A REVIEW OF THE KJELDAHL DETERMINATION OF ORGANIC NITROGEN

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I. INTRODUCTION

During the fifty-odd years which have passed since the inception of Kjeldahl's (57) method for determining organic nitrogen, considerable progress has been made. That this is true is borne out by the amount of literature on the subject.

Originally, the method was designed for the brewing industry as an aid in following protein changes in grain during germination and fermentation. Its use soon became widespread and can now be regarded as an important tool of analytical chemistry. Fundamentally, it is a wet oxidation employing concentrated sulfuric acid as a digestion medium, and resulting in the formation of ammonium sulfate which is subsequently distilled with an excess of alkali. The ammonia thus formed is determined by any one of several available methods.

Prior to Kjeldahl's method, however, concentrated sulfuric acid had been used for preliminary treatment of organic material before completing the determination of nitrogen by the now obsolete Will–Varrentrapp method. Wanklyn (140) had used alkaline permanganate for the determination of protein nitrogen, and Kjeldahl, following along this line, concluded that the formation of ammonia would take place more easily in an acid medium. Experimental evidence showed this to be the case, and that the use of concentrated sulfuric acid was necessary.

Many improvements and modifications of the method followed, after its value was established. Primarily devised for the determination of protein

nitrogen, the method has been extended to include the determination of various other forms of nitrogen.

From the abundance of available data, any attempt at correlation must be made through the medium of classification, and in the following data, the component parts of the Kjeldahl method will be discussed under their respective headings.

II. DIGESTION MEDIA

In Kjeldahl's original method, sulfuric acid alone was used as a digestion medium. The principal objections to this procedure were the length of time for digestion, and the necessity of using a small sample in order not to prolong the digestion. To increase the severity of the reaction and reduce the digestion time, Gunning (48) in 1899 proposed the use of potassium sulfate as a means of raising the boiling point of the digestion mixture. This procedure is standard practice. However, one fact should be borne in mind,—namely, that when the composition of the residue approaches that of the acid sulfate, the tendency is to lose ammonia. The residue in the flask after digestion should not solidify on cooling.

P. A. W. Self (119) stated that ammonia is almost completely volatile under these conditions, and, further, that if 25 cc. of sulfuric acid and 10 g. of potassium sulfate are used in the digestion, at least 15 g. of sulfuric acid should remain in the flask, approximately 6.7 g. of acid being consumed to form the acid sulfate. This loss of ammonia was confirmed by Carpiaux (14). Prolonged boiling and too rapid distillation of acid should be avoided. It may sometimes be necessary to stop the digestion in order to add more acid.

The amount of acid necessary to digest 1 g. of organic matter varies, of course, with the type of material under examination. It has been found (119) by experiment that 1 g. of carbohydrate requires approximately 7.3 g. of sulfuric acid for complete oxidation, 1 g. of protein requires 9.0 g., and 1 g. of fat 17.8 g. of sulfuric acid.

The use of sodium sulfate as a substitute for potassium sulfate has been suggested and reported upon from time to time. Latshaw (62) made a comparison of the two, and showed that when an equivalent amount of sodium sulfate was used, no difference in results was noticed. On the other hand, Brill and Agcaoli (12) were unsuccessful in their attempt to substitute one for the other. Various other workers (4, 25, 78, 97, 136), however, have reported favorably upon its use.

An investigation of the Kjeldahl method by Phelps (98) showed that the proportion of either sulfate to sulfuric acid must be carefully regulated in order not to cause volatilization of ammonia, and, with highly refractory compounds, the use of sodium sulfate is not recommended unless the

conditions can be very closely controlled. Later work by Daudt (21) confirmed the fact that the acid-sulfate ratio was important. A comparison of lithium, sodium, and potassium sulfates (68) showed that the potassium salt was most effective.

The effect of sodium pyrophosphate in the determination of nitrogen in gelatin has been investigated (8), and this salt has been shown to be less efficient than potassium sulfate. The substitution of most of the potassium or sodium sulfate (ten-sixteenths) by dibasic potassium phosphate (K_2HPO_4) (40) shortened considerably the digestion time of samples containing protein nitrogen. Low results, however, were obtained when all the alkali sulfate was replaced by the phosphate.

Phosphoric acid (33, 34, 35, 66, 67, 110, 126, 145) in combination with sulfuric acid has also been used as a digestion medium in both macro and micro Kjeldahl methods. Rapid digestions are possible, and such combinations are sometimes helpful where complete digestion by ordinary methods is impossible.

