Kjeldahl Digestion Method Formulated by a Criterion Related to the Oxidation Number of Nitrogen

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A comprehensive Kjeldahl digestion method was formulated based on the criteria that 1) compounds having only nitrogens of oxidation number −III are digested by using H_2SO_4 with increased K_2SO_4 without the presence of any other oxidizing agent, and that 2) alternative compounds having nitrogens of oxidation number greater than −III are digested by using H_2SO_4 with K_2SO_4 and the reducing agent Zn. This formulation resulted in a satisfactory nitrogen determination of almost all types of organic nitrogen compounds, including those compounds for which it is known to be difficult to analyze using the conventional Kjeldahl method, for example pyridines, pyrazolones, 1,2-diazines, and 1,2,3-triazoles.

The Kjeldahl method has been one of the most popular methods for nitrogen analysis in a variety of fields since it was developed in 1883 by Kjeldahl. Meanwhile, further studies concerning digestion reagents have been desired by many workers. Among the recently proposed reagents, ZrO_2,[1] TiO_2,[2] $PdCl_2$,[3] H_2SO_5,[4] for instance, have been effectively used for reinforcing the principal oxidizing agent, sulfuric acid. Despite these numerous studies concerning auxiliary digestion reagents, a number of nitrogen compounds, including pyrazolones, 1,2-diazines, and 1,2,3-triazoles, remain refractory to Kjeldahl digestion.

The efforts to search for particular digesting agents that are effective for certain Kjeldahl-digestion-infeasible compounds have seemed to be made depending on the experimental approach in response to each occasional need. Comprehensive point of view that is commonly available for nitrogens of all bonding forms for indicating appropriate digestion system has not been established.

We previously found a method of Kjeldahl digestion wanted for cyanide nitrogen and cyano nitrogen in cyano complexes, in which digestion takes place while keeping H_2SO_4 in the condensed state as much as possible, from the initial stage of digestion.[5,6] We subsequently extended the object of our digestion studies to nitrogen compounds which possess a wider variety of bonding forms, and conclusively established a comprehensive Kjeldahl digestion method formulated based on a criterion related to the oxidation number of nitrogen.

This criterion involves classifying the nitrogen compounds into two types, type-1 and type-2, with regard to the bonding form of nitrogen, and bestowing different digestion systems that are proper for the respective types. Type-1 compounds include only nitrogens of oxidation number −III possessing N–C and/or N–H bonds; type-2 compounds include nitrogens with oxidation numbers greater than −III possessing, for instance, N–N and/or N=N bonds whether or not they include other nitrogens having the oxidation number −III.

Type-1 compounds were digested in the present work by using only concd H_2SO_4 with K_2SO_4, without adding any auxiliary oxidizing agents. The presence of a greater amount of K_2SO_4 could bring about an elevation of the temperature of the digestion mixture, which was thought to be the most important condition in this case regarding the proceeding of digestion.

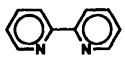
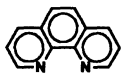
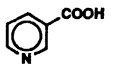
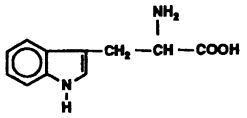
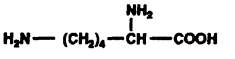
The digestion of type-2 compounds requires the presence of reducing agents in addition to oxidizing agents. Without a reducing agent the nitrogen having an oxidation number greater than −III tends to be oxidized to nitrogen gas or nitrogen oxide and lost, although not all amount of it is oxidized. In this work, type-2 compounds were digested by heating with Zn powder and K_2SO_4 in dil H_2SO_4 prepared by diluting the concd H_2SO_4 with an equal amount of H_2O. The compounds were reduced with Zn in dil H_2SO_4 in the initial stage, followed by oxidation of its carbon chains, this oxidizing reaction taking place in situ with increasing K_2SO_4–H_2SO_4-concentration in the course of digestion.

