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83 Total Nitrogen[1]

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83–1 INTRODUCTION

The determination of total nitrogen in soils and other complex heterogeneous materials containing several forms of N presents many difficulties, and with soils these difficulties are increased by the inadequacy of knowledge concerning the forms of N present and by the low N content of the material under analysis. The total-N content of soils ranges from <0.02% in subsoils to >2.5% in peats; the surface layer of most cultivated soils contains between 0.06 and 0.5% N.

Two methods have gained general acceptance for determination of total-N: the Kjeldahl method, which is essentially a wet-oxidation procedure, and the Dumas method, which is fundamentally a dry-oxidation (i.e., combustion) technique.

In the Kjeldahl method, the N in the sample is converted to ammonium (NH_4^+) by digestion with concentrated H_2SO_4 containing substances which promote this conversion, and the ammonium is determined from the amount of NH_3 liberated by distillation of the digest with alkali. In the original Kjeldahl (1883) procedure, H_2SO_4 alone was employed for digestion, $KMnO_4$ being used to complete the oxidation of organic matter. However, the extensive investigations of the Kjeldahl technique stimulated by the great practical importance of this remarkably simple method of determining N have shown that both the speed and completeness of conversion of organic-N to ammonium by digestion with H_2SO_4 can be increased by adding salts to raise the temperature of digestion, or by adding catalysts to promote oxidation of organic matter. In practically all Kjeldahl methods now employed, K_2SO_4 or Na_2SO_4 is used to raise the temperature of digestion, and catalysts such as selenium, mercury, or copper, are used to promote oxidation of organic matter.

In the classical Dumas method of determining N, the sample is heated

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with copper oxide at a high temperature (usually above 600°C.) in a stream of purified CO_2, and the gases liberated are led over hot Cu to reduce nitrogen oxides (mainly N_2O) to N_2, and then over copper oxide to convert CO to CO_2. The N_2-CO_2 mixture thus obtained is collected in a nitrometer containing concentrated alkali, which absorbs the CO_2, and the volume of N_2 gas is measured.

The standard Dumas method is a time-consuming and complicated procedure, and the comparatively rapid and simple Kjeldahl method has been used almost exclusively for determination of N in soils. However, Dyck and McKibbin (1935) analyzed 26 organic soils by Dumas and Kjeldahl methods, and found that with every sample tested, the Dumas method gave a considerably higher N value. Similar results were obtained with organic soils in comparative work reported by Bremner and Shaw (1958), but they found that Dumas and Kjeldahl methods gave practically identical results with mineral soils. These findings have been confirmed by a recent comparison of the total-N values obtained in analysis of mineral and organic soils when Kjeldahl analysis was performed by the method described in section 83–3, and Dumas analysis was performed using the Coleman Model 29 Nitrogen Analyzer.[2]

The higher values obtained with organic soils by the Dumas method may be partly due to occlusion of atmospheric N_2 by organic matter (see Bremner and Shaw, 1958), but it seems likely that they are due mainly to incomplete combustion in the Dumas analysis with formation of methane or other hydrocarbons instead of CO_2. The Dumas method has been found to give erroneously high results with several substances, including pyrimidines, natural oils, and amides of long-chain fatty acids. Van Meter et al. (1951) found that high values obtained in Dumas analysis of shale oil and its fractions were due to formation of hydrocarbons (notably methane) during Dumas combustion, and Steyermark (1961) has found that substances containing long aliphatic chains often give very high results when analyzed by the Dumas method owing to the formation of methane instead of carbon dioxide during the combustion (see also Kirsten, 1947; Holt and Hughes, 1955).

It is commonly assumed that Dumas methods are superior to Kjeldahl procedures in that they are universally applicable, but this assumption is erroneous. The customary Dumas methods fail with many compounds, particularly heterocyclic compounds which tend to form nitrogenous chars that are difficult to burn (see Alford, 1952). The Kjeldahl methods commonly used also fail to recover various forms of N. For example, the Kjeldahl

[2] The author is indebted to the Wilkens-Anderson Co. for assistance in the analyses by the Coleman Nitrogen Analyzer. This analyzer provides an improved, automatic method of Dumas analysis which is simpler and more rapid than the standard Dumas procedure, and it may prove useful for total-N analysis of soils. Sternglanz and Kollig (1962) have described several modifications of this method which improve its precision and accuracy and permit its use for analysis of refractory compounds.

procedures generally employed for determination of total-N in soils fail to recover organic-N in refractory heterocyclic compounds such as nicotinic acid and pyridine and in certain compounds containing N-N and N-O linkages. This raises serious doubt concerning the reliability of these methods, because the identity of a considerable fraction of soil N is still obscure. Further doubt arises from a survey of data reported in the literature on the determination of N in soils. For example, Alves and Alves (1952) compared the effectiveness of various catalysts in Kjeldahl digestion of soils by studying the effects of varying the period of digestion with each catalyst, and found that the maximal value obtained, and the period of digestion required to attain this value, depended greatly upon the catalyst employed. Apart from the wide variation in the results obtained with different catalysts, the most disturbing finding in this work was that N was lost with each of the catalysts tested when digestion was continued beyond the time required to achieve the maximal value.

However, recent work (Bremner, 1960) has provided good evidence that conventional types of Kjeldahl methods are satisfactory for determination of total-N in soils provided certain precautions are observed. This evidence was obtained by taking advantage of the fact that numerous modifications of customary Kjeldahl methods have been developed which greatly extend the scope of the Kjeldahl procedure and permit inclusion of almost all combined forms of N. Application of a selection of these modified Kjeldahl methods to a variety of soils containing from 0.03 to 2.7% N showed that with each of the soils tested, the results by the various modified methods were identical, and in close agreement with the results obtained by conventional methods. This work showed that several Kjeldahl methods which have been proposed for determination of total-N in soils, including the procedure recommended by the Assoc. Offic. Agr. Chemists (1955), are unsatisfactory, but that good results can be obtained with these methods by increasing the period of digestion specified. It also indicated that very little of the N in the soils examined was in the form of highly refractory organic-N compounds or of compounds containing N-N or N-O linkages. This investigation left little reasonable doubt that the Kjeldahl method recommended here (section 83–3) is satisfactory for total-N analysis of most soils, because none of the other methods tested gave higher values, and there are few, if any, naturally occurring combined forms of N which cannot be determined by one or another of these methods. It should be emphasized, however, that this work was designed to test the accuracy of conventional Kjeldahl methods in analysis of soils containing the usual low levels of nitrate and nitrite. Such methods do not include nitrate or nitrite, and they must be modified to include these if the soils under analysis contain significant amounts of nitrate or nitrite, or if, as in some tracer investigations using N^{15}-labeled compounds, it is essential to recover even trace amounts of nitrate or nitrite. Many investigations of N transformations

in soils, particularly studies of denitrification, have been vitiated by the use of Kjeldahl methods which fail to recover nitrate or nitrite.

No comprehensive review of the literature on the Kjeldahl method is available, but much of the important literature and information concerning the Kjeldahl procedure is discussed in articles by Kirk (1950), Lake et al. (1951), Middleton and Stuckey (1951), McKenzie and Wallace (1954), and Baker (1961). Literature on the use of the Kjeldahl method for determination of N in soils has been reviewed by Bremner (1960).

83–2 PRINCIPLES

83–2.1 General

The Kjeldahl procedures generally employed for determination of total-N involve two steps: (*1*) digestion of the sample to convert the N to ammonium; (*2*) determination of the ammonium in the digest. The digestion is usually performed by heating the sample with H_2SO_4 containing substances which promote oxidation of organic matter and conversion of organic-N to ammonium, the substances generally favored being salts such as K_2SO_4 or Na_2SO_4, which increase the temperature of digestion, and catalysts such as Hg, Cu or Se, which increase the rate of oxidation of organic matter by H_2SO_4. The ammonium in the digest is usually determined by titration of the ammonia liberated by distillation of the digest with alkali, or, when the digest contains Hg, with alkali containing sodium thiosulfate, sodium sulfide, or zinc dust.

The two-step Kjeldahl procedure described has proved satisfactory for total-N analysis of most of the nitrogenous compounds known to occur in soils and plant materials, but it does not give accurate results with compounds containing N-N and N-O linkages (e.g., azo-, nitroso- and nitrocompounds, hydrazines, hydrazones, oximes, pyrazolones, isooxazoles, 1,2-diazines, 1,2,3-triazines, nitrites, nitrates). However, most of these compounds can be analyzed successfully if the customary two-step Kjeldahl procedure is modified by inclusion of some form of pretreatment. The methods of pretreatment found to be most generally successful in Kjeldahl analysis of organic compounds containing N-N and N-O linkages are the hydriodic acid reduction method introduced by Friedrich and his co-workers (Friedrich, 1933; Friedrich et al., 1933) and the zinc-iron reduction method of Steyermark et al. (1958). Bremner (1960) found that inclusion of these pretreatments did not affect the results obtained in total-N analysis of mineral and organic soils by conventional Kjeldahl methods, which suggests that very little, if any, of the N in soils is in the form of organic compounds containing N-N or N-O linkages. However, soils sometimes contain significant amounts of inorganic-N in the form of nitrate or nitrite,

which contain N-O linkages, and this N is not recovered quantitatively by the two-step Kjeldahl procedures generally employed for determination of total-N. The pretreatments which have been employed for inclusion of nitrate, nitrite and other forms of N in Kjeldahl analysis of soils are discussed below.

