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Interferences by Organic Nitrogen Compounds in Ammonium-N Determination

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Abstract

Organic nitrogen compounds were evaluated for interferences in NH_4-N determination in water, 2M KCl and 0.5M K_2SO_4 at NH_4-N level of 0 and 1.0 mg l^{-1}. The results revealed that organic nitrogen compounds interfere negatively in NH_4-N determination using the Technicon AutoAnalyzer II. Soil extracting solutions like 2M KCl and 0.5M K_2SO_4 further increased interferences by these compounds. These results suggested that a pre-treatment step either distillation or gas phase dialysis should be included to reduce the interferences caused by the organic nitrogen compounds in the NH_4-N determination by the Technicon AutoAnalyzer II.

Introduction

Organic nitrogen compounds are thought likely to occur in soil solutions and extracts. Sowden (1956) found that the amino acids in soil were in the order glycine > aspartic acid, glutamic acid, alanine > threonine, serine, proline, valine, leucin > isoleucine > tyrosine, phenylalanine > methionine or cystine. Solutions of potassium chloride and potassium sulphate are used for extraction of availbale nitrogen from soils. This available nitrogen is measured from soil extracts and acid digests by manual or automated techniques (Bremner and Hauck, 1982; Bremner and Mulvaney, 1982 and Keeny and Nelson, 1982). The colorimetric methods of NH_4-N determination in aqueous solutions based on the Berthelot colour reaction either catalysed or without catalyst have been reviewed by searle (1984).

White and Gosz (1981) reported that amino acids added to the KCl extracts of soil were found to contribute a significant positive interference to the automated determination of ammonium. They modified the Technicon Industrial Method No. 98-70 W by using 110 g l^{-1} NaOH instead of 200 g NaOH l^{-1} in the phenol reagent (which lowered the pH to 12.5 in the reagent and 12 in the final solution) and by lowering the temperature from 90 C to 60°C in the heating bath for the colour development. Modification of the method yielded results identical to steam distillation analysis. The original method was found to over estimate inorganic ammonium nitrogen in extracts of forest floor material by 17-26 percent. Rowland (1983) concluded from his study that amino acids interfere, but only contribute a small error (11%) in soil extractable nitrogen. Similarly Burton *et al.* (1989) found small but significant errors due to amino acid interference in the ammonium determination by authomated method from soil extracts.

Searle (1990) concluded that the Berthelot reaction can be used for measuring ammonium ions in the presence of appreciable concentrations of amino acids, provided that the reagents and reaction conditions used are carefully chosen to limit hydrolysis. This is best achieved by using the nitroprusside catalyst reaction, which enables the use of low sample volume, low reagent concentration and low reaction temperature.

Considering the above facts it was, therefore, decided to conduct experiment to investigate the possible interferences by organic nitrogen compounds in combination with known concentration of NH_4-N in water, 2M KCl and 0.5M K_2SO_4 in NH_4-N determination using the Berthelot colour reaction Technicon AutoAnalyzer II.

Materials and Methods

Experiment was conducted to investigate the interferences by 17 amino acids, galactosamine, glucosamine and urea in the laboratory of the Department of AFE, University of Glasgow during 1991.

Washing of glassware: Beakers, stirring rods and volumetric flasks were first washed with hot water and soaked overnight in a 2 percent solution of Decon 90 (Decon Laboratories limited). These were then washed with hot water, rinsed twice with deionized water and finally dried in an oven at 70 C.

Reagents: Analar grade reagents and deionized water were used throughout.

Alkaline Phenol: Sodium hydroxide (22.5 g) was dissolved in about 800 ml deionized water in 1 litre dark glass bottle and the resulting solution was degassed. Fifty gram phenol was weighed in 1 litre beaker and approximately 600 ml sodium hydroxide solution was added and stirred with a glass rod to dissolve the phenol. The solution was returned to the bottle and the volume was made to 1 litre with degassed water and mixed gently.

