free amine, either aniline or nitrotoluidine. No mention has been made of the possibility of a mixture which would contain the condensations of aniline and chloral and nitrotoluidine and chloral because the crude products, and also the purified products, which have been obtained by this work, have always shown very constant melting points and the behavior towards acids has always been the same.

From the foregoing work it has, therefore, been concluded that the mixed-type condensation product has been prepared in which one molecule of aniline and one molecule of nitrotoluidine are condensed with one molecule of chloral, according to the following reactions: $CCl_3CHO + C_6H_5NH_2 = CCl_3CHOH.NHC_6H_5$, and $CCl_3CHOH.NHC_6H_5 + C_7H_5N_2O_2 = C_{15}H_{14}N_3O_2Cl_3 + H_2O$.

The subsequent decomposition of this condensation product, giving 5-chlorine-3-nitro-4-toluidine, would probably take place in the following two steps, for the reason that nitrotoluidine and chloral will form an addition product which breaks down into the same chloral derivative, without the presence of either free or combined aniline: $C_{15}H_{14}N_3O_2Cl_3 \longrightarrow CCl_3CHOH.NHC_6H_3NO_2CH_3 + C_6H_5NH_2$, and $CCl_3CHOH.NHC_6H_3NO_2CH_3 + HCl \longrightarrow C_7H_8N_2O_2Cl$ + aldehyde derivative.

BOSTON, MASS.

THE DETERMINATION OF TOTAL SULPHUR IN ORGANIC MATTER.

BY HERMAN SCHREIBER.

Received June 7, 1910.

Introduction.

A great many methods have been proposed for the determination of total sulphur in organic matter, but probably only two of these are either easy to manipulate or accurate, namely, the Barlow-Tollen,[1] or absolute method, and the Osborne,[2] or peroxide method. When the latter is applied to solid material, however, it leaves much to be desired in the way of ease of manipulation and speed, and has absolutely no claim to exactness of detail. It is true that the water used for moistening the sample is measured and the sodium carbonate is weighed, but the amount of sodium peroxide added varies with the material analyzed and with the rate at which the reagent is added. The amount of acid which must be added after fusion is also an unknown factor; it varies in each case which necessitates making the solution alkaline again, and then acid. These, however, are not all of the difficulties encountered. The fusions have a tendency to burn and blow out of the crucible, and this happens most frequently when the determinations must be rapidly made, for the

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[1] THIS JOURNAL, **26**, 341 (1904).

[2] U. S. Dept. **Agr., Bureau** of Chemistry *Bull.* **107,** revised, p. 23.

peroxide method has a dignity all its own and will brook no haste or impatience. All of these objections were keenly realized when it became necessary to make about 100 sulphur determinations in a recent research.

An ideal method for this determination should embody several features: First, it must not call for excessive amounts of reagents, nor such as will interfere with the precipitation of the barium sulphate or contaminate the precipitate; second, the reagents employed must serve two purposes, they must destroy all of the organic matter and all of the sulphur must be fixed and oxidized to sulphate; third, the method must be fairly rapid and not require a large amount of practice, so that an analyst can make the determination from time to time without great delay and difficulty.

It is difficult to outline a method which will meet all of these conditions, but after making quite a number of experiments the following procedure was evolved, which seems to fulfil the requirements more nearly than the peroxide method.

The Proposed Method.

Weigh 1 gram of material in a nickel crucible of 100 cc. capacity, add 10 cc. of a solution made by dissolving 100 grams of sodium nitrate and 150 grams of sodium hydroxide in 500 cc. of water. Then add 5 grams of crystallized magnesium nitrate and stir with a platinum rod, making sure that the mass is thoroughly mixed and that the sample is broken up as much as possible. Wash down the material adhering to the stirring rod and sides of the crucible with the smallest possible amount of water. (This is essential since the addition of much water will prolong the subsequent heating unnecessarily.) Heat for one hour on a hot plate covered with a thin sheet of asbestos paper, keeping the temperature at about 130°. Then put the cover on the crucible, tilting it in such a fashion as to leave an opening for the steam to escape and heat further for one hour at from 150–160°, or until the material is entirely dry. If the fusions begin to bump, lower the heat so that the covers will not be jarred down tight on the crucibles, and the material lost by frothing. When the mass is entirely dry, put the covers on tight and heat gradually until the temperature reaches 180°, then heat for thirty-five minutes, maintaining the temperature at about 180–200°. These temperatures were determined by laying the thermometer down on the hot plate.