83–2.2 Pretreatment of Sample

Bal (1925) found that the total-N values obtained by Kjeldahl analysis of some Indian soils containing a high percentage of clay were increased significantly when the soils were treated with water before digestion. He also noted that the residues from digestion of these soils with H_2SO_4 were much coarser and darker in color when the pretreatment with water was omitted, and that the effect of this treatment was markedly greater with the clay fractions of the soils than with the silt or fine silt fractions. From these and other observations, he concluded that the soils contained some material which cemented the soil particles together and protected organic matter inside the particles from the action of H_2SO_4, and that this cementing material was not readily soluble in concentrated H_2SO_4 but was easily dissolved by dilute H_2SO_4. Several workers (e.g., Srinivasan, 1932; Walkley, 1935; Ashton, 1936) have confirmed Bal's observations regarding the effect of treating clay soils with water before Kjeldahl analysis. Walkley (1935) found that ball-milling of such soils before analysis for total-N had an even greater effect than treatment with water, and that it also increased the values obtained in analysis for carbon. He deduced that the lower N values obtained with these soils when the treatment with water was omitted were not due to the presence of cementing materials insoluble in concentrated H_2SO_4, but to failure of the soil crumbs to disperse in this acid. Since recent work has shown that a considerable proportion of the N in some soils (particularly subsoils) is in the form of ammonium trapped in the lattices of clay minerals, these observations by Bal and Walkley suggest that the clay soils they examined contained ammonium (and possibly organic) N within the lattices of clay minerals, and that this N was not determinable by the Kjeldahl method unless the soils were first ball-milled to destroy the clay lattices or were treated with water to permit expansion of the lattices during treatment with sulfuric acid.

This explanation of the effect of the water treatment is not supported by experience using the Kjeldahl method described in section 83–3, because it has been found that the results obtained by this method with soils containing large amounts of clay and clay-fixed ammonium are not affected if the pretreatment with water is omitted (Bremner and Harada, 1959; Bremner, 1959, 1960). However, the period of digestion in this method is considerably longer than that generally adopted in Kjeldahl analysis of soils, and

it is possible that the lower values obtained by Bal and other workers when pretreatment with water was omitted were due to the use of short periods of digestion which did not effect the release of ammonium- and organic-N associated with clay minerals.

The possibility that clay-fixed ammonium is not determinable by customary Kjeldahl methods even if a pretreatment with water and a prolonged period of digestion are employed has been investigated by Bremner and Harada (1959), Bremner (1960), and Bremner et al.[3] They found that ammonium fixed on addition of ammonium to soils and clay minerals was recovered quantitatively by the Kjeldahl method described in section 83–3, and that the results obtained by this method with soils containing large amounts of clay and of indigenous clay-fixed ammonium were not increased by pretreatment of the samples with HF to destroy clay minerals before Kjeldahl digestion. However, Stewart and Porter (1963) recently found that this pretreatment led to a significant increase in the values obtained in Kjeldahl analysis of three clay soils containing large amounts of indigenous clay-fixed ammonium. Current evidence therefore suggests that ammonium fixed on addition of ammonium compounds to soils and clay minerals is readily determinable by customary Kjeldahl methods, but that the indigenous clay-fixed ammonium in some soils is not recovered quantitatively by these methods.

Soils present unusual analytical problems in that besides containing organic-N in a variety of combinations and ammonium trapped in the lattices of minerals, they sometimes contain significant amounts of nitrate and nitrite. The Kjeldahl methods usually employed for determination of total-N do not effect quantitative recovery of nitrate- or nitrite-N, but they normally include some of this N. Total-N in soils containing nitrate and nitrite cannot therefore be calculated by assuming that the N recovered by these methods represents (organic + ammonium)-N, and adding (nitrate + nitrite)-N as determined by separate procedures. The recoveries of nitrate and nitrite in Kjeldahl analysis of soils by customary methods are highly variable, and they appear to depend largely upon the amounts of water and organic matter in the sample. High recoveries can be obtained with organic soils, and loss of nitrate during digestion can be reduced by addition of organic matter in the form of glucose (Bremner and Shaw, 1958).

Three modifications of the Kjeldahl method have been used for analysis of soils containing nitrate and nitrite: the alkaline reduction modification of Davisson and Parsons (1919); the salicylic acid modification introduced by Cope (1916); and the permanganate, reduced-iron modification of Olsen (1929).

In the alkaline reduction modification, the sample is heated in a Kjeldahl flask with Devarda's alloy and alkali to reduce nitrate and nitrite to NH_3, and the NH_3 liberated is collected in H_2SO_4 in an absorption tower con-

[3] Bremner, J. M., Silva, J. A., and Waring, S. A. 1962. Unpublished work. Department of Agronomy, Iowa State University, Ames, Iowa.

nected to the Kjeldahl flask. When reduction is judged complete, the H_2SO_4 in the absorption tower is transferred to the Kjeldahl flask and used for Kjeldahl digestion of the sample. This is a tedious and complicated procedure necessitating the use of a large amount of alkali for distillation of the digest, and its application to soils has been very limited. It was originally designed for inclusion of nitrate, and its accuracy when used to include nitrite does not appear to have been tested.

In the salicylic acid modification, the sample is treated with salicylic acid dissolved in concentrated H_2SO_4, and the nitro compounds formed by reaction of salicylic acid with nitrates in acid medium are reduced to the corresponding amino compounds by heating the mixture with sodium thiosulfate or zinc dust before Kjeldahl digestion. The identity of the nitro compounds formed in the procedure has not been fully established, but work by Stalcup and Williams (1955) indicates that the main product of nitration is 5-nitrosalicylic acid, and that small amounts of 3-nitrosalicylic acid are also formed. The main defect of the salicylic acid method is that it is affected by water. The maximal amount of water which can be tolerated does not appear to have been determined. Piper (1944) states that nitration of salicylic acid does not take place if more than a trace of water is present, but Bremner and Shaw (1958) found that 50 to 75% of nitrate- and nitrite-N added to 5-g. samples of soil treated with 10 ml. of water was recovered by a salicylic acid modification of the Kjeldahl method. The drying of soil samples to eliminate interference by water in analysis by the salicylic acid modification of the Kjeldahl method involves the risk of loss of N; and extensive loss of ammonium, nitrate and nitrite can occur if the practice of drying at 105°C. is followed (see Bremner and Shaw, 1958). Another problem concerning use of the salicylic acid method for inclusion of nitrate and nitrite in Kjeldahl analysis of soils is that there appears to be no evidence that this method permits quantitative recovery of nitrite.

In the Olsen (1929) modification of the Kjeldahl method, the sample is treated before Kjeldahl digestion with potassium permanganate and sulfuric acid to oxidize nitrite to nitrate, and then with reduced iron to reduce nitrate to ammonium, the reduction being effected by the nascent hydrogen formed by reaction of the iron with the sulfuric acid used in the permanganate treatment. This method has received little attention, but recent work (Bremner and Shaw, 1958) has shown that it gives excellent results. It is directly applicable to moist or waterlogged soils and permits quantitative recovery of both nitrate and nitrite.

83–2.3 Digestion

In the literature on the Kjeldahl method, the term digestion is used to describe the process of heating with H_2SO_4 to convert organic-N to ammonium, the adjective refractory is applied to organic-N compounds which

are resistant to digestion, and the term clearing is used to describe the stage in digestion at which the digested material ceases to lose color and is free from char or other suspended material. Soil digests do not clear in the usual sense of the term, as they generally contain a considerable amount of suspended material.

Recent work (Bremner, 1960) has shown that the most important factor in Kjeldahl digestion of soils is the temperature of the treatment with H_2SO_4, which is controlled largely by the amount of K_2SO_4 (or Na_2SO_4) employed. If the concentration of K_2SO_4 is low (e.g., 0.3 g. per ml. of H_2SO_4), it is necessary to digest the sample for several hours to ensure accurate results; if it is high (e.g., 1.0 g. per ml. of H_2SO_4), short periods of digestion are adequate. Catalysts have a marked effect on the rate of digestion when the salt concentration is low, but have practically no effect when the salt concentration is high, and satisfactory Kjeldahl analysis of soil can be performed without the use of catalysts by digestion for a comparatively short time with H_2SO_4 containing a high concentration of K_2SO_4 (e.g., 1 g. of K_2SO_4 per ml. of H_2SO_4).