Experimental

Reagents. The reagents used as samples were of reagent grade guaranteed to be above 98% purity by Aldrich Chemical Co., Tokyo Kasei Kogyo Co. or Wako Pure Chemical Ind. The digestion agents, K_2SO_4 and concd H_2SO_4, were both of superior grade. The reduction reagent, Zn powder, was of commercial grade reagent which was prepared for the determination of nitrogen oxides. For capturing ammonia and titrating it, respectively, boric acid of superior grade and amidosulfuric acid of standard grade (99.91%) for volumetric analysis were used. The inorganic reagents were all purchased from Wako Pure Chemical Ind.

Procedure. A portion of a sample including about 1 mg of nitrogen was weighed into a digestion flask. After the digestion reagents were added to the sample in the flask, the flask was placed on an electric furnace (Model ME-6, Shibata Co.), and heating immediately commenced. The time was recorded after the fuming of sulfuric acid. The ammonia was distilled by the use of a Parnas–Wagner's distillation apparatus for 4 min at a rate about 5 $ml\,min^{-1}$ from an alkalinized mixture into 5 ml of a 2% boric acid solution containing a MR-BCG indicator. Titration was performed with 0.01 $mol\,dm^{-3}$ aqueous amidosulfuric acid. A mixture containing the same reagents, but not the sample compound, was used as a blank.

Table 1. Effects of K_2SO_4 on the Digestion of Type-1 Compounds Known to Be the Most Difficult to Undertake Kjeldahl Digestion

Compound[a]	Digestion time/h	N % obtained with various amount of K_2SO_4 supplied			N % calcd
		1.5 g[b]	2.0 g[b]	4.0 g[b]	
Pyridine 	1		6.1	11.2	
	2		6.8	17.2	
	3		9.6	17.5	17.71
2,2′-Bipyridine 	1	1.5	4.3	12.8	
	2	4.4	7.5	17.9	
	3	4.8	10.4	17.9	17.94
1,10-Phenanthroline·H_2O 	1	5.0	7.6	14.0	
	2	7.1	9.8	14.0	
	3	8.0	11.2	14.0	14.14
Nicotinic acid 	1	1.0	1.5	8.7	
	2	1.7	3.6	11.3	
	3	2.5	4.8	11.3	11.38
Tryptophan 	1	8.8	10.3	13.5	
	2	9.6	11.8	13.5	
	3	10.6	13.0	13.5	13.72
Lysine·HCl 	1	10.5	11.2	15.2	
	2	11.4	13.1	15.2	
	3	12.0	14.0	15.2	15.34

a) Digestion was performed on the 9 samples each prepared by taking 2 ml from the same solution which contained about 1 mg of nitrogen per 2 ml. b) For every digestion, 2 ml of concd H_2SO_4 was used.

