A Rapid Determination of Nitrogen in Organic Compounds by the Iodic Acid Decomposition Method

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The applications of the characteristic behavior of iodic acid in strong phosphoric acid to chemical analysis have been investigated, and the volumetric methods of determining elementary carbon[1] such as charcoal, graphite etc. and determining oxidation values[2] of organic compounds have been already reported by the author and his coworkers. In the course of these studies it was found that nitrogen contained in organic substances can be easily determined by the iodic acid-strong phosphoric acid reagent. If an organic compound containing nitrogen is heated with iodic acid in strong phosphoric acid, the compound is decomposed and the nitrogen in it is liberated. Consequently, after the decomposition mentioned above, sending this nitrogen with carbon dioxide into an azotometer, which is filled with potassium hydroxide solution, the nitrogen can be determined gas-volumetrically. This method is, so to speak, the wet Dumas method; namely, the combustion tube in the case of the Dumas method is replaced by the special reaction vessel in which samples are decomposed.

Apparatus.—The apparatus necessary in this method is shown in Fig. 1. It is mainly composed of three parts, reaction vessel A, azotometer B, and carbon dioxide generator C. All of these are hand-made in addition to a Kipp's apparatus for production of carbon dioxide.

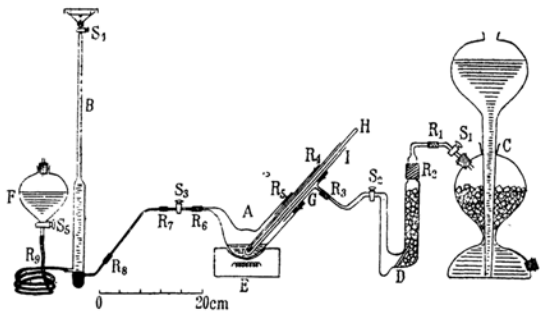

Fig. 1. Apparatus.

A: Reaction vessel.
B: Azotometer.
C: CO_2 generator.
D: $CaCO_3$-filled guard tube.
E: Electric heater.
F: Leveling vessel.
G: Gas-introducing tube.
H: Thermometer.
I: Thermometer-protecting tube.
R_1-R_9: Rubber tube.
S_1-S_5: Stopcock.

Into the reaction vessel a gas-introducing tube G and a thermometer H, which is covered with a thermometer-protecting tube I, are inserted. These tubes are attached to the reaction vessel with suitable rubber tubes as shown in Fig. 1.

The measuring-tube of the azotometer is made of a commercial buret, which is graduated at intervals of 0.02 ml. and has a graduated capacity of 5 ml. It is connected at the bottom by means of a rubber tube with a leveling vessel F.

Between the reaction vessel and the Kipp's apparatus there is a calcium carbonate-filled guard tube D for catching particles of hydrochloric acid.

The reaction vessel is heated with an electric heater, the temperature of which is regulated with a transformer.

Reagents. — *Strong phosphoric acid.* Strong phosphoric acid prepared by heating and evaporating commercial extra pure grade orthophosphoric acid (d=1.7) until its temperature reached 300°C was used.

Potassium iodate.—Extra pure grade potassium iodate was used.

Carbon dioxide. This was produced from calcium carbonate (marble) and hydrochloric acid (1: 1) in a Kipp's apparatus. To obtain the carbon dioxide in the purest condition the operation of putting these substances into a Kipp's apparatus should be carried out in the same manner as that used in the case of the micro-Dumas method.

Potassium hydroxide. A 30% solution of potassium hydroxide was used.

Procedure.—Weigh accurately a 10 to 50 mg. sample, depending upon its nitrogen content, into a small weighing tube; choose the sample weight so that the volume of the nitrogen formed will be 1 to 5 ml. Previously heat gently in a reaction vessel a mixture of 3 to 5 ml. of strong phosphoric acid and two or three times as much of the theoretically required amount of potassium iodate until the added potassium iodate disperses into the acid homogeneously. After cooling the contents of vessel, put the weighing tube containing the sample into the vessel along the inside wall of it.

Insert a gas-introducing tube, a thermometer, and a thermometer-protecting tube into the reaction vessel, and connect the reaction vessel with a carbon dioxide generator and a guard tube. Connect a rubber tube R_7 with a suction pump, open the stopcock S_3, and suck up the air present

1) T. Kiba, S. Ohashi, T. Takagi and Y. Hirose, *Japan Analyst*, 2, 446 (1953).
2) S. Ohashi, This Bulletin, 28, 171 (1955).

between S_2 and S_3. Now, close the stopcock S_3 and open the stopcock S_2 to fill the container with carbon dioxide. Repeat these operations several times to make sure that no air remains in the container. Finally fill it with carbon dioxide. Next, connect the rubber tube R_7 with an azotometer and open the stopcock S_2, S_3 and S_4 to let carbon dioxide flow through it for two or three minutes. Close the stopcock S_3 and immediately raise the leveling vessel F to fill the azotometer with the potassium hydroxide solution. Then close the stopcock S_4.

