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Elimination of Interferences of Organic Nitrogen Compounds by Dialysis Inclusion with Technicon AutoAnalyzer II in Ammonium-N Determination

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Abstract

The dialysis system was included in the main manifold for ammonium-N determination of the Technicon AutoAnalyzer II. Studies using this system indicated that only traces of $\text{NH}_4\text{-N}$ were present as impurities in organic nitrogen compounds such as urea, aspartic acid, lysine and histidine. Positive and negative interferences in the $\text{NH}_4\text{-N}$ determination were attributed to hydrolysis or participation of the organic nitrogen compounds in the colorimetric reaction. These positive and negative interferences were eliminated by gas dialysis. The level of random variability measured with dialysis was consistent with random variability due to solution preparation and to the system complexity. The dialysis system was further developed for the determination of $\text{NH}_4\text{-N}$ in Kjeldhal digests. Dialysis use with Technicon AutoAnalyzer II did not disturb the precision of the system.

Introduction

There are chemical interferences caused by certain organic compounds in the ammonium-N determination by the automated Berthelot reaction. The interference can be eliminated by including distillation or gas phase dialysis as a pre-treatment step (Crowther and Evans, 1980). Flow injection analysis (FIA) has become a modern technique rather than continuous flow analysis (CFA). The gas dialysis using PTFE membrane has been included as pre-treatment in the FIA system (Aoki *et al.*, 1986; Martin and Mayerhoff, 1986; and Willason and Johnson, 1986). Hara *et al.* (1988) developed a gas dialysis concentrator which continuously gave about a ten fold increase in the concentration of ammonium ions recovery in water analysis. Schulze *et al.* (1988) proposed that gas separation is more satisfactory than the routine methods such as nesslerization and the Berthelot reaction for determining of ammonium in seawater.

Keeping in view the above facts a PTFE gas dialysis system was developed and included with the Technicon AutoAnalyzer II system of ammonium-nitrogen determination to eliminate the interferences caused by organic nitrogen compounds.

Materials and Methods

The study was carried out to investigate the elimination of interferences of organic nitrogen compounds by dialysis inclusion with Technicon AutoAnalyzer II in Ammonium-N determination in the Department of AFE, University of Glasgow, Scotland, UK. The glassware used in this experiment was 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 .

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). The method is applicable to water samples and a wide range of soil extractant solutions, acid digests of plant or soil material. The schematic diagram of the flow system is shown in Fig. 1. The dialysis system was included with AutoAnalyzer Manifold for determining $\text{NH}_4\text{-N}$ as shown in Fig. 2.

1M NaOH: Forty gram sodium hydroxide was dissolved in 500 ml of deionized water in 1 litre volumetric flask. After cooling, the volume was made up to the mark with deionized water.

2M HCl: One hundred and seventy ml concentrated HCl was diluted with deionized water and volume was made up to the mark in 1 litre volumetric flask.

1M HCl: Eighty five ml concentrated HCl was diluted with deionized water in 1 litre volumetric flask up to the mark.

0.01M HCl: Ten ml of 1M HCl acid were pipetted into 1 litre volumetric flask and volume was made up to the mark with deionized water.

Gas Dialysis membranes

Tetrafluoroethene (TFE) 1" x 600: This was a product (Part No. 14-831-3006) of Fisher Scientific Co; USA.

Polytetrafluoroethene (PTFE) 1" x 600: This was a product (Military Specification) of Gortex W.L. and Associates, New York, Delaware, USA.

Dialysis blocks: These were 3" and 6" dialyzers of the Technicon Corporation, Newyork, USA.

Results and Discussion

Twenty organic compounds were evaluated for their interference potential in the determination of ammonium-N using the dialysis system with the Technicon AutoAnalyzer II. The results are shown in Table 1.

Table 1 shows the interferences caused by the organic nitrogen compounds in water without dialysis and chemical interferences caused by the organic nitrogen compounds in ammonium-N determination using the dialysis system with the Technicon AutoAnalyzer II. If there were ammonium-N impurities in the organic nitrogen compounds, they would show positive interferences in the ammonium-N measurement even with dialysis.

