Danish State Research on Soil and Plant Sciences **Report No. 1323.**

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Determination of ammonium in plants

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Summary

Two methods for determination of ammonium in plants are described. The plant sample is extracted by a calcium sulphate solution containing phenylmercuric acetate to preserve the extracts.

The ammonium is determined by steam distillation with a glycine-sodium hydroxide buffer solution pH 10.6 followed by a titration or directly by an ammonia electrode. The steam distillation apparatus used is designed in a way, which makes it easy to divide the sample into portions and distill the portions one by one in quick succession. Using the technique described 45 seconds is sufficient for distilling off at least 0.2 mg of ammonium nitrogen from a volume of 10 ml.

The interference from different alkali labile nitrogen compounds including glutamine was found to be less than 1 % for both methods. The methods give quantitative recovery of ammonium added to extracts from different species of plants.

Key-words: Ammonium determination, plants.

Resumé

I nærværende beretning er beskrevet to metoder til bestemmelse af ammoniumkvælstof i planter.

Plantematerialet blev ekstraheret med en calciumsulfat-opløsning tilsat phenylmercuriacetat for at inaktivere enzymer og mikroorganismer under ekstraktion og opbevaring. Ammoniumindholdet i ekstrakten bestemtes ved dampdestillation med en glycin-natriumhydroxid-stødpudeopløsning (pH 10,6) efterfulgt af en titrering, e ler direkte ved hjælp af en ammoniak-elektrode efter tilsætning af natriumhydroxid til ekstrakten.

Da reduktion af destillationstiden til et minimum er af afgørende betydning for at mindske interferensen fra labile kvælstofforbindelser, anvendtes et destillationsapparat, konstrueret på en måde, som gør det let at dele prøven i portioner og destillere de enkelte delprøver en for en i hurtig rækkefølge. Dette indebærer, at den tid, prøven som helhed er underkastet destillation, reduceres til den tid, som kræves for afdestillation af ammoniakken i en enkelt delprøve. Ved anvendelse af den beskrevne destillationsteknik fandtes, at destillation i 45 sec. var tilstrækkeligt til afdestillation af en ammoniakmængde svarende til mindst 0,2 mg kvælstof i en delprøve på 10 ml.

Interferensen fra forskellige alkalilabile kvælstofforbindelser fandtes at være mindre end 1 % for begge metoder. Ligeledes giver begge metoder en kvantitativ genfindelse af ammoniumkvælstof tilsat ekstrakter fra forskellige plantearter.

Ved bestemmelse af ammoniumindholdet i plantemateriale indeholdende 0,03–0,16 % ammoniumkvælstof fandtes lidt lavere værdier ved anvendelse af ammoniakelektroden end ved anvendelse af destillationsmetoden, medens det modsatte var tilfældet ved bestemmelse af indholdet i materiale indeholdende ca. 0,5 % ammoniumkvælstof.

Introduction

For studies of ammonium absorption by plants and nitrogen metabolism in plants it is of interest to determine the ammonium content. However, determination of ammonium in biological materials is frequently attended with difficulties because extracts from such materials usually contain substances which interfere with the method. When using colorimetric methods coloured and turbid extracts may cause problems, whereas the problems of using distillation methods chiefly are due to interferences from alkali-labile nitrogen compounds present in the extracts.

In order to decrease the liberation of ammonia from labile nitrogen compounds, magnesium oxide and different buffer solutions have been used for the distillation. Distillation in vacuo (*Pucher et al.*, 1935) and the Conway diffusion technique (*Bremner* and *Shaw*, 1955) have been used as well.

For determination of ammonium in soil extracts *Bremner* and *Keeney* (1965) elaborated a method which – by means of steam distillation with magnesium oxide for a short time – made it possible to distill off up to 2 mg ammonium nitrogen from a sample volume of 20 ml without significant interference from several nitrogen compounds including amides and hexosamines. Results obtained by means of this method for determination of ammonium in soil extracts and water samples were found to be identical with results obtained by an ammonia electrode (*Banwart et al.*, 1972).