Results and Discussion

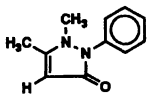
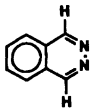
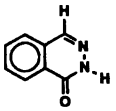
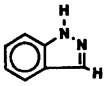
Analysis of Type-1 Compounds. Each compound containing about 1 mg of nitrogen was digested for 1 to 3 h with 2 ml of concd H_2SO_4 and 1.5, 2 or 4 g of K_2SO_4. Table 1 gives the results of analyses of the compounds known to be the most difficult to undertake Kjeldahl digestion, such as pyridine, 2,2′-bipyridine, 1, 10-phenanthroline, nicotinic acid, tryptophan, and lysine. In the case of digestion using 2 ml of concd H_2SO_4 and 4 g of K_2SO_4, the values of the analyzed nitrogen converged to a definite value within 3 h, as shown in the Table 1. Thus, even those digestion-refractory compounds proved to have been analyzed well by the digestion. From these results, it has become apparent that the quantity of K_2SO_4 in a digestion solution is the important condition concerning the digestion of type-1 compounds. The temperatures attained by the addition of 1.5, 2, and 4 g K_2SO_4 to 2 ml of concd H_2SO_4, respectively, were 326, 332, and 360 °C. With regard to most of the type-1 compounds, including diamine compounds, nitrogen could be satisfactorily recovered by digestion for 1 h with 2 ml of concd H_2SO_4 and 1.5 g of K_2SO_4. Purine and uracil, which have plural nitrogens apart from each other in the heterocyclic ring, could also be digested with 2 ml of concd H_2SO_4 and 1.5 g of K_2SO_4 for 1 h. Diamine compounds formerly had to be digested by heating for a long time with H_2SeO_3 and H_2SO_4.[7] The analytical results concerning purine, uracil and ethylenediamine sulfate are given in Table 4 (jumping over Tables 2 and 3). The method of digesting with H_2SO_4 and K_2SO_4 kept those nitrogens having an oxidation number of −III from being oxidized, and thus lost. In the digestion of the type-1 compound, the presence of auxiliary oxidizing agents proved not to be necessary. Although they could sometimes reduce the amounts of K_2SO_4 demanded, they occasionally further oxidized the resulting ammonium, which was therefore lost.[8] The reliable effectiveness of "the amounts" of K_2SO_4 in the proceeding of digestion was respected prior to expecting an aid of any other auxiliary oxidizing agents, which could have a positive effect for some compounds, but could not be assured to be free from having a negative effect on some other compounds.

The analytical method previously reported by us for the cyanide nitrogen and the cyano nitrogen in cyano complexes, by digesting with H_2SO_4 in the most condensed state possible, can be assigned to be included in the method for type-1.

The nitrile compound, for example 1,2-phthalodinitrile, also proved to be satisfactorily analyzed by the digestion method for type-1 (Table 4).

Analysis of Type-2-Compounds. Compounds antipyrine, phthalazine, phtharazone, 1,2,3-benzotriazole, and indazole were tested. Each compound, con-

Table 2. Effects of the Presence of Zn on the Digestion of Type-2 Compounds Having N–N Linkages in the Heterocyclic Ring

Compound[a]		Digestion time/h	N % obtained under different conditions of Zn-addition: Digestion without Zn[b]	Digestion by the delayed Zn-addition[c]	Digestion method presented by this paper[d]	N % calcd
Antipyrine		1	12.8	12.9	14.2	
		2	13.2	13.3	14.6	
		3	13.2	13.4	14.7	14.89
Phthalazine		1	13.7	17.6	21.2	
		2	13.7	20.0	21.3	
		3	13.8	20.1	21.3	21.53
Phthalazone		1	1.9	1.9	18.9	
		2	1.9	1.9	19.0	
		3	1.9	1.9	19.0	19.17
1,2,3-Benzotriazole		1	11.7	11.7	34.9	
		2	11.7	11.7	34.9	
		3	11.7	11.7	34.9	35.28
Indazole[e]		1	2.7	2.8	22.8	
		2	2.8	3.1	23.4	
		3	2.8	3.1	23.4	23.72

a) Digestion was performed on the 9 samples each prepared by taking 2 ml from the same solution which contained about 1 mg of nitrogen per 2 ml. b) The sample solution was digested with 2 g of K_2SO_4 and 2 ml of concd H_2SO_4. c) After the digestion for 1 h with 2 g of K_2SO_4 and 2 ml of concd H_2SO_4, 2 ml of H_2O and 300 mg of Zn were added. d) The sample solution was digested with 300 mg of Zn, 2 g of K_2SO_4 and 2 ml of concd H_2SO_4. In some cases the foam evolution arose from the digestion mixture at the commencement of heating. Care was taken to keep the foam from running out of the flask by controlling the heating during the foam evolution. e) To this sample, 500 mg instead of 300 mg of Zn was used.

