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A Theoretical Consideration on Chemical Reactions in the Kjeldahl Digestion

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The decomposition of organic nitrogen compounds by Kjeldahl digestion was discussed and some experiments were carried out. The relationship between the amount of sulfuric acid consumed and the amount of ammonia produced at various stages including final ones of the decomposition was examined. As a result, chemical reaction of decomposition was evaluated, and the temperature at the initial stage reveals one of the important factors which determine the ratio of oxidative action of hot concentrated sulfuric acid to reductive action of sulfur dioxide produced in the reaction system. It is concluded that, if the temperature of initial stage is adjusted properly, it is possible to decompose mostly nitro and azo compounds into ammonia by sulfuric acid containing potassium sulfate without reductive pretreatment which has previously been believed to be indispensable.

Since publication of the Kjeldahl method for nitrogen analysis,[1] a number of studies have been carried out on its applications and improvements, and this method, together with the Dumas', is extensively used in various fields for the determination of nitrogen. As reported previously,[2] however, many problems must be solved before this method is applicable to all types of nitrogen compounds. This statement is supported, for example, by a series of many years' studies by Bradstreet.[3]

As is known, the most important and fundamental of the Kjeldhal method is the digestion of nitrogen compounds with hot concentrated sulfuric acid, and a detailed study of this step is necessary for its application and its improvement. The decomposition reaction is extremely complicated and only very few studies concerning kinetics by Bredig *et al.*[4] and Schwab *et al.*[5] have been made. These studies gave some light on the rate of decomposition, but not on the applicability of this method.

This paper deals, experimentally as well as theoretically, with the thermal decomposition in concentrated sulfuric acid and the applicability of the Kjeldahl method.

Theoretical and Experimental

Thermal decomposition of organic compounds without nitrogen in concentrated sulfuric acid can be expressed by Eq. (1) as known previously;

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[1] J. Kjeldahl, Z. *anal. Chem.*, **22**, 336 (1883).

[2] Y. Morita and Y. Kogure, *Nippon Kagaku Zasshi* (*J. Chem. Soc. Japan, Pure Chem. Sect.*), **88**, 1179 (1967).

[3] R. B. Bradstreet, *Ind. Eng. Chem., Anal. Ed.*, **10**, 696 (1938); *ibid.*, **12**, 657 (1940); *Chem. Revs.*, **27**, 331 (1940); *Anal. Chem.*, **21**, 1012 (1949); *ibid.*, **26**, 185 (1954); *ibid.*, **26**, 235 (1954); *ibid.*, **29**, 944 (1959); *ibid.*, **32**, 114 (1960).

[4] C. Bredig and J. W. Brown, Z. *physik. Chem.*, **46**, 502 (1903).

[5] G. M. Schwab and E. Schwab-Agallidis, *J. Am. Chem. Soc.*, **73**, 803 (1951); *Angew. Chem.*, **65**, 418 (1953); *Mh. Chem.*, **86**, 341 (1955); *ibid.*, **88**, 288 (1957).

$$C_nH_mO_l + (2n+m/2-l)\cdot H_2SO_4 \rightarrow n\cdot CO_2 + (2n+m/2-l)\cdot SO_2 + (2n+m-l)\cdot H_2O \quad (1)$$

where n, m, and l denote the numbers of atoms C, H, and O, respectively.

On the other hand, oxidative decomposition of various organic nitrogen compounds in a chromic acid-sulfuric acid mixture can be expressed by Eq. (2). This is derived from Eq. (1) by taking into account the nitrogen contained and the type of linkage of nitrogen shown in the studies of Jurecek *et al.*[6] as well as the present author[7];

$$C_nH_mO_lN_{d=a+b+c} + (2n+m/2-l-1.5a+2.5b)\cdot O \rightarrow n\cdot CO_2 + (m/2-3a-b)\cdot H_2O + a\cdot NH_3 + b\cdot HNO_3 + c/2\cdot N_2 \quad (2)$$

where n, m, l, and $d(=a+b+c)$ denote the numbers of atoms C, H, O, and N, respectively, and a, b, and c denote respectively the numbers of nitrogen atoms present in the functional groups are shown in Table 1.

In such oxidative decomposition, the decomposition products are ammonia, nitric acid, and nitrogen molecule or a mixture whose proportion depends upon the nitrogen functional groups, and the consumption of oxigen varies with these groups.[6-7]

It is desirable in the Kjeldhal digestion to convert all the nitrogen atoms into ammonia and not to react as shown in Eq. (2).

