http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journals of Biological Sciences, 2 (3): 813-816, 1999 *Research Article*

Investigation of Interfering Reaction Conditions in Ammonium-N Determination using the Berthelot Colour Reaction

Muhammad Ayyaz Khan and T.H. Flower*
Department of Agronomy, Faculty of Agriculture, Gomal University, D.I. Khan, NWFP, Pakistan
*Department of AFE, University of Glasgow, G12 8 QQ, Scotland, UK

Abstract

Solutions of three amino acids (serine, glycine and proline) were analyzed under varying reaction conditions for their interferences in ammonium-N determination using the Berthelot indophenol colour reaction. The results regarding the changes made in the reaction conditions revealed that freshly prepared reagents should be used in studies investigating interferences by amino acids. Room temperature should be stable or reagents bottles should be placed in a water bath having a constant temperature of 25°C. The hypochlorite reagent should be freshly prepared at a concentration of 50 ml l^{-1}.

Introduction

The available nitrogen is measured from soil extracts and total nitrogen from kjeldhal digests by manual or automated techniques (Bremner and Hauck, 1982; Keeny and Nelson, 1982). The calorimetric methods of ammonium-N determination in aqueous solution based on the Berthelot colour reaction either catalysed or without catalyst have been reviewed by Searle (1984). There is evidence about interferences due to the presence of amino acids in Samples during nitrogen determination by calorimetric methods from the clinical research. Forgan-Smith *et al*. (1976) described the Berthelot indophenol reaction as a reliable method for the estimation of ammonia generated by renal tissue slices in Krebs-Ringer bicarbonate solution containing glutamine concentration not exceeding 0.5 mmol/l in the final solution to be assaysed.

Reaction conditions can change the extent of interference by amino acids and the sensitivity of the specific method of ammonium-N using the Berthelot reaction. For instance, very low hypochlorite cocentrations may result in negative interference through reactions between amino acids and the hypochlorite source (Searle, 1984). Adamsen *et al*. (1985) reported that glycine and creatine gave apparent ammonium recovery, this would be due to increased breakdown of these compounds in the higher pH of the reaction.

Keeping in view the above mentioned facts it was necessary to optimize those conditions which may have an effect on the Berthelot indophenol colour reaction.

Materials and Methods

Experiments were conducted to investigate the interference lever of reaction conditions in Ammonium-N determination using the Technicon AutoAnalyzer II in the AFE Laboratory, University of Glasgow. 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 micocomputer which was used for the measurement of peak heights and calculation of results. The reagents bottles were also put in a separate water bath with a constant temperature of 25°C. The schematic diagram of the flow system is shown in the 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 a 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 11.2 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.

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 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.