Table 3. Effects of H_2O on the Digestion of Type-2 Compounds

Compound[a]	N % obtained by various modes of H_2O addition[b]: None	0.5 ml	1.0 ml	2.0 ml	N % calcd
Antipyrine	13.0	13.0	14.8	14.8	14.89
Phthalazine	19.4	21.1	21.1	21.3	21.53
Phthalazone	12.0	18.8	18.8	18.8	19.17
1,2,3-Benzotriazole	26.4	34.9	34.9	34.9	35.28
Indazole	5.0	10.0	22.4	23.4	23.72

a) The sample containing about 1 mg of nitrogen was digested. b) To the samples, 0—2 ml of H_2O, 300 mg (or 500 mg for indazole) of Zn, 2 g of K_2SO_4 and 2 ml of concd H_2SO_4 were added and then digested for 3 h.

taining about 1 mg of nitrogen, was digested with 2 ml of H_2O, 300 mg (or 500 mg for indazole) of Zn powder, 2 g of K_2SO_4 and 2 ml of concd H_2SO_4. These type-2 compounds were known to be difficult to analyze using the conventional Kjeldahl method because of having N–N linkages in the heterocyclic ring. The analytical results concerning type-2 compounds stated above according to the proposed digestion are given in Table 2; they show that these compounds were demonstrated to be well analyzed. The contribution of Zn as a reducing agent and the necessity of its presence from the initial stage of digestion were demonstrated by the results of digestions carried out in both the absence and delayed presence of Zn, which are given in Table 2.

The reduction with Zn in this method was carried out in dil H_2SO_4, not in HCl. If carried out in HCl, the resulting ammonium would be turned into nitrogen gas, and would be lost; an additional cumbersome procedure would thus become inevitable in order to remove the HCl off at any cost prior to the oxidation process.[9] The H_2SO_4, as was in a dilute state during the early stage, reacted with Zn to take part of the reducing of the nitrogen compounds. Meanwhile being gradually concentrated during the course of heating, the H_2SO_4 became capable of acting as an oxidizing agent in situ. Hitherto, such reducing agents as Zn–Fe in an HCl soln,[10] H_3PO_2,[11] etc. sometimes had to be used, but were accompanied by a troublesome procedure.

Table 4. Analytical Results Found for the Compounds of Various Types

Compound	N % obtained by various condition			N % calcd
	K_2SO_4 (4 g)[a]	Zn (300 mg), K_2SO_4 (2 g)[b]	Zn (300 mg), K_2SO_4 (2 g+2 g)[c]	
Isoniazid	10.4	28.5	30.2	30.65
Pyridine	17.5	15.4	17.5	17.71
Phthalazone	1.0	19.0	19.0	19.17
Pyridine + Phthalazone	9.5	14.6	18.2	18.41[d]
1-Nitronaphthalene	—	8.06	—	8.09
Azobenzene	—	15.1	—	15.38
3-Nitrosophenol	—	11.4	—	11.38
Hydroxylamine sulfate	—	17.0[e]	—	17.07
3-Amino-5-methylisoxazole	—	28.1	—	28.56
Cyclohexanone 2,4-dinitrophenylhydrazone (Primary standard for elemental analysis)	—	19.9	—	20.14
Purine	46.7[f]	—	—	46.65
Uracil	24.9[f]	—	—	25.00
Ethylenediamine sulfate	17.7[f]	—	—	17.71
1,2-Phthalodinitrile	21.9[f]	—	—	21.87

a) The sample added with 2 ml of H_2O was digested with 4 g of K_2SO_4 and 2 ml of concd H_2SO_4 for 3 h. b) The sample added with 2 ml of H_2O was digested with 300 mg of Zn, 2 g of K_2SO_4 and 2 ml of concd H_2SO_4 for 3 h. c) After the digestion with 300 mg of Zn, 2 g of K_2SO_4 and 2 ml of concd H_2SO_4 for 1 h, additional 2 g of K_2SO_4 was supplied and the digestion was continued further 2 h. d) The sample solution containing 2.28 mg of pyridine and 2.64 mg of phthalazone in 2 ml was analyzed. e) To this sample, 500 mg instead of 300 mg of Zn was used. f) The sample was digested with 1.5 g of K_2SO_4 and 2 ml of concd H_2SO_4 for 1 h.