The steam distillation method of *Bremner* and *Keeney* (1965) seemed to be attractive because it is quick, without significant interference from several nitrogen compounds, and does not require expensive equipment. For the purpose of using this method for determination of ammonium in plant materials preliminary investigations were carried out by distillation of samples containing glutamine, which may be present in plant materials in quantities. The results of these investigations showed that about 3 per cent of the amide nitrogen was liberated as ammonia by distillation for 3 minutes, and that the amount liberated was proportional to the time of distillation. These findings, and the fact that the time required for distilling off the ammonia is proportional to the sample volume indicates that it may be possible to decrease the interference from labile nitrogen compounds by dividing the sample into portions and distilling these one by one.

In the present paper a steam distillation apparatus is described. By means of this the sample is easily divided into portions and the portions distilled one by one in quick succession. Results obtained by this method are compared with results obtained when an ammonia electrode is used.

Methods

Determination of amonium by distillation

The distillation apparatus used is shown in Fig. 1. The apparatus is designed so that the sample is retained in the distillation vessel by the steam pressure. This implies that by shutting off the steam flow the sample is quickly removed from the distillation vessel. To minimize the condensation in the distillation vessel this is enclosed in a glass jacket.

The condenser and the distillation unit are connected by a spherical joint, and the other parts by rubber tubings. Pinchcocks were used as closing mechanism. A 5 liter flask containing distilled water and pumice is used as steam generator. The flask is heated by an electric heating mantle, and the power supply to the mantle is controlled by a variable transformer.

Titration equipment

Titration was carried out using a titrator (Radiometer type TTT 2) connected to an autoburette (Radiometer type ABU 13) fitted with a 2.5 ml burette. Conventional titration is suitable too.

Reagents

Calcium sulphate-phenylmercuric acetate solution ($CaSO_4$-PMA). Dissolve 3.44 g of $CaSO_4$, $2H_2O$ and 10 mg of phenylmercuric acetate in 2000 ml of water.

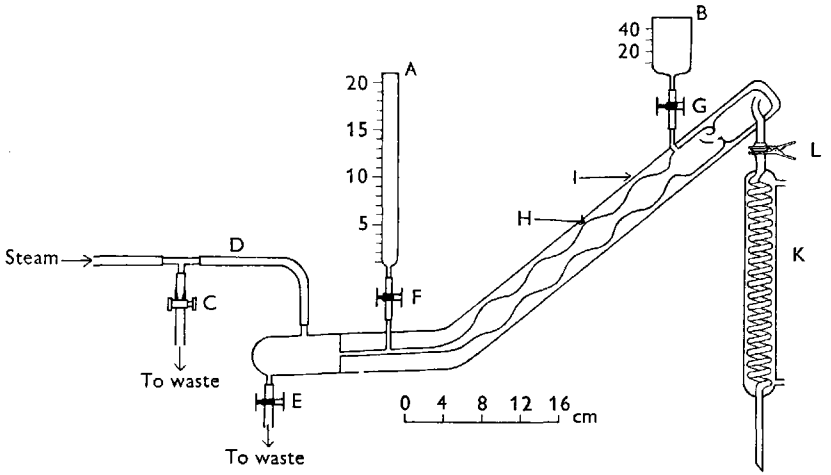

Fig. 1. Steam distillation apparatus. A burette for buffer solution; B funnel for sample; C, E, F, G rubber tubings with pinchcocks; D rubber tubing; H distillation vessel; I glass jacket; K condenser; L spherical glass joint (S 19/9).

Glycine-sodium hydroxide buffer solution pH 10.6. Dissolve 3.755 g of glycine in 100 ml of water (0.5 *M*) and adjust the pH to 10.6 by 0.5 *M* sodium hydroxide.

Boric acid solution (2 %). Dissolve 10 g of boric acid in 500 ml of water.

0.0143 N Hydrochloric acid.

Standard ammonium solution. Dissolve 0.382 g of ammonium chloride in 500 ml of water. This solution will contain 0.2 mg of ammonium nitrogen per ml.
All chemicals: A.R. quality.

Procedure

Place 0.25 g of dry plant material in a 100 ml bottle, and add 50 ml of $CaSO_4$-PMA solution. Shake the bottle by a mechanical shaker for 30 minutes, and filter the resulting suspension (*Munktell* 00).The extractant contains phenylmercuric acetate to inhibit enzymatic and micro biological activity during extraction and storing (*Douglas* and *Bremner*, 1970; *Kyllingsbæk*, 1975). Stored in a refrigerator the extracts are stable for at least 5 days.