Heat the liquid in the reaction vessel A on a small electric heater. During the decomposition reaction shake well the reaction vessel to decompose the sample completely, and keep the temperature of the reactants below 260°C, above which iodic acid is decomposed, liberating oxygen, and leading to a serious error. The end of the decomposition reaction can be easily recognized by the ceasing of the liberation of iodine. Remove the electric heater and transfer the gas in the reaction vessel into the azotometer by carefully opening the stopcock S_2. A part of the iodine liberated is sent into the azotometer and absorbed into the potassium hydroxide solution. Drive the gas in the vessel by letting the carbon dioxide flow, until the gas introduced into the azotometer becomes so-called micro-bubbles.

After standing fifteen minutes, raise the leveling vessel until the solution in it stands at exactly the same height as that in the azotometer. Read the volume of nitrogen, the thermometer, and the barometer.

The percentage of the nitrogen present is computed according to the following equation:

$$\log N\% = \log v + \log g + (1 - \log w).$$

In this equation, $N\%$ represents the percentage of the nitrogen, v the volume (ml.) of the nitrogen measured at t°C and p mm Hg, and g the weight (mg.) of 1 ml. of the nitrogen at t°C and p mm Hg, and w the weight (mg.) of the sample used for the analysis. The value of p mm Hg is obtained by subtracting the aqueous vapor pressure of the 30% potassium hydroxide solution at t°C from the atmospheric pressure measured by means of the barometer.

Results and Discussion

Analyses of various known organic compounds containing nitrogen were made with this method. These results are summarized in Table I.

TABLE I

DETERMINATION OF NITROGEN IN VARIOUS KNOWN ORGANIC COMPOUNDS

Substance	Nitrogen Content %	Sample Weight mg.	Nitrogen Content Found %	Nitrogen Content Deviation %
Ammonium chloride NH_4Cl	26.18	15.2	26.68	+0.50
		12.8	26.59	+0.41
		17.8	25.63	−0.55
		16.6	25.62	−0.56
		10.8	26.18	0.00
Ammonium oxalate $(COONH_4)_2 \cdot H_2O$	19.71	25.7	19.46	−0.25
		21.5	19.44	−0.27
		24.9	19.06	−0.62
		10.4	19.53	−0.18
		15.9	19.56	−.015
Acetanilide $C_6H_5NHCOCH_3$	10.36	34.3	10.34	−0.02
		27.6	10.17	−0.19
		19.5	10.40	+0.04
		19.7	10.36	0.00
		26.5	10.03	−0.33
Phthalimide $C_6H_4(CO)_2NH$	9.52	18.0	9.23	−0.29
		18.7	9.22	−0.30
Phenacetin $C_2H_5OC_6H_5NHCOCH_3$	7.82	26.1	7.74	−0.08
		44.6	7.76	−0.06
		39.6	7.79	−0.03
		34.4	7.74	−0.08
		32.8	7.91	+0.09
Thionalide $C_{10}H_7NHCOCH_2SH$	6.45	23.2	6.33	−0.12
		11.9	6.28	−0.18
		18.5	6.34	−0.11
		24.1	6.53	+0.08

TABLE I (Contd.)