The amount of H_2O present for diluting H_2SO_4 during the initial stage of digestion in this analysis is important; its effects on the digestion of type-2 compounds discussed above are given in Table 3.

The digestion method using Zn, K_2SO_4, and H_2SO_4 enabled a satisfactory determination of the nitrogen of most of the type-2 compounds, including nitro-compounds, azo-compounds, and hydrazides as ordinary instances. Isoniazid, a species of hydrazides, was digested in the same system with a slight alteration, i.e. by supplying an additional 2 g of K_2SO_4 1 h after starting the digestion, and continuing digestion for an additional 2 h, since it included pyridine-nitrogen in addition to the N–N nitrogen in the same molecule. The addition of 4 g of K_2SO_4 at the beginning of heating was not recommended, since it would elevate the temperature of the solution higher during the early stage of heating, so that an undesired oxidation of the nitrogens having higher oxidation numbers would be caused, regardless of the presence of the reducing agent. The digestion method appropriate for isoniazid was applicable to the analysis for such a mixture as that of phthalazone (type-2) with pyridine (type-1). The analytical results for these compounds are given in Table 4, accompanied by those of a few other examples of type-2 compounds, including cyclohexanone 2,4-dinitrophenylhydrazone, a standard compound for elemental analysis. The method for isoniazid should also naturally be applied to the analysis of samples containing unknown constituents. Only the analysis of 3-indazolinone, among the type-2 compounds tested, has not yet been successful. Samples containing some oxidizing agent which could oxidize the resulted ammonium, for example, Ru(III), could not be analyzed by this method.

The values of the mean, the standard deviation (S.D.) and the standard error of the mean (S.E.) for 5 analyses of the compounds listed in Tables 1 and 2, and of the standard compound, cyclohexanone 2,4-dinitrophenylhydrazone, are given in Table 5.

In this work, it was intended that all types of organic nitrogen compounds could be subjected to the digestion by using an oxidizing system comprising H_2SO_4 and K_2SO_4, and excluding the presence of all other auxiliary oxidizing agents. The oxidizing function of this system was considered to be of a definite sort and to be a variable in its intensity, monotonously increasing with the increasing amount of K_2SO_4 used. Accordingly, all nitrogen compounds were to be arranged in the order of decomposition-infeasibility, and all nitrogens in the compounds in the order of oxidation-refractoriness under exposure to this specific type of oxidizing function. It should be noted that the nitrogens of different oxidation states in compounds should assume different behaviors under the same oxidizing function.

Due to the difference in the oxidation-refractriness, the nitrogens which were not to be oxidized but digested to ammonium, could be discriminated from such nitrogens, which were more or less apt to be oxidized by this system to be partly lost as nitrogen gas or nitrogen oxides. The nitrogens of oxidation number −III proved

Table 5. The Values of the Mean, the Standard Deviation (S.D.) and the Standard Error of the Mean (S.E.) for Analyses of the Compounds Listed in Tables 1 and 2, and of the Standard Compound, Cyclohexanone 2,4-Dinitrophenylhydrazone

Compound	N % mean[a)]	S.D.[b)]	S.E.[c)]	N % calcd
Pyridine[d)]	17.6	0.05	0.02	17.71
2,2′-Bipyridine[d)]	17.8	0.05	0.02	17.94
1,10-Phenanthroline·H_2O[d)]	14.0	0.10	0.04	14.14
Nicotinic acid[d)]	11.3	0.10	0.04	11.38
Tryptophan[d)]	13.6	0.05	0.02	13.72
Lysine·HCl[d)]	15.1	0.10	0.04	15.34
Antipyrine[e)]	14.7	0.05	0.02	14.89
Phthalazine[e)]	21.4	0.10	0.04	21.53
Phthalazone[e)]	18.9	0.15	0.07	19.17
1,2,3-Benzotriazole[e)]	34.9	0.05	0.02	35.28
Indazole[e)]	23.3	0.10	0.04	23.72
Standard compound[e)]	19.9	0.10	0.04	20.14

