[CONTRIBUTION FROM THE CHEMICAL LABORATORY AND THE BOTANICAL LABORATORY OF THE UNIVERSITY OF MICHIGAN.]

THE IODOMETRIC DETERMINATION OF AMINO NITROGEN IN ORGANIC SUBSTANCES.

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It has been shown by Artmann and Skrabal[1] and independently by E. Rupp and E. Rossler,[2] that ammonia can be accurately determined by oxidation to nitrogen in alkaline solution by means of standard hypobromite solution, the excess of the latter being determined by adding potassium iodide and acid and titrating the liberated iodine with thiosulfate. Since this method is more rapid and convenient than the usual distillation with alkali, it seemed probable that it could be advantageously applied in a modification of the Kjeldahl method, thereby shortening the usual procedure and obviating the necessity of a distillation apparatus.

Since the material must first be decomposed by digestion with hot conc. sulfuric acid until the carbon disappears, attention was directed towards shortening this part of the process. Hot conc. perchloric acid (60 to 70%) either alone or in the presence of sulfuric acid, oxidizes organic matter of all sorts very rapidly. An attempt was made, therefore, to substitute this for sulfuric acid, but although it leaves nothing to be desired as regards the destruction of organic matter, it also causes oxidation of the ammonium salt, thereby giving low results. The organic matter is not always completely destroyed when the solution is colorless; in this case it is oxidized by the hypobromite, giving high results. This is well shown in the following table.

It is obvious that it is practically impossible to avoid loss of nitrogen by this method if the organic matter is completely oxidized. Only by adding the perchloric acid drop by drop, cooling before each addition, and heating afterwards, is it possible to avoid an excess, with consequent oxidation of ammonia. This procedure is too tedious to be of any value.

Conc. hydrogen peroxide (20 to 40%) was tried, but this, too, evidently caused oxidation of ammonia, since the results were always low.

Potassium persulfate was suggested by Scott and Myers[3] in the determination of nitrogen in urine. This was found to give excellent results. However, it is absolutely necessary that it be added *dry* to the cool, *concentrated* acid solution in which the organic matter has been charred, since if any water is present, the results are always low, some ammonia apparently being oxidized. Under these conditions a considerable excess of oxidizing agent does no harm, although from 5 to 10 times the weight

[1] Artmann and Skrabal, *Z. anal. Chem.*, **45**, 5 (1907).

[2] Rupp and Rossler, *Arch. Pharm.*, **243**, 104 (1905).

[3] Scott and Myers, THIS JOURNAL, **39**, 1044 (1917).

of organic matter is usually sufficient, The solution quickly becomes colorless and the excess is decomposed by heating, with the evolution of oxygen and ozone. Most of the persulfate on the market contains ammonium salts which are very difficult to remove.

TABLE I.—OXIDATION WITH PERCHLORIC ACID.

Expt.	Material.	H_2SO_4 Cc.	$HClO_4$ Cc.	Time of boiling. (Minutes.)	N found. G.	N present. G.	Error in % total N.
1......	0.2 g. sugar + 0.0677 g. $(NH_4)_2SO_4$	..	15	till colorless	0.0156	0.0144	+8.5
2......	0.2 g. sugar + 0.0904 g. $(NH_4)_2SO_4$	..	15	till colorless	0.0206	0.0192	+7.3
3......	0.2 g. sugar + 0.0712 g. $(NH_4)_2SO_4$	..	15	5	0.0144	0.0151	—4.6
4......	0.2 g. sugar + 0.0444 g. $(NH_4)_2SO_4$	..	15	5	0.00927	0.00942	—1.6
5......	0.0775 g. $(NH_4)_2SO_4$	15	..	30	0.0164	0.0164	0.0
6......	0.0596 g. $(NH_4)_2SO_4$	10	5	30	0.00564	0.01264	—55.4
7......	0.2 g. sugar + 0.0572 g. NH_4ClO_4	..	15	till colorless	0.00832	0.00682	+22.0
8......	0.0746 g. NH_4ClO_4	..	20	30	0.00666	0.00889	—25.1
9......	0.4366 g. NH_4ClO_4	10	10	45	0.0324	0.0521	—37.8
10......	0.4684 g. NH_4ClO_4	..	25	120	0.0528	0.0558	—5.4
11......	0.1302 g. acetanilid	..	15	till colorless	0.0138	0.0129	+7.6
12......	0.1138 g. acetanilid	..	15	till colorless	0.0119	0.0113	+5.6
13......	0.1568 g. acetanilid	..	25	till colorless	0.0166	0.0155	+7.1
14......	0.1518 g. acetanilid	..	25	till colorless	0.0163	0.0150	+8.7
15......	0.1598 g. acetanilid	..	15	30	0.0157	0.0158	—0.9
16......	0.2098 g. acetanilid	..	15	30	0.0199	0.0208	—4.0
17......	0.1768 g. acetanilid	10	10	20	0.0100	0.0175	—42.7
18[a].....	0.2206 g. acetanilid	15	10 drops	5	0.0214	0.0218	—2.2
19[b].....	0.1754 g. acetanilid	15	$KClO_4$	5	0.0162	0.0174	—6.9
20[b].....	0.1670 g. acetanilid	15	$KClO_4$	5	0.0166	0.0165	+0.6