Substance	Nitrogen Content %	Sample Weight mg.	Nitrogen Content Found %	Nitrogen Content Deviation %
Urea	46.63	9.5	46.0$_5$	−0.5$_8$
NH_2CONH_2		9.2	47.2$_5$	+0.6$_2$
		10.6	46.5$_4$	−0.0$_9$
		10.0	46.2$_7$	−0.3$_6$
		10.6	45.8$_7$	−0.7$_4$
Thiourea	36.81	13.4	36.4$_3$	−0.3$_5$
NH_2CSNH_2		23.3	36.2$_5$	−0.5$_5$
		10.9	36.2$_7$	−0.5$_4$
		14.8	36.8$_8$	+0.0$_7$
		12.3	36.0$_8$	−0.7$_3$
Methyl orange	12.84	16.5	12.7$_8$	−0.0$_5$
$(CH_3)_2NC_6H_4NNC_6H_4SO_3Na$		14.0	12.7$_2$	−0.1$_2$
		24.7	12.8$_5$	+0.0$_1$
		14.0	12.9$_3$	+0.0$_9$
		23.2	12.5$_5$	−0.2$_8$
Methyl red	15.61	23.8	15.9$_9$	+0.3$_8$
$(CH_3)_2NC_6H_4NNC_6H_4COOH$		17.8	15.5$_8$	−0.0$_3$
		17.8	15.5$_3$	−0.0$_8$
		15.4	15.8$_2$	+0.2$_1$
		21.0	15.6$_5$	+0.0$_4$
Dithizone	21.85	23.6	21.2$_3$	−0.6$_2$
$C_6H_5NHCSNNC_6H_5$		23.2	21.0$_2$	−0.8$_3$
		15.5	21.8$_8$	+0.0$_3$
		14.0	21.7$_8$	−0.0$_7$
		15.0	21.7$_7$	−0.0$_8$
DL-Tryptophane	13.72	15.5	13.1$_4$	−0.5$_8$
$C_8H_6N\cdot CH_2CH(NH_2)COOH$		23.8	13.5$_5$	−0.1$_5$
		10.6	13.3$_0$	−0.4$_2$
L-Histidine hydrochloride	20.05	17.4	19.8$_7$	−0.1$_8$
$C_3H_3N_2\cdot CH_2CH(NH_2HCl)COOH\cdot H_2O$		20.7	20.2$_7$	+0.2$_2$
		27.2	19.7$_5$	−0.3$_0$
		20.8	19.5$_9$	−0.4$_5$
1-Phenyl-3-methyl-	16.08	36.50	16.31	+0.23
pyrazolone-(5)		32.94	16.35	+0.27
$CH_3C_3H_2ON_2\cdot C_6H_5$		99.86	15.86	−0.22
		26.93	16.08	0.00
		33.36	16.39	+0.31
2-Aminobenzothiazole	18.65	9.82	18.39	−0.26
$C_7H_4NS\cdot NH_2$		16.76	18.58	−0.07
		22.19	18.24	−0.41
		21.31	18.17	−0.48
Nitron	17.94	19.0	18.1$_3$	+0.1$_9$
$C_{20}H_{16}N_4$		22.4	18.1$_7$	+0.2$_3$
		19.0	17.7$_9$	−0.1$_5$

Two ammonium salts were also analyzed. As pure samples as possible were selected or prepared and dried in the calcium chloride or sulfuric acid desiccator.

Not only the nitrogen in ammonium salts (ammonium chloride and ammonium oxalate) and amines (acetanilide, phthalimide, phenacetin, thionalide, urea, and thiourea), but also in azo-compounds (methyl orange, methyl red, and dithizone), a pyrrole (DL-tryptophane), pyrazols (L-histidine hydrochloride and 1-phenyl-3-methyl-pyrazolone-(5)), and a thiazole (2-aminobenzothiazole) were determined by means of this method. The nitrogen in the complicated compound such as nitron was also quantitatively determined. The precision and accuracy of the data are within ±3%.

Since it was assumed that the nitrogen in amino acids might be accurately estimated with this method, several amino acids (glycocoll, DL-α-alanine, DL-α-amino-n-valeric acid, L-leucine, DL-isoleucine, L-cystine, and arginine hydrochloride) were analyzed. Analyses for these compounds gave satisfactory results as shown in Table II.

For the compounds containing the already oxidized-form nitrogen such as nitro- or nitroso- radicals this method can not be used.

Uric acid and theobromine, which are derivatives of purine, atropine and lacto-flavine, which have complicated rings containing nitrogen, and antipyrine gave very low results for nitrogen with this method. The reason why this method can be applied to 1-phenyl-3-methyl-pyrazolone-(5), but not to antipirine, although these compounds have similar pyrazol rings, has not yet been determined.