Complexing Reagent: Fifty gram potassium sodium tartrate and 50 g sodium citrate were dissolved in 800 ml deionized water and degassed. Sodium nitroprusside (1.2 g) was weighed in 100 ml beaker. Fifty ml of degassed water was added to the beaker and stirred gently with a magneti

stirrer. The resulting solution was added to the citrate-tartrate solution. Thirty percent Brij-35 (0.5 ml) was added and volume was made to 1 litre. The solution was then mixed gently.

Sodium hypochlorite solution (0.5%): Fifty ml sodium hypochlorite solution (12% w/v available chlorine) was diluted to 1 litre with degassed deionized water and mixed gently.

Ammonium-N standard stock solution (1000 mg N l^{-1}): Ammonium sulphate was dried for an hour at 110°C in the oven and cooled in a desiccator. Dried ammonium sulphate (4.718 g) was dissolved in deionized water and the volume was made to 1 litre. The solution was stored at 2°C.

NH_4-N Working solution (100 mg N l^{-1}): Ten ml of 1000 mg l^{-1} NH_4-N stock solution was pippeted into 100 ml flask by a bulb pipette and the volume was made with deionized water upto the mark.

NH_4-N working Standard (1 mg N l^{-1}): One ml of 100 mg l^{-1} NH_4-N working solution was added to 100 ml volumetric flask and the volume was made with deionized water.

Zero NH_4-N working solution: Deionized water 2M KCl and 0.5M K_2SO_4 were analysed as zero NH_4-N solutions with their appropriate set of samples.

Table 1: Number of nitrogen atoms and molecular weight of organic nitrogen compounds

Organic compounds	No. of nitrogen atoms	Molecular Weight
Alanine	1	89.09
Arginine	4	210.70
Asparagine	2	150.10
Aspartic acid	1	133.10
Glutamic acid	1	147.10
Glutamine	2	146.10
Glycine	1	75.07
Histidine	3	209.60
Isoleucine	1	131.20
Leucine	1	131.20
Lysine	2	183.70
Methionine	1	149.20
Phenylalanine	1	165.20
Proline	1	115.10
Serine	1	105.10
Threonine	1	119.20
Valine	1	117.10
Galactosamine	1	215.60
Glucosamine	1	215.60
Urea	2	60.06