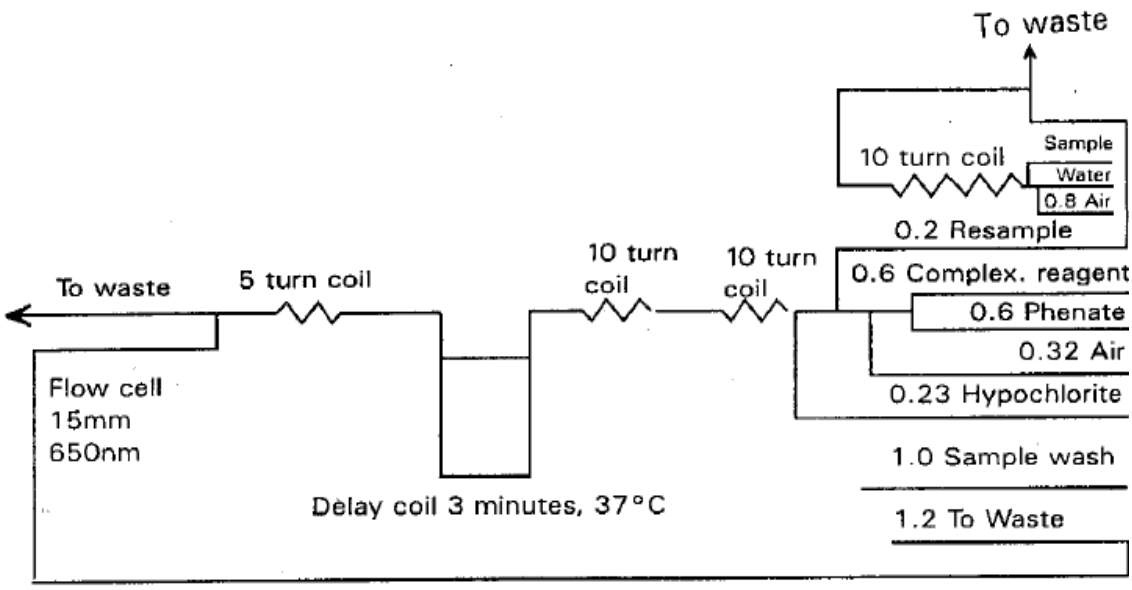

Fig. 1: Auto Analyzer Manifold For Determining NH_4-N

NH_4-N working solutions (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_e-N working Standard (1 mg N l^{-1}): One ml of 100 mg NH_4-N working solution was added to 100 ml volumetric flask and the volume was made math 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 weights of 3 amino acids (Serine, Glycine and Proline) 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}.

Amino acids samples without added NH_4-N: One ml of each amino acid 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}.

Amino acids samples with added NH_4-N: One ml of NH_4-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 three 100 ml volumetric flasks for each amino acid. These were then diluted with water to-produce solutions containing 1 mg l^{-1} NH_4-N and 10 mg l^{-1} organic nitrogen.

Procedure: The ammonium-NI manifold as shown in Fig. 1 was used for NH_4-N determination in water and also to determine the possible interference of amino acids. 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 solution were analysed for the determination of NH_4-N in organic N solution with and without NH_4-N in water using 0 and 1 mg l^{-1} NH_4-N working standards and blank solutions. Possible interferences were found by making changes in reaction conditions.

Results and Discussion

Effect of reagent age: Solutions of three amino acids were studied for their interferences in water for their interferences in NH_4-N determination using one day and seven days old reagents. The results obntained are presented in Table 1. Evidently the age of the reagents did not have much effect on the interference by amino acids in ammonium determination. However, freshly prepared reagents gave more reproducible results.

Effect of temperature: The effect of temperature 0, 20, 40 and 60°C was evaluated for its influence on the interferences due to amino acids in ammonium-N determination. The temperature was arranged by water from a controlled temperature water bath flowing through

Table 1: Effect of reagent age on interferences caused by amino acids

		Effect in (mg l^{-1}) N of organic compounds at two ammonium concentrations					
		Water		2 M KCl		0.5 M K_2SO_4	
Reagent age (days)	Amino acid	0	1	0	1	0	1
1	Serine	0.03*	-0.01**	0.02	-0.04	0.02	-0.05
	Glycine	0.07	-0.01	0.05	-0.08	0.05	-0.10
	Proline	0.02	-0.01	0.00	-0.13	0.00	-0.07
7	Serine	0.04	-0.01	0.03	-0.02	0.03	-0.03
	Glycine	0.10	-0.01	0.07	-0.06	0.07	-0.04
	Praline	0.04	-0.00	0.02	-0.12	0.02	-0.03

Table 2: Effect of reagent temperature on interferences caused by amino acids

		Effect in (mg l^{-1}) N of organic compounds at two ammonium concentrations					
		Water		2 M KCl		0.5 M K_2SO_4	
Temperature (°C)	Amino acid	0	1	0	1	0	1
0	Serine	0.03*	-0.04**	0.03	-0.02	0.03	-0.02
	Glycine	0.10	-0.02	0.10	-0.04	0.10	-0.02
	Proline	0.02	+0.01	0.02	-0.10	0.02	-0.01
20	Serine	0.04	+0.01	0.04	-0.04	0.03	-0.03
	Glycine	0.10	-0.03	0.07	-0.08	0.07	-0.07
	Praline	0.03	-0.01	0.02	-0.13	0.01	-0.05
40	Serine	0.04	-0.01	0.02	-0.10	0.02	-0.09
	Glycine	0.08	-0.03	0.04	-0.19	0.04	-0.16
	Proline	0.02	-0.02	0.00	-0.23	0.00	-0.15
60	Serine	0.02	-0.06	0.01	-0.24	0.00	-0.18
	Glycine	0.08	-0.08	0.00	-0.63	0.00	-0.38
	Praline	0.02	-0.05	0.00	-0.44	0.00	-0.40

