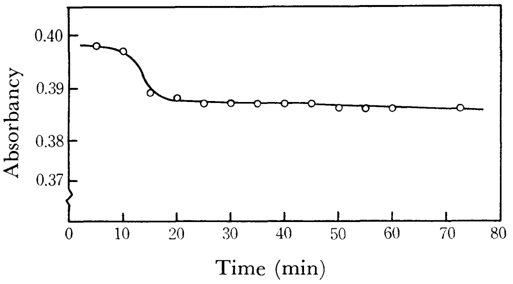


Fig. 4. The change of the absorbancy in the colored solution of methylene blue.

Reduction Time in Strong Phosphoric Acid. At the beginning, the detected amounts of sulfur fluctuated a great deal. The reduction time in strong phosphoric acid, one of the causes of fluctuation, was examined. The results are shown in Fig. 3. It was found that the sample should be heated for 20 to 30 min at 300°C to reduce sulfate to hydrogen sulfide completely.

Stability of the Color of Methylene Blue Solution. The stability of the color of methylene blue solution under room light, which has not been given in literature, was examined to simplify the analytical procedure. From the result, it is obvious that the absorbance has a constant value from 25 to 45 min after the color development. Thus the measurement should be carried out within 30 min after the solution was stood in room light at room temperature.

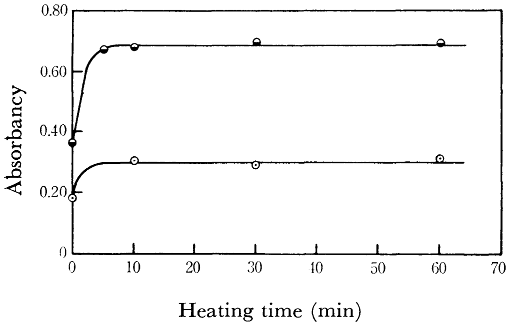


Fig. 3. The relation between absorbancy and heating time.
⊙ 0.5 m*l* of standard solution of sodium sulfate
◒ 1.0 m*l* of standard solution of sodium sulfate
The time when the temperature in the electric furnace reached 300°C is plotted as zero.

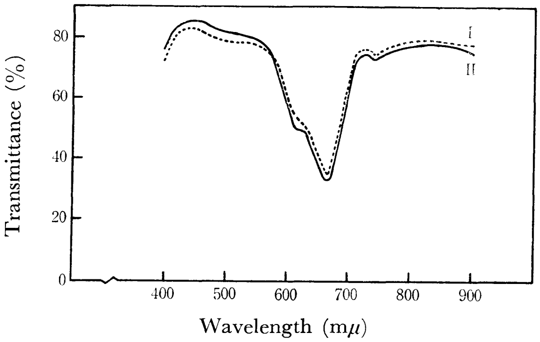


Fig. 5. The absorption spectrum curve of methylene blue solution.
I methylene blue solution prepared from a standard solution of sodium sulfide
II methylene blue solution prepared from a standard solution of sodium sulfate

Absorption Curve. Kiba and his co-workers[14] proposed that the absorbance of methylene blue solution should be measured at 655 mμ. Others[15] recommend 740 or 640 mμ as a proper wave length. Because of this uncertainty the absorption curve of methylene blue was reexamined with a Hitachi EPU-2A type spectrophotometer. The results are shown in Fig. 5. From the figure, it may be stated that the maximum absorption exists at 662.5 mμ rather than 655 mμ. Accordingly, the absorbance of methylene blue should be measured at 662.5 mμ.

Calibration curves were obtained by using sodium sulfide and sodium sulfate. These curves give linear relationship in the concentration range from 0 to 40 μg/50 m*l*. The molar absorptivity calculated from the slope of these calibration curves agreed well with the value reported by Johnson and Nishita.[16]

Table 2. The molar absorptivity

Sulfur compounds	Molar absorptivity	
	present study	Johnson and Nishita
Sodium sulfide	34100	34000
Sodium sulfate	33800	—

Interference of nitrate on the absorbance was pointed out by Kiba and his co-workers.[14] Accordingly, the interference of silver nitrate used on the visualization of spots was examined by using potassium tetrathionate as a sample. No influence was found on the determination of sulfur. The sulfur derived from silica gel can be disregarded, because only 0.4 μg of sulfur is contained in about 20 mg of silica gel.

The Determination of Sulfur. The results obtained for the developed spots by the above procedure are shown in Table 3. Since the measured values are obtained 10 to 30% lower than the theoretical values, it is impossible to determine directly a minute amount of sulfur. Therefore, an internal standard method, in which the amount of polythionate sulfur is compared with that of thiosulfate sulfur determined iodimetrically, was examined by using a mixed solution containing an equal amount of potassium trithionate and tetrathionate. The results are shown in Table 4.

Table 3. Results by the direct method

Sample volume added (μl)	Sulfur amounts(μg)		Concentration of sample (mol/*l*)
	added	detected	
1.94	13.6	9.4	0.0756
2.91	20.4	13.6	0.0730
3.40	23.9	16.5	0.0757
3.88	27.2	20.7	0.0834
4.37	30.7	24.2	0.0865
4.37	30.7	27.2	0.0974
4.37	30.7	26.4	0.0945
5.82	41.0	31.7	0.0850

sample solution: 0.1 mol/*l* (f=1.099) solution of sodium thiosulfate

Table 4. Results by the internal standard method

Sample No.	Sulfur amounts detected (μg)			Mole ratios	
	S_2	S_3	S_4	S_3/S_2	S_4/S_2
I	16.2	6.4	5.3	0.205	0.164
	16.1	5.6	4.9	0.230	0.152
	11.2	4.8	—	0.288	—
	14.0	5.1	—	0.243	—
	12.8	—	4.5	—	0.176
II	9.2	10.6	7.1	0.738	0.386
	7.9	9.6	5.2	0.810	0.329
	8.5	9.2	6.7	0.722	0.394

Amount of sulfur added (μg) and theoretical mole ratios

	S_2	S_3	S_4	S_3/S_2	S_4/S_2
I	16.0	6.2	5.3	0.250	0.166
II	9.6	10.4	6.4	0.722	0.333

S_2, S_3 and S_5 represent thiosulfate, trithionate and tetrathionate, respectively.

The measured values fluctuate within 10 to 20%. The cause of the fluctuation may be mainly due to the imperfect collection of visualized spots and could be diminished by careful technique. Thus this method may be applied to semi-quantitative determination of low valence sulfur compounds such as polythionates.

The authors wish to thank Professor Tsugio Sawaya of the Laboratory of Industrial Analytical Chemistry for very valuable advice and help.

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15) D. F. Bolz, "Chemical Analysis" Vol. III; "Colorimetric Determination of Non-Metals," Interscience Publishers, New York (1958), p. 273.

16) C. M. Johnson and H. Nishita, *Anal. Chem.*, **24**, 736 (1952).