DETERMINATION OF TOTAL NITROGEN IN PLANTS AND PLANT SOLUTIONS: A COMPARISON OF METHODS WITH MODIFICATIONS

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The analyst who has had considerable experience in the determination of nitrogen is impressed by the fact that some substances are analyzed with more ease and accuracy than other substances. Many factors are involved, not the least of which is the combination of the various forms of nitrogen in the sample to be analyzed. For example, if we consider the determination of total nitrogen in whole plants (peas, wheat, barley, etc.) including the residual nutrient solutions in which they grew, amino-, amide-, and some ammonia-nitrogen are present in the plants in addition to nitrate- and possibly ammonia-nitrogen in the residual solutions. In such a case the determination of total nitrogen presents problems that challenge the accuracy of the various methods used.

Two methods are used generally: (1) some modification of the Devarda method, and (2) the official salicylic-thiosulphate method (Assoc. Off. Agr. Chemists, '21, p. 8; I, 28). The latter method has been criticized severely by several investigators whose data have been interpreted as indicating the limitations and defects of any method based on the reduction of nitrates in acid medium. Further reference will be made to these criticisms after the presentation of data. On the other hand, the Devarda method for total nitrogen in the presence of organic matter is time-consuming, since (1) there is a preliminary alkaline distillation with the alloy to collect the ammonia obtained from the reduction of the nitrates and other substances acted upon in the process (Allen, '15; Davisson, '18), and (2) the organic matter and remaining nitrogen must be subjected to a Kjeldahl digestion followed by a second distillation into the same or a second lot of standard acid. Unfortunately, then, neither of these methods is

determined. The official salicylic-thiosulphate method was used. Extremely inaccurate results were obtained. From nutrient solutions containing respectively 400, 300, 200, 100, 50, 25, and 10 mgs. of nitrogen per 950 cc., only 68, 67, 30, 62, 62, 54, and 27 per cent of the nitrogen was recovered. From solutions containing smaller quantities of nitrogen than 10 mgs. the amounts recovered were usually less than for the blank, that is, there was a loss of nitrogen from the reagents used. A total of about 60 determinations was made, and there was no agreement among the results obtained. The greatest losses of nitrogen occurred from those samples containing whole plants in addition to the residual nutrient solutions. In several cases there was a visible evolution of nitrogen dioxide fumes, and duplicate determinations varied widely. These data tend to corroborate the statements of those investigators who have criticized the official salicylic-thiosulphate method.

In spite of these criticisms, however, it was thought advisable to attempt some modifications that might overcome the difficulties encountered. Several possibilities were tried, and from the results of these tests it was tentatively determined that the inaccuracy of the method was due, primarily, to the presence of water at some stage during the process of acid digestion. Certain details of manipulation seemed to influence the determination to a limited degree.

A modification of the official method was devised, and its accuracy for the determination of total nitrogen was tested out on the various forms of nitrogen. For purposes of comparison, simultaneous determinations on samples from the same stock, measured by the same pipettes and at the same temperature, were made by a modification of the Devarda method. The procedure for this comparison method was as follows: (The procedure for the modified official method will be given later.)

I. *If organic matter is not present.*—Place the sample in an 800-cc. Kjeldahl flask; add 1.0 gm. of Devarda alloy [illegible]

a total volume of 150 cc.; add 6 cc. of 10 per cent sodium hydroxide; connect to the distillation apparatus and distill into standard acid at slow boiling for 1 hour. Titrate the standard acid to neutrality.

II. *If organic matter is present.*—Proceed as in I; continue distillation to as low a volume as is safe; disconnect. Add 45 cc. of concentrated suphuric acid and 10 gms. of anhydrous sodium sulphate; digest for 1 hour after the copper color appears (the mixture remains milky due to aluminum and zinc precipitates); cool; make up to an estimated volume of 400 cc. and distill as for the modified official method.

All the determinations reported were made of the following forms of nitrogen:

1. Nitrate-nitrogen as $NaNO_3$ which was twice recrystallized from Merck's blue-label grade. The solution used was such that 1 cc. was equivalent to 1 mg. of nitrogen.

2. Ammonia-nitrogen as $(NH_4)_2SO_4$ which was recrystallized from Merck's blue-label grade. The solution used was such that 1 cc. was equivalent to 1 mg. of nitrogen.