The clearing of the digestion mixture is not necessarily an indication that all the nitrogen has been converted to ammonia. The possibility of the formation of amines has been investigated. Villiers and Moreau-Talon (139) stated that too energetic oxidizing agents, under the influence of temperature, concentration, and time of heating, promote the formation of amines. Gortner and Hoffman (44) found that approximately 7 per cent of the distillate from a Kjeldahl-Gunning digestion could be amine nitrogen, and also that magnesium, calcium, strontium, and barium salts influenced the amount of amines formed.

Many workers have recommended an after-boil ranging from 0.5 to 3 hr., in special cases even longer than this. In contradiction to this, it has been shown (5) that in the case of rice or wheat flour, oatmeal, and bone meal, it is not necessary to continue digestion after the solution has cleared. On the other hand, the period of after-boil in determining nitrogen in coal varies from 18 min. to 235 hr. An investigation by Crossley (19) into the relative merits of mercuric oxide and selenium as digestion catalysts brought out the fact that a definite minimum time of after-boil was necessary.

III. OXIDIZING AGENTS

Regardless of the fact that sulfuric acid is a strong oxidizing medium, it is often necessary to increase the severity of the reaction. Two substances that have merited a great deal of attention are potassium permanganate and hydrogen peroxide.

From the evidence presented by various authors, the suitability of permanganate is questionable. Siegfried and Weidenhaupt (121) have stated

that if the permanganate is added in small amounts to the digestion mixture and then brought to a boil, there is no danger of loss of ammonia. With certain substances, it is essential to boil after each addition. Oxidation is complete if there is no decolorization after boiling for 3 min. In the determination of nitrogen in coal, Fieldner and Taylor (31) reported no loss of ammonia, and that no modification for nitrates or nitro compounds was necessary. Cochrane (16, 17) also found that lower results were obtained without the use of permanganate. In the analysis of soil and grass, Ashton (4) found that the addition of 5 g. of permanganate after the clearing of the digestion mixture gave good results with soils, but low results with grass.

On the other hand, Frear, Thomas, and Edmiston (38) found a loss of nitrogen to occur if permanganate is added immediately after the source of heat is removed, but if the temperature is allowed to fall 100°F. (in approximately 2 min.), no loss of nitrogen is observed if the permanganate is added at this time.

Phelps (99), Paul and Berry (97), and Beet (6) stated that permanganate gives low results or that it is unnecessary. Its use has been discontinued, owing to the uncertainties involved.

Hydrogen peroxide has been used for both macro and micro digestions. The reaction is more or less violent and needs to be handled carefully. The conditions under which the peroxide is added vary somewhat. Kleeman (58) and Heuss (51) treated the sample with 25 cc. of hydrogen peroxide and added 40 cc. of concentrated sulfuric acid. Koch and McMeekin (60) reported that the addition of 30 per cent peroxide to concentrated acid causes a very rapid oxidation with complete retention of nitrogen as ammonia. A 20-min. heating with sulfuric acid and 5 per cent of acid containing sulfur trioxide was recommended by Saccardi (112), after which peroxide to the amount of 10 per cent of the sulfuric acid is added. Provvedi (105) noted that digestion took place in 45 min., but attributed the activity of the hydrogen peroxide to other agents reacting with the sulfur dioxide produced by the reduction of the sulfuric acid.

The fact that no loss of nitrogen has been reported with the use of peroxide makes its use acceptable as an oxidizing agent, albeit a very active one.

Brief mention should be made of perchloric acid. Parker and Terrill (95) have used perchloric acid successfully in the Kjeldahl determination of nitrogen in leather, and Mears and Hussey (75) have reported that it aids digestion, decolorization taking place in a very short time.

On the other hand, LeTourneur-Hugon and Chambionnot (64) stated that while perchloric acid appreciably shortens digestion time, all methods of using it are not suitable. They recommended addition of the acid, a few drops at a time, during boiling, whereby complete decolorization is effected in a few minutes.

IV. CATALYSTS

The search for catalysts to produce a further increase in the velocity of the reaction has led experimenters through a large part of the Periodic System.

One of the earliest catalysts was platinic chloride, used by Ulsch (137) in 1886, who reported that it was satisfactory except when excessive amounts were used. Anderson (2) found that a loss of nitrogen occurred when platinic chloride was used as a catalyst in the determination of nitrogen in urine, milk treated with pepsin or trypsin, old albumin solutions, or hydrolyzed casein, but not in determinations with milk, serum, or fresh albumin solutions. He suggested that this loss might be due to the combination of the chlorine set free with the amino groups, and that this combination cannot take place with nitrogen in peptide combinations. He also found that nitrogen compounds containing chlorine could be accurately determined.

