Microcolorimetric Determination of Organic Sulfur by the Tin(II)-Strong Phosphoric Acid Reduction Method

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It has long been desired by organic, biological, and medical chemists that some rapid and accurate methods for the determination of minute amounts of sulfur in organic and biological materials might be developed. The determination of organic sulfur ordinarily consists of two steps, i.e. the oxidation of the sulfur to sulfate and the estimation of the sulfate. However, oxidation of organic sulfur requires time, skill and patience even by the use of an elaborate apparatus and powerful reagents, and, furthermore, reliable estimation of the sulfate is not always easy, whether it is worked out colorimetrically or turbidimetrically. If the oxidation of organic sulfur can be completed under mild conditions with the use of an open vessel, and if the resulting sulfate can be reduced to hydrogen sulfide in some simple way, the determination of the organic sulfur will become easier and more sensitive. Although various oxidizing and reducing procedures have been proposed for this purpose[1-11], they are so

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far too complicated to be readily applicable to routine works.

In this laboratory strong phosphoric acid has been used for the last five years for many analytical purposes with excellent results, and in a previous paper[12] the authors reported a new rapid colorimetric method for the determination of minute amounts of sulfate, in which the tin(II)-strong phosphoric acid, a new powerful reducing agent first described by the authors, was used[13]. In the present investigation, this method has been extended to the determination of organic sulfur of the order of micrograms by means of preliminary oxidation of organic sulfur with chromium(VI)-strong phosphoric acid, a new powerful oxidizing agent which is composed of potassium dichromate and strong phosphoric acid[14]. The rapidity and the simplicity of the original method are still retained in this procedure, by which 1 to 35 micrograms of organic sulfur can be estimated colorimetrically.

Apparatus

Apparatus for Oxidation, Reduction and Absorption.—The vessel for the oxidation of organic sulfur to sulfate is a test-tube of fused silica, which can be used also as the reaction vessel for the reduction of the sulfate. The test-tube should be made of fused silica, since unexpectedly high values of sulfur were occasionally obtained when glass vessels were used, owing to minute amounts of sulfur in the glass.

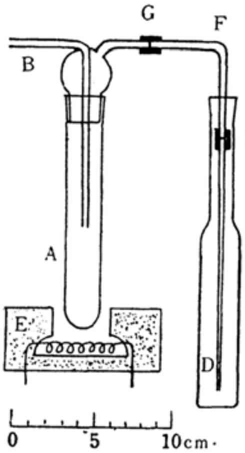

Fig. 1. Apparatus for oxidation, reduction and absorption.
A: Reaction vessel of fused silica
B: Glass cap fitted to a reaction vessel
C: Absorbing vessel
  a: The 35 ml. mark
  b: The 50 ml. mark
D: Detachable delivery tube (4 mm. in outside diameter)
E: Electric heater
F: Gas leading tube
G, H: A short piece of rubber tubing

A set-up of the apparatus for the reduction of the sulfate to hydrogen sulfide and the absorption of the hydrogen sulfide evolved is shown in Fig. 1. Six gas-washing bottles (not shown) are placed between a Kipp's apparatus for the production of carbon dioxide and the inlet arm of the reaction vessel (A). Solutions used for the purification of the carbon dioxide are described below. A bent glass tube (F) is attached to the outlet arm of the reaction vessel with a short piece of rubber tubing (G). A detachable delivery tube (D) is connected with another rubber tubing to the bent tube (F) and inserted into the absorbing vessel (C). Since some zinc sulfide adheres to that part of the tube immersed in the solution, it must be disconnected and allowed to remain in the absorbing vessel during the development of the methylene blue color.

Photoelectric Colorimeter.—A "Hirama" photoelectric colorimeter with 1-cm. cells and a filter of 655 mμ was used throughout this work.