3. Amino-nitrogen as glycine, an Eastman Kodak Company product. The solution used was such that 1 cc. was equivalent to 0.91 mg. of nitrogen.

4. Amide-nitrogen and amino-nitrogen as asparagine, a Merck product. The solution used was such that 1 cc. was equivalent to 0.95 mg. of nitrogen.

5. Total organic plant nitrogen as found in 6-day-old wheat seedlings; 10 such seedlings produced 7 mgs. of nitrogen with but little variation from this average. It would have been somewhat more accurate had smaller seeds been used, in which case the larger number used would have appreciably decreased the variation from the average.

The acid and alkali used in titration were standardized against benzoic acid obtained from the U. S. Bureau of Standards (sample No. 39B). A total of 190 determinations, including the preliminaries, was made. The results obtained were subjected to a statistical analysis, the data of which, expressed as average

TABLE I

RECOVERY OF TOTAL NITROGEN

(average percentages and probable error)

Sample determined	Recovery by Devarda method		Recovery by modified method		No. of trials
	Sample first evptd. to dryns.	Sample plus water	Sample first evptd. to dryns.	Sample plus water	
50 mgs. nitrate-N.	97.7 ±.19	98.4 ±.03	100.4 ±.06	62.2 ±.38	8
100 mgs. nitrate-N.	68.5*	72.4* 99.9‡ ±.24	99.2 ±.10	17.8 ±.81	13 3
50 mgs. nitrate-N. 10 mgs. ammonia-N.	98.5 ±.23	99.4 ±.22	98.0 ±.06	36.7 ±1.80	12
50 mgs. nitrate-N. 10 mgs. amino-N.	97.7 ±.14	99.4 ±.04	99.3 ±.14	51.7 ±4.03	12
50 mgs. nitrate-N. 10 mgs. {amino-N. amide-N.	97.5 ±.09	96.3 ±.10	99.3 ±.28	47.6 ±.29	15
50 mgs. nitrate-N. 7 mgs. plant-N.†	99.6 ±.09	98.8 ±.09	100.2 ±.32	64.6 ±.23	12
50 mgs. nitrate-N. 7 mgs. plant-N.† 0.5 cc. H_2SO_4	These samples were not determined, as during evaporation heavy NO_2 fumes were given off.				
50 mgs. nitrate-N. 7 mgs. plant-N.†‖	100.3 ±.56		99.7 ±.69		14
50 mgs. nitrate-N. 7 mgs. plant-N.† 1 cc. N/10 NaOH	99.2 ±.52		99.1 ±.62		6
50 mgs. nitrate-N. 0.5 gm. sucrose	98.1 ±.64	99.5 ±.00	92.7 ±.90	33.2	10

* 1.0 gm. of Devarda alloy used.

† Supplied as the nitrogen content of ten 6-day-old wheat seedlings.

‡ 2.0 gms. of Devarda alloy used.

‖ This solution was adjusted to neutrality prior to determination.

have not been determined—it is recommended, however, that no more than just a trace of free water be present).

2. When the sample is practically dry the official method as here modified is somewhat more accurate for the determination of total nitrogen than the Devarda method used.

3. The modified method is accurate for the determination of amino-, amide-, ammonia-, nitrate-, and total plant-nitrogen and combinations of these forms of nitrogen in plants and plant solutions.

4. If sugar is present in abundance a slight loss of nitrate-nitrogen may occur, due to the reducing action of the sugar. This loss would be very slight in actual practice since the nitrate-nitrogen content of plants is small.

The procedure for the modified official method as used in this investigation is as follows:

Place the sample in an 800-cc. Kjeldahl flask; adjust to neutrality or make slightly alkaline; if water is present evaporate *just to* dryness on a water bath under vacuum. Add 35–40 cc. of salicylic acid mixture (1.0 gm. of salicylic acid to 30 cc. of concentrated nitrogen-free sulphuric acid); mix thoroughly and allow to stand for at least an hour with occasional shaking (if organic matter is present, stopper tightly with a rubber cork and allow to stand over night). Add 5 gms. of sodium thiosulphate and heat for 5 minutes with a low flame; cool; add 7–10 gms. of anhydrous sodium sulphate and a pinch of copper sulphate. Digest for an hour at the boiling point after the solution clears; just before the solution solidifies dilute to an estimated volume of 400 cc.; cool completely. Add a small piece of paraffin, 100 cc. of a saturated solution of sodium hydroxide, and a piece of mossy zinc; connect immediately to the distillation apparatus and distill 150–200 cc. over into standard acid during a period of 1 hour. Titrate the standard acid to neutrality with standard alkali and calculate the amount of nitrogen present.