Kjeldahl methods which involve short periods of digestion using a high concentration of K_2SO_4 would appear to have advantages, because speed of analysis is usually an important consideration, and most of the difficulties and dangers associated with Kjeldahl digestion (bumping, spattering, etc.) increase with the period of digestion. However, the use of a high concentration of K_2SO_4 has the complication that the digest is likely to solidify on cooling,[4] which means that considerable time may be required to take up the digest with water before distillation with alkali. Digests from Kjeldahl methods using more than about 0.8 g. of K_2SO_4 per ml. of H_2SO_4 usually solidify on cooling, and when solidification occurs it is often necessary to reheat the Kjeldahl flask after addition of water to disintegrate the solid cake of digest. Bumping usually occurs during this reheating even if the flask is swirled continuously, and it is sometimes so violent that it causes fracture of the Kjeldahl flask and loss of the digest. Another disadvantage of methods using high concentrations of K_2SO_4 is that considerable frothing occurs during digestion of organic soils, so that although the period of digestion required after clearing is considerably shorter than with methods using low concentrations of K_2SO_4, the clearing time is much longer. A further disadvantage of these methods is that serious frothing also occurs during removal of water when the pretreatment with water recommended by Bal (1925) for Kjeldahl analysis of clay soils is used. However, both of these difficulties can be minimized by adding the K_2SO_4 after a preliminary digestion with H_2SO_4.

[4] It is often stated (or implied) that solidification of a digest on cooling indicates that loss of nitrogen occurred during digestion, but this is not true. When Kjeldahl digestion is performed by the method of McKenzie and Wallace (1954) using 1 g. of K_2SO_4 per ml. of H_2SO_4, the digest always solidifies on cooling, and this is one of the best methods available for Kjeldahl analysis of refractory nitrogen compounds.

The main defect of methods using high concentrations of K_2SO_4 is that they will stand less abuse than methods using low concentrations, because the risk of loss of N during Kjeldahl digestion is more serious when the initial concentration of K_2SO_4 is high. Loss of N occurs when the temperature of digestion exceeds about 400°C., and this temperature is attained when the concentration of K_2SO_4 is about 1.3 to 1.4 g. per ml. of H_2SO_4. This is much higher than the concentration usually employed for Kjeldahl digestion of soil (0.22 to 0.33 g. of K_2SO_4 per ml. of H_2SO_4), and appreciably above the concentration required for rapid Kjeldahl digestion of refractory N compounds such as tryptophan and nicotinic acid (1.0 g. of K_2SO_4 per ml. of H_2SO_4). However, loss of H_2SO_4 occurs during Kjeldahl digestion, and this leads to an increase in the salt concentration and the temperature of digestion. Under the conditions normally employed for Kjeldahl digestion, loss of H_2SO_4 by volatilization and decomposition is very small if a properly designed digestion stand is employed, but a significant amount of H_2SO_4 is consumed during Kjeldahl digestion of soil in oxidation of organic matter and reactions with the mineral constituents. Determinations of the amounts of H_2SO_4 consumed during Kjeldahl digestion of mineral and organic soils have shown that there is practically no danger of loss of N due to rise in temperature caused by consumption of H_2SO_4 when the low-salt-concentration Kjeldahl methods recommended here are used, even if the amount of soil taken for analysis is twice the amount specified (Bremner, 1960). This does not hold for Kjeldahl methods involving high concentrations of K_2SO_4; and, if such methods are adopted, consumption of H_2SO_4 must be taken into consideration, and allowed for if necessary, particularly if the methods are modified to include nitrate or nitrate plus nitrite.

If sufficient information is available regarding the soils to be analyzed, acid consumption during Kjeldahl digestion can be calculated fairly accurately from the data in Table 83–1, which shows the amounts of concentrated H_2SO_4 consumed during Kjeldahl digestion by various soil constituents and by reagents used in modifications of the Kjeldahl method to include nitrate. The acid-consumption value of the organic fraction of soils is much higher than the corresponding values of the inorganic soil constituents, and acid consumption during Kjeldahl digestion of organic soils can exceed 6 ml. per g. of soil. The fact that ferric and aluminum oxides have higher values than other inorganic soil constituents suggests that soils rich in sesquioxides may require special attention in Kjeldahl analysis. Piper (1944) apparently encountered difficulties in Kjeldahl analysis of ferruginous and lateritic soils, because he recommends that the amount of H_2SO_4 normally employed for macro-Kjeldahl digestion of soils be increased for their analysis.

Calculations from Table 83–1 show that about 9.7 ml. of concentrated H_2SO_4 are consumed in Kjeldahl digestion of the 1 g. of salicylic acid and 5 g. of sodium thiosulfate used in the customary salicylic acid modification

Table 83–1. Amounts of H_2SO_4 consumed by various materials during Kjeldahl digestion (Bremner, 1960).

Material	Acid consumption
	ml. $36N$ H_2SO_4/g. material
Soil organic carbon	10.0
Soil organic matter	5.8*
Al_2O_3	1.63
Fe_2O_3	1.04†
Clay	0.60
$CaCO_3$	0.55
Silt	0.30
Sand	0
Salicylic acid	6.76
$Na_2S_2O_3 \cdot 5H_2O$	0.58
Reduced iron	1.50†

* Calculated from organic carbon value on assumption that soil organic matter contains 58% carbon.
† Calculated on assumption that digestion product is ferric sulfate.

of the macro-Kjeldahl method of soil analysis to include nitrate. This is a very significant consumption of H_2SO_4, considering that only 30 ml. of H_2SO_4 are generally employed in this method, and it illustrates the importance of making allowance for H_2SO_4 consumption when Kjeldahl methods involving high concentrations of K_2SO_4 are modified to include nitrate.

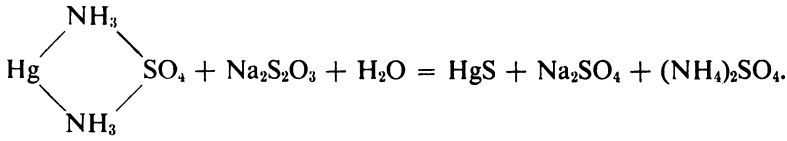
Consumption of H_2SO_4 is not the only factor to be considered in gauging the risk of loss of N during Kjeldahl digestion of soil, because soils differ from most materials analyzed by the Kjeldahl method in having a high mineral content. The reaction of H_2SO_4 with the mineral constituents of soils leads to the formation of salts, and they increase the temperature of digestion. However, most of the salts formed during Kjeldahl digestion of soils (calcium sulfate, ferric sulfate, aluminum sulfate, etc.) are not very soluble in concentrated H_2SO_4, and their effect on the temperature of digestion is probably very small compared with the effects of the salts formed by Kjeldahl digestion of the reagents employed in modifications of the Kjeldahl method to include nitrate and nitrite. The influence of these reagents on the temperature of digestion does not appear to have been studied, but there seems little doubt that the sodium thiosulfate used in the salicylic acid modification of the Kjeldahl method has a marked effect on the temperature of digestion.

Sodium sulfate is often used instead of K_2SO_4 to raise the temperature of digestion, but the author has found that this increases spattering during Kjeldahl digestion of soils.

The catalysts most frequently employed for Kjeldahl digestion are those containing Se, Hg and Cu. Their efficiency in producing rapid clearing in Kjeldahl digestion of soil decreases in the order Se > Hg > Cu. Selenium

is not significantly more effective than Hg when catalysis of both clearing and conversion of organic-N to ammonium are considered, but both Se and Hg are considerably more effective than Cu. Considerable controversy exists concerning reports that Se causes loss of N when used as a catalyst in Kjeldahl digestion, but a critical evaluation of the literature on this subject indicates that Se is a safe and effective catalyst when properly used, and that loss of N occurs only if it is used in excessive quantity, or with large amounts of K_2SO_4 or Na_2SO_4, and digestion is fairly prolonged. Recent work (Bremner, 1960) has confirmed these conclusions and has shown that no loss of N occurs during Kjeldahl digestion of soils by the selenium-catalyzed methods recommended here even when digestion is continued for as long as 12 hours.