A catalyst which has been used since the beginning of the Kjeldahl method is mercury, either in the form of the metal, oxide, iodide, or sulfate, or in combination with various other compounds. From the point of view of speed, it is one of the most efficient catalysts. In 1885 Wilfarth (142) reported on a number of compounds used as digestion catalysts. He found that, while mercuric oxide was fast and effective, its tendency was to hold back the ammonia upon distillation. This is, of course, to be expected, since mercury forms complexes with ammonia. It is necessary, therefore, to convert the mercury to some compound that will cause no interference. This is accomplished by the use of alkali sulfide (22, 142), sodium thiosulfate (22, 104), monosodium phosphate, and potassium xanthate (90). Even potassium arsenate (55) has been used. Of these compounds, probably potassium sulfide (or sodium sulfide) and sodium thiosulfate are the most common. Of the other compounds studied by Wilfarth, the copper oxide was stated to be less efficient than mercury, while ferric oxide, bismuth trioxide, stannic oxide, lead dioxide, and Pb_3O_4 were not recommended.

Phelps and Daudt (100) (1919), studying the determination of nitrogen in refractory organic compounds, reported that mercuric oxide was superior to cupric sulfate, but that alum, nickel sulfate, zinc chloride, manganous chloride, manganese dioxide, and tungstic, molybdic, titanic, and vanadic acids could not be recommended.

The use of mercurous iodide has been suggested by Sborowsky and Sborowsky (116). They claimed that carbonaceous material is oxidized much more readily than if mercury alone is used as the catalyst. This was confirmed by Richards (109), who used it in the Kjeldahl digestion of leather and coal.

In refutation of the statement of Sborowsky and Sborowsky, Hassig

(50) stated that the digestion is not hastened by the use of mercurous iodide, and that there is the disadvantage of the sublimation of the iodide in the neck of the flask.

Copper, alone, or as the oxide or sulfate, is another example of a catalyst universally accepted for the Kjeldahl determination. The efficiency claimed for it is less than that of mercury, hence it is necessary to increase the digestion time. The efficiency of copper sulfate, mercuric oxide, and potassium sulfate in sulfuric acid as a digestion mixture was confirmed by Trescott (135) and was recommended for adoption as an official method by the Association of Official Agricultural Chemists.

Arnold and Widemeyer (3), in 1892, reported that the use of a mixture of copper sulfate and mercuric oxide shortened the digestion time, and later Bredig and Brown (11) (1903) claimed that the mixed catalyst was more efficient than either one used alone. Powdered copper (56), also, has been used, but it seems reasonable to assume that there is no particular advantage to be gained over copper sulfate, since this compound is formed on addition of the copper to the sulfuric acid. There is also the distinct disadvantage of having to prepare the pure copper.

A more recent catalyst is selenium, used as such, or in the form of the dioxide (SeO_2) or the oxychloride ($SeOCl_2$), and either alone or in combination with various other catalysts.

First mention of selenium was made by Lauro (63), who used both selenium and selenium oxychloride as catalysts in the determination of nitrogen in flour. Rich (108) reported on the use of selenium oxychloride in combination with copper in the determination of total protein, and stated that it reduced the total time of the analysis by at least half an hour, as compared with copper alone.

A series of determinations made on a high-protein flour and on ground bran by Sandstedt (114), using (*1*) copper and mercuric oxide and (*2*) copper and selenium, showed that digestion was complete in 45 min. with copper and selenium and in 1 hr. with copper and mercuric oxide. This author stated that there appears to be greater danger of losing nitrogen by extremely long digestion with selenium than with other catalysts. An added advantage of selenium is that it is unnecessary to add a precipitant before distilling.

Comparative experiments by Crossley (19) with selenium and with mercuric oxide showed that the time of digestion was appreciably shortened. Messman (78), Tennant, Harrell, and Stull (133), and Belov and Pakhomova (7) also confirmed the efficiency of selenium.

Osborn and Krasnitz (93) stated that while selenium, or selenium oxychloride, may claim a slight advantage over copper sulfate, there is no advantage over mercuric oxide. The combination of selenium with mercuric oxide is more efficient than either alone. They recommended

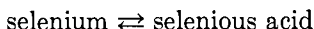
the use of elemental selenium rather than the oxychloride. Further comparisons were made by these authors on a wide variety of substances to determine the relative catalytic speeds of selenium, mercuric oxide, selenium and mercuric oxide, and selenium and copper sulfate. Selenium and mercuric oxide was found to be 25 per cent faster than mercuric oxide; selenium alone, and selenium and copper sulfate were less effective than mercuric oxide. Extending the digestion period increases the danger of loss of nitrogen in the following order: mercuric oxide, selenium, and selenium and mercuric oxide. They stated that this loss can be obviated by the addition of larger quantities of sulfuric acid.