Subsequent to this investigation 380 determinations were made by the above method with an accuracy equivalent to that here reported. Some of the samples determined had a total nitrogen content as high as 400 mgs., in which case the amount of

were made on samples composed of whole plants of wheat, barley, or peas, plus the residual nutrient solutions in which they grew. If this modified method be in error all attempts to locate that error have failed and any suggestions or criticisms are welcome.

Attention to detail is essential to accurate determinations; for this reason it is well to mention a few details of manipulation that have been found of value:

1. It is the habit of some analysts to wash down the neck of the Kjeldahl flask during the process of digestion; in the interests of personal safety and certainty of results this should be avoided. Such a practice introduces water into the sample and operates against the advantages of a previous evaporation under vacuum. If sulphur collects in the neck of the flask it can be removed easily by heating gently and uniformly the neck of the flask in the open flame; by using a strong hot flame during the last hour of digestion the accumulation of sulphur is almost entirely avoided.

2. If excess solid organic matter is present in the sample it may be necessary to increase the amount of salicylic acid mixture used in order to maintain a liquid condition of the contents during the first part of digestion.

3. The open flame should be allowed to come in contact with only that portion of the flask which is covered by the solution. Use an asbestos ring to prevent this (Paul and Berry, '21).

4. In the presence of organic matter, the tendency of the digesting mixture to foam and spew out presents an irritating problem. This loss by foaming has been entirely overcome in this laboratory by allowing the acid to thoroughly disintegrate the solid portions of organic matter, without heat, over an approximate 12-hour period It is advisable to redistribute the acid by occasional shaking. It is necessary to stopper the flasks tightly with rubber corks to prevent the absorption of ammonia fumes. The practice used by the author has been to add the

inclined position by 2 notched wooden supports and connected to an ordinary water filter-pump by a series of 5 corks (rubber) and 4 Y-tubes. A 3-liter safety bottle is placed between the filter-pump and the flasks on the water bath to prevent the entrance of water into the flasks when, for any reason, the water pressure becomes reduced. In operation, a partial vacuum is quickly developed and ebullition proceeds at a rapid rate, providing all rubber connections are sufficiently thick-walled to withstand the vacuum developed. Evaporate the sample just to dryness, not to an ash-dry condition. Release the vacuum slowly before removing the flasks; if released rapidly the flasks will crack.

6. A few Pyrex glass beads or small angular pieces of broken Pyrex glass placed in the flask will facilitate evaporation and subsequent digestion; they may be used over and over again.

7. "Bumping" during distillation is a question of concentration and relative abundance of insoluble substances present—at a dilution of 400 cc. practically no "bumping" was experienced.

8. To determine the correct amount of alkali to use in distillation the following test is of value: When ready to distill, add 2 drops of phenolphthalein indicator; add the paraffin, sodium hydroxide, and zinc; after the flask is connected to the distillation apparatus and the flame is adjusted, shake the flask vigorously and if the correct amount of alkali has been added the pink color of the indicator will flash through the solution for 1 to 2 seconds and disappear. If the pink color lasts for more than 2 seconds it is advisable to add more alkali; if the color disappears in less than 1 second a useless excess of alkali is present. Once adjusted the amount of alkali remains practically constant as long as the amount of acid used in digestion is not varied.

In the first part of this paper reference was made to certain criticisms against various methods which are based on the reduction of nitrates in acid medium, and it was also shown that

It is pertinent to this investigation, therefore, although no attempt has been made to go into the literature, that all such criticisms which did come to notice[1] be considered. In doing so it is to be remembered: (1) that the accuracy of the modified official method here proposed has been tested out for plants and nutrient solutions only, (2) that, while no difficulty is anticipated in using this method on other biological substances in watery medium (for example, soil extracts), no broad generalizations are advanced until these tests are actually made, (3) that a criticism of the principle upon which this method is based, that is, the acid reduction of nitrates, is also an indirect criticism of the method here used, though the particular method under criticism may be that of Jodlbauer (Metge, '18, p. 30), Förster (Krische, '06, p. 71), etc.