a) The mean, $\bar{x}$, was calculated for 5 times of determinations. b) The standard deviation, s, was calculated by the formula,

$$s = \sqrt{\left\{\sum (x - \bar{x})^2\right\}/(5-1)}$$

in which x is the value for individual determination. c) The standard error of the mean, m_o, was calculated by the formula,

$$m_o = s/\sqrt{5}.$$

d) The sample was digested with 4 g of K_2SO_4 and 2 ml of concd H_2SO_4 for 3 h. e) The sample added with 2 ml of H_2O was digested with 300 mg (500 mg for indazole) of Zn, 2 g of K_2SO_4 and 2 ml of concd H_2SO_4 for 3 h. Indazole was proved to be especially sensitive to the contact with concd H_2SO_4, so the concd H_2SO_4 had to be added with stirring under the special attention. Otherwise, a part of nitrogen would be lost owing to the oxidation by H_2SO_4 before being reduced by Zn.

not to be oxidized, but to be satisfactorily digested to ammonium, even those which were contained in the more digestion-refractory compounds being proved to be feasible by using a larger amount of K_2SO_4. Such nitrogens as these having oxidation numbers greater than −III in a molecule, proved to belong to the kind apt to be oxidized and thus lost. Thus, for the protection of such nitrogens in the digesting process, the reducing agent, Zn, was used in dil H_2SO_4, in order to keep them from being oxidized until the molecule was digested and all nitrogens in the molecule turned to ammonium due to the gradually growing oxidizing effect of the system, $H_2SO_4+K_2SO_4$, with an increasing concentration of H_2SO_4 in the process of heating.

Zinc, a useful reducing agent that is applicable to various important processes in analytical chemistry, was the most desirable reducing agent for the proposed method of analysis, due to its chemical properties as well as the method of its actual usage. The Zn^{2+} ion formed in the digestion solution has the electron configuration with the outer shell filled ($3d^{10}$), being different from those of the ions of transition metals commonly used as a reducing agent, such as Fe. It would accordingly be of a property that is least provable to have a particular catalytical contribution on the oxidation of any particular nitrogens in molecules. Under the protection of this reducing agent, the commencement and proceeding of the desired digesting reaction were performed continuously without any interception, leading to the accomplishment of this analytical method schemed on a framework of consideration accounting the oxidation-reduction effects of the exclusively selected oxidizing system and reducing agent.

In the framework of the consideration presented above, several basic presumptions rendered from our studies on Kjeldahl analysis were comprehended, which can be stated as follows:

(1) Concd H_2SO_4 can break the carbon chain and oxidize at least partially those nitrogens having an oxidation number greater than −III which are thus lost. The role of the reducing agent, Zn, was presumed to keep those nitrogens from being oxidized.

(2) The addition of K_2SO_4 was presumed to afford an elevated temperature and/or the fused state to the digestion mixture to accelerate the decomposition of carbon chains, without oxidizing those nitrogens having an oxidation number of −III.

These presumptions were found to hold due to the virture of the satisfactory results of this work.

Conclusion. A comprehensive Kjeldahl digestion system which exclusively uses H_2SO_4, K_2SO_4, and Zn was formulated on the basis of a criterion related to the oxidation number of nitrogen. The nitrogens in organic compounds were classified based on the criterion into two types, and proper digestion systems for the respective types were bestowed. Type-1 compounds including only nitrogens having an oxidation number of −III were digested by using concd H_2SO_4 with increased K_2SO_4 without the presence of any other oxidizing agent. Type-2 compounds having nitrogens with oxidation numbers above −III were digested by using H_2SO_4 with K_2SO_4 and the reducing agent Zn.

By having proved the satisfactorily sure analytical results obtained for the considered nitrogen compounds through the proposed method, the great defect of Kjeldahl digestion of failing in the analysis for certain significant types of nitrogen compounds is thought to have been removed.

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