Reagents

Tin(II)-Strong Phosphoric Acid.—Prepare from 250 g. of strong phosphoric acid and 50 g. of tin(II) chloride dihydrate of extra pure grade as described in the previous paper[13].

Chromium(VI)-Strong Phosphoric Acid.—Prepare from potassium dichromate and diluted tin(II)-strong phosphoric acid by the following precaution. Even in the case of extra pure grade, potassium dichromate and phosphoric acid always contain minute amounts of sulfate as impurity, which may give occasionally too high values in the determination of sulfur by this method. In the present study, the authors used potassium dichromate of the purest reagent grade supplied by the Osaka Goverment Industrial Research Institute, which contained as little as 0.0015 % of sulfate. A commercial reagent should be recrystallized twice from sulfur-free water. Sulfates contained in commercial phosphoric acid could be removed as follows: add 1 g. of tin(II) chloride dihydrate of pure grade into 100 g. of strong phosphoric acid and heat it to 300°C just as in the preparation of tin(II)-strong phosphoric acid. By this treatment, the sulfates contained in the phosphoric acid is removed as hydrogen sulfide during the heating. This will be called "Diluted Tin(II)-Strong Phosphoric Acid" in the following description. In an Erlenmyer flask, dissolve 12g. of potassium dichromate of the purest grade in 100 g. of the diluted tin(II)-strong phosphoric acid by heating on a steam bath. The chromium(VI)-strong phosphoric acid thus prepared can be stocked in a stoppered vessel without dehydrant, but it is advisable to prepare the

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[12] T. Kiba and I. Kishi, This Bulletin, **30**, 53 (1957).
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[14] S. Ohashi, ibid., **28**, 645 (1955).

reagent soon before use.

Carbon Dioxide.—Purify the carbon dioxide produced in the Kipp's apparatus by passing it through five gas-washing bottles containing, respectively, water, 1% potassium permanganate in 10% sodium carbonate solution, vanadium(II) sulfate in sulfuric acid (see below), 5% barium chloride, and zinc acetate absorbing solution (see below). Place a sixth bottle, an empty one, for the purpose of equalizing the flow and of intercepting floating liquid droplets.

Vanadium(II) Sulfate Solution.—Dissolve 2g. of ammonium vanadate in 200ml. of water, add 20 ml. of 6 N sulfuric acid, and shake vigorously with 3% zinc amalgam in an atmosphere of carbon dioxide until the violet color of vanadium(II) appears. Transfer the solution quickly into a gas-washing bottle containing a small amount of the amalgam. This is employed to remove oxygen completely from the carbon dioxide.

Zinc Acetate Absorbing Solution.—Dissolve 20 g. of zinc acetate dihydrate of extra pure grade in 1 l. of redistilled water. If necessary, add two or three drops of glacial acetic acid to clarify the solution, but the pH of the solution should not be below 5. The redistilled water is prepared by distilling ordinary distilled water with a small amount of sodium hydroxide in an all-glass still.

***p*-Amino Dimethylaniline Solution.**—Dissolve 1.4 g. of *p*-amino dimethylaniline sulfate crystals of pure grade in 200 ml. of 1 : 1 sulfuric acid.

Ferric Chloride Solution.—Dissolve 10 g. of ferric chloride hexahydrate in 100 ml. of distilled water.

Standard Sulfate Solution —Dissolve 4.4299g. of unhydrous sodium sulfate of extra pure grade in 1 l. of sulfur-free water. Take 20 ml. of this solution with a pipet and dilute it to 1 l. so that the final solution contains 20 micrograms of sulfur in 1 ml. Pipet accurately the required volume of this solution for making the standard calibration curve for the colorimetric determination of sulfur.

Barium Chloride Solution.—Dissolve 1 g. of barium chloride dihydrate in 100 ml. of sulfur-free water, and use 0.5 ml. of the solution in each test.