Now set the crucible (with the cover on tight) into a round hole in a piece of asbestos board, so that about 1.5 inches of the lower part of the crucible shall project below the asbestos board as Lunge[1] has directed, the asbestos board to be laid flat. Heat with the Bunsen burner for half an hour, allowing the flame to just touch the bottom of the crucible during the first fifteen minutes, and then with the full heat during the last fifteen minutes. (Never let the inner cone of the Bunsen burner

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[1] *Chemisch-Technische Untersuchungs Methoden*, 1, 428 (1904).

strike the crucible.) During the first five minutes of heating with the full flame keep the crucible in an upright position, then remove the cover and tilt the crucible so as to fuse any material which may have crept up the sides. Then return the crucible to the upright position, replace the cover, and heat for five minutes more. When the fusion has solidified, and before it has entirely cooled, place the crucible in a 600 cc. beaker with 150 cc. of distilled water and cover with a watch glass. Put the crucible cover in the beaker, beside the crucible, and slightly rotate and tilt the beaker so that all parts of the crucible shall be touched by the water. Run in 13 cc. of hydrochloric acid (specific gravity 1.19) from a burette and again rotate the beaker slightly. Tip the crucible so that the other side comes in contact with the acid liquor, allow it to stand a few minutes, and remove the crucible and cover, washing the liquid adhering to them back into the beaker by means of distilled water. Any fused material adhering to the sides of the crucible can easily be removed with a stirring rod. Place the beaker on the steam bath and heat for about half an hour and then let it stand in the cold over night. Filter and wash the insoluble residue. Heat the filtrate on the steam bath or otherwise, and precipitate with a 10 per cent. barium chloride solution.

In all of the determinations here reported the solutions were precipitated by heating on the steam bath, adding 10 cc. of barium chloride to the solution quickly, and stirring as soon as the precipitate began to form. After adding the barium chloride the solutions were allowed to remain on the steam bath about one hour, and were then removed to the table and stood in the cold over night, after replacing any water lost by evaporation. All filtrations, except the solution of the fusions of the barium sulphate with sodium carbonate, were filtered through S and S blue ribbon filter paper. The fusions of the barium sulphate were filtered through white ribbon filter paper and all precipitates were washed with cold water.

In this method a crucible loses from 0.3–1.3 grams of its weight in each determination, an average of 0.9 gram as calculated from 20 determinations. By the peroxide method the crucible loses from 1.2–2 grams of its weight in a fusion, an average of 1.4 grams based on 13 determinations. Using a griddle hot-plate, 14 by 18 inches, 18 fusions can easily be made in a day. The only way in which a determination can be lost is by heating too rapidly on the hot-plate, thus causing it to boil over or spatter.

Description of Samples and Analytical Results.

Total sulphur was determined by both the peroxide method and the proposed method on five samples of dried white of eggs, three of which were made from the same lot of eggs by boiling with various amounts of copper sulphate, washing free of copper, and drying and grinding; one was a sample of commercial dried albumin; and one was the white of eggs

TABLE 1.—COMPARISON OF THE RESULTS BY THE PEROXIDE AND BY THE PROPOSED METHOD FOR THE DETERMINATION OF TOTAL SULPHUR.