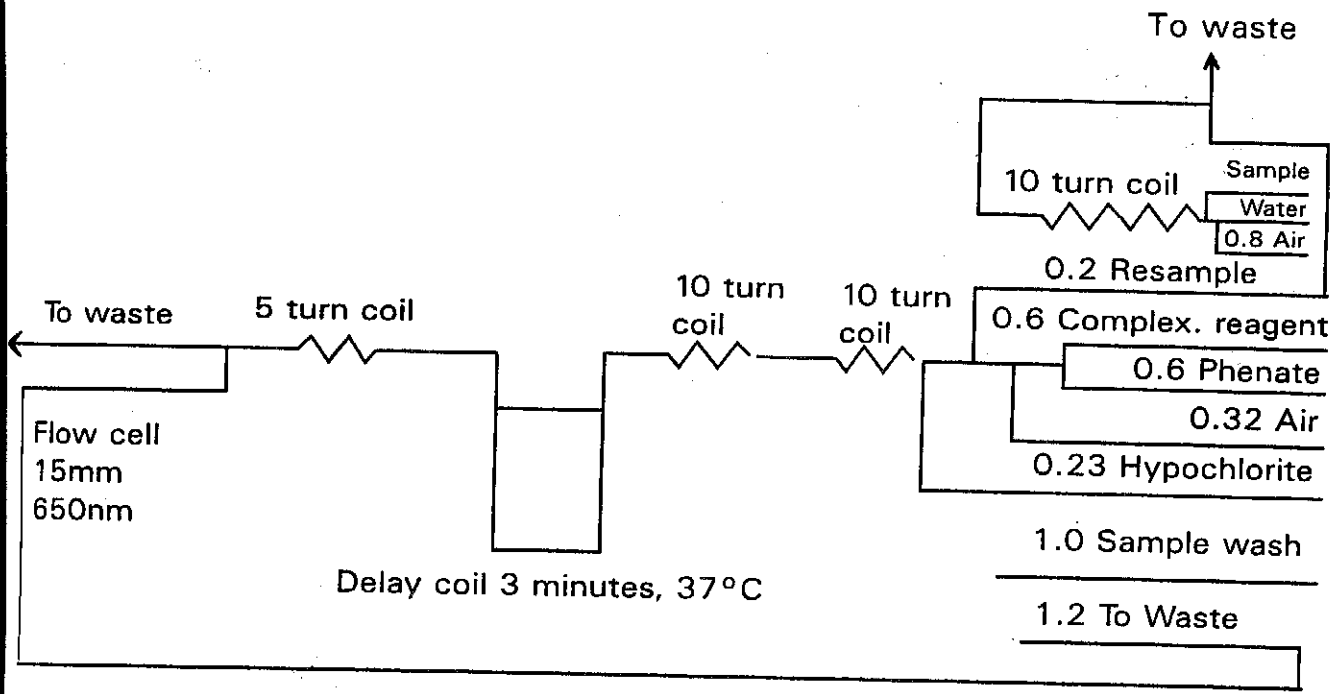

Fig. 1: AutoAnalyzer Manifold for Determining NH_4-N

Stock solutions of organic nitrogen compunds: The required weights of 17 amino acids (galactosamine, glucosamine and urea) were transferred to 25 ml volumetric flasks carefully and volume was made to the mark with deionized water. The strength of each solution was 1000 mg N l^{-1}.

Organic compound samples without added NH_4-N: One ml of each organic nitrogen compund stock solution (1000 mg N l^{-1}) was diluted separately into 100 ml volumetric flasks with water. Each solution contained 10 mg N l^{-1}.

Organic compound sample with added NH_4-N: One ml of NH_4-N (100 mg N l^{-1}) and 1 ml of organic compound solution (1000 mg N l^{-1}) were added together by an automatic pipette into three 100 ml volumetric flasks for each compound. These were then diluted with water to produce solutions containing 1 mg l^{-1} NH_4-N and 10 mg l^{-1} organic nitrogen.

Organic Compounds: The organic compounds studied for their possible interferences in ammonium-N determination in water, 2M potassium chloride and 0.5M potassium sulphate are shown in Table 1.

Determination of Ammonium-N: Ammonium nitrogen was measured by a modification of the indophenol green method using a complexing reagent to prevent interferences due to the precipitation of hydroxides in the reagent system. With the inclusion of a sodium nitroprusside catalyst, the sensitivity of the method was such that ammonium could be determined in the range of 0 to 1 ppm and with care 0 to 0.1 ppm (Brown, 1973). This method is applicable to water samples and a wide range of soil extractant solutions and acid digests of plant or soil material.

Procedure: The ammonium-N manifold as shown in the figure was used for NH_4-N determination in water and also to determine the possible interference of organic nitrogen compounds. The samples were run at the rate of 40 per hour and the colour was developed in the water bath at 38°C. The colour intensity was measured at 650 nm. the calibration graph for NH_4-N is linear from 0 to 5 mg NH_4-N. The solutions were analysed for the determination of NH_4-N in organic N solution with and without NH_4-N in water 2M KCl and 0.5M K_2SO_4 using 0 and 1 mg l^{-1} NH_4-N working standards and blank solutions.

Results and Discussion

Experiment was conducted to investigate the interferences by 17 amino acids, galactosamine, glucosamine and urea. These compounds were evaluated for interferences in ammonium-N determination in water, potassium chloride and potassium sulphate solution at ammonium-N level of 0 and 1.0 mg l^{-1} (Table 2).