After the completion of this investigation a reference to the work of Allen ('15) became available. Allen's work was very carefully done and is referred to often. In his summary, referring to reduction methods, he concludes that "of such procedures only the modified Devarda and aluminium reduction methods gave promise of meeting our requirements." The data presented indicate that these methods (that is, 2 Devarda methods and 1 aluminium reduction method) were the only ones actually tested out by Allen ('15). He rejected the acid methods of nitrate reduction, apparently upon the evidence presented by Mitscherlich and Herz ('09), as follows:

"Mitscherlich and Herz conducted an extended investigation on the perfection of an accurate Kjeldahl method which would include all forms of nitrogen, in course of which they studied, among other sources of error, the question of the reduction of nitric nitrogen. Using phenolsulfonic acid and zinc dust, sodium hydroxide, and zinc-iron dust, Jodlbauer's method, and Förster's method, they were unable to obtain the theoretical amount of ammonia from nitrate." (Allen, '15, p. 522).

Reference was then made to the work of Mitscherlich and Herz ('09), whose data is an excellent tribute to accurate procedure.

For the purposes of this study, however, attention must be called to certain facts pertaining to their report ('09):

1. Of the 40 pages devoted to the report, only 12 (pages 307–318) are concerned with a study of any method based on the reduction of nitrates in acid medium.

2. Through their data they give no detailed statement of procedure for any of the many methods they used, except for that modification of the Devarda method which they recommend (Mitscherlich and Herz, '09, p. 280). They refer to the other methods by name only (for example, Förster, Jodlbauer, etc.), and it is logical to assume, therefore, that the procedures for the methods used were those in common use at the time. For example, the Förster method in use at that time was in part as follows:

"0.5 g Salpeter (50 ccm der Lösung 10:1000) werden in einem Kjeldahlkolben mit 15 ccm einer 6%igen Phenolschwefelsäure oder mit 15 ccm einer 6%igen Salizylsäure-Schwefelsäure vermischt, . . ." etc. (Kirsche, '06, pp. 71–72).

The procedure for the Jodlbauer method is very similar:

". . . : 250 ccm Wasser werden mit 25 ccm Phenolschwefelsäure im Kjeldahl-Kolben versetzt und nach Zugabe einiger Sandkörnchen möglichst weit eingedampft. . . ." etc. (Metge, '18, p. 20, no. 13).

Attention is called to the presence of water in the samples determined by these methods.

3. Finally, attention must be called to the fact that the determinations upon which Mitscherlich and Herz ('09) based their conclusions and to which Allen ('15) referred were made upon samples in water solutions or upon soil extracts, some of which were very dilute. Most of the tables presented have headings similar to the following examples:

"Analyse einer Kaliumnitratlösung mittels der Zink-Eisenstaub-Reduktionsmethode" [table 37, p. 316].

"Versuche zur quantitativen Reduktion des Salpeterstickstoffs einer Kaliumnitrat-Lösung" [table 38, p. 317].

"Stickstoffbestimmung in einem Bodenextrakt nach der Zinkeisenstaub-Reduktionsmethode" [table 36, p. 315].

Ammoniakmengen entsprechend ccm N/50 H_2SO_4 im Destillat festgestellt:" [table 34, page 313].

In a few cases the solutions apparently were evaporated and theoretical recovery of nitrogen was not obtained (Mitscherlich and Herz, '09, p. 316). In most of these cases some acid was added (table 32, p. 312; table 35, p. 314) prior to evaporation and in the other cases no statement can be made due to the absence of specific details of procedures.

From a study on the determination of nitrites and nitrates in plants Strowd ('20) concluded that "the determination of nitrates in plants by finding the difference between the Kjeldahl-Gunning-Arnold method and the Kjeldahl method modified to include nitrates is unsatisfactory." Strowd attempts to explain this discrepancy on the basis that "appreciable amounts of nitrate were apparently reduced without zinc and salicylic acid." These deductions were based on 2 determinations only (Strowd, '20, table 1); one being a determination of the nitrate-nitrogen content of a "Pure $NaNO_3$ solution," and the other a "Pure $NaNO_3$ solution + nitrate-free plant extract." By calling attention to these facts it is not implied that the difference between the nitrogen obtained by the Kjeldahl-Gunning-Arnold method and the modified official method, as here reported, would be accurate for the determination of nitrate-nitrogen only—this is another problem. The purposes of this investigation are served by calling attention to the presence of water in the samples determined (Strowd, '20).