It is difficult to evaluate the extensive literature on the effectiveness of different catalysts in Kjeldahl digestion of organic-N compounds, but if effects on both rate of clearing and of conversion of organic-N to ammonium are considered, there is considerable evidence that Hg is the most effective single catalyst. However, the use of Hg as a catalyst has been discouraged by the practical difficulties encountered in the determination of ammonium in digests containing Hg. When a digest containing Hg^{2+} is treated with alkali, some of the ammonium in the digest reacts with the mercuric oxide precipitated by the alkali to form a mercury-ammonium complex, and the ammonium in this complex is not readily liberated by distillation with alkali. To avoid low results in the determination of ammonium, it is necessary to add sodium sulfide or thiosulfate to precipitate Hg^{2+} as HgS, or Zn dust to reduce the HgO to metallic mercury. The reaction involved in the decomposition of the mercury-ammonium complex by thiosulfate has been represented as follows (Clark, 1943):



$$\begin{matrix} & NH_3 & \\ Hg & & SO_4 \\ & NH_3 & \end{matrix} + Na_2S_2O_3 + H_2O = HgS + Na_2SO_4 + (NH_4)_2SO_4.$$

Numerous difficulties have been experienced with the techniques used to prevent interference by mercuric oxide in the determination of ammonium. For example, it has been found that they can lead to the evolution of H_2S, to the appearance of metallic mercury in the distillate, to serious bumping when the Hg is precipitated as the sulfide, and to the formation of a black deposit in the condenser of the distillation apparatus. McKenzie and Wallace (1954) found that when sodium thiosulfate was used to eliminate interference by mercuric oxide, the success of the method was dependent upon the ratio of thiosulfate to mercuric oxide. Some red mercuric oxide was precipitated when the $Na_2S_2O_3 \cdot 5H_2O/HgO$ ratio was 3, whereas black mercuric sulfide was always precipitated when the ratio was 5. These ob-

servations have been confirmed, and it has been further observed that if a considerable excess of alkali is used for distillation after precipitation of Hg as the sulfide, the mixture turns yellow, and metallic mercury distills into the flask used to collect the ammonia (see Clark, 1943; Bremner, 1960). The precise reasons for some of the difficulties experienced in distillation of ammonium from digests containing Hg are still obscure, but the author has found that these difficulties can always be overcome by some modification of the prescribed method of distillation. This suggests that it should not be difficult to develop a satisfactory Hg-catalyzed method for Kjeldahl digestion of soils. Efforts in this direction seem desirable, because apart from the fact that Hg is a highly effective catalyst, the use of Hg as catalyst apparently does not involve the risk of loss of N associated with the use of Se.

Kjeldahl methods in which digestion is performed using 0.6 to 0.7 g. of K_2SO_4 per ml. of H_2SO_4 have distinct advantages over methods employing lower concentrations of K_2SO_4 in respect to both speed and completeness of digestion of organic-N (particularly if Hg is used as catalyst), and they appear to be free of some of the defects of methods employing higher concentrations of K_2SO_4 (e.g., frothing, solidification of digest, risk of loss of N). However, experience with these methods is presently too limited to permit an evaluation of their suitability for soil analysis, and their accuracy when modified to include nitrate and nitrite in Kjeldahl analysis of soils has not been studied. In contrast, the Kjeldahl method recommended here has been found to give highly reproducible results with a wide variety of soils, and to give excellent recoveries of nitrate and nitrite when modified as described in section 83–5.

It has been found that the use of sealed tubes permits digestion at temperatures above 400°C. without loss of N and allows complete oxidation of heterocyclic nitrogen compounds without addition of the substances usually employed to promote conversion of organic-N to ammonium in digestion with H_2SO_4 (e.g., White and Long, 1951; Grunbaum et al., 1952). The use of sealed tubes for digestion also eliminates losses of N through bumping and prevents contamination with ammonia from laboratory air, and Stevenson (1960) has found that these advantages make the sealed-tube technique particularly suitable for Kjeldahl analysis of rocks, silicate minerals, and subsurface soils.

83–2.4 Determination of Ammonium (NH_4^+)

Various colorimetric, titrimetric and gasometric methods for direct determination of ammonium in Kjeldahl digests have been proposed, but the determination is usually performed by the more reliable and accurate Kjeldahl procedure involving estimation of the NH_3 liberated by distillation

of the digest with strong alkali. In the distillation method which has been most commonly employed, the NH_3 liberated by distillation is collected in an accurately measured amount of standard mineral acid (usually H_2SO_4), and determined by titration of the excess acid with standard alkali (usually NaOH) using indicators such as methyl red, bromocresol green, and methyl red-methylene blue mixtures. This method gives excellent results, but Winkler's (1913) modification in which the NH_3 is distilled into H_3BO_3 and titrated with standard H_2SO_4 is equally accurate, and it has the advantage that it is a direct method requiring only one standard reagent, whereas the original procedure is a difference method requiring two standard reagents. Neither the volume nor the strength of the H_3BO_3 solution used to collect the distillate needs to be known accurately, because the ammonium borate formed by reaction of NH_3 with H_3BO_3 is titrated back to H_3BO_3 when the distillate is titrated with H_2SO_4:

$$NH_3 + H_3BO_3 = NH_4^+ + H_2BO_3^-$$
$$H^+ + H_2BO_3^- = H_3BO_3.$$

A further advantage of the Winkler modification is that a sufficiently large excess of H_3BO_3 can be used to ensure complete absorption of NH_3 even if the amount liberated is larger than anticipated. The use of H_3BO_3 solution containing the titration indicator has additional advantages, because the color changes which occur when the distillate is collected in H_3BO_3-indicator solution serve to indicate both the presence of sufficient alkali in the digest for distillation of NH_3 to occur and the completion of the NH_3 distillation. Several indicators, including methyl orange (Winkler, 1913), Congo red (Winkler, 1913), bromophenol blue (Scales and Harrison, 1920), and methyl red (Meeker and Wagner, 1933) have been employed for the titration with standard mineral acid using Winkler's modification, but the general experience has been that the sharpest end-points are obtained using mixed indicators such as tetrabromophenol blue-methyl red (Stover and Sandin, 1931), methylene blue-methyl red (Meeker and Wagner, 1933), bromocresol green-methyl red (Ma and Zuazaga, 1942), and bromocresol green-paranitrophenol-new coccine (Sher, 1955).

The NH_3 liberated by distillation of Kjeldahl digests using Winkler's method is usually determined by titration of the distillate with H_2SO_4, but the titration can be performed with sulfamic acid (see Wagner et al., 1952) or potassium biiodate (see McKenzie and Wallace, 1954), and these reagents have the advantage that they can be used as primary standards.

The distillation of NH_3 from Kjeldahl digests presents few difficulties apart from those encountered with digests containing Hg (see section 83–2.3), and most of these difficulties can be readily overcome by some modification of the method of distillation. For example, the problems caused by bumping during distillation of Kjeldahl digests can be eliminated by the use of steam to liberate the NH_3.

Three macro-Kjeldahl methods for soil analysis are described below. The first is described as the regular method to indicate that it is applicable in the usual situation in which the soil sample contains only a small quantity of nitrate- or nitrite-N, and it is not necessary to include this N. The second is a salicylic acid modification of the regular method to include nitrate,[5] and the third an Olsen-type modification of the method to include both nitrate and nitrite. A semimicro-version of the regular Kjeldahl method is also described.

83–3 REGULAR MACRO-KJELDAHL METHOD[6]

83–3.1 Special Apparatus

1. Macro-Kjeldahl digestion flasks (350 or 500 ml.).
2. Macro-Kjeldahl digestion stand.
3. Macro-Kjeldahl distillation apparatus.

83–3.2 Reagents

1. Sulfuric acid (H_2SO_4), concentrated.[7]
2. Potassium sulfate (K_2SO_4).
3. Copper sulfate ($CuSO_4 \cdot 5H_2O$).
4. Selenium.
5. Sodium hydroxide (NaOH), approximately 10*N*: Place 4.2 kg. of NaOH in a heavy-walled 10-liter Pyrex flask, add 4 liters of water, and swirl the flask until the alkali is dissolved. Cool the solution with a rubber stopper in the neck of the flask to prevent absorption of atmospheric CO_2, and allow it to stand for several days to permit any Na_2CO_3 present to settle. Siphon the clear supernatant liquid into a large Pyrex bottle which contains about 1.5 liters of CO_2-free water and is marked to indicate a volume of 10 liters, and make the solution to 10 liters by addition of CO_2-free water. Then swirl the bottle vigorously to mix the contents, and fit the neck with some arrangement which permits the alkali

[5] This modification is included because it may be more convenient than the Olsen-type modification for total-N analysis of air-dried soil samples known to contain significant amounts of nitrate and little or no nitrite. However, for reasons outlined in section 83–2.2, the Olsen-type modification appears preferable for most analytical work requiring inclusion of nitrate or nitrite.

[6] Bremner and Shaw (1958), Bremner (1959, 1960).