The results in Table 2 show positive interferences by amino acids at 0 mg l^{-1} ammonium-N addition in water, potassium chloride and potassium sulphate. These interferences ranged from 0 to 0.17 mg l^{-1}. These results show positive interference by some amino acids while others did not show colour development using the Berthelot reaction. The positive interferences might be due to either ammonium-N impurities in the amino acid samples, their participation in the reaction or their hydrolysis under the reaction conditions to produce ammonium-N. However, it is not possible to distinguish between positive chemical interferences and ammonium-N impurities. Amino acids with small molecular weights showed small positive interferences which disappeared in the amino acids with the larger molecular weights (either branched or straight chain). The amino acid sample solutions were prepared on the basis of molecular weight divided by the number of N atoms in their molecules. Therefore, all these solutions had same molarity. However, it is not as simple to make a clear comparison of the concentrations of amino acids such as glycine and arginine which contain different forms of N in their molecules.

There are some effects of the functional groups. For instance, the comparison between the carboxylic acid and amide containing amino acids shows that the carboxylic acids caused more interference than the corresponding amides. The ring structures either aromatic or heterocyclic showed no interference at all. The largest interference which was due to galactosamine again might be due to either its hydrolysis or ammonium-N impurities.

These results lead to the conclusion that there is no breakdown of the amino acids under the present reaction conditions as the interferences are shown by the short chain unsubstituted amino acids such as glycine and not by those having functional groups which might more easily hydrolyse to yield ammonium-N such as lysine or glutamine.

The addition of the amino acids, galactosamine and glucosamine caused negative interferences at 1.0 mg l^{-1} ammonium-N in water, potassium chloride and potassium sulphate. Only urea interfered positively. There was little difference in its interference between water and the salt solutions. However, in the case of the amino acids negative interference increased between water and the salt solutions. The negative interferences by the unsubstituted amino acids were inversely proportion to their molecular weights (glycine to isoleucine, see Table 2). On the other hand, the interferences by the amino acids having hydroxyl or carboxylic groups in their molecules such as serine, threonine, aspartic and glutamic acids increased with an increase in the molecular weight. As far as the effect of amides on the interference is concerned, the amino acids with amide groups showed less interference than the corresponding carboxylic acids. Of the five remaining amino acids, arginine and histidine showed low negative interferences compared with lysine, proline and phenylalanine. Galactosamine and glucosamine showed almost similar interferences at this concentration

Table 2: Interferences by organic nitrogen compounds.

	Effect in (mg l^{-1}) N of organic compounds at two ammonium concentrations					
	0	1	0	1	0	1
Organic compounds	Water		2M KCl		0.5M K_2SO_4	
Glycine	0.03*	-0.09**	0.03	-0.21	0.03	-0.21
Alanine	0.02	-0.07	0.02	-0.19	0.02	-0.19
Valine	0.00	-0.03	0.00	-0.10	0.00	-0.13
Leucine	0.01	0.00	0.00	-0.09	0.00	-0.07
Isoleucine	0.00	-0.03	0.00	-0.10	0.00	-0.12
Serine	0.02	-0.05	0.01	-0.12	0.01	-0.14
Threonine	0.00	-0.08	0.00	-0.19	0.00	-0.20
Methionine	0.00	-0.23	0.00	-0.38	0.00	-0.40
Aspartic acid	0.04	0.00	0.04	-0.09	0.08	-0.08
Glutamic acid	0.03	-0.05	0.03	-0.18	0.02	-0.14
Asparagine	0.01	-0.01	0.02	-0.04	0.01	-0.04
Glutamine	0.02	0.00	0.02	-0.03	0.02	-0.03
Lysine	0.02	-0.04	0.02	-0.09	0.02	-0.09
Arginine	0.00	-0.01	0.00	-0.04	0.00	-0.02
Phenylalanine	0.00	-0.12	0.00	-0.13	0.00	-0.16
Proline	0.00	-0.07	0.00	-0.18	0.00	-0.19
Histidine	0.00	-0.03	0.00	-0.04	0.00	-0.06
Urea	0.02	+0.02	0.09	+0.01	0.10	+0.02
Galactosamine	0.17	-0.01	0.09	-0.07	0.10	-0.06
Glucosamine	0.02	-0.02	0.01	-0.06	0.01	-0.06