The catalytic action of red mercuric oxide, selenium, selenium dioxide–copper, and selenium oxychloride in the determination of protein in wheat was discussed by Snider and Coleman (127), who found that when from 75 to 250 mg. of selenium are substituted for 0.5 g. of mercuric oxide, low values for nitrogen are obtained in the evaluation of the crude protein content of wheat, and, further, that a combination of 0.3 g. of selenium dioxide and 0.05 g. of copper in place of 0.5 g. of mercuric oxide reduced the time of determination by about 15 to 20 per cent. Goswami and Ray (45) used selenium and yellow mercuric oxide as a catalyst for the digestion of milk, whey, lymph, and protein-containing substances, and reported a substantial reduction in time, as compared with other catalysts. Selenium oxychloride (0.2 cc.) showed no advantage over mercuric oxide. A combination of 0.1 g. nickel and 0.1 g. selenium, while inducing a rapid clearing of the digestion mixture, gave low protein determinations.

It has been suggested by Illarionov and Soloveva (53) that the catalytic effect of selenium and tellurium is due to the formation of H_2SeO_3 and H_2TeO_3 in the hot sulfuric acid, and that these acids act as carriers of oxygen. The catalytic effect is proportional to the quantity of selenium or tellurium used. In a recent paper, Sreenivasan and Sadasivan (130) showed that the efficiency of selenium as a catalyst was greatly increased by the addition of mercuric oxide. They explained this in the following manner: The catalytic action of selenium in the presence of mercuric oxide depends upon the reaction

$$\text{selenium} \rightarrow \text{selenious acid} \rightleftarrows \text{selenic acid}$$

As long as oxidizable material is present, the reaction proceeds to the right, and goes to completion when all organic matter has been oxidized. Selenium alone proceeds according to the following reaction:



$$\text{selenium} \rightleftarrows \text{selenious acid}$$

Since the speed of the reaction in either direction shows no pronounced difference, the efficiency as a catalyst is much less than with mercuric oxide.

In a comparison of ferrous sulfate–selenium (9) and copper sulfate–selenium, it was found that the former possessed a slight advantage.

Davis and Wise (23)[1] have stated that selenium does not appear to be as universally adaptable to general laboratory conditions as mercury, and that its use in combination with catalysts, especially mercury, is to be discouraged.

Copper selenite dihydrate as a single catalyst has been used by Schwoegler, Babler, and Hurd (118), who found that the digestion time was considerably reduced.

Many other authors have presented definite evidence that selenium alone, or in combination, is an effective catalyst.

The preceding statements do not by any means cover the field of catalysts. This is extensive, and in many instances the catalysts mentioned exert a specific action. Brief mention will be made of a few.

The use of vanadium pentoxide as a catalyst was recommended by Oefele (92), who regarded it as an oxygen-carrier. Marino and Gonelli (74) reported that it shortened digestion time. In the Kjeldahl digestion of flour, Parri (96) found that the digestion time was considerably reduced when a mixture of 0.1 g. of vanadium pentoxide and 0.5 g. of cupric oxide was used as a catalyst. With either catalyst alone, the time was 6 hr., but a mixture of the catalysts required only 2.2 hr. for clearing. The addition of oxides of vanadium, nickel, and antimony as catalysts in the analysis of piperidine by Brill and Agcaoli (12) was found to be unsatisfactory. The work of Margosches and Lang (69) showed vanadium to be of doubtful efficiency. These same authors used tungsten (WO_3) and copper oxide, also ceric oxide, as catalysts with fairly satisfactory results.

Metallic cadmium was reported by Saiko-Pittner (113) to be a very satisfactory catalyst for pyramidon.

Dakin (20) first used potassium persulfate, $K_2S_2O_8$, which, by its decomposition, increases the effect of the sulfuric acid. The following year Milbauer (80) confirmed this, and years later, both Pittarelli (101) and Wong (147) reported on its efficiency.

Manganese dioxide (132) and potassium perchlorate (39) have been used, the latter giving satisfactory results in the digestion of leather.