TABLE 1. VARIOUS FUNCTIONAL GROUPS CONTAINING NITROGEN INVOLVED IN OXIDATIVE DECOMPOSITION PRODUCTS BY THE CHROMIC ACID-SULFURIC ACID MIXTURE

Functional group		Decomposition product	
$-CONH_2$	(Carbamoyl group)	a	NH_3
$-OCN$	(Cyanato group)		
$-NCO$	(Isocyanato group)		
$-SCN$	(Thiocyanato group)		
$-NCS$	(Isothiocyanato group)		
$-CN$	(Cyano group)		
$-NC$	(Isocyano group)		
$-NH_2$	(Amino group)		
$=NH$	(Imino group)		
$=N-$	(Nitrogen heteroatom without N-N linkage)		
$-NO_2$	(Nitro group)	b	NHO_3
$-NOOH$	(Isonitro group)		
$-NHOH$	(Hydroxyamino group)		
$=NOH$	(Hydroxyimino group)		
$-NHNH_2$	(Hydrazino group)	c	N_2
$-N=N-$	(Azo group)		
$=N-N=$	(Azino group)		
$-\overset{+}{N}\equiv N$	(Diazonium group)		

But the actual decomposition reaction is mainly oxidation accompanied by reduction, dehydration, hydrolysis, substitution, and other complicated reaction, and can not be simply expressed by Eq. (2).

For example, the nitro group of 3-nitrosalicylic acid is quantitatively converted into nitric acid when oxidized with a chromic acid-sulfuric acid mixture,[7] but yields a calculated amount of ammonia when decomposed with hot concentrated sulfuric acid.[2] Thus, the Kjeldahl digestion does not proceed in accordance with Eq. (2).

Now, if the Kjeldahl digestion is the following oxidation-reduction reaction,

$$C_n + 2n\cdot H_2SO_4 \rightarrow n\cdot CO_2 + 2n\cdot SO_2 + 2n\cdot H_2O$$

$$H_m + m/2\cdot H_2SO_4 \rightarrow m/2\cdot SO_2 + m\cdot H_2O$$

$$O_l + l\cdot SO_2 + l\cdot H_2O \rightarrow l\cdot H_2SO_4$$

$$N_{d=a+b+c} + 1.5d\cdot SO_2 + 3d\cdot H_2O \rightarrow d\cdot H_2SO_4 + d/2\cdot (NH_4)_2SO_4$$

the sum of these reactions can be written as follows.

$$C_nH_mO_lN_d + (2n+m/2-l-d)H_2SO_4 \rightarrow n\cdot CO_2 + (2n+m/2-l-1.5\text{d})\cdot SO_2 + (2n+m-l-3d)\cdot H_2O + d/2\cdot (NH_4)_2SO_4 \quad (3)$$

If the decomposition of nitrogen compounds proceeds ideally according to Eq. (3), Eq. (4) can be obtained.

$$\frac{x}{w} = \frac{(2n+m/2-l-d)\cdot H_2SO_4}{C_nH_mO_lN_d}(=K_1) \quad (4)$$

x = amount of sulfuric acid consumed.

w = weight of sample.

K_1 = a proportional constant characteristic of a given sample.

As the Kjeldahl method does not fit the determination of nitrogen in all nitrogen compounds, it is obvious that Eqs. (3) and (4) have not universal validity.

In order to study this subject more closely, Eq. (4) must be replaced by Eq. (5) which is applicable to most cases.

$$\frac{x}{w} = K_1\alpha = K_1' \quad (5)$$

α = a factor which depends upon the mode of decomposition.

The value of K_1' is experimentally given and α can be calculated from Eq. (4).

When $\alpha = 1$, Eq. (5) becomes identical with Eq. (4), but when $\alpha > 1$, it does not so and in this case

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[6] M. Jureček, *Mikrochim. Acta*, **1962**, 926; *ibid.*, **1962**, 1101; *ibid.*, **1963**, 643; V. Novák, P. Kozák, P. Matousek and M. Jureček, *Collect. Czechoslov. Chem. Commun.*, **28**, 487 (1963); *ibid.*, **28**, 3443 (1963).

[7] Y. Morita and Y. Kogure, *Nippon Kagaku Zasshi* (*J. Chem. Soc. Japan, Pure Chem. Sect.*), **86**, 87 (1965); *ibid.*, **86**, 508 (1965); *ibid.*, **86**, 601 (1965).

nitric acid or nitrogen molecule is produced by the decomposition. Some examples are now given below.