*Each value is the mean of two replicates; ** This is a corrected value by deduction the interference (mean of two replicates) caused by each amino acid at 0 mg l^{-1} N.

ammonium-N. Methionine was the amino acid which suppressed the colour development most strongly in this study. It inhibited colour formation by up to 23 Percent in water, 38 Percent in 2M potassium chloride and 40 percent in the 0.5M potassium sulphate.

These results show that many of the amino acids, and particularly methionine, if present in soils and extracted by potassium salts can cause errors of up to 40 percent in the determination of available ammonium-N by the Berthelot reaction. There is evidence of larger interferences by the amino acids in the literature. White and Gosz (1981) compared an automated indophenol method (Technicon Industrial Method No. 98-70W) with steam distillation (Bremner, 1965) for the determination of ammonium-N in 2M KCl extracts of forest floor samples. They found that amino acids in 2M KCl contributed significant positive interferences to the automated method. This method overestimated ammonium-N in extracts from 17-26 per cent. To minimize the interferences by the amino acids, they modified the method by using 110 g l^{-1} sodium hydroxide instead of 200 g l^{-1} in the phenol reagent (which lowered the pH to 12.5 in the reagent and to 12.0 in the final solution). They also lowered the temperature from 90°C to 60°C for the colour development step. Their modification yielded results identical to steam distillation analysis.

Rowland (1983) reported that amino acids such as glycine, glutamic acid, alanine, leucine and aspartic acid at a concentration of 1 mg l^{-1} interfered in the ammonium-N determination by the nitroprusside catalysed indophenol reaction at low temperature (Technicon Industrial Method No. 329-74W) and showed an apparent recovery compared with steam distillation of up to 47 percent of the amino acids nitrogen. He concluded from these results that interference was not due to contamination of ammonia in the reagent but the breakdown of the amino acids in solution. He also found levels of interference which ranged from 1.3 to 17.1 percent in the extracts of 19 soils having low ammonium content (below 4.0 mg l^{-1} extractable ammonium-N). There were significant correlations between the interference level and the loss on ignition and the total nitrogen content of the soil. He concluded that release of ammonia due to breakdown of organic fractions in soil extracts seem to occur at the temperature and pH of nitroprusside catalysed reaction. Attempts to reduce the interference levels by adjusting the pH of the reaction, inclusion of a buffer or by reduction of the heating period in the colour formation step did not produce significant reduction in the interference.

Burton *et al.* (1989) analysed amino acids in 2M KCl solutions containing ammonium-N at 20 mg l^{-1} by both an automated Berthelot procedure (Technicon Industrial Method No. 98-70W) and by distillation with magnesium oxide (Bremner, 1965). They also analysed 2M KCl soil extracts by both steam distillation and an automated indophenol method. Less than 2 percent of the organic

Table 3: Comparison of different reaction conditions.

Source	Method	Temperature (°C)	NaOH in reagent (g l^{-1})	NaOH in final solution (g l^{-1})
White and Gosz (1981)	Technicon Industrial Method 98-70W	90	200	41
White and Gosz (1981)	Technicon Industrial Method 98-70W modified	60	110	22
Rowland (1983)	Technicon Industrial Method 329-74 W	37	20	13
Burton et al. (1989)	Technicon Industrial Method 98-70W	90	200	41
Searle (1990)	Technicon Industrial Method 98-70W	90	200	41
Searle (1990)	Method A	45	45	11
Searle (1990)	Method B	30	10	5
Brown (1973)	Present Method	37	22.5	8

Table 4: Percentage recovery of amino acid nitrogen as ammonium-N.