In a survey made on the comparative digestion times of (*1*) cupric sulfate, (*2*) mercuric oxide, (*3*) cupric sulfate, mercuric oxide, and selenium, and (*4*) cupric sulfate, mercuric oxide, selenium, and hydrogen peroxide, Poe and Nalder (102) found that the addition of peroxide appreciably decreased the time of clearing. A comparison of several catalysts (89) used in the digestion of oil-cake samples showed mercuric oxide to be

[1] Report of the Sub-Committee on Selenium as a Kjeldahl Catalyst in the Cereal Laboratory.

more efficient than selenium, and copper sulfate less efficient than either of these.

The accelerating and retarding action of various elements was studied by Ranedo (106), who showed that elements in the third and fourth groups of the Periodic System retard attack considerably. If accelerator elements are present, this effect is overcome. Selenium and sufficient platinum are among the most active accelerators.

The effect of each of thirty-nine metals on the determination of nitrogen in a gluten flour, studied by Osborn and Wilkie (94), showed that mercury was the most satisfactory. Of the thirty-nine metals, ten or twelve catalyzed the digestion, and the best catalysts appear to be mercury, tellurium, titanium, iron, and copper. Under less violent conditions selenium, molybdenum, vanadium, tungsten, and silver were found suitable. Larger amounts of selenium and vanadium interfere with the accuracy of the determination. Platinum also interferes.

Milbauer (81, 82, 83, 84, 85, 86) has made extensive investigations on the oxidation of organic substances with sulfuric acid. The mechanism of the digestion was studied through the medium of simple substances. The relative activities of the catalysts employed varied with the temperature and the type of compound being oxidized. Experiments performed with sucrose and a large number of metals as catalysts showed that selenium dioxide and mercuric sulfate (1:1) and selenium dioxide and cupric sulfate (3:1) were most effective. Mixtures of the catalysts are less satisfactory. In a recent communication, this author (87) has shown that, of twenty-five catalysts, mercuric sulfate–selenium promotes the most rapid digestion when the ratio of mercury to selenium is 4:1. A further reduction of time is obtained by the addition of an oxidizing agent. Addition of phosphorus pentoxide to the mercury–selenium catalyst reduces digestion time to 3.5 per cent of the time required with concentrated sulfuric acid alone. Equilibrium states and the reaction of the Kjeldahl digestion in a current of gases have also been studied by Milbauer.

V. DISTILLATION AND DETERMINATION OF AMMONIA

The preparation of the digestion mixture for distillation, and subsequent absorption of the ammonia evolved, is largely mechanical, rather than chemical, in nature. The numerous manipulative details will not be discussed here, since these can usually be left to the operator's discretion. No space will be devoted to discussion of the various modifications of the apparatus.

One of the first means of recovering the ammonia was by steam distillation. This has been more or less a controversial subject, inasmuch as

some investigators have reported high values due to entrainment of alkali which was carried over into the absorption flask. Aeration, also, has been employed as means of driving off the ammonia. However, low results are obtained unless a large excess of alkali is used. Generally speaking, heat distillation is probably the most common means of ammonia recovery.

An investigation by Merlo (77) on the removal of ammonia by aeration showed that, at room temperature, the ammonia from 20 cc. of 5 per cent ammonium chloride solution was completely removed in 1.5 hr., and at 40°C. in 45 min. The rate at which air was passed through the solution was 600 to 700 liters per hour. Kober and Graves (59) stated that the amount of ammonia left in the distillation residue after aeration for an hour is negligible, and that the completeness of the distillation is independent of heat. An absorption flask devised by Sjöquist (125) allows addition of standard acid without opening the system. The following results were obtained by aeration:

TIME	AMMONIA RECOVERED
	per cent
20 min.	12.0
60 min.	32.5
4 hr.	84.5
9 hr.	98.5
11 hr.	100.0

Experiments were carried out by Falk and Lugiura (30), according to conditions stated by Kober (59), and a comparison made of aeration and heat distillation methods. For many substances the aeration method gave low results, but subsequent steam distillation raised the values so that there was satisfactory agreement with those obtained by heat distillation. A series of experiments with pure ammonium sulfate showed that distillation by the aeration method was incomplete unless a suitable excess of strong alkali was present.

Meldrum, Melempy, and Meyers (76), studying the recovery of ammonia by aeration at different temperatures, stated that there is an advantage to be gained with respect to the time required.

A successful installation for steam distillation and its advantages have been described by Adriano (1) and also by Green (47).

There now remains only the determination of ammonia to be considered. A variety of methods is at hand. The most common procedure is to distill the ammonia into an excess of standard acid (hydrochloric or sulfuric), and titrate the remaining acid with standard alkali, the difference in the two titrations being calculated to nitrogen.