[7] A convenient arrangement for storing and dispensing the concentrated H_2SO_4 and NaOH solution required for macro-Kjeldahl analysis of soil has been described by Piper (1944). The H_2SO_4 can also be dispensed conveniently by the automatic burettes designed for use with the standard 5-pint acid bottles used in the chemical industry.

to be stored and dispensed with protection from atmospheric CO_2 (see Piper, 1944; Jackson, 1958).

6. Boric acid-indicator solution: Place 80 g. of pure boric acid (H_3BO_3) in a 5-liter flask marked to indicate a volume of 4 liters, add about 3,800 ml. of water, and heat and swirl the flask until the H_3BO_3 is dissolved. Cool the solution, and add 80 ml. of mixed indicator solution prepared by dissolving 0.099 g. of bromocresol green and 0.066 g. of methyl red in 100 ml. of ethanol. Then add 0.1*N* NaOH cautiously until the solution assumes a reddish purple tint (pH ca. 5.0), and make the solution to 4 liters by addition of water. Mix the solution thoroughly before use.
7. Sulfuric (or hydrochloric) acid (H_2SO_4 or HCl), 0.05*N* standard.

83–3.3 Procedure

Place a sample containing about 10 mg. of N in a dry macro-Kjeldahl flask, add 20 ml. of water; and, after swirling the flask for a few minutes, allow it to stand for a further 30 minutes. Then add 10 g. of K_2SO_4, 1 g. of $CuSO_4 \cdot 5H_2O$, 0.1 g. of Se and 30 ml. of concentrated H_2SO_4, and heat the flask cautiously on the digestion stand. When the water has been removed and frothing has ceased, increase the heat until the digest clears, and thereafter boil the mixture gently for 5 hours. Regulate the heating during this boiling so that the H_2SO_4 condenses about one-third of the way up the neck of the flask.

After completion of digestion, allow the flask to cool, and add about 100 ml. of water (slowly, and with shaking). Then cool the flask under a cold-water tap, and transfer the contents to a 1-liter conical (or 800-ml. Kjeldahl) flask for distillation. As far as is practicable, retain any sandy residue in the digestion flask during this transfer, because sand can cause severe bumping during Kjeldahl distillation. Four washings of the sandy residue with 50 ml. of water are usually adequate for quantitative transfer of the ammonium in the digest.

To determine the ammonium-N liberated by digestion, place a 500-ml. Erlenmeyer flask containing 50 ml. of H_3BO_3-indicator solution under the condenser of the distillation apparatus so that the end of the condenser is below the surface of the H_3BO_3. Then hold the distillation flask at a 45° angle, add a teaspoonful of pumice, and pour about 150 ml. of 10*N* NaOH down the neck so that the alkali reaches the bottom of the flask without mixing appreciably with the digest. Attach the flask as quickly as possible to the distillation apparatus, mix the contents thoroughly by swirling, and immediately commence distillation. Regulate the heating to prevent suck-back of H_3BO_3 and to minimize frothing or bumping during distillation, and check that the flow of cold water through the condenser is sufficient to keep the temperature of the distillate $<35°C$. When about 150 ml. of distillate have

been collected, lower the receiver flask so that the end of the condenser is above the surface of the distillate; and, after rinsing the end of the condenser with water, remove the flask and stop distillation. Determine ammonium-N in the distillate by titration with 0.05*N* H_2SO_4 using a 25-ml. burette graduated at 0.1-ml. intervals (1 ml. 0.05*N* $H_2SO_4 \simeq 0.7$ mg. ammonium-N). The color change at the end-point is from green to pink.

83–4 MACRO-KJELDAHL METHOD TO INCLUDE NITRATE[8]

83–4.1 Special Apparatus

Same as in section 83–3.1.

83–4.2 Reagents

1. Reagents 1 to 7 described in section 83–3.2.
2. Salicylic acid-sulfuric acid mixture: Dissolve 50 g. of salicylic acid in 2 liters of concentrated H_2SO_4.
3. Sodium thiosulfate: Powder crystals of $Na_2S_2O_3 \cdot 5H_2O$ to pass a 20-mesh screen.

83–4.3 Procedure

Place a sample containing about 10 mg. of N in a dry macro-Kjeldahl flask, add 40 ml. of salicylic acid-sulfuric acid mixture, and swirl the flask until the acid is thoroughly mixed with the soil. Allow the mixture to stand several hours (or overnight), add 5 g. of sodium thiosulfate through a dry thistle funnel having a long stem that reaches down into the bulb of the Kjeldahl flask, and heat the mixture cautiously on the digestion stand until frothing has ceased. Then cool the flask, add 20 ml. of water, 10 g. of K_2SO_4, 1 g. of $CuSO_4 \cdot 5H_2O$ and 0.1 g. of Se, and proceed as described in section 83–3.3 for digestion and determination of ammonium by the regular method.

83–5 MACRO-KJELDAHL METHOD TO INCLUDE NITRATE AND NITRITE[9]

83–5.1 Special Apparatus

Same as in section 83–3.1.

[8] Salicylic acid version of regular method (section 83–3) modified to include treatment with water recommended by Bal (1925).

[9] Olsen (1929) method as modified by Bremner and Shaw (1958).

83–5.2 Reagents

1. Reagents 1 to 6 described in section 83–3.2.
2. Potassium permanganate ($KMnO_4$) solution: Dissolve 50 g. of $KMnO_4$ in 1 liter of water, and store the solution in an amber bottle.
3. Dilute sulfuric acid (H_2SO_4): To 1 liter of water in a 4-liter Pyrex flask add slowly, and with continuous shaking, 1 liter of concentrated H_2SO_4.
4. Reduced iron:[10] Use a finely divided product, and sieve it to remove any material which does not pass a 100-mesh screen.
5. *n*-octyl alcohol.
6. Sulfuric (or hydrochloric) acid (H_2SO_4 or HCl), 0.01*N* standard.

83–5.3 Procedure

Place a sample containing about 10 mg. of N in a dry macro-Kjeldahl flask, add sufficient water to bring the total water content to around 10 ml.; and, after swirling the flask for a few minutes, allow it to stand for a further 30 minutes. Then add 10 ml. of $KMnO_4$ solution; and, after swirling the flask for about 30 seconds, hold it at a 45° angle, and pipette 20 ml. of dilute H_2SO_4 down the neck using a pipette with a fine tip to ensure slow addition of the acid. Swirl the flask continuously during this operation; and, when the addition of acid has been completed, allow the flask to stand for 5 minutes, and add 2 drops of octyl alcohol. Then add 5 $\pm$0.1 g. of reduced iron through a dry thistle funnel having a long stem that reaches down into the bulb of the Kjeldahl flask; and immediately place a 25-ml. Erlenmeyer flask in inverted position in the neck of the Kjeldahl flask, and swirl the flask to bring the iron into contact with the acid. Allow the flask to stand until the initial strong effervescence has ceased (about 15 minutes), and then heat it gently on the digestion stand so that the mixture just boils. Keep the Erlenmeyer flask in the neck of the Kjeldahl flask during this process to limit the loss of water; and, after heating the mixture for 45 minutes, turn off the heater, allow the Kjeldahl flask to cool, and remove the Erlenmeyer flask. Then add 10 g. of K_2SO_4, 1 g. of $CuSO_4 \cdot 5H_2O$, 0.1 g. of Se, and 30 ml. of concentrated H_2SO_4, and heat the flask cautiously on the digestion stand. When the water is removed and frothing ceases, increase the heat until the mixture assumes a yellowish-green color, and complete the digestion by boiling the mixture gently for 5 hours.

After completion of digestion, allow the Kjeldahl flask to cool, and add about 150 ml. of water, slowly and with shaking. If the digest has solidified

[10] A satisfactory product is supplied by The British Drug Houses Ltd., Poole, Dorset, England. This can be obtained in the USA from The Ealing Corporation, 33 University Road, Cambridge 38, Mass.

on cooling, and the solid cake of digest does not disintegrate during addition of water, heat the flask cautiously with vigorous swirling until the digest is in a fluid condition. Then cool the flask under a cold-water tap, and transfer the diluted digest by means of a funnel to a 500-ml. volumetric flask. Rinse the Kjeldahl flask 4 times with about 70 ml. of water to complete the transfer, and rinse the stem of the funnel with water until the level of the liquid in the volumetric flask is about 2 cm. below the graduation mark. Then allow the flask to stand; and, when it has reached room temperature, add water to make 500 ml., and mix the contents thoroughly.