Adjust with the variable transformer the power supply to the heating mantle, so that 10–12 ml of distillate is collected per minute. Then the apparatus should be steamed out for about 10 minutes before use. Place the glycine-sodium hydroxide buffer solution in the burette A and the sample (e. g. 20 ml) in the funnel B. Remove the condensation water from the distillation vessel by shutting off the steam flow (move the pinchcock at C to D), and open the pinchcock at E. Close the pinchcock at E again, and turn on the steam (move the pinchcock back from D to C). Let in 1 ml of the buffer solution to the distillation vessel by opening the pinchcock at F, and then 10 ml of the sample by opening the pinchcock at G. Start a stop watch, and place a flask, which contains 5 ml of the boric acid solution, under the condenser. Shut off the steam after 45 seconds by moving the pinchcock from C to D, and remove the solution from the apparatus by opening the pinchcock at E. Repeat the procedure immediately; close at E, turn on the steam, let in 1 ml buffer at F and 10 ml of the sample at G. Start the stop watch, distill for 45 seconds, and remove the solution from the apparatus. After distillation of the last

10 ml of the sample the apparatus is steamed out for about 1 minute to collect the residual ammonia.

Determination of ammonium by ammonia electrode

An ammonia electrode Orion Model 95-10 connected to a Specific Ionmeter Orion Model 404 was used for determination of ammonium nitrogen by the electrode-method.

Reagents

Calcium sulphate-phenylmercuric acetate solution ($CaSO_4$-PMA). See above.

0.25 N sodium hydroxide. Dissolve 5 g of NaOH in 500 ml of water.

Standard ammonium solution. See above.

Procedure

Extraction of the plant material was carried out as described above.

Determination of ammonium by the electrode-method was carried out as described by *Jensen* (1975). Prepare two ammonium chloride standards in the concentration range of the unknown solutions to be measured, the first standard solution with a concentration value of c ppm ammonium N and the second with a concentration value of 1/5 c ppm ammonium N.

Set the Function Switch on the ionmeter to monovalent anion position. Set Slope Indicator to 100 % and Temperature Compensator to the temperature of the solution. Pipet into a 50 ml beaker 30 ml of the first standard and then 3 ml of 0.25 *N* sodium hydroxide solution. Place the beaker on a magnetic stirrer, and stirr the solution at moderate speed. Place the electrode in the solution. After 3 minutes turn the Calibration Control until the needle points exactly to 100 on the logarithmic scale. Remove the electrode, rinse with distilled water, and blot with absorbent tissue. Place the electrode in the second standard as described for the first standard. After 3 minutes turn Temperature Compensator until the needle points exactly to 20 on the logarithmic scale. Move the Slope Indicator until the arrow of the Temperature Compensator points to the temperature of the solution. Repeat the calibration procedure. Add 3 ml sodium hydroxide to 30 ml of the unknown solution, place the electrode in this solution as described for the standards. Read the result on the logarithmic scale after 3 minutes and calculate the content of ammonium in the solution. Samples and standards should be at the same temperature.

Results and Discussion

The instruction manual for the ammonia electrode states that the electrode is subject to interference from volatile amines and Hg^{++}. This is in agreement with results from investigations of *Banwart et al.* 1972, who found a marked interference from methylamine, ethylamine, and Hg^{++}. The interference from the amines was detected in solutions containing 1 ppm ammonium N as ammonium chloride and 10 ppm of the two amines respectively, and the interference from Hg^{++} in a similar ammonium chloride solution made 0.09 *M* with respect to Hg^{++}. No interference was detected when solutions of ammonium chloride were made 0.09 *M* with respect to the following cations and anions: Na^+, K^+, Ca^{++}, Cu^{++},Fe^{+++}, Al^{+++}, NO_3^-, NO_2^-, Cl^-, SO_4^{--}, HCO_3^-, and CO_3^{--}. According to results mentioned, a risk of interference from Hg^{++} exists when phenylmercuric acetate is used for preservation of the extracts, but at the concentration recommended in the present paper no interference was found.

As previously mentioned distillation with different buffer solutions have been used for determination of ammonium. In the present work a buffer solution which makes the solution for distillation alkaline to about pH 10.5 was used. At this pH about 90 % of the ammonium nitrogen is in ammonia form.