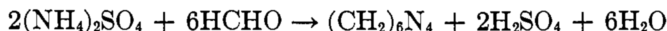
In the presence of a comparatively large volume of water and insufficient standard acid, it is possible to absorb all the ammonia without loss. Such a method was suggested by Neumann (91), who measured out slightly less than the amount of acid actually needed, and, after distillation, titrated the excess of ammonia with the same standard acid. This obviates the necessity of two standard solutions.

Along the same line is the boric acid method proposed by Winkler (145). Boric acid is an extremely weak acid and does not cause a color change with the indicators used. However, ammonia is fixed by it, and as such can be titrated directly with acid. Scales and Harrison (117), and also Spears (129), confirmed Winkler's method, and recommended bromophenol blue as an indicator. An excess of 4 per cent boric acid was used. (This represents a saturated solution of the acid.) On the other hand, Staver and Sandin (131) use a 2 per cent boric acid solution and a mixed indicator of methyl red and tetrabromophenol blue. Exhaustive experiments, performed by Sapegin and Ometov (115) on the determination of nitrogen in leather, indicated that absorption of the ammonia in 2 per cent boric acid was the most satisfactory. A mixed indicator of methylene blue and methyl red was used. Brecker (10) also used the preceding indicator and stated that methyl red alone may be used. The stability of boric acid prepared with pure water and kept in Pyrex bottles has been established by Eisner and Wagner (28).

A point to be mentioned is the presence of amines in the distillate. Erdmann (29) has suggested the presence of mono-, di-, or tri-methylamine when the compound under examination contains the groups $CH_3N=$, CH_3NH- or $(CH_3)_3N=$. In a study of the Kjeldahl method, Villiers and Moreau-Talon (139) stated that the use of too energetic oxidizing agents which might break down the ammonia should be avoided. Temperature, duration of heating, and concentration also have their effect on the formation of amines. These authors distilled the ammonia into an excess of dilute hydrochloric acid, evaporated the distillate to dryness, and weighed the ammonium chloride. Chlorine was subsequently determined by either Mohr's or Volhard's method. The two results should agree, and discordant results indicate amine formation. A preliminary paper by Gortner and Hoffman (44) stated that the distillate from a Kjeldahl-Gunning digestion contains amines to the extent of 7 per cent of the nitrogen. Magnesium, calcium, barium, and strontium salts influence the amount of amines present.

A method of considerable interest is the so-called "formol titration" (46, 87, 111, 120, 123, 148), which dispenses with the distillation of ammonia. Briefly, if a neutral ammonium salt is treated with formaldehyde,

hexamethylenetetramine and free acid are formed according to the following equation:

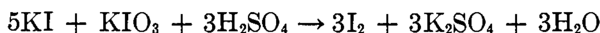


$$2(NH_4)_2SO_4 + 6HCHO \rightarrow (CH_2)_6N_4 + 2H_2SO_4 + 6H_2O$$

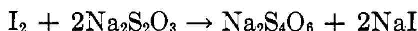
The liberated acid is titrated with standard alkali.

The use of Nessler's reagent gives us a colorimetric method of determining small quantities of ammonia in the distillate. It has been employed in microanalysis (60) and in the analysis of biological materials (36). Chiles (15) states that if a protective colloid such as gum arabic is employed in the nesslerization of ammonia in the presence of alkali sulfates, higher concentrations of ammonia can be determined.

Kjeldahl originally proposed the iodometric determination of the excess standard acid after distillation of the ammonia. The reaction takes place in the following manner:



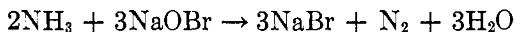
$$5KI + KIO_3 + 3H_2SO_4 \rightarrow 3I_2 + 3K_2SO_4 + 3H_2O$$



$$I_2 + 2Na_2S_2O_3 \rightarrow Na_2S_4O_6 + 2NaI$$

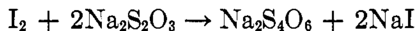
According to Wilson and Mattingley (144), since carbon dioxide is usually present in the distillate, it is advisable to boil it off before adding the iodide–iodate solution. Owing to this sensitivity toward carbon dioxide, the method has been discarded. However, Michaelis and Maeda (79) have stated that the iodometric method applied to the microchemical determination of nitrogen is to be preferred to the acidimetric method, since the pH of the end point is ideal for the ammonia determination, and because the danger of changing the end point by carbon dioxide absorption is much less than in acidimetric determinations.