Procedure

When the sample is a solution, pipet accurately an aliquot containing 1 to 35 micrograms of sulfur. When it is a solid, take an accurately-weighed quantity containing the same range of sulfur. Put the measured sample into the test-tube of fused silica. Add 0.5 ml. of the barium chloride solution and evaporate the contents to dryness at the bottom of the vessel in a water-bath. Pour 1 ml. of the chromium (VI)-strong phosphoric acid over the residue from a pipet. Dip the vessel into a steam-bath till the content appears homogeneous. Place the vessel on an electric heater (E) (Fig. 1) and heat gently for about ten minutes to decompose the organic matter completely. The color change of the content from red to green shows the end of the reaction. Cool the content and pour 5 ml. of the tin(II)-strong phosphoric acid into the vessel. Fill the absorbing vessel with 35ml. of the zinc acetate solution. Connect each part of the apparatus as shown in Fig. 1., and pass carbon dioxide very rapidly for five minutes to expel the air in the reaction and the absorption vessel. The passage of the gas should be rapid enough to cause strong agitation of the surface of the liquid in the absorption vessel. The end of the gas delivery tube should reach to the bottom of the absorption vessel. At the end of five minutes, reduce the flow of the gas to a rate of one bubble per second. Heat the reaction vessel on the small electric heater (E), which is regulated by a variable transformer, till white fumes of phosphoric acid appear. Continue the heating for about fifteen minutes. It is unnecessary to keep the temperature strictly constant but it should be high enough to ensure that the content in the reaction vessel exhibits a white turbidity near the end of the fifteen minutes. Stop the heating and continue to pass carbon dioxide about five minutes.

Remove the absorption vessel and keep it in a thermostat for several minutes at 24°C. Add quickly 1.5 ml. of *p*-aminodimethylaniline solution using a rapid delivery pipet, stopper the vessel and shake it vigorously, and then quickly add 0.5 ml. of ferric chloride solution. Stopper and shake vigorously the vessel again. After keeping the vessel in the thermostat at 24°C. for fifteen minutes, remove the delivery tube from the solution, washing down the adhering solution into the vessel with distilled water. Add more distilled water till the liquid reaches the 50 ml. mark. Read the absorbancy of the solution in the colorimeter at 655 mμ within thirty minutes. According to the authors' experience, main sources of error in this method seem to be imperfect removal of oxygen from the carbon dioxide and careless delay in adding the colorization reagents viz., *p*-aminodimethylaniline and ferric chloride.

A standard calibrating curve, showing the relation between the amounts of sulfur taken and the absorbancies measured, should be prepared by carrying out the procedure with the standard sodium sulfate solution in the range 1 to 40 micrograms of sulfur. Estimate the amount of organic sulfur in the sample analyzed by comparing the absorbancy measured with this calibration curve. Use a mixture of the absorbing solution and the reagents of the definite amounts as reference for the photoelectric measurement.

Results and Discussion

Behavior of Chromium(VI)-Strong Phosphoric Acid.—This oxidizing agent is so powerful that all organic compounds, even coal, are completely oxidized at a temperatures below 200°C. Moreover, it has the advantageous property that all of the chromium(VI) remaining unchanged can be decomposed to chromium(III) easily by further heating, so that no excess of chromium(VI) will remain and consume the tin(II)-strong phosphoric acid in the succeeding reduction process. This chemical property of chromium(VI)-strong phosphoric acid is very interesting and advantageous not only for this purpose but for many other oxidizing processes.

