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**AN AUTOMATED METHOD FOR DETERMINING
NITRATE-NITROGEN IN SOIL EXTRACTS**

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SUMMARY

A method is described in which nitrate is determined automatically in 2N KCl extracts of calcareous soil. Nitrate is reduced to nitrite with hydrazine and copper catalyst under alkaline conditions at 37°C. The nitrite formed is reacted with sulphanilamide in acid solution to produce a diazonium salt. This salt is then coupled with N-1-naphthylethylenediamine dihydrochloride to form a soluble coloured compound. The absorbance of the coloured solution is measured at 520 m m.

The method has advantages over current published methods in that problems associated with precipitation of calcium and magnesium ions were overcome by buffering the sample stream at a pH level that allowed for the conversion of substantial amounts of nitrate to nitrite but prevented precipitation. In addition, the method has removed the need for a dialyser to overcome interfering cations. The method is precise, accurate, sensitive and rapid. The operating range is 0 to 4 p.p.m. $\text{NO}_3\text{-N}$ (solution) with a coefficient of variation of 0.7%.

I. INTRODUCTION

Nitrogen is the plant nutrient most commonly found deficient in the soils of the wheat growing areas of Queensland. Knowledge of the nitrate-nitrogen level in the top 60 cm of soil before planting is used on the Darling Downs for nitrogen fertilizer recommendations. Many research programmes have been undertaken to provide a parameter for prediction of nitrogen fertilizer requirements and subsequently to improve the precision of prediction. Such programmes generate large numbers of samples which require analysis for nitrate-nitrogen.

In the past, Bremner and Keeney's (1966) distillation method was employed. For large-scale sampling, this procedure is time-consuming and results in a considerable time delay between sample collection and obtaining a result. Consequently, it was decided that the whole analytical undertaking should be automated.

Numerous methods have been proposed for the determination of microgram quantities of nitrates in natural waters, sewerage, blood and soil extracts. Most methods for determining low concentrations of nitrate are based on its reduction to nitrite with subsequent colorimetric determination. In the colour reaction, the nitrite ion reacts with sulphanilamide and N-1-naphthylethylenediamine dihydrochloride in acid conditions to form a violet-red soluble dye. It was decided to use this procedure and to apply it to automatic analysis on an AutoAnalyser.

The reduction of nitrate to nitrite can be accomplished using various agents. Hydrazine (Mullins and Riley 1955), zinc (Chow and Johnstone 1962, Litchfield 1967), cadmium (Bernard and Macchi 1966), cadmium amalgam (Morris and Riley 1963) and copperized cadmium (Armstrong, Stearns and Strickland 1967, Henriksen and Selmer-Olsen 1970) have been used.

Reduction columns prepared using zinc, cadmium, copperized cadmium and cadmium amalgam suffer from various limitations. All columns require time-consuming preparation and washing steps in their manufacture as well as careful maintenance to preserve their efficiency.

Liberation of gas bubbles (Henriksen and Selmer-Olsen 1970) and compaction of the metallic particles (Karlsson and Torstensson 1975) in the column during sample passage seriously affect the precision of the methods when used in automated systems. Incorrectly prepared columns are also capable of reducing the nitrite formed to lower oxidation states.

The use of a liquid reductant is therefore a much more attractive proposition since it can be added in the same state of activity for each sample and its efficiency remains constant throughout any given batch. Hydrazine is the most promising liquid reductant.

Five automated methods were selected to determine whether they would be suitable for the determination of nitrate-nitrogen in 2N KCl extracts of the alkaline cracking clay soils found within the Darling Downs region of Queensland.

This paper describes a method which was found to be precise, sensitive, accurate and rapid for these soils.