Table 3: Effect of hypochlorite concentrations on interferences caused by amino acids

		Effect in (mg l^{-1}) N of organic compounds at two ammonium concentrations					
		Water		2 M KCl		0.5 M K_2SO_4	
Hypo-chlorite (ml^{-1})	Amino acid	0	1	0	1	0	1
25	Glycine	0.05*	-0.05**	-0.03	-0.35	0.02	-0.33
	Praline	0.00	-0.04	-0.06	-0.33	-0.02	-0.29
50	Glycine	0.12	-0.03	0.09	-0.05	0.09	-0.08
	Praline	0.03	-0.01	0.02	-0.03	0.02	-0.05

*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 l^{-1} N

a 1.2 cm plastic pipe. The reagents lines were wrapped around this pipe several times to ensure that the reagents reached the required temperature before entering the the system manifold. The temperature of 20, 40 and 60°C be were obtained through the water bath thermostat. 0°C was maintained by adding ice to the water bath. The results are shown in Table 2. At zero ammonium-N, the amino acid positive interference remained almost unaffected in water, but it was reduced in salt solutions with increase in temperature. However, an increase in temperature resulted in increased negative interferences by amino acids at 1.0 mg l^{-1} ammonium-N in potassium chloride and sulphate solutions but not in water. These results point out that any fluctuation in room temperature will lead to the variation in results. This problem of inconsistency in the results can overcome by placing the reagent bottles in a water bath with a constant temperature of 25°C.

Effect of hypochiorite concentration: The effect of two hypochiorite concentrations is shown in Table 3. Two solutions of hypochlorite reagent were prepared by dissolving separately 25 ml and 50 ml hypochlorite in a litre of deionized water. The same test solutions of amino acids were run sequentially using these hypochlorite solutions. All

other conditions and reagents were kept constant. The results of the two hypochlorite concentrations shown in Table 3 indicate that amino acids at zero ammonium-N addition caused small negative interferences in salt solutions at low hypochlorite concentration. In water, however, their interferences were positive. Only glycine showed a positive interference in potassium sulphate, Use of 50 ml per litre hypochlorite resulted in positive interferences by these amino acids at zero ammonium-N concentration in all three solutions.

At 1.0 mg l^{-1} ammonium-N, both amino acids interfered negatively at both hypochlorite concentrations. Addition of amino acids to all three solutions at 1.0 mg l^{-1} ammonium-N and low hypochlorite concentration resulted in strong negative interferences. At 50 ml hypochlorite per litre, these negative interferences were much reduced. It can be suggested from these results that 50 ml per litre of hypochlorite is the optimum rate to be used. These results lead to the point of important consideration that reaction conditions have an effect on interferences caused by amino acids. Similar results have been reported by Forgan-Smith *et al*. (1976), Searle (1984) and Adamsen *et al*. (1985) who all considered about the level of interferences caused by the fluctuation in reaction conditions.

References

Adamsen, F.J., D.S. Bigelow and G.R. Scott, 1985. Automated methods for ammonium, nitrate and nitrite in 2 *M* KCl-phenylmercuric acetate extracts of soil. Commun. Soil Sci. Plant Anal., 16: 883-898.

Bremner, J.M. and R.D. Hauck, 1982. Advances in Methodology for Research on Nitrogen Transformations in Soils. In: Nitrogen in Agricultural Soils, Agronomy, Stevenson, F.J. (Ed.). 22nd Edn., American Society of Agronomy, Madison, WI., USA., pp: 467-502.

Forgan-Smith, J., B.D. Slaughter and R.B. Cross, 1976. The estimation of ammonia in kidney slices in the presence of added glutamine and other amino acids. Clin. Chim. Acta, 69: 139-141.

Keeny, D.R. and D.W. Nelson, 1982. Inorganic Forms of Nitrogen. In: Methods of Soil Analysis, Part-2, Black, C.A. (Ed.). American Society of Agronomy, Madison, WI., USA., pp: 643-698.

Searle, P.L., 1984. The Berthelot or indophenol reaction and its use in the analytical chemistry of nitrogen: A review. Analyst, 109: 549-568.