Number of sample.	Description of sample.	Peroxide method.[1]					Proposed method.[1]					Peroxide method averages.		Proposed method averages.		Difference between columns B and C.
			First precipitation.		Reprecipitation.			First precipitation.		Reprecipitation.[2]						
		Amount of sample. Grams.	Grams of sulphur.	Per cent. of sulphur.	Grams of sul. phur.	Per cent. of sulphur.	Amount of sample. Grams.	Grams of sulphur.	Per cent. of sulphur.	Grams of sulphur.	Per cent. of sulphur.	A. Before fusion. Per cent.	B. After fusion. Per cent.	C. Before fusion. Per cent.	D. After fusion. Per cent.	(B-C.) Per cent.
1	White of egg	1.0035	0.0162	1.61	..	..	1.0045	0.0151	1.50	..	..	1.64	..	1.54	..	..
		0.9955	0.0166	1.67	..	..	1.0025	0.0159	1.58	..	..					
2	White of egg	0.9978	0.0167	1.67	..	..	1.0015	0.0145	1.45	..	..	1.67	..	1.49	..	..
		1.0025	0.0167	1.67	..	..	0.9995	0.0153	1.53	..	..					
3	White of egg	1.0148	0.0163	1.61	..	..	1.0100	0.0160	1.58	..	..	1.64	..	1.58	..	..
		1.0330	0.0172	1.67	..	..	1.0075	0.0158	1.57	..	..					
51	White of egg	1.0110	0.0153	1.51	..	..	1.0055	0.0139	1.38	..	..	1.49	..	1.35	..	..
		1.0035	0.0146	1.46	..	..	1.0160	0.0133	1.31	..	..					
54	White of egg	1.0046	0.0131	1.30	..	..	1.0045	0.0133	1.32	..	..	1.35	..	1.29	..	..
		1.0000	0.0138	1.38	..	..	1.0023	0.0125	1.25	..	..					
	Blank	....	0.0017	..	..	..	..	0.0004	..	..	..	..	..	..	..	..
A	Mustard (ground)	1.0015	0.0124	1.24	0.0122	1.22	1.0105	0.0120	1.19	0.0111	1.10	1.24	1.22	1.19	1.10	+0.03
		1.0030	0.0124	1.24	0.0122	1.22	1.0035	0.0119	1.19	0.0111	1.10					
B	Mustard (ground)	1.0040	0.0130	1.30	0.0128	1.28	1.0050	0.0146	1.45	0.0124	1.23	1.28	1.28	1.32	1.22	—0.04
		1.0255	0.0128	1.25	0.0130	1.27	1.0050	0.0118	1.18	0.0121	1.20					
C	Mustard (ground)	1.0025	0.0128	1.28	0.0120	1.20	1.0040	0.0120	1.20	0.0111	1.10	1.26	1.22	1.21	1.10	+0.01
		1.0075	0.0124	1.23	0.0124	1.23	1.0213	0.0125	1.22	Lost	..					