[a] Charred with H_2SO_4, then $HClO_4$ added and boiled for 5 minutes.

[b] Charred with H_2SO_4, then $KClO_4$ added and boiled for 5 minutes.

Preparation of the Standard Hypobromite Solution.

A convenient concentration is 0.6 *N*, of which one cc. corresponds to 2.8 mg. of nitrogen. To a cold solution of 30 g. of sodium hydroxide in 800 cc. water, add slowly, with constant stirring, 50 g. of bromine and keep the solution well cooled. This solution should be kept in the dark, and the lower the temperature the slower is its rate of change to bromate and bromide, some oxygen being liberated. At 0° to 5° the change in one week was negligible, but at room temperature it was necessary to standardize the solution each day. The following table shows the rate of change of two different solutions.

TABLE II.—STABILITY OF AN ALKALINE SOLUTION OF SODIUM HYPOBROMITE.

Date.	Temp. ° C.	Cc. of 0.1 N thiosulfate equivalent to 10 cc. of solution.
Aug. 19	—3	74.39
Aug. 24	—1	73.58[a]
Aug. 25	—1	73.58
Aug. 28	—1	73.59
Aug. 29	+9	73.39
Aug. 30	0	72.99
July 27	22	66.15
July 29	22	64.87
July 30		
7 P. M.	22	63.92
7 A. M.	22	63.82
Aug. 13	22	57.34
Aug. 16	22	56.14[b]

[a] During this interval the temperature rose to 10° for a day.

[b] 22 cc. of this amount corresponded to bromate present.

Details of the Method.

Weigh out into a short-necked 300-cc. Kjeldahl flask an amount of the sample corresponding to about 10 or 15 mg. of nitrogen. Add 15 cc. of conc. sulfuric acid and heat until the organic matter is completely charred and the rapid evolution of sulfur dioxide has ceased. This usually requires from 5 to 15 minutes. Cool, and to the dark solution add through a thistle tube solid potassium persulfate free from ammonium salts, equal to 10 times the weight of the sample. This method of adding the persulfate is used to prevent any of the latter from sticking to the side of the flask. For the reason mentioned above, it could not be washed in with water. Warm very gently with constant swirling until the solution is colorless. This usually requires about one minute. The heating should take place so slowly that there is no evolution of bubbles of oxygen while the solution is still dark colored. Sometimes it is necessary to make more than one addition of potassium persulfate to oxidize all the carbon, and occasionally material is found that does not require as much as was recommended. After the carbon is completely oxidized, heat the acid to gentle boiling for five minutes to destroy the excess of persulfate, which decomposes with the evolution of oxygen and ozone. Cool, add 70 cc. of water and boil to remove any sulfur dioxide formed during the previous heating of the acid. *Cool,* add a drop or two of aqueous methyl orange or methyl red solution and neutralize with a solution of sodium hydroxide (about 10 N), then add 2 drops more. Cool under running water frequently during this process to avoid loss of ammonia.

The ammonia is now ready for titration. Add 10 cc. of 0.6 N hypobromite solution, sufficient so that not over $^2/_3$ of it is reduced by the ammonia, and let stand for 5 minutes. Evolution of nitrogen takes place thus:

$$2NH_3 + 3NaBrO = 3NaBr + N_2 + 3H_2O.$$

Add 5 g. of potassium iodide and 6 cc. of hydrochloric acid (sp. gr. 1.19). Titrate the liberated iodine with 0.2 *N* sodium thiosulfate solution, using starch as indicator.

