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Interference of Amino Acids in Ammonium-N Determination using the Berthelot Colour Reaction

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Abstract

Solutions of 10 amino acids in water, 2M KCl and 0.5M K_2SO_4 were analysed to determine their possible interference using the Berthelot colour reaction. The results confirmed the suspicion that amino acids present in samples analysed by colorimetric methods could result in over or under estimate of NH₄-N in soils. All amino acids showed positive interference at zero NH₄-N concentration. However, these amino acids in water and soil extracting solutions (2 M KCl and 0.5 M K₂SO₄) showed negative interference at 1 mg l⁻¹ NH₄-N.

Introduction

Amino acids are thought likely to occur in soil solutions and extracts. Lowe (1973) examined the amino acid distribution in hydrolysates from L, F and H horizons of forest humus layers in British Columbia and reported that amino acid-N content of horizon samples ranged from 0.07 to 0.95 percent. Particularly amino acids are suspected to cause interference in ammonium determination by colorimetric methods. The common solutions used for extraction of available nitrogen from soils are either 2 M KCl or $0.5 \text{ M K}_2\text{SO}_4$. The available nitrogen is measured from soil extracts and total nitrogen from Kjeldhal digests by manual or automated method (Bremner and Mulvaney, 1982). 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 acids interference in the ammonium determination by automated method for soil extracts. Searle (1990) concluded that the reaction of Berthelot can be used for measuring NH₄-N in the presence of appreciable concentration of amino acids, provided that the reagents and reaction conditions used are carefully chosen to limit hydrolysis. Considering the importance of presence of amino acids in the soil solutions, it was imperative to conduct experiments to know level of interference caused by amino acids in the determination of N₄-N by the Berthelot colour reaction using the Technicon AutoAnalyzer II.

Materials and Methods

Experiment was conducted to determine the interference of amino acids in ammonium nitrogen determination using the fierthelot colour reaction in the Laboratory of Department of AFE, University of Glasgow, Scotland, UK. The Technicon AutoAnalyzer II was used for the analysis of ammonium-N. Ten amino acids were selected to use in this experiment. The Technicon AutoAnalyzer II consisted of sampler, proportioning pump, a water bath with constant temperature and colorimeter equipped with either 530 or 650 or 880 nm filters and phototubes. Results of the samples were recorded with a single pen chart recorder. This system was connected to a BBC microcomputer which was used for the measurement of peak heights and calculations of results. The reagents bottles were also put in a separate water bath with a constant temperature of 25° C.

Ammonium nitrogen was measured by a modification of the indophenol green method using a complexing reagent to prevent interference 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 solution and acid digests of plant or soil material. The flow system is shown in Fig. 1.

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

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Fig. 1: AutoAnalyzer Manifold For Determining NH₄-N

Sodium hypochiorite 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 I^{-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 $N I^{-1}$): Ten ml of 1000 mg $I^{-1} NH_4$ -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 I⁻¹): One ml of 100 mg I⁻¹ NH₄-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 was analysed as zero NH_4 -N solution with a set of samples.

Working solutions of Amino acids: The required weight of 10 amino acids were transferred to 25 ml volumetric flasks carefully and volume was made to the mark with deionized water. The strength of each stock solution was 1000 mg N I^{-1} .

Amino acids samples withotjt added NH₄-N: One ml of each

amino acid stock solution (1000 mg N I^{-1}) was diluted separately into 100 ml volumetric flasks with deionized water. Each solution contained 10 mg N I^{-1} .

Amino acids sample with added NH₄-N: One ml of NH₄-N (100 mg N l⁻¹) and 1 ml of amino acid solution (1000 mg N l⁻¹) were added together by an automatic pipette into 100 ml volumetric flasks for each amino acid. These were then diluted with deionized water to produce solution containing 1 mg l⁻¹ NH₄-N and 10 mg l⁻¹ organic nitrogen.

Procedure: The ammonium-N manifold as shown in Fig. 1 was used for NH₄-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 NH₄-N is linear from 0 to 5 mg NH₄-N. The solutions were analysed for the determination of NH₄-N in organic N solution with and without NH₁-N in water using 0 and 1 mg l⁻¹ NH₄-N working standards and blance solutions.

Results and Discussion

It is clear from the results that all amino acids showed positive interference at zero ammonium-N concentration. Glycine showed greater interference than all other amino acids in water and 2 M KCI. It remained equal with alanine in K_2SO_4 .

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	Effect in (mg I^{-1}) N of organic compounds at two ammonium concentrations					
	0	1	0	1	0	
Amino Acids	Water		2 M KCI		0.5 M K ₂ SO ₄	
Alanine	0.12*	-0.04**	0.14	-0.08	0.12	-0.17
Glutamic acid	0.02	-0.02	0.09	-0.10	0.07	-0.17
Glycine	0.21	-0.03	0.24	-0.10	0.12	-0.22
Histidine	0.04	-0.03	0.02	-0.04	0.02	-0.07
Methionine	0.10	-0.16	0.02	-0.26	0.01	-0.35
Phenylalanine	0.05	-0.06	0.05	-0.11	0.03	-0.15
Proline	0.09	-0.02	0.03	-0.08	0.02	-0,15
Serine	0.10	-0.05	0.09	-0.11	0.06	-0.18
Threonine	0.10	-0.03	0.08	-0.10	0.06	-0.18
Valine	0.03	-0.02	0.03	-0.10	0.02	-0.19

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

Amino acids in water and soil extracting solutions (2 M KCI and 0.5 M K₂SO₄) showed negative interferences at 1.0 mg I^{-1} NH₄-N. Methionine suppressed most strongly the colour development due to added NH₄-N. This resulted in the negative interference of -0.16, -0.26 and -0.35 in water, 2 M KCI and 0.5 M K₂SO₄, respectively (Table 1). It is very clear that the negative interfernce by amino acids has increased in the salt solutions commonly used in soil extracting research work. Probably this is due to the effect of chloride and sulphate ions on the chemistry of the Berthelot reaction. This confirmed the suspicion that amino acids present in sample analysed by calorimetric methods could result in over or underestimate of NH₄-N in soils. These results are in accordance with those of Rowland (1983), Burton et al. (1989) and Searle (1984) who pointed out the interference caused by amino acids during the automated method of NH₄-N determination. It can be concluded from these results that ammino acids cause interferences in NH₄-N determination by the Berthelot (1859) indophenol colour reaction.

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