D	Mustard (ground).....	1.0135	0.0158	1.56	0.0157	1.55	1.0120	0.0133	1.32	0.0137	1.35	1.52	1.51	1.40	1.37	+0.11
		1.0000	0.0147	1.47	0.0146	1.46	1.0060	0.0148	1.47	0.0139	1.38					
E	Mustard (ground).....	1.0080	0.0142	1.41	0.0140	1.39	1.0017	0.0133	1.33	0.0124	1.24	1.35	1.34	1.32	1.17	+0.02
		1.0035	0.0129	1.29	0.0128	1.28	1.0000	0.0131	1.31	0.0109	1.09					
	Blank...............	..	0.0009	..	0.0011	..	..	0.0004	..	0.0007	..	..	..	..	..	..
7931	Valve rubber.........	1.0000	0.0435	4.35	0.0426	4.26	1.0135	0.0435	4.29	0.0417	4.12	4.35	4.27	4.27	4.10	±0.00
		1.0155	0.0441	4.34	0.0432	4.27	0.9990	0.0425	4.25	0.0407	4.07					
7934	Valve rubber.........	1.0000	0.0438	4.38	0.0430	4.30	1.0075	0.0415	4.12	0.0397	3.94	4.35	4.26	4.21	3.95	+0.05
		1.0025	0.0432	4.31	0.0422	4.21	1.0055	0.0432	4.30	0.0397	3.95					
7935	Valve rubber.........	1.0045	0.0324	3.23	0.0305	3.04	1.0045	0.0286	2.85	0.0276	2.75	3.19	2.92	2.88	2.77	+0.04
		1.0040	0.0316	3.15	0.0281	2.80	1.0075	0.0292	2.90	0.0281	2.79					
7937	Valve rubber.........	0.9990	0.0435	4.35	0.0428	4.28	1.0055	0.0417	4.15	0.0401	3.99	4.30	4.21	4.14	3.99	+0.07
		1.0010	0.0425	4.25	0.0414	4.14	1.0035	0.0413	4.12	0.0400	3.99					
8136	Tubing.............	1.0040	0.0846	8.43	..	..	1.0230	0.0805	7.87	0.0771	7.54	8.44	8.38	7.82	7.50	..
		1.0080	0.0852	8.45	..	..	1.0660	0.0827	7.76	0.0794	7.45					
		..	..	..	..	..	1.0035	0.0798	7.95	..	..	..	..	8.20	..	..
		..	..	..	..	..	1.0135	0.0820	8.09	..	..					
	Tubing[3]............	..	..	8.12	..	..	..	..	..	0.0000	..	8.06	..	..	..	..
		..	..	8.00	..	..	..	..	..	..	..					
	Blank...............	..	0.0022	..	0.0028	..	..	0.0002	..	0.0000	....	..	..	..	..	..

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[1] Results given have been corrected for blank.

[2] After fusing barium sulphate with sodium carbonate.

[3] Determination by E. W. Boughton, Contracts Laboratory.

boiled in the shell, dried, and ground. Sulphur was also determined on four samples of mustard seed, and one sample of ground mustard. *A* is pure white mustard, *B* is black mustard containing 64 per cent. of charlock, *C* is commercial ground mustard, *D* is pure brown mustard, and *E* is commercial ground seed. These samples were furnished by the Microchemical Laboratory. Sulphur was also determined on five samples of vulcanized rubber furnished by the Contracts Laboratory.

The 60 determinations were made without repeating any, except No. 8136, unless a loss occurred by the leaking of the crucible. This happened in three instances only, so that the work represents fairly well the results which the two methods will give under the same conditions without repetition and selection of results. Twelve determinations were made at a time with each kind of material, two determinations on each sample, and two blanks. The results obtained are given in Table 1. The peroxide fusions were not hurried, but were allowed to fuse for three or four hours. No. 8136 is a sample of rubber tubing which was not ground very finely, and which had been found to contain about 50 per cent. of free sulphur. These facts probably account for the discrepancies in the determination.

Discussion of Results.

Skinner[1] comparing the Barlow-Tollen's and peroxide methods found that the latter gave slightly higher results in five out of seven cases, the difference averaging about +0.11 per cent. of SO_4 or +0.036 per cent. of sulphur. The proposed method gives sulphur results about 0.1 per cent. lower than the peroxide method, the average of the 12 values which were lower by the proposed method would thus be about 0.064 per cent. less than by the absolute method. Both of these discrepancies are well within the experimental error. Also it should be noted that the difference between the figures obtained by fusing the barium sulphate in the peroxide method and reprecipitating, and those resulting from the first precipitation by the proposed method (as given in columns B and C, Table 1) varies from —0.04 to +0.11 per cent. In six out of twelve cases the figures obtained by the first precipitation in the proposed method are nearer the values obtained by fusing and reprecipitating the barium sulphate in the peroxide method than they are to the results of the first precipitation in the peroxide method. The peroxide method gives a very high blank as compared to the proposed method; with the former this blank amounted to from 0.009–0.0028 per cent. of sulphur and in the latter it amounts to from 0–0.0007 per cent. As the blanks by the peroxide method are very high an error would be introduced if they were not run in each set. This is very clearly demonstrated in the determinations of sulphur in sulphuric acid solutions as given in Table 2.