But the dialysis results (column 3) show that the chemical interferences caused by organic compounds (without dialysis) as shown in column 1 were mostly removed or were much reduced after inclusion of the dialysis step in the system. Small effects shown by aspartic acid, lysine and histidine may be due to trace ammonium impurity. Only urea showed clear evidence of the presence of ammonium-N impurity as its positive interference remained unchanged. The effects in column 1 are not, therefore, due to ammonium-N impurities, but are probably due to the hydrolysis of the organic nitrogen compounds or their taking part in the Berthelot reaction. Glutamine gave an odd result with the dialysis system. There was low possibility of glutamine hydrolysis because the pH of the final mixture solution in the donor stream was 12.0. Its greater positive interference might be due to some ammonium-N cross contamination during the sample preparation at this time of analysis.

The addition of organic nitrogen compounds showed very small effects on the ammonium-N measurement at 1.0 mg l⁻¹ ammonium-N. The chemical interferences were mostly reduced with the inclusion of the dialysis in the ammonium system of the Technicon AutoAnalyzer II. For example, the negative interferences by methionine and phenylalanine were reduced from 23 and 12 per cent to 0 per cent and 1 per cent, respectively. The small effects shown in the column 4 on the ammonium-N recovery showed both positive and negative deviations. These small effects are probably not chemical interferences but are due to the random error caused by the solution variability and system complexity. This study showed a small level of variability in the ammonium-N measurement of the same solution of approximately the same magnitude as in column 4 of Table

digests vary in their acid content due to acid evaporation during the digestion and reaction of acid with the sample. Therefore, even after the dilution of the digests with deionised water, still there is variation in the final concentration of the acid of the samples. This acidity variation of the samples could cause variation in the pH of the final mixture reaction during the colour development. Consequently, it will affect the colour formation among the samples. It was, therefore, appropriate to test the developed dialysis system shown in the Fig. 2 for the analysis of the Kjeldahl digests to eliminate the acidity effect on the colour development during the Berthelot colour reaction.

Table 1: Interferences by organic nitrogen compounds in water.
Effect in (mg l⁻¹) N of organic compounds at two ammonium concentrations

Organic compounds	Without dialysis		With dialysis	
	0	1	0	1
	--(mg l ⁻¹)--		--(mg l ⁻¹)--	
Glycine	0.03*	-0.09**	0.00	+0.02
Alanine	0.02	-0.07	0.00	+0.01
Valine	0.00	-0.03	0.00	+0.01
Leucine	0.01	0.00	0.00	-0.03
Isoleucine	0.00	-0.03	0.00	-0.01
Serine	0.02	-0.05	0.00	0.00
Threonine	0.00	-0.08	0.00	0.00
Methionine	0.00	-0.23	0.00	0.00
Aspartic acid	0.04	0.00	0.02	-0.02
Glutamic acid	0.03	-0.05	0.00	0.00
Asparagine	0.01	-0.01	0.00	+0.01
Glutamine	0.02	0.00	0.10	-0.04
Lysine	0.02	-0.04	0.01	-0.02
Arginine	0.00	-0.01	0.00	-0.02
Phenylalanine	0.00	-0.12	0.00	+0.01
Proline	0.00	-0.07	0.00	0.00
Histidine	0.00	-0.03	0.01	-0.02
Urea	0.02	+0.02	0.02	-0.03
Galactosamine	0.17	-0.01	0.03	+0.01
Glucosamine	0.02	-0.02	0.00	+0.01

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

Factors like dilution and neutralization of the digests, temperature rise due to the reaction of acid and sodium hydroxide and the donor flow rate may have influence on the ammonia transfer through the hydrophobic membranes used in the dialysis. Therefore, the system shown in the Fig. 2 was subjected to different modifications for its applicability for Kjeldahl digests. The initial objective of these changes was to perform the dilution, neutralization and dialysis in a single step but this was not achieved because of unstable flow through the donor stream of the

Evaluation of the Dialysis System analysing Kjeldahl digests: Kjeldahl digests contain high acid concentration and high ammonium-N content. These digests can be diluted and neutralized with sodium hydroxide before the determination of ammonium-N by the Berthelot reaction. However, the