Another iodometric method, more or less general, makes use of alkaline hypobromite:



$$2NH_3 + 3NaOBr \rightarrow 3NaBr + N_2 + 3H_2O$$

$$NaOBr\ (excess) + 2KI + 2HCl \rightarrow 2KCl + NaCl + I_2 + H_2O$$



$$I_2 + 2Na_2S_2O_3 \rightarrow Na_2S_4O_6 + 2NaI$$

Willard and Cake (143) were the first to apply the above reaction to the Kjeldahl process. Various other investigators (42, 65, 103, 107, 134) have employed it, principally in the micro determination of nitrogen.

A little-used modification suggested by Sors (128) consists in exactly neutralizing the acid digest and adding a known excess of standard alkali. The ammonia is boiled off, and the remaining alkali titrated. The difference in titration represents the equivalent of ammonia that was present.

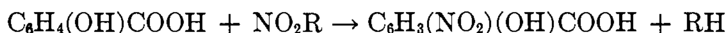
VI. APPLICATION OF THE METHOD TO THE MORE COMPLICATED COMPOUNDS

Up to this point, we have considered the nitrogen to be easily available upon oxidation. In other words, a great many natural products and pure organic nitrogen compounds have their nitrogen in basic form which is easily split off during digestion to form ammonium sulfate. There exists, however, a number of compounds whose nitrogen is present in other than a basic form, examples of which are nitrates, nitro, nitroso, and azo compounds, and compounds containing ring nitrogen. In order to rationalize the method and make it generally applicable to the many existing forms of nitrogen, a great deal of attention has been and is being given to those classes of compounds whose behavior is decidedly refractory.

In the year 1886 Jodlbauer (54) introduced the important principle of addition of phenols to the digestion mixture. These phenols, which are readily nitrated, convert the nitrogen into a form more easily reducible. For a reducing agent, Jodlbauer used zinc dust, with platinum chloride as a catalyst.

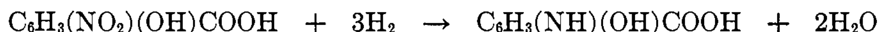
The next advance was made by Forster (37), who substituted sodium thiosulfate for zinc, and used a mixture of phenol in sulfuric acid.

Cope (18), in 1916, after an extended use, reported salicylic acid to be a very satisfactory substitute for phenols. This reagent is now generally accepted, although phenol is still used. The following equations illustrate the various phases of the conversion of a nitro compound into ammonium sulfate, the first step being the formation of nitrosalicylic acid:



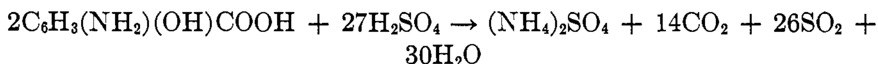
$$C_6H_4(OH)COOH + NO_2R \rightarrow C_6H_3(NO_2)(OH)COOH + RH$$

After this conversion has been effected, a suitable reducing agent is added which transforms the nitrosalicylic acid into aminosalicylic acid:



$$C_6H_3(NO_2)(OH)COOH + 3H_2 \rightarrow C_6H_3(NH)(OH)COOH + 2H_2O$$

Now that the nitrogen has been reduced to the amino form, it can be converted easily to ammonium sulfate:



$$2C_6H_3(NH_2)(OH)COOH + 27H_2SO_4 \rightarrow (NH_4)_2SO_4 + 14CO_2 + 26SO_2 + 30H_2O$$

In addition to the two reducing agents mentioned, iron (52), zinc and iron (27), stannous chloride–tin (61), sodium hyposulfite (54, 103) ($Na_2S_2O_4$), sulfur (26), and hydriodic acid (40) have all been used.

Mention of more notable examples of compounds which are extraordinarily resistant to oxidation includes antipyrine, pyramidon, pyridine, quinoline, and isoquinoline. Many others must be subjected to special treatment before yielding their nitrogen as ammonia. It can be seen,

then, that any attempt at generalization must prove more or less useless. Fleury and Levaltier (34) have studied the gases evolved during digestion, and have stated that when low values are obtained, considerable nitrogen is liberated, and some carbon monoxide.

Margosches and Vogel (70), from their work on nitro compounds, established the fact that the position of substituent groups exerted a definite influence on the digestion. They found that when an OH or RO group was in the ortho-position to the nitro group, quantitative results could be obtained. Further experiments (68) with the mononitrophenols, mono nitrobenzoic acid, and mononitrocinnamic acids showed that the sulfuric acid digestion takes place in two steps. With ortho compounds, the earlier stage is characterized by a black solution, during which time most of the nitrogen is converted to ammonium acid sulfate, the remainder being converted by the time the solution is decolorized. The para compounds give a brown solution and practically no nitrogen is converted to the ammonium salt during this time.