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[1] U. S. Dept. Agr., Bureau of Chemistry, *Bull.* 116, p. 92.

Sulphuric Acid Solutions.

To determine the effect of the salts in solution on the precipitation of sulphuric acid, fusions were made by the peroxide method and by the proposed method, definite volumes of dilute sulphuric acid being added to the solutions and blanks run at the same time. The fusions were made exactly as in the case of the total sulphur determinations given in Table 1. Then they were dissolved, an excess of 2 cc. of hydrochloric acid was added, the solution was filtered, and the measured amount of dilute sulphuric acid added. The sulphuric acid was precipitated with barium chloride solution in the same manner as in the other determinations. The results are given in Table 2.

TABLE 2.—DETERMINATION OF SULPHUR IN DILUTE SULPHURIC ACID SOLUTIONS BY FOUR DIFFERENT METHODS.

Quantity of solution used. cc.	By precipitating from distilled water after adding 2 cc. excess of concentrated hydrochloric acid. Gram.	By weighing as ammonium sulphate by Lunge's method. Gram.	By precipitating from a solution containing a fusion.	
			By the proposed method. Gram.	By the peroxide method. Gram.
10 of solution A........	0.0140	0.0142	0.0141	0.0127
	0.0141	0.0142	0.0141	0.0147
25 of solution A........	0.0352	0.0350	0.0362	0.0340
	0.0351	0.0349	0.0361	0.0355
10 of solution B........	0.0799	0.0795	0.0832	0.0811
	0.0800	0.0796	0.0832	0.0805
Blanks[1]................	..	..	0.0001	0.0024

The proposed method gives results 0.1 per cent. too high when 3.5 per cent. of sulphur is in solution and 0.3 per cent. too high when 8 per cent. of sulphur is present. This occlusion of salts evidently compensates for an error which would cause the result to be low, as can be seen from Table 1 and the experimental data.

Lunge and Stierling[2] found that in the precipitations of sulphuric acid in aqueous solution and in the presence of salts, the quick addition of the barium chloride solution gave erroneous data in aqueous solution and correct results in the presence of salts while the slow addition of the precipitant gave just the opposite.

The proposed method employs about 7.6 grams of salt (calculated as chlorides) and the peroxide method 15 grams or more.

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[1] Corrected for in values given.

[2] See also Allen and Johnston, THIS JOURNAL, 32, 617, where they state, "A very good uncorrected determination may be obtained by precipitating rapidly but it is due to partial compensation of variable errors, and is not in any case so reliable as one which is made by slow precipitation and corrected as described." This agrees with Lunge and Stierling's conclusion on page 350 of their article, 6th Intern. Congress Applied Chem., 1906, p. 347.

Preliminary Experiments.

The preliminary experimental work leading up to this method consisted in testing the various reagents which have been proposed in methods for the determination of sulphur, varying the temperature, reagents, and time and manner of treatment. The 22 experiments made involved 160 single determinations exclusive of those that were lost on account of faulty manipulation, or seemed valueless from the nature of the reaction.

Determinations were made by using mixtures of reagents composed of from 1.25–3 grams of sodium hydroxide, 5–15 cc. of water, 0.5–1 gram of sodium carbonate, 1–2 grams of sodium nitrate, 1–3 grams of magnesium oxide, and from 3–10 grams of crystallized magnesium nitrite.