Results of Analysis.—Analyses of some known organic compounds containing sulfur were carried out by this method. Each sample was first dried in an Abderhalden's apparatus at a suitable temperature for the compound, and weighed accurately. The weighed sample was dissolved in a definite volume of water, in some cases with the aid of a small amount of hydrochloric acid. An aliquot from the solution was employed for the analysis. By the measurement of the absorbancy of the methylene blue color produced in each case, the amount of sulfur in the sample tested was calculated by means of the equation:

$$x=0.047+0.01478y$$

where x is the absorbancy measured and y is the amount of sulfur in micrograms. The equation was derived from many experimental data obtained by carrying out the procedure with sodium sulfate as the standard. The numerical values in the equation, as a matter of course, were calculated by the method of the least square from the experimental data. The constant, 0.047 represents the sulfur contained as an impurity in the reagents used, viz., potassium dichromate and the diluted tin(II)-strong phosphoric acid. The numerical values in the equation will depend on the sulfur contained in the reagents as well as on the spectral purity of the photoelectric colorimeter employed. The results obtained with some organic compounds are listed in Tables I to VI.

TABLE I

DETERMINATION OF SULFUR IN SULFUR GUANIDINE

Sample taken μg.	Sulfur contained μg.	Absorbancy measured	Sulfur found μg.	Recovery of sulfur %
0.	0.	0.047	0.	
13.4	2.	0.0762	1.98	99.0
40.1	6.	0.1355	5.99	99.8
66.8	10.	0.1948	10.00	100.0
100.2	15.	0.2688	15.01	100.1
133.6	20.	0.3469	20.02	100.1
167.0	25.	0.4160	24.97	99.9
200.6	30.	0.4896	29.95	99.8
233.9	35.	0.5630	34.91	99.7

TABLE II

DETERMINATION OF SULFUR IN METHYL ORANGE

Sample taken μg.	Sulfur contained μg.	Absorbancy measured	Sulfur found μg.	Recovery of sulfur %
0.	0.	0.047	0.	
20.4	2.	0.0760	1.96	98.0
61.3	6.	0.1353	5.97	99.5
102.1	10.	0.1946	9.97	99.7
153.1	15.	0.2686	14.99	99.9
204.2	20.	0.3545	19.99	99.9
255.2	25.	0.4276	24.94	99.8
306.3	30.	0.5010	29.91	99.7
357.3	35.	0.5745	34.88	99.7

TABLE III

DETERMINATION OF SULFUR IN *l*-CYSTINE

Sample taken μg.	Sulfur contained μg.	Absorbancy measured	Sulfur found μg.	Recovery of sulfur %
0.	0.	0.047	0.	
7.5	2.	0.0745	1.86	93.0
22.5	6.	0.1482	5.90	98.3
37.5	10.	0.2078	9.93	99.3
56.2	15.	0.2819	14.95	99.6
74.9	20.	0.3415	19.93	99.7
93.7	25.	0.4198	24.95	99.8
112.4	30.	0.5030	29.90	99.7
131.1	35.	0.5760	34.84	99.5

TABLE IV

DETERMINATION OF SULFUR IN *l*-METHIONINE

Sample taken μg.	Sulfur contained μg.	Absorbancy measured	Sulfur found μg.	Recovery of sulfur %
0.	0.	0.047	0.	
9.3	2.	0.0746	1.87	93.5
27.9	6.	0.1350	5.95	99.2
46.5	10.	0.1938	9.93	99.3
69.8	15.	0.2685	14.99	99.9
93.1	20.	0.3419	19.95	99.8
116.3	25.	0.4150	24.90	99.6
139.6	30.	0.4890	29.90	99.7
162.9	35.	0.5626	34.88	99.6

TABLE V

DETERMINATION OF SULFUR IN THIOUREA

Sample taken μg.	Sulfur contained μg.	Absorbancy measured	Sulfur found μg.	Recovery of sulfur %
0.	0.	0.047	0.	
4.7	2.	0.0748	1.88	94.0
14.2	6.	0.1340	5.89	98.2
23.7	10.	0.1930	9.88	98.8
35.6	15.	0.2670	14.88	99.2
47.5	20.	0.3410	19.21	95.6
59.4	25.	0.4050	24.22	96.9
71.2	30.	0.4884	29.86	99.5
83.1	35.	0.5284	34.80	99.4