Nitrogen source amino acids	Technicon method* method 98-70W	Method 1 searle A	Method 2 searle B	Method 3 Brown
Threonine	94	22	4	0.0
Glycine	70	25	4	0.3
Alanine	36	15	1	0.2
Glutamic acid	19	11	1	0.3
Methionine	17	11	1	0.0
Leucine	12	6	1	0.0
Phenylalanine	10	12	1	0.0

* Burton *et al.* (1989). The value is percent apparent recovery of 20 mg N l^{-1}.

1. Searle (1984). The value is percent apparent recovery of 20 mg N l^{-1}.
2. Searle (1990). The value is percent apparent recovery of 20 mg N l^{-1}.
3. Brown (1973). Method followed in present study. The value is percent apparent recovery of 10 mg N l^{-1}.

nitrogen present in the various solutions was detected as ammonium by steam distillation. With the automated indophenol procedure, the apparent recovery of amino nitrogen as ammonium varied from 0 to 94 percent. Except for threonine, the percentage apparent recovery tended to be inversely related to the molecular weights of the amino acids.

The automated indophenol method compared with steam distillation showed apparent recovery as ammonium in 2M KCl extracts of soil samples. Amino acid interference may be particularly important in perturbed soil samples and in studies of the inter-conversion of organic and inorganic forms of nitrogen. Therefore, they recommended distillation for ammonium determination in such samples to avoid over or under estimation of the different forms of nitrogen.

Searle (1990) suggested that the Berthelot reaction can be used for measuring ammonium ions in the presence of appreciable concentration of amino acids, provided that the reagents and reaction conditions used are carefully chosen to limit hydrolysis. He suggested that this is best achieved by using the nitroprusside catalysed reaction, which enables the use of low sample volumes, low reagent concentrations and low reaction temperatures.

The present method (Brown, 1973) is a nitroprusside catalysed indophenol reaction at a low temperature of 37°C. The sodium hydroxide concentration in the phenol reagent was 22.5 g l^{-1} and in the final reaction mixture solution was 8.0 g l^{-1}. Moreover, Table 3 shows the comparison of the reaction conditions of the different methods as discussed above.

The above quoted research work indicated that the amino acids or other organic compounds are more or less hydrolysed due to the high pH of the reagents in the nitroprusside catalysed reaction and the high temperature in the methods without a nitroprusside catalyst. The results show that hydrolysis during the Berthelot colour reaction results in the release of ammonia which causes errors in the determination of ammonium-N of soil extracts and other biological samples. The modifications of the methods did not eliminate the positive interferences. However, only the methods of Searle (1990) reduced the positive interferences to much extent. No one has tried to investigate the inhibition effect of amino nitrogen on the ammonium recovery when added to certain ammonium-N concentration except White and Gosz (1981). The results of the present study suggest that the hydrolysis rate is very low compared with the lowest hydrolysis of amino acids reported by Searle (1990) (Table 4).

Therefore, it can be assumed that amino acids are not hydrolysed during the Berthelot colour reactions unless the amino group on the α-carbon is more readily hydrolysed in the low molecular weights amino acids. Table 4 further explains that organic compounds are not hydrolysed during the present method (Brown, 1973).

The results presented in Table 2 show that addition of the amino acids to 1.0 mg l^{-1} ammonium-N caused a negative interferences in water, 2M KCl and 0.5M K_2SO_4. This effect of the amino acids on the apparent recovery

ammonium-N seems worse in the potassium salt solutions compared with water. The amino acids present in the ammonium-N solution inhibited the Berthelot colour formation acting as reaction inhibitors, for example methionine inhibited the ammonium colour formation up to 40 per cent.

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