The treatment of the material with the reagents varied from drying on the steam bath and burning to heating at from 80–230° for from one and a half to five hours before burning. By these means values were obtained which ranged, for instance, from 1.13–1.45 per cent. of sulphur on a sample which contained 1.56 per cent. by the peroxide method, and from 3.91–4.50 per cent. on a rubber containing 4.17 per cent. by the peroxide method. Of 75 determinations, each the average of two duplicates, the peroxide value was the highest except in three instances. When the preliminary heating was continued for from two and a half to five hours, rising above 175° for at least thirty minutes of that time, it was noticed that the values usually approached the peroxide value by 0.1 per cent.

These preliminary determinations all indicate that adding reagents in solution, drying, and then ashing causes a loss of sulphur and that the substance must receive a definite preliminary heating before the sulphur can be absorbed by the alkaline reagent. This was noted after repeated experiments had been made with modifications of Eschka's method and it seemed probable that when the alkaline reagent was added in solution, the mixture dried down, and a coating consisting of an alkaline oxidizing medium was formed, all of the sulphur would be absorbed. This idea was substantiated by subsequent experiments.

Potassium salts were not tried in any of the experiments as Lunge and Stierling[1] have found that potassium chloride dissolves twice as much

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[1] The determination of sulphuric acid by means of barium chloride in the presence of interfering substances. Report of the Sixth International Congress of Applied Chemistry, 1906, p. 347. Allen and Johnston, THIS JOURNAL, 32, 606, state that the increased loss is due, not entirely to the slightly greater solubility of the barium sulphate in potassium chloride, but to the loss of "free sulphuric acid" from the precipitate on ignition or more exactly, the loss due to the volatilization of the "free sulphuric acid" is greater than that due to the solubility of the barium sulphate in the potassium chloride present. However, in their article in *J. Ind. Eng. Chem.*, **2**, 199 they state that in rapid precipitation "the quantity of free sulphuric acid is lowered and under certain conditions practically vanishes."

barium sulphate as sodium chloride does. The use of sodium carbonate was discontinued after the twelfth experiment as Barlow[1] has shown that as far as the absorption of sulphur is concerned soda can be omitted when magnesia is present.

The values obtained by these different methods rarely approached those by the peroxide method nearer than 0.1 per cent., which suggested that the latter may be too high. It was therefore decided to examine a new lot of samples by the peroxide method, fuse the barium sulphate obtained with soda, reprecipitate, and determine the sulphur by the proposed method. The results obtained are given in Table 1.

It appears that the first precipitation of the sulphuric acid by this method gives values very nearly approaching those obtained by fusing the barium sulphate obtained by the peroxide method and reprecipitating. The differences between the two sets of results thus obtained range from —0.04 + 0.11 per cent. (an average of 0.04 per cent.) as given in the last column of Table 1; these differences are within the experimental error.

THE INVERSION OF CANE SUGAR BY INVERTASE. V. THE DESTRUCTION OF INVERTASE BY ACIDS, ALKALIS, AND HOT WATER.

BY C. S. HUDSON AND H. S. PAINE.

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Measurements of the Rate of Destruction.—In a previous publication[2] it was shown that invertase is destroyed by both acids and alkalis. At the temperature of 30° the destructive action became noticeable first at the acid concentration of 0.01 normal and rapidly increased with the acid strength, becoming almost instantaneous at 0.05 normal; the alkaline destruction began a little below 0.01 normal and became almost instantaneous at 0.045 normal. It is to be supposed that at lower temperatures these rates will all be smaller and that at such temperatures a stronger acidity or alkalinity will be required for a noticeable destructive action. On the other hand, at higher temperatures the rates of destruction will doubtless be greater and the destructive action will be noticeable for weaker concentrations of acidity and alkalinity. At a sufficiently high temperature the acid and alkaline ions of water itself will doubtless cause a noticeable destructive action of pure water on invertase. It has long been known that hot water destroys invertase and other enzymes; as these views appear to correlate this destruction by hot water with the destruction by acids and alkalis at low temperatures, measurements were made for the purpose of tracing the destructive

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[1] Losses of sulphur on charring, THIS JOURNAL, 26, 354 (1904).

[2] THIS JOURNAL, 32, 774 (1910).