TABLE II

DETERMINATION OF NITROGEN IN SOME AMINO ACIDS

Substance	Nitrogen Content %	Sample Weight mg.	Nitrogen Content Found %	Nitrogen Content Deviation %
Glycocoll NH_2CH_2COOH	18.66	15.3	18.8_5	$+0.1_9$
		24.0	18.1_4	-0.4_2
		21.9	18.3_9	-0.2_7
		19.1	18.8_5	$+0.2_0$
		25.6	18.3_8	-0.2_8
DL-α-Alanine $CH_3CH(NH_2)COOH$	15.76	25.5	15.6_3	-0.1_3
		22.1	15.9_0	$+0.1_4$
		18.1	15.5_6	-0.2_0
		26.2	15.7_6	0.0_0
		25.6	15.3_6	-0.4_0
DL-α-Amino-n-valeric acid $CH_3CH_2CH_2CH(NH_2)COOH$	11.96	30.9	11.6_8	-0.2_8
		20.3	11.8_1	-0.1_5
		18.6	11.5_4	-0.4_2
		35.6	11.7_6	-0.2_0
		16.3	12.0_4	$+0.0_8$
L-Leucine $(CH_3)_2CHCH_2CH(NH_2)COOH$	10.68	21.1	10.6_0	-0.0_8
		18.3	10.5_9	-0.0_9
		21.2	10.9_9	$+0.3_1$
		13.3	10.6_7	-0.0_1
		31.1	10.5_3	-0.1_5
DL-Isoleucine $CH_3CH_2CH(CH_3)CH(NH_2)COOH$	10.68	23.1	10.9_0	$+0.2_2$
		18.3	10.9_7	$+0.2_9$
		19.2	10.6_5	-0.0_3
		15.7	10.2_8	-0.4_0
L-Cystine $(-SCH_2CH(NH_2)COOH)_2$	11.66	20.9	11.3_7	-0.2_9
		20.9	11.4_7	-0.1_9
		28.1	11.6_3	-0.0_3
Arginine hydrochloride $NH_2C(NH)NHCH_2CH_2CH_2$-$CH(NH_2HCl)COOH$	26.60	14.3	26.4_1	-0.1_9
		14.4	26.8_5	$+0.2_5$
		12.3	25.8_9	-0.7_1
		13.7	26.6_9	$+0.0_9$
		18.5	25.8_0	-0.8_0

As has been indicated above this iodic acid decomposition method can be applied to many species of organic compounds containing nitrogen, but not to all species.

The compounds having relatively low melting points such as diphenylamine, p-dimethylaminobenzaldehyde, and azobenzene, the melting points of which are 54, 74, and 68°C, respectively, can not be analyzed with this method, because these compounds are partially volatilized during the decomposition reaction.

It was shown by means of experiments for nicotinic acid and oxine that the nitrogen in pyridine and quinoline ring can not be liberated with this reagent at all. Since the nitrogen in amino-form can be determined with this method as mentioned above, it was presumed that for the two nitrogens of nicotinic amide only the nitrogen in amino-form

TABLE III
DETERMINATION OF NITROGEN IN NICOTINIC AMIDE

Substance	Half the Nitrogen Content %	Sample Weight mg.	Nitrogen Content Found %	Nitrogen Content Deviation %
Nicotinic amide $C_5H_4N \cdot CONH_2$	11.57	14.8	11.9_3	$+0.3_6$
		21.2	11.6_9	$+0.1_2$
		19.5	11.6_8	$+0.1_1$
		13.8	11.9_4	$+0.3_7$

might be measured. As might have been expected half the total nitrogen could be determined as indicated in Table III.

The advantages of this method are as follows: (1) Since the iodic acid decomposition of organic compounds in strong phosphoric acid is a wet reaction, the velocity of the decomposition in this method is much more rapid than in the micro-Dumas method. The time required for one analysis is about thirty to forty minutes. If this method is modified from semi-micro to micro method in the future, the analysis time will be more shortened. (2) The progress of the decomposition reaction can be watched through the glass and the end of its reaction can be easily recognized. Namely, it is indicated by means of the ceasing of the bubble formation and clearing of the liquid. (3) The apparatus is very cheap and all of it can be made by oneself.

The defects of this method are the following: (1) Similarly to the case of the Kjeldahl method, not all types of compounds containing nitrogen can be analyzed with this method as mentioned above. (2) The precision and accuracy of the data in this method are slightly worse than the micro-Dumas or Kjeldahl method. This is probably owing to the imperfection of the apparatus. The improvements of these defects for this method and the modification of this method to the micro scale are the remaining problems for the future.

Summary

A new method has been developed to permit the rapid determination of nitrogen in organic compounds. The sample is decomposed with iodic acid-strong phosphoric acid reagent in a glass reaction vessel, and the liberated nitrogen is collected in an azotometer filled with potassium hydroxide solution by the flow of pure carbon dioxide. From the volume of the nitrogen thus collected the percentage of the nitrogen in the sample can be readily determined. This method is, so to speak, the wet modification of the Dumas method.

For various types of compounds containing nitrogen, ammonium salts, amine, azo-compound, pyrrol, thiazole, etc., satisfactory results were obtained by means of this method. The nitrogen in a 10 to 50 mg. sample can be determined for thirty to forty minutes with a simple and inexpensive apparatus.

But volatile compounds, nitro- or nitroso-compounds, compounds having purine, pyridine, quinoline, and other complicated rings, etc., can not give good results.

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