To determine ammonium in the digest, place a 250-ml. Erlenmeyer flask containing 10 ml. of H_3BO_3-indicator solution under the condenser of the distillation apparatus so that the end of the condenser is in contact with the H_3BO_3. Then mix the contents of the volumetric flask thoroughly by inverting the flask several times, and immediately transfer an aliquot (usually 50 or 100 ml.) of the diluted digest to a 500-ml. Kjeldahl flask containing two glass beads (to prevent bumping) and a few drops of liquid paraffin (to prevent frothing). Add sufficient water to give a total volume of about 200 ml., hold the Kjeldahl flask at a 45° angle, and pour sufficient 10*N* NaOH down the neck to ensure an excess of about 10 ml. of 10*N* NaOH. The amount of alkali required depends upon the amount of H_2SO_4 consumed during digestion and the volume of the aliquot taken for distillation. It is convenient to use 25 ml. of 10*N* NaOH for a 50-ml. aliquot, and 50 ml. for a 100-ml. aliquot. Immediately after addition of the alkali, connect the flask to the distillation apparatus, swirl the flask to mix the contents, and commence distillation. Regulate the heating so that about 10 ml. of distillate are collected per minute, and check that the flow of cold water through the condenser is sufficient to keep the temperature of the distillate below 35°C. When about 100 ml. of distillate have been collected, lower the receiver flask so that the end of the condenser is above the surface of the distillate, rinse the end of the condenser with water, remove the receiver flask, and stop distillation. Determine ammonium-N in the distillate by titration with 0.01*N* H_2SO_4 using a 25-ml. burette graduated at 0.02-ml. intervals (1 ml. 0.01*N* $H_2SO_4 \simeq 0.14$ mg. ammonium-N). The color change at the end-point is from green to pink.

83–6 COMMENTS ON MACRO-KJELDAHL METHODS

83–6.1 Preparation of Sample

Jackson (1958) recommends that soil samples for macro-Kjeldahl analysis be ground to pass a 0.15-mm. (100-mesh) screen to ensure complete oxidation of the organic matter within the small aggregates. It does not appear to be necessary to follow this recommendation to obtain accurate re-

sults in macro-Kjeldahl analysis of mineral soils, because analyses of a wide range of mineral soils by the macro-Kjeldahl method described in section 83–3 have not revealed significant differences between the results obtained using samples ground to pass 100- and 32-mesh screens. Bremner and Shaw (1958) analyzed 14 mineral soils which varied in texture from a coarse sandy loam to a heavy clay loam, and in N content from 0.07 to 0.54%, by the methods described in sections 83–3 and 83–5, and obtained highly consistent results using 5-g. samples ground to pass a 0.5-mm. (32-mesh) screen. Reproducible results were also obtained with most of the soils using samples ground to pass a 2-mm. (9-mesh) screen, the exceptions being soils containing large amounts of medium and coarse sand. Organic soils must be ground more finely than mineral soils to ensure that a representative sample is obtained. It is recommended that soils containing $<0.5\%$ N be ground to pass a 32-mesh screen, that soils containing 0.5 to 1.0% N be ground to pass a 60-mesh screen, and that soils containing $>1.0\%$ N be ground to pass a 100-mesh screen.

83–6.2 Digestion

Many types of gas- and electrically-heated digestion stands for macro-Kjeldahl analysis are available commercially, and comment on their suitability for soil analysis must be limited to the following general statements: (*1*) The gas or electric heaters should have individual controls which permit rapid adjustment of the heat, and they should provide sufficient heat to cause brisk boiling of a mixture of 30 ml. of concentrated H_2SO_4 and 30 g. of K_2SO_4. At full heat the gas heaters on a digestion stand found satisfactory by the author for macro-Kjeldahl analysis of soil brought 100 ml. of water in a 350-ml. Pyrex Kjeldahl flask to a rolling boil in approximately 3.5 minutes. (*2*) The design should be such that the Kjeldahl flask is supported at an angle $<45°$ from the horizontal and is exposed to direct heat only in the region occupied by liquid during digestion. Serious loss of N can occur during digestion if the flask is exposed to the heater above the level of the liquid. (*3*) The insulation and venting should be such that a mixture of 30 ml. of concentrated H_2SO_4 and 10 g. of K_2SO_4 can be boiled continuously for several hours with the H_2SO_4 condensing about one-third of the way up the neck of the digestion flask.

Whatever type of digestion stand is used, it is essential to have some arrangement for effective disposal of the acid fumes formed during digestion. Jackson (1958) has described the fume-disposal system used at the University of Wisconsin for macro-Kjeldahl digestion of soils.

During the process of digestion, the Kjeldahl flasks should be rotated at intervals to dislodge any material adhering to the walls and bring it into contact with the acid. This is particularly important with clay soils, because

clay promotes spattering, and the spattered material may adhere so tenaciously to the walls of the flask that it is not washed down into the digestion mixture by the condensing H_2SO_4.

Bumping during Kjeldahl digestion of soils can usually be eliminated by addition of glass beads or other materials which promote smooth boiling, but severe bumping which cannot be eliminated in this way is sometimes encountered during Kjeldahl digestion of sandy soils. Kjeldahl methods involving short periods of digestion using a high concentration of K_2SO_4 may prove useful for analysis of these soils, because the severity of bumping usually increases with the period of digestion. The danger of loss of N due to spattering and bumping during digestion is considerably reduced if the Kjeldahl flask is supported at an angle $<45°$ from the horizontal.

Repeated tests by Bremner (1959, 1960), using a wide variety of soils, have failed to reveal a single case in which the pretreatment with water recommended by Bal (1925) has affected the results of Kjeldahl analysis by the regular method described here, and it seems very doubtful if this pretreatment is necessary when the regular method is used. However, its inclusion does not add materially to the time required for the analysis, and it may prove to be a safeguard against low results in analysis of certain clay soils. In the salicylic acid modification of the Kjeldahl method to include nitrate, the water cannot be added before treatment of the soil sample with the salicylic acid-sulfuric acid mixture, because water affects the recovery of nitrate by this treatment.

In some salicylic acid modifications of the Kjeldahl method, Zn is used instead of sodium thiosulfate for reduction of the nitro-compounds formed by reaction of nitrates with salicylic acid. If such modifications are adopted, it is essential to use Zn dust; granulated Zn is unsatisfactory.

The 5-hour period of digestion after clearing specified in the three methods described need not be adopted if it is not essential to obtain highly accurate results. The ammonium-N produced by digestion of soils for 2 hours is rarely less than 98% of the amount formed by digestion for 5 hours; and, with most soils, the results of analyses using 2- and 5-hour periods of digestion are identical. A 1-hour period of digestion is adequate for routine analysis of soils and for other analytical work which does not require high accuracy.

83–6.3 Determination of Ammonium

In some macro-Kjeldahl methods recommended for soil analysis (e.g., Assoc. Offic. Agr. Chemists, 1955; Jackson, 1958) the transfer of the soil digest for distillation of ammonium is eliminated by connecting the digestion flask directly to the distillation apparatus, but serious bumping is often encountered when this technique is used, particularly with sandy soils.

Granulated Zn is frequently added before distillation of Kjeldahl digests with alkali, as it reacts with hot alkali to liberate hydrogen, and this promotes smooth boiling and decreases the danger of the distillate sucking back into the distillation flask. However, Perrin (1953) found that the use of Zn to prevent bumping in distillation of digests containing Cu led to carryover of alkali into the distillate, apparently because Cu greatly increases the volume of hydrogen generated by Zn during the distillation with alkali. Campbell and Hanna (1937) have cautioned against the use of Zn in combination with Se, as it may give rise to noxious fumes of H_2Se.

It is often advantageous to make the digest to volume and distill an aliquot. This minimizes the danger of bumping or frothing during distillation, permits replication of the ammonium determination, and allows this determination to be performed by methods which may be more convenient than the macro-Kjeldahl distillation technique, e.g., by steam distillation using the Hoskins apparatus (see section 83–7) or by microdiffusion methods (see McDonnell and Murphy, 1953; Black, 1957). It is preferable to distill an aliquot of the digest obtained in the method recommended to include nitrate and nitrite, because serious bumping can occur when the entire digest is distilled.

To be satisfactory, a macro-Kjeldahl distillation apparatus should have a spray-trap which removes all trace of alkali from the distillate, and a water condenser which cools so effectively that the temperature of the distillate does not exceed about 35°C. It is desirable that the base of the water jacket on the condenser be fitted with a trap to prevent water condensing on the external surface of the condenser from entering the receiver flask, and that the end of the condenser be fitted with a detachable glass tube (about 25 cm. long) having a bulb about 4 cm. in diameter near the end attached to the condenser. The latter arrangement facilitates washing of the condenser tip before titration of the distillate and reduces the risk of suck-back during distillation. If suck-back does occur, the analysis can be saved by adding the residual H_3BO_3 to the distillation flask and redistilling into a fresh lot of H_3BO_3.