Further work by Margosches, Kristen, and Scheinost (71) was conducted on mono-, di-, and tri-nitrophenols, nitroanisoles, nitrophenetoles, nitrobenzoic acids, nitrobenzyl alcohols, and nitroanilines, using four modifications of the Kjeldahl method; viz., with potassium sulfate, with cupric oxide, with mercuric oxide, and without catalysts. Most of the compounds gave low results by all modifications. From the data obtained, the authors concluded (*1*) that if an hydroxyl group is ortho to the nitro group, it is usually easy to convert the nitrogen quantitatively to the ammonium salt; (*2*) that if the hydroxyl group is para to the nitro group, it is practically impossible to do so; (*3*) that a methyl group meta to the nitro group gives results close to the theoretical.

It is, therefore, necessary to resort to some means of effecting a reduction of the nitrogen of these refractory compounds to some other form that will result in complete conversion on subsequent digestion with sulfuric acid. Margosches and Kristen (72) applied Flamand and Prager's (32) modification for azo compounds. The sample is dissolved in ethyl alcohol and reduced with zinc and hydrochloric acid, followed by the ordinary digestion with sulfuric acid. The amounts of zinc, hydrochloric acid, and sulfuric acid should be increased according to the number of nitro groups present. A continuation of these studies (73) included such compounds as *m*-nitrobenzenesulfonic acid, mononitrotoluenes, and dinitrobenzenes. Nitrogen determinations (Flamand–Prager modification) were run using cupric oxide or cupric sulfate, mercuric oxide, or combinations of these as catalysts. The results were tabulated and showed that with sulfuric acid alone approximately correct results were obtained with 2,4-, and 2,6-dinitrotoluenes and with 1,2,4,6-dinitroxylene. Ad-

dition of potassium sulfate or mercuric oxide gave low results in every case, while addition of cupric oxide or cupric sulfate gave results higher than the theoretical. Low results were thought to be due to volatilization or sublimation in the digestion flask. While these modifications made possible the determination of nitrogen in some compounds hitherto classed as refractory, they cannot be considered of general application.

The preliminary reduction of osazones of the disaccharides makes it possible to use the Kjeldahl method for these compounds, although glucosazone and mannose phenylhydrazone give high results. Dorfmüller (24) stated that satisfactory results for the osazones of disaccharides and mannose phenylhydrazone are obtained by a preliminary reduction with sodium hyposulfite in sodium carbonate solution, although glucosazone gives low results. There is no reliable general method for this type of compound, and each must be considered as an individual case.

Weizmann, Yopf, and Kirzors (141) have recommended the use of fuming sulfuric acid (7 per cent sulfur trioxide) and zinc as a reducing agent for nitro compounds, and have reported good results on *o*- and *p*-nitrophenols, nitronaphthalene, and picric acid.

Another method for the reduction of nitro and azo compounds is Simek's (122) use of saturated sodium hydrosulfite solution. The sample is refluxed for 30 min., which is usually sufficient for most substances, although trinitrodihydroxybenzene requires longer. The excess sodium hydrosulfite is decomposed, the water carefully driven off, and the digestion continued in the usual manner.

The use of hydriodic acid and phosphorus (41), which has been applied to the microchemical determination, is helpful in reducing hydrazine, nitro, nitroso, and azo derivatives prior to digestion. Volatile substances, or those liberating nitrogenous decomposition products, must be reduced with hydriodic acid in a sealed tube. Diazo compounds, if first coupled with phenol to form a stable azo derivative, can be analyzed satisfactorily by this method. The use of pure dextrose (49) has been found to be helpful in determining nitrogen in azo and nitro compounds.

From the data already presented, the obvious conclusion is that there is no general method available by which all types of nitrogen can be determined. Certainly progress has been made, however, inasmuch as the nitrogen of many compounds can now be determined quantitatively through the medium of preliminary reduction, or addition of specific reagents.

VII. THE RELATION OF MICROCHEMISTRY TO ORGANIC NITROGEN

The micro Kjeldahl determination does not differ, in principle at least, from the macro method,—aside from constructional and manipulative

details. It has given invaluable aid to biological and physiological research, where only small quantities of materials are available. Much of the technique was devised and many of the principles of microanalysis were laid down by Fritz Pregl, to whom necessity was the mother of invention in the matter of analyzing extremely small samples.

Numerous articles are available, dealing with modifications of the method, technique, and apparatus. Since the micro method is, as stated above, the same in principle as the macro method, the same precautions apply. Any further discussion would seem unnecessary and superfluous.

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