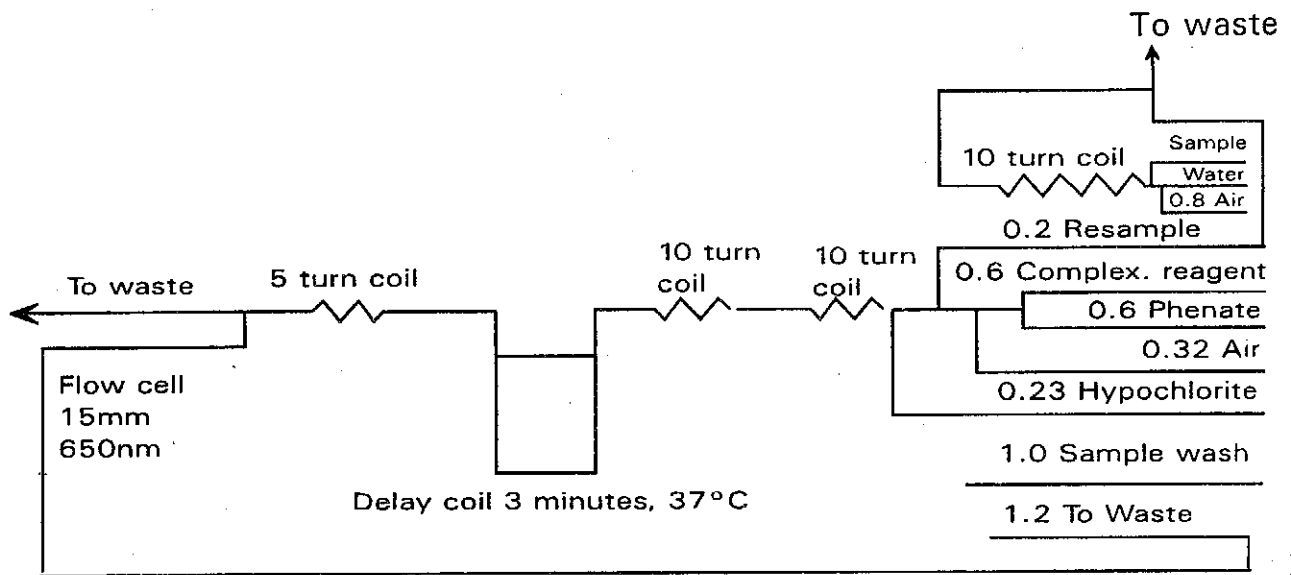


Fig. 1: AutoAnalyzer manifold for determining $\text{NH}_4\text{-N}$

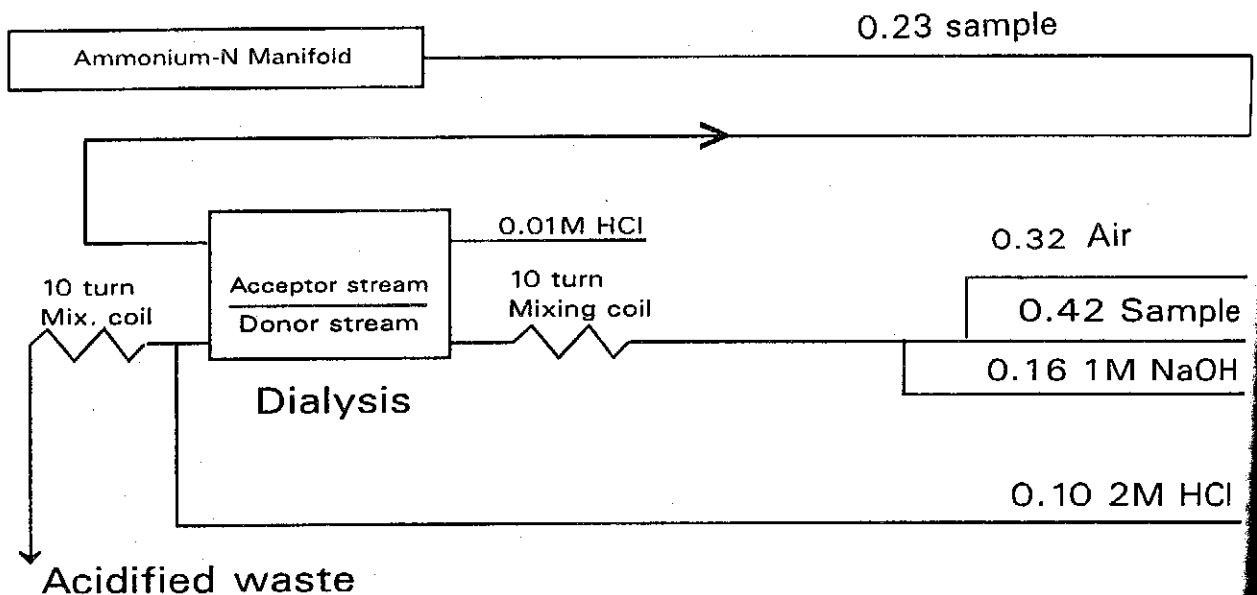


Fig. 2: Developed dialysis system

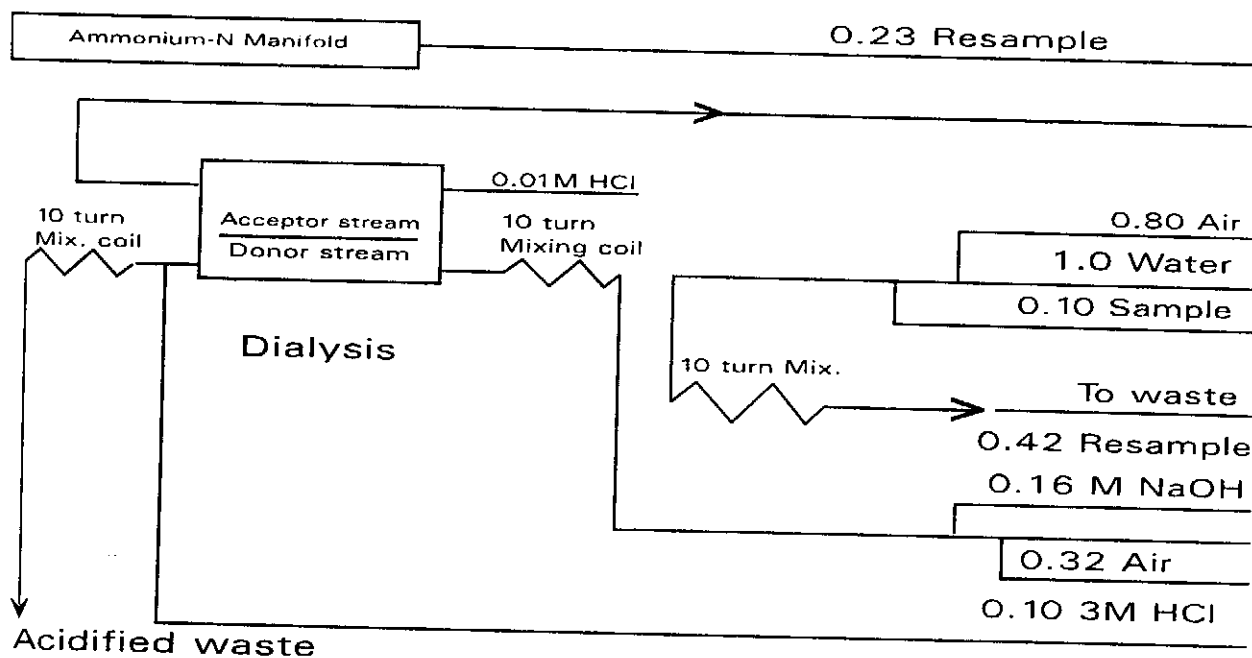


Fig. 3: Dialysis system for total nitrogen measurement

dialysis system. So, a separate dilution step was included with the dialysis system and the neutralization and dialysis were performed in the second step as shown in the Fig. 3. Fifty Kjeldahl digests of grass were analysed for their ammonium-N content with and without dialysis. It is evident from these results that the two methods showed very good correlation ($y = 0.04 + 1.00x$, $r^2 = 1.00$). Although a paired T test showed a significant difference ($p < 0.001$) between the two methods, the means differ only by 0.05 percent. It appears that the acid variation in the digests did not show adverse effect on the determination when using simple dilution.

Table 2: Precision of the Dialysis system.

	Without dialysis	With dialysis
Mean	2.84	2.77
Standard deviation	0.0230	0.0153

Precision of the Final Dialysis System: The same Kjeldahl digest was analysed in 20 individual sample cups with and without the dialyses. The mean and the standard deviation are shown in Table 2.

Table 2 shows that both methods used for ammonium-N determination in Kjeldahl digest gave very low standard deviations. Therefore, dialysis use with the Technicon AutoAnalyzer II did not disturb the precision of the system.

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