It is customary when using HCl or H_2SO_4 for collection of ammonia to boil the distillate for a few minutes to remove CO_2 before titration with alkali. This is not permissible when H_3BO_3 is used, because ammonia is lost if the H_3BO_3 solution is boiled (see Markley and Hann, 1925; Wingo et al., 1950). However, interference due to CO_2 should not occur if reasonable precautions are taken to ensure that the NaOH solution used for distillation is protected from atmospheric CO_2 during its preparation and storage.

The sharpness of the end-point obtained in the titration with standard acid to determine ammonium in distillates collected in H_3BO_3 depends upon the quality of the H_3BO_3, the strength of the H_3BO_3 solution, and the indicator. The better the quality of the H_3BO_3, and the more dilute the H_3BO_3 solution consistent with complete retention of ammonia, the sharper is the

end-point (see Yuen and Pollard, 1953). Various indicators have been used for the titration (see section 83–2.4), but most workers favor the bromocresol green-methyl red indicator mixture recommended by Ma and Zuazaga (1942). The author's experience is that the sharpness of the end-point obtained with bromocresol green-methyl red mixtures depends upon the source of the indicators employed, and that it can vary with different batches of indicator from the same source. It may be necessary, therefore, to alter the recommended proportions of the constituents of mixed indicators to obtain a satisfactory end-point.

The 50 ml. of 2% H_3BO_3 solution used in the macro-Kjeldahl methods described should effectively absorb about 50 mg. of ammonia-N (see Scales and Harrison, 1920; Yuen and Pollard, 1953). This is about five times the amount which will be liberated if the recommendations concerning sample size in analysis by these methods are followed.

83–6.4 Miscellaneous

The H_2SO_4 (or HCl) solutions employed for determination of ammonium in the methods described can be standardized satisfactorily using borax, anhydrous sodium carbonate, and other alkaline reagents (see Vogel, 1951). The use of tris(hydroxymethyl)aminomethane [2-amino-2(hydroxymethyl)-1,3-propanediol] is recommended, because it is now available commercially (under the trade name of THAM)[11] as a highly purified reagent which can be used as a primary acidimetric standard. This reagent has a high equivalent weight, is not appreciably hygroscopic, and is readily soluble in water. It is often stated that aqueous solutions of this substance do not absorb CO_2, but recent work has shown that they absorb CO_2 at a slow rate and should be protected from atmospheric CO_2 (Bates and Hetzer, 1961).

The only reagent employed in the methods described which is likely to contain a significant quantity of N is the reduced iron used in the Kjeldahl method to include nitrate and nitrite. Appreciable amounts of N have been detected in several batches of the product recommended, but this N is allowed for by the control analyses, and highly consistent results are obtained in these analyses using 5-g. samples of the finely divided material specified.

The procedures described can be tested using $(NH_4)_2SO_4$, $NaNO_2$, and KNO_3 to check the recovery of ammonium-N, nitrite-N and nitrate-N, respectively, and diphenylguanidine, acetanilide, or cystine to check the recovery of organic-N. Acetanilide and cystine specially purified for use as standards in N analysis can be obtained from the National Bureau of Standards, Washington, D. C.

[11] A technical data pamphlet describing the use of THAM as a primary acidimetric standard can be obtained from Fisher Scientific Co., 1458 N. Lamon Ave., Chicago, Ill.

83–7 SEMIMICRO-KJELDAHL METHOD[12]

83–7.1 Special Apparatus

1. Micro-Kjeldahl digestion flasks (30 or 50 ml.).
2. Micro-Kjeldahl digestion stand.
3. Hoskins (1944) steam distillation apparatus. This apparatus is available commercially (A. Gallenkamp & Co. Ltd., Sun Street, London, England) with interchangeable micro- and macro-distillation chambers. The macro-unit is required for the method described here. A modified macro-unit specially designed by Edwards and Bremner[13] for distillation and determination of ammonium in nitrogen isotope-ratio analysis is shown in Fig. 83–1. The main modifications of the Hoskins design in this appara-

[12] Bremner (1960).

[13] Edwards, A. P., and Bremner, J. M. 1961. Unpublished work. Department of Agronomy, Iowa State University, Ames, Iowa.

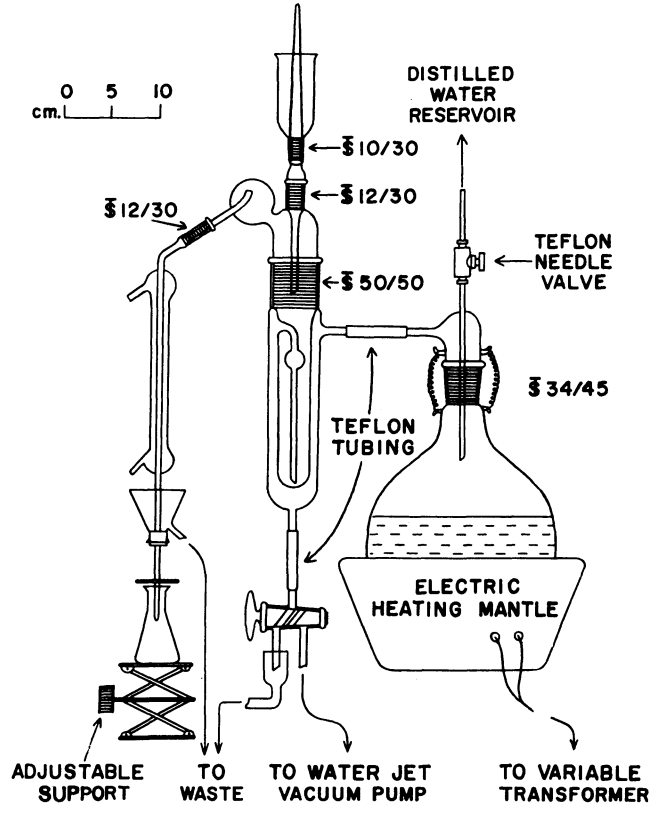


Fig. 83–1. Modified Hoskins steam-distillation apparatus.[13]

tus are as follows: (*a*). The base of the funnel of the apparatus is fitted with a standard-taper (10/30) ground joint and funnel plug instead of a stopcock. (*b*). The condenser and spray trap are connected by a standard taper (12/30) ground joint. (*c*). The base of the water jacket on the condenser is fitted with a condensate trap. (*d*). The tube at the base of the steam jacket of the distillation chamber is connected by teflon tubing to a three-way stopcock which is connected to a water-jet vacuum pump. (*e*). The steam is generated by electrical heating of distilled water in a 5-liter flask which is connected to a reservoir of distilled water.

83–7.2 Reagents

1. Reagents 1, 5 and 6 described in section 83–3.2.
2. Potassium sulfate-catalyst mixture: Prepare an intimate mixture of 100 g. of K_2SO_4, 10 g. of copper sulfate ($CuSO_4 \cdot 5H_2O$), and 1 g. of Se. Powder the reagents separately before mixing, and grind the mixture in a mortar to powder the cake which forms during mixing.
3. Sulfuric (or hydrochloric) acid (H_2SO_4 or HCl), 0.01*N* standard.

83–7.3 Procedure

Place a sample containing about 1 mg. of N in a dry micro-Kjeldahl flask, add 2 ml. of water, and after swirling the flask for a few minutes, allow it to stand for a further 30 minutes. Then add 1.1 g. of K_2SO_4-catalyst mixture and 3 ml. of concentrated H_2SO_4, and heat the flask cautiously on the digestion stand. When the water has been removed and frothing has ceased, increase the heat until the digest clears, and thereafter boil the mixture gently for 5 hours. Regulate the heating during this boiling so that the H_2SO_4 condenses about one-third of the way up the neck of the digestion flask.

After completion of digestion, allow the flask to cool, and add about 20 ml. of water (slowly, and with shaking). Then swirl the flask to bring any insoluble material into suspension, and transfer the contents to the distillation chamber of the Hoskins apparatus through the funnel of the apparatus. Rinse the Kjeldahl flask 3 times with about 9 ml. of water to complete the transfer. Add enough water to the distillation chamber to bring the level of the solution to a mark made previously to indicate a volume of 50 ml., and close the stopcock connecting the funnel and distillation chamber. Add 5 ml. of H_3BO_3-indicator solution to a 50-ml. Erlenmeyer flask which is marked to indicate a volume of 35 ml., and place the flask under the condenser of the distillation apparatus so that the end of the condenser is about 4 cm. above the surface of the H_3BO_3. Then add 20 ml. of 10*N* NaOH to the funnel of the apparatus, and run the alkali slowly into the distillation

chamber by opening the funnel stopcock. When about 1 ml. of alkali remains in the funnel, rinse the funnel rapidly with about 15 ml. of water; and, after allowing enough of this water to run into the distillation chamber to bring the level of the solution to a mark made previously to indicate a volume of 80 ml., close the funnel stopcock, and immediately commence distillation by closing the steam by-pass tube at the base of the steam jacket of the distillation chamber.[14] When the distillate reaches the 35-ml. mark on the receiver flask, stop the distillation by opening the steam by-pass tube, rinse the end of the condenser, and determine ammonium-N in the distillate by titration with 0.01*N* H_2SO_4, using a 10-ml. burette graduated at 0.01-ml. intervals (1 ml. of 0.01*N* $H_2SO_4 \simeq 0.14$ mg. of ammonium-N). The color change at the end-point is from green to pink.