Two gramms of concentrated sulfuric acid is put into a micro Kjeldahl flask containing sample, and heated by 100 W electric heater with a variable resistor. When the solution in the flask becomes light brown, 0.5 g of potassium sulfate is added and the mixture is heated until it turns clear and colorless. In this way the decomposition is completed.

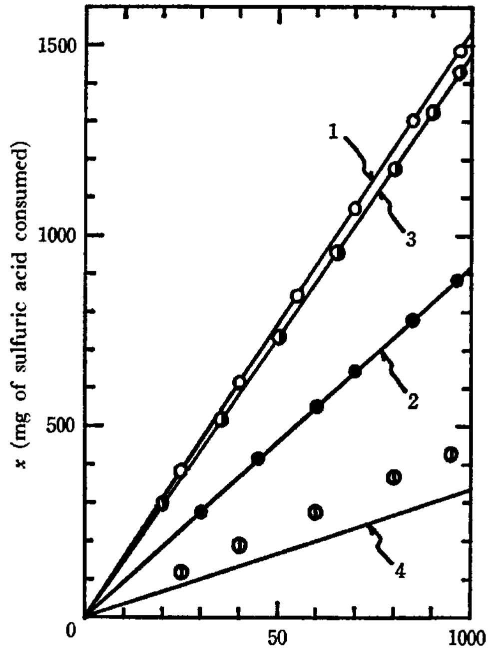


Fig. 1. Amount of sulfuric acid consumed *vs.* weight of sample in Kjeldahl digestion.

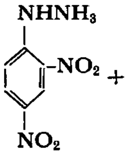
○, ●, ◑, ⦶: Observed ——: Calculated
○, 1: Aniline ●, 2: Nitrobenzene
◑, 3: Azobenzene ⦶, 4: 2,4-DNPH

The mixture is then diluted with water, boiled to drive off SO_2 and the remaining sulfuric acid is titrated. The blank test is made and the actual amount of sulfuric acid consumed is obtained by subtracting the blank value from the titrated one. In Fig. 1, mg of sulfuric acid consumed (x) is plotted against mg of sample (w).

As shown in Fig. 1, Eq. (4) is not applicable to 2,4-dinitro-phenylhydrazine (2,4-DNPH) alone. Consequently, the decomposition of this compound can not be expressed by Eq. (3), but corresponds to the case where $\alpha > 1$ in Eq. (5). Four possible decomposition reactions of 2,4-DNPH are shown below, and the values of α calculated for each reaction are shown in Table 2.

Table 2. Calculated values of α in decomposition of 2,4-DNPH and observed ones

Mode of reaction	I	II	III	IV	Observed
α	1.00	1.29	2.00	2.29	1.29



$$7\cdot H_2SO_4 \rightarrow 6\cdot CO_2 + 5\cdot SO_2 + 2\cdot H_2O + 2(NH_4)_2SO_4 \qquad (I)$$

$$9\cdot H_2SO_4 \rightarrow 6\cdot CO_2 + 8\cdot SO_2 + 8\cdot H_2O + (NH_4)_2SO_4 + N_2 \qquad (II)$$

$$14\cdot H_2SO_4 \rightarrow 6\cdot CO_2 + 13\cdot SO_2 + 12\cdot H_2O + (NH_4)_2SO_4 + 2\cdot HNO_3 \qquad (III)$$

$$16\cdot H_2SO_4 \rightarrow 6\cdot CO_2 + 16\cdot SO_2 + 18\cdot H_2O + 2\cdot HNO + N_2 \qquad (IV)$$

It appears from Table 2 that in the decomposition of 2,4-DNPH, (II) may occur and this assumption is supported by the measurements of ammonia described below.

In this way, it is possible to interpret the reaction at the completion of the decomposition in terms of the amount of sulfuric acid consumed.

Because of the complex nature of the decomposition reaction, it is necessary to elucidate the steps involved to clarifiy the overall decomposition. For this purpose, the relationship between the amount of sulfuric acid consumed (x_t) and that of ammonia produced (y_t) after *t*hr must be considered.

If the amount of sulfuric acid consumed in Eq. (3) and the amount of ammonia produced are assumed to be proportional at all time, the degree of decomposition is x_t/x which is equal to y_t/y. Therefore, the equation

$$y_t = \frac{y}{x} x_t = K_2 \cdot x_t \qquad (6)$$

must be valid.