The hypobromite is conveniently standardized against pure ammonium sulfate, or against standard thiosulfate after the addition of iodide and acid, as above.

If iron is present, the liberation of iodine by the ferric salt may be prevented by the addition of phosphoric acid, one cc. (sp. gr. 1.7) being sufficient for several mg. of iron.

TABLE III.

Expt.	Substance.	Weight Sample. G.	N found. %.	N present. %.
1.	α-Alanine	0.0816	15.83	15.73
2.	"	0.1332	15.72	15.73
3.	Acetanilid A	0.2493	10.21	10.22
4.	"	0.1394	10.22	10.22
5.	0.2 g. sugar + $(NH_4)_2SO_4$	0.0790	21.10	21.20
6.	"	0.0786	21.05	21.20
7.	"	0.0585	21.06	21.20
8.	"	0.0642	21.32	21.20
9.	$(NH_4)_2SO_4$ alone	0.0552	21.09	21.20
10.	Benzidine	0.1040	15.16	15.20
11.	"	0.1510	15.21	15.20
12.	Acetanilid B	0.1423	10.42	10.37
13.	"	0.1723	10.31	10.37
14.	"	0.2206	10.44	10.37
15.	Acetanilid C	0.3086	9.95	9.90
16.	"	0.1268	9.90	9.90
17.	Michler's ketone	0.2154	10.12	10.13[a]
18.	Gelatin A	0.1000	16.95	16.94
19.	"	0.0932	16.88	16.94
20.	"	0.1328	16.93	16.94
21.	Gelatin B	0.1110	16.70	16.71
22.	Peptone	0.2804	13.01	13.00
23.	"	0.2984	13.00	13.00
24.	"	0.1888	13.02	13.00
25.	"	0.1888	13.06	13.00
26.	Wheat Flour	0.3076	2.15	2.11
27.	"	0.3118	2.05	2.11
28.	"	0.3642	2.18	2.11
29.	"	0.4608	2.16	2.11
30.	Sawdust	0.2192	1.53	1.53
31.	"	0.3164	1.56	1.53
32.	"	0.2076	1.47	1.53
33.	"	0.2378	1.47	1.53
34.	Egg Albumen	0.1474	12.82	12.70
35.	"	0.1728	12.72	12.70
36.	"	0.2859	12.61	12.70
37.	"	0.2580	12.64	12.70

[a] Combustion method.

Precautions.

Potassium persulfate often contains ammonium salts and retains traces even after 3 recrystallizations. In such a case it is necessary to run a blank and apply a correction for the amount used. In running this blank sugar free from nitrogen must be added to reduce most of the persulfate; otherwise some of the ammonium will be oxidized.

It is very essential that there be no water present during the oxidation of the organic matter by the persulfate, since in the presence of water some of the ammonia is oxidized.

A very large excess of persulfate gives low results.

A number of organic substances were analyzed by this method, the results being shown in the preceding table. Some of these substances which were not absolutely pure or of uncertain composition, were analyzed by the official Kjeldahl method (Expts. 12 to 37), and the results are compared. Ten cc. of approximately 0.6 N hypobromite solution was added in nearly every case. The thiosulfate was standardized against pure potassium dichromate.

Some preliminary experiments indicate that this method can be used for the determination of nitrogen in coal, but this work has not been completed.

Summary.

1. The Kjeldahl method of determining nitrogen can be made more rapid by adding potassium persulfate to the charred solution of organic matter in conc. sulfuric acid, complete oxidation occurring very quickly.

2. The ammonium salt formed may be accurately and quickly determined by adding to the neutralized solution an excess of standard hypobromite, and determining the excess by adding iodide and acid, and titrating the liberated iodine with thiosulfate.

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OXIDATION OF THE GRIGNARD REAGENT.

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If the general reactions of organic chemistry were named in the order of their importance from the standpoint of synthetic work the Grignard reaction would be one of the first on the list. Although only 20 years have passed since Grignard's discovery was announced there have appeared more than 500 papers devoted to applications of this reaction.

The formation of phenols through the oxidation of aryl magnesium halides mentioned by Bodroux[1] and Wuyts[2] seemed to us worthy of fur-

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[1] Bodroux, *Bull. soc. chim.*, [3] 31, 33 (1904).

[2] Wuyts, *Compt. rend.*, 148, 930 (1909).