A 0.5 *M* solution of glycine adjusted to pH 10.6 with a 0.5 *M* solution of sodium hydroxide was found to be satisfactory. A similar buffer solution made from borax and sodium hydroxide was found to be unsatisfactory, be-

Table 1. Liberation of ammonia N from different nitrogen compounds using different methods for determination of ammonium

Nitrogen compounds	mg N per sample		ammonia N liberated as % of amino or amide N		
	amino N	amide N	ammonia electrode	distillation 45 sec.[1])	distillation 3.5 min.[2])
urea	0	0.400	0.1	x	0.3
asparagine	0.200	0.200	x	x	0.4
glutamine	0.200	0.200	0.7	0.6	3.3
glucosamine	0.200	0	0.1	0.5	1.1
allantoine	0	0.200	0.1	x	x
citrulline	0.200	0.200	x	x	x

1) New distillation apparatus shown in Fig. 1.
2) Conventional steam distillation.
x: < 0.05

cause use of this solution gave rise to liberation of ammonia from glucosamine.

Using the distillation technique described, it was found that 45 seconds was sufficient to distill off at least 0.2 mg of ammonium nitrogen from a volume of 10 ml.

The interference caused by liberation of ammonia from different nitrogen compounds is illustrated by Table 1. In addition to the nitrogen compounds specified in the table all solutions analysed contained 1 ppm of ammonium N. From the results in the table it is seen that the interference is almost the same for the ammonia electrode-method and for distillation for 45 seconds as described. From the table it is also seen that conventional steam distillation for 3.5 minutes gives rise to a considerably higher interference. In a similar investigation no interference was found from the following amino acids: lysine, histidine, arginine, aspartic acid, threonine, serine, glutamic acid, glycine, alanine, proline, hydroxy proline, valine, isoleucine, leucine, methionine, cysteine, tyrosine, phenylalanine, and tryptophane.

However, the errors introduced by liberation of ammonia from labile nitrogen compounds will consequently depend on the ratio of labile nitrogen compounds to ammonium in the extracts examined. From Table 1 it is seen that the ammonia liberated from glutamine, one of the most alkali-labile nitrogen compounds present in plants, is less than 1 per cent of the amide nitrogen when ammonia electrode or distillation for 45 seconds is used. This means that less than 1 per cent of the glutamine extracted together with the ammonium gives rise to interference. Accordingly interference from labile nitrogen compounds do – in most cases – hardly influence the results obtained using these methods.

Results from experiments in which dry matter from ammonium nitrate treated barley plants was extracted for different periods of time showed no influence of the time of extraction in the range 10 to 60 minutes. In the present work the plant material was extracted for 30 minutes. The extracts were found to be stable for at least 5 days when stored in refrigerator at 5–7° C.

The applicability of the two methods for determination of ammonium in extracts from different plant species is illustrated by the results in Table 2. The plant material used in the investigation contains only trace of ammonium nitrogen. It is seen from the table that the methods give almost quantitative recovery of ammonium added to the extracts examined.

Analysing plant material with a low content of ammonium, the results found seem to be slightly lower using the ammonia electrode than using distillation, whereas the opposite seems to be the case when plant material with a higher

Table 2. Recovery of ammonium N as % of ammonium N added to extracts from different species of plants

	added mg ammonium N	barley plants	rye-grass	field bean (leaves)	beet (leaves)	beet (roots)
distillation 45 sec.	0.02	100.2	102.2	98.2	99.0	102.2
	0.10	103.2	102.2	98.2	100.5	101.1
	0.20	100.7	100.4	99.4	99.1	99.3
ammonia electrode	0.02	103.6	101.5	99.1	101.9	102.8
	0.10	101.1	103.1	98.8	100.4	100.2
	0.20	104.2	104.1	99.2	100.8	100.8

Table 3. Standard deviation for results from determination of ammonium in dry matter from barley plants

Average ammonium N contents as % of dry matter		s		s %		number of
distillation	electrode	distillation	electrode	distillation	electrode	samples
0.030	0.027	0.001	0.001	3.8	3.7	6
0.065	0.063	0.003	0.002	4.3	3.5	5
0.163	0.160	0.002	0.002	1.4	1.3	7
0.528	0.548	0.012	0.012	2.2	2.1	6

content of ammonia is analysed. This is seen from Table 3, which also illustrates the precision of the methods. From duplicate analyses of samples of dry matter from ammonium nitrate treated barley plants standard deviations were calculated according to

$$s^2 = \frac{\Sigma d^2}{2p};$$

d is the difference between duplicate determinations and p is the number of samples.

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Manuskript modtaget den 3. august 1976.