Equation (6) shows that the ratio of the amount of sulfuric acid consumed (x) to the amount of ammonia produced (y) at the completion of the decomposition is constant for a given sample, and K_2 can be determined by measuring x_t and y_t at various stages of decomposition. A comparison of these observed values with those calculated ones on the basis of Eq. (3) will give some insight into the mechanism of the reaction. It is clear from this experiments that the decomposition which is obeyed Eq. (6) always includes the chemical reaction shown by Eq. (3). However, Eq. (6) can not be applicable to acid amides and nitriles which yield ammonia on acid hydrolysis.

Next, some example are shown below; the relationship between observed x_t and y_t at various stages of decomposition of typical organic nitrogen compounds is shown in Fig. 2. Conveniently, if y_t is plotted against x_t/x, $x_t/x = 1$ means that the decomposition is completed and the solution becomes colorless and clear, whereas, $x_t/x < 1$ means that the mixture is incompletely decomposed and the solution develops varying colors. The degree of decomposition can be controlled by adjusting the time of heating.

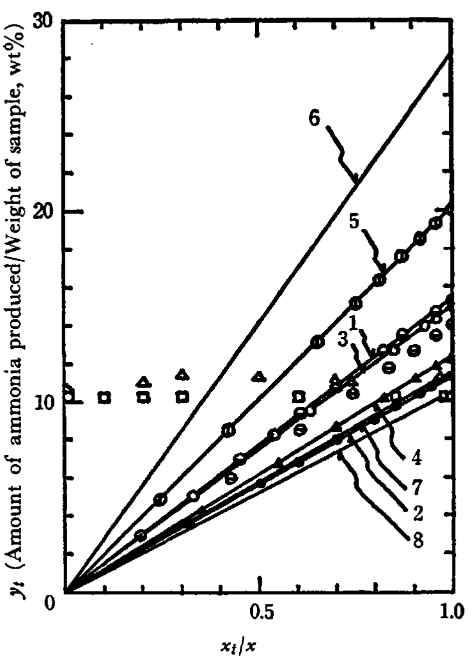


Fig. 2. Amount of ammonia produced *vs.* digestion degree in Kjeldahl digestion.

○, ●, ◒, ▲, ⦶, ⊖, △, □: Observed
——: Calculated
○, 1: Aniline ●, 2: Nitrobenzene
◒, 3: Azobenzene
▲, 4: Cyclohexanone oxime
⦶, 5: 2,4-Dinitrophenylhydrazone of cyclohexanone ⊖, 6: 2,4-DNPH
△, 7: Benzamide □, 8: Anisonitrile

As shown in Fig. 2, aniline, nitrobenzene, cyclohexanone oxime, azobenzene, and 2,4-dinitrophenylhydrazone of cyclohexanone are decomposed in accordance with Eq. (6), and a complete agreement is obtained between observed and calculated values. It may be concluded that Eq. (3) is applicable to the main decomposition reaction of these compounds. It is found that 2,4-DNPH are decomposed according to Eq. (6). However, the reaction (II) mentioned earlier is the main reaction of this compound because that y at $x_t/x=1$ is 50% of the calculated value and the observed value of α in Eq. (5) supports the validity of Eq. (II). Contrarily, anisonitrile and benzamide are not decomposed in accordance with Eq. (6).

All amino compounds unexceptionally converted into ammonia by the oxidative decomposition as shown in Table 1. By the reductive preteatment, nitro and azo compounds are believed to be analyzed quantitatively, but nitrobenzene, azobenzene, and other nitro compounds are found by the author to be analyzed without the above-mentioned treatment as shown in Fig. 2.

This finding indicates that oxidation and reduction may occur side by side in the course of reaction depending upon the condition, and that the compounds containing nitro and azo groups are reduced preferentially.

On the other hand, a concentrated sulfuric acid mainly reacts oxidatively with a reducing functional group such as the hydrazino group. Consequently, when the hydrazino group becomes less reductive by substitution to form hydrazono group like 2,4-dinitrophenylhydrazone of cyclohexanone, the reductive effect of hydrazono group is the same as that of azo group.

The reduction reaction for nitro and azo groups is accompanied by the carbonization of organic samples at the initial stage of the decomposition and takes place slowly as the decomposition proceeds. Therefore, it is desirable to heat the sample initially at a temperatures as low as possible and to suppress excessive oxidation reaction and after appropriate decomposition, to raise the temperature to increase the oxidative action of sulfuric acid.

It may be concluded that nitro and azo compounds can be decomposed quantitatively into ammonia by sulfuric acid containing potassium sulfate similarly as amino compounds, and the reductive pretreatment hitherto made is found to be needless. Furthermore, failures in the past endeavor to decompose these compounds seem mostly to be due to a lack of the study on the heating condition.