For the determination of total nitrogen in plants Gallagher ('23, p. 67) concludes that "nitrate reductions are invariably essential in dealing with Kjeldahl estimations of plant products, since the plant contains nitrates at nearly all stages of growth." He recommends a Devarda method to accomplish this and discards the principle of acid reduction of nitrates on a purely theoretical basis as follows:

"In the estimation of nitrates by means of acid reducing agent a

Under certain conditions, and for the method (Ulsch) involved this may be a true statement of fact, but it is not true as a generalized criticism of the reduction of nitrates in acid medium. In acid medium, for example, concentrated sulphuric acid, in the absence of water, and in the presence of either phenol or salicylic acid, the above generalization probably does not hold true. Skeleton equations to illustrate the transformation of the nitric-acid radicle into ammonia may be somewhat as follows, in the case of phenol:

(1) $C_6H_5.OH + HNO_3 = C_6H_4.OH.NO_2 + H_2O$

(2) $C_6H_4.OH.NO_2 + 3H_2 = C_6H_4.OH.NH_2 + 2H_2O$

(3) $C_6H_4.OH.NH_2 + NaOH = C_6H_4.OH.ONa + NH_3$

A very similar transformation takes place with salicylic acid; the reduction process is hastened by the addition of zinc or sodium thiosulphate. By equation (1) it is evident that nitric acid is responsible for the nitrification of the phenol- or salicylic-acid molecule. The nitric acid is obtained from the action of the sulphuric acid on the nitrate present:

(4) $NaNO_3 + H_2SO_4 = NaHSO_4 + HNO_3$

If water is present in the sample to be determined it is probable that the nitric acid will be diluted below the point at which it can quantitatively nitrify the phenol or salicylic-acid molecule; if so, the instability of the nitric-acid molecule will be manifest and there will be a loss of nitrogen-dioxide gas:

(5) $4HNO_3 = 2H_2O + 4NO_2 + O_2$

In fact, in many cases when even small amounts of water are present, visible amounts of nitrogen-dioxide fumes may be given off. The above generalization by Gallagher ('23, p. 64) is based on the reported data of 2 determinations only; the first one being the analysis of ". . . . a *solution*[1] containing nitrate only, . . . ," and the second being the analysis of ". . . . a *solution* containing

SUMMARY

For the determination of total nitrogen in plants and plant solutions the official salicylic-thiosulphate method has proved inadequate. The inaccuracies of this method are demonstrated to be due, primarily, to the presence of water in the sample under analysis. A modification of the official salicylic-thiosulphate method is proposed and certain details of manipulation are discussed. Under the conditions of this investigation this proposed modified method has proved to be approximately twice as rapid and just as accurate as a modified Devarda method used for comparison purposes. Some of the criticisms advanced against the principle of acid reduction of nitrates are reviewed.

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LITERATURE CITED

Allen, E. R. ('15). The determination of nitric nitrogen in soils. Jour. Ind. and Eng. Chem. **7**: 521–529. 1915.

Association of Official Agricultural Chemists ('21). Official and tentative methods of analysis. Washington, D. C., 1921.

Burrell, R. C., and Phillips, T. G. ('25). The determination of nitrate nitrogen in plants. Jour. Biol. Chem. **65**: 229–234. 1925.

Davisson, B. S. ('18). Ammonia and nitric nitrogen determinations in soil extracts and physiological solutions. Jour. Ind. and Eng. Chem. **10**: 600–605. 1918.

Gallagher, P. H. ('23). Estimation of nitric-nitrogen and total-nitrogen in plant tissue extracts. Jour. Agr. Sci. **13**: 63–68. 1923.

Krische, P. ('06). Das agrikulturchemische Kontrollwesen. Leipzig, 1906.

Metge, G. ('18). Laboratoriumsbuch für Agrikulturchemiker. Halle (Saale), 1918.

EXPLANATION OF PLATE

PLATE 19

Apparatus used for the evaporation of the samples under vacuum.