83–7.4 Comments

Semimicro-Kjeldahl methods have obvious advantages, particularly when a limited amount of sample is available for analysis; and, when properly used for soil analysis, they give highly reproducible results which are in close agreement with the results obtained using macro-Kjeldahl procedures. However, the advantages of semimicro-methods are offset by the fact that it is necessary to use finely ground soil to control sampling error, and macro-methods may be more convenient if the sample for analysis is not limited and a finely ground sample is not required for other types of analyses. Highly consistent results can be obtained in semimicro-Kjeldahl analysis of many mineral soils using material ground to pass a 60-mesh sieve, but it is generally necessary to use more finely ground material to obtain reproducible results in semimicro-analysis of organic soils or of mineral soils containing a high percentage of coarse sand. It is recommended that soils containing $<1\%$ N be ground to pass a 100-mesh (0.14-mm.) screen, and that soils containing $>1\%$ N be ground to pass a 150-mesh (0.105-mm.) screen.

Digestion stands for semimicro-Kjeldahl analysis of soils should meet the requirements previously outlined for macro-Kjeldahl digestion stands (see section 83–6.2), excepting those relating to the performance of the gas or electric heaters. To be satisfactory, a micro-Kjeldahl digestion stand should have heaters which permit brisk boiling of a mixture of 3 ml. of concentrated H_2SO_4 and 3g. of K_2SO_4, and the insulation and venting should be such

[14] Before use the distillation apparatus should be steamed out to remove traces of ammonia, and the rate of steam generation should be adjusted so that about 6 ml. of distillate are collected per minute. The flow of cold water through the condenser of the apparatus should be such that the temperature of the distillate obtained using this rate of distillation does not exceed 25°C. It is not necessary to interrupt the flow of steam to the steam jacket of the distillation chamber before addition of the digest and alkali, but the by-pass tube of the steam jacket must be kept open during these additions.

that a mixture of 3 ml. of H_2SO_4 and 1 g. of K_2SO_4 can be boiled continuously for several hours with the H_2SO_4 condensing about one-third of the way up the neck of the digestion flask. At full heat the heaters on a micro-Kjeldahl digestion stand found satisfactory by the author for soil analysis brought 25 ml. of water in a 50-ml. Pyrex Kjeldahl flask to a rolling boil in approximately 2.5 minutes.

Several types of steam-distillation units for determination of ammonium are available commercially, and most of these can be employed successfully to determine ammonium in soil digests. The macro-version of the Hoskins unit is recommended, because it has proved to be the most convenient and reliable of the various commercial steam distillation units used by the author for determination of ammonium in Kjeldahl analysis of soils, and detailed information is available concerning its use for determination of ammonium (see Bremner, 1960). Its main advantages over the much-favored Markham (1942) apparatus are that it has a large distillation chamber (80 ml. of liquid can be safely distilled) and a very efficient spray-trap. The Parnas-Wagner (1921) micro-Kjeldahl steam distillation apparatus has similar advantages, and it is recommended that this apparatus be used if the Hoskins apparatus is not available. The steam-distillation apparatus described in section 84–3.3.1 (Fig. 84–1) can also be employed, and with this apparatus the transfer of the digest for distillation can be eliminated by performing the digestion in a Kjeldahl flask which can be connected directly to the distillation apparatus for determination of ammonium.

Considerable time can be saved by employing two Hoskins units with their steam by-pass tubes connected to a water-jet vacuum pump as shown in Fig. 83–1, because the chambers of the units can then be emptied and washed very rapidly by suction. With this arrangement it is not necessary to turn off the supply of steam between successive analyses to obtain the vacuum required to empty and wash the distillation chamber, and it is easily possible to perform 80 distillations in a normal working day.

It has been found convenient to replace the 500-ml. steam-generator flask supplied with the Hoskins apparatus by a 5-liter flask, and to use an electric heating mantle[15] for generation of steam (see Fig. 83–1). With this arrangement the desired rate of distillation is readily obtained using a variable transformer to adjust the input to the heating mantle, and once this adjustment has been made it is rarely necessary to alter it during a series of distillations to maintain the desired rate of distillation. A small amount of H_2SO_4 should be added to the steam generator flask to trap any ammonium-N in the distilled water used to generate steam. It is also advisable to add pumice or glass beads to eliminate bumping in the steam generator flask, because bumping can cause the liquid in the distillation chamber to siphon into the steam jacket.

[15] Suitable mantles can be obtained from Glas-Col Apparatus Company, Terre Haute, Ind.

Unsatisfactory results are sometimes obtained if water condensing on the external surface of the condenser of the steam distillation apparatus enters the ammonia receiver flask, and a trap for this condensate (see Fig. 83–1) or a collar of filter paper should be attached to the base of the water jacket on the condenser to prevent this. It is not necessary to distill with the end of the condenser under the surface of the H_3BO_3.

The method described is a semimicro-version of the regular macro-Kjeldahl procedure described in section 83–3, and like the macro-method, it does not include nitrate or nitrite. However, it can be readily modified to include nitrate or nitrate plus nitrite by the use of semimicro-versions of the pretreatments employed in the salicylic acid and Olsen modifications of the regular macro-Kjeldahl methods described in sections 83–4 and 83–5.

The comments in section 83–6.2 regarding the period of digestion required for soil analysis by the regular macro-Kjeldahl procedure apply to the semimicro-version of the method. The results by the semimicro-method are not markedly affected if the period of digestion specified is reduced to 2 hours, and the same results can be obtained by semimicro-methods which involve short periods of digestion using a high concentration of K_2SO_4 (see Bremner, 1960). The merits and defects of these alternative methods are discussed in section 83–2.3.

83–8 ADDENDUM

83–8.1 Dumas Analysis of Soils (see section 83–1)

Stewart et al. (1963) have recently employed gas chromatography to show that for soil and plant samples on which micro-Dumas results were significantly higher than those obtained by the Kjeldahl procedure, a gas identifiable as methane is present together with N_2 in the micro-Dumas nitrometer. They believe that with proper instrumentation to secure complete combustion, micro-Dumas analyzers can compete favorably with standard Kjeldahl procedures for ease and simplicity of operation, and can be made equally reliable for measuring the total-N content of soils and plant materials.

83–8.2 Salicylic Acid Modification of the Kjeldahl Method (see section 83–2.2)

A recent study of this method [16] has shown that it permits quantitative determination of both nitrate and nitrite in samples containing appreciable amounts of water. The maximal amount of water which can be tolerated

[16] Cheng, H. H., and Bremner, J. M. 1963. Unpublished work. Department of Agronomy, Iowa State University, Ames, Iowa.

has not been determined, but analyses by a semimicro-version of the salicylic acid method (using 0.1 g. of salicylic acid, 3 ml. of concentrated H_2SO_4, and 0.5 g. of sodium thiosulfate) have shown that recoveries of nitrate and nitrite by this procedure are quantitative in the presence of 0.6 ml. of water, and exceed 95% in the presence of 1 ml. of water. It has also been found that quantitative recovery of nitrate can be obtained by this procedure if either the salicylic acid or the sodium thiosulfate is omitted, and that it is not necessary to add salicylic acid to obtain quantitative recovery of nitrite.

83–8.3 Effects of Air-Drying Soils Before Total-N Analysis (see section 83.2)

A recent investigation[17] of the effects of drying soil samples after addition of different amounts of ammonium, nitrate, and nitrite showed that air-drying did not cause significant loss of nitrate from the soils tested, but that it sometimes led to extensive loss of ammonium and nitrite. Loss of inorganic nitrogen on air-drying was particularly marked with samples containing both ammonium and nitrite, presumably because these react during air-drying to liberate molecular nitrogen ($NH_4^+ + NO_2^- = N_2 + 2H_2O$). Also, experiments using $NaN^{15}O_2$ showed that air-drying of neutral and acidic soils containing nitrite induces a chemical reaction between nitrite and soil organic matter in which some nitrite-N is converted to gaseous forms of nitrogen and some is fixed by soil organic matter in a form such that it is nonexchangeable and is not readily released by treatments with hot acid or alkali.

[17] Bremner, J. M., Führ, F., and Keeney, D. R. 1963. Unpublished work. Department of Agronomy, Iowa State University, Ames, Iowa.

83–9 LITERATURE CITED

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