TABLE VI

DETERMINATION OF SULFUR IN CRESOL RED

Sample taken μg.	Sulfur contained μg.	Absorbancy measured	Sulfur found μg.	Recovery of sulfur %
0.	0.	0.047	0.	
23.9	2.	0.0755	1.93	96.5
71.6	6.	0.1356	5.99	99.5
119.3	10.	0.1948	9.99	99.9
178.9	15.	0.2690	15.02	100.1
238.5	20.	0.3388	19.74	98.7
298.2	25.	0.4165	25.00	100.0
357.8	30.	0.4900	29.97	99.9
417.4	35.	0.5635	34.95	99.9

In the column under "Recovery of sulfur" is given the ratio in percentage of sulfur found to the amount taken in each case.

Preliminary Elimination of Sulfur from the Reagents.—As described in the above section, strong phosphoric acid, prepared from commercial orthophosphoric acid (D=1.7), always contains a minute amount of sulfur as impurity. In order to eliminate the sulfur the authors have prepared the diluted tin(II)-strong phosphoric acid as described above. The amounts of sulfur contained in the untreated strong phosphoric acid and in the diluted tin(II)-strong phosphoric acid were determined by carrying out the same reduction procedure. The results are shown in Table VII, which shows that the sulfur contained in the original strong phosphoric acid was expelled almost completely by treating with as little as 1 g. of tin(II) chloride dihydrate. The contents of sulfur in potassium dichromate reagent were estimated by the same procedure, and the results are tabulated all together in Table VII.

TABLE VII

SULFUR CONTENT IN THE REAGENTS AND ITS ELIMINATION

Reagent	Amount taken ml.	Absorbancy measured	Sulfur found in 1 ml. of the reagent μg.
Strong phosphoric acid	1	0.2229	15.1
	1	0.1852	12.5
Diluted tin(II)-strong phosphoric acid (A)	1	0.011	0.74
	1	0.013	0.87
Diluted tin(II)-strong phosphoric acid (B)	1	0.013	0.87
	1	0.013	0.87
Mixture of 100 g. of tin(II)-strong phosphoric acid (A) and 6 g. of $K_2Cr_2O_7$ (C)	1	0.027	1.82
	1	0.024	1.62
Mixture of 100 g. of tin(II)-strong phosphoric acid (B) and 12 g. of $K_2Cr_2O_7$ (C)	1	0.044	2.98
	1	0.050	3.38
Mixture of 100 g. of tin(II)-strong phosphoric acid (A) and 6 g. of $K_2Cr_2O_7$ (D)	1	0.062	4.19
	1	0.051	3.45

Note:

Diluted tin(II)-strong phosphoric acid (A) was prepared from 100 g. of strong phosphoric acid and 1 g. of tin(II)-chloride dihydrate; (B) from 100 g. of the acid and 5 g. of the salt.

$K_2Cr_2O_7$ (C) was a standard reagent supplied by Osaka Goverment Industrial Research Institute; (D) was a commercial reagent recrystallized in the laboratory.

Summary

A simple and rapid method for the determination of minute amounts of organic sulfur was established by using the oxidizing action of chromium(VI)-strong phosphoric acid and the reducing power of tin(II)-strong phosphoric acid. Organic sulfur was oxidized to sulfate by the former in a test-tube of fused silica and the sulfate was reduced to hydrogen sulfide by the latter in the same vessel. The hydrogen sulfide evolved was absorbed in zinc acetate solution and estimated colorimetrically by the ordinary methylene blue method. The absorbancy of the colored solution was measured at 655 $m\mu$ with a photoelectric colorimeter using 1-cm. cells. The sulfur content of the sample was estimated by comparing the absorbancy value with the standard curve prepared with sodium sulfate. Some organic compounds containing sulfur were analyzed by this method and satisfactory results were obtained. However, analysis of the miscellaneous organic substances such as biological specimens has not been undertaken and the problems for this purpose remain to be solved in future.

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