II. EXPERIMENTAL

Evaluation of published methods

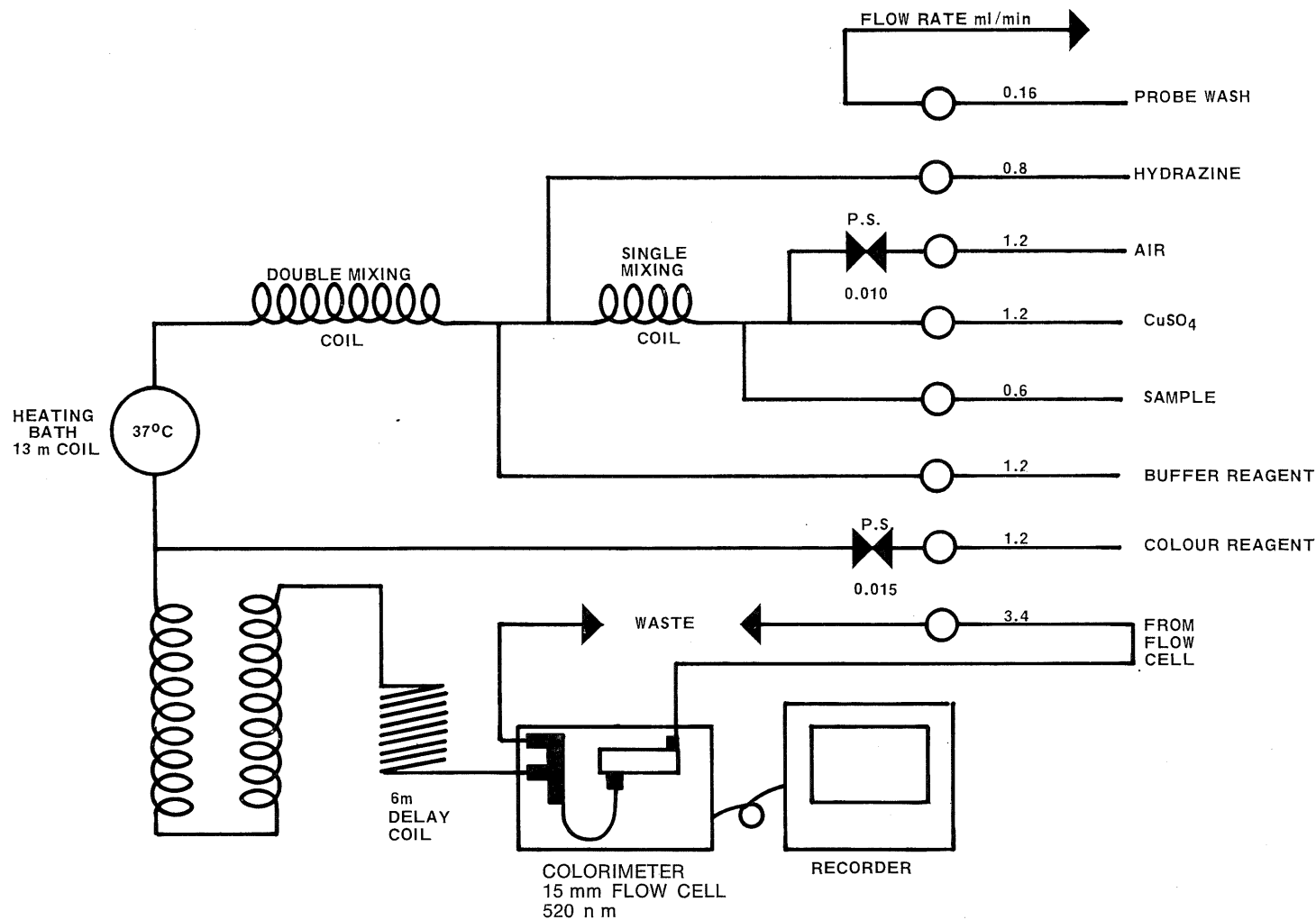
The five methods selected were those of Britt (1962), Henriksen (1965), Kamphake, Hannah and Cohen (1967), Henzell, Vallis and Lindquist (1968), and Hamm, Radford and Halstead (1970). Since the reaction requires a high pH for optimum nitrate reduction, all methods except that of Henriksen (1965) gave heavy precipitates of calcium and magnesium salts in the sample stream when sodium hydroxide reagent was added. Serious stream disruption and erroneous peak heights occurred.

Henriksen's method, although appearing to be satisfactory, required numerous reagent solutions as well as the assembly of an extensive manifold system. However, he observed that reasonable conversion of nitrate to nitrite could be accomplished without calcium and magnesium precipitation when the pH of the reduction stream was in the range 9.5 to 9.7. This pH range was maintained using a sodium hydroxide-phenol buffer solution. Unfortunately this buffer solution is very unstable and needs to be prepared daily. Phosphate and borate buffer solutions and different manifold arrangements were examined to select a combination which provided sensitivity, stability, reproducibility and simplicity of construction without the use of a dialyser.

III. MATERIALS AND METHODS

Apparatus

A Technicon AutoAnalyser I (Technicon Corporation, Tarrytown N.Y.) with a manifold construction as shown in figure 1 was used. Sample time was 75 s with a wash time between samples of 65 s thus enabling a throughput of approximately 25 per hour. The nitrate reduction coil was held at 37°C (Kamphake, Hannah and Cohen 1967) in a commercially available constant temperature bath.



P.S. – PULSE SUPPRESSOR

Figure 1. Flow diagram for the automatic analysis of nitrate in soil extracts on the AutoAnalyser.

Reagents

STOCK COPPER SOLUTION. Dissolve 3.91 g cupric sulphate A.R. in 1 litre of distilled water.

WORKING COPPER SOLUTION. Dilute 25 ml stock solution to 4 litres with distilled water. Add 4 ml Brij 35 wetting agent.

HYDRAZINE SULPHATE. Dissolve 1.2 g hydrazine sulphate A.R. in 2 litres of distilled water. This solution is stable for 1 month.

BUFFER SOLUTION. Dissolve 45 g sodium tetraborate decahydrate A.R. and 5 g sodium hydroxide A.R. in 1 500 ml distilled water. Dilute to 2 litres with distilled water when dissolved.

COLOUR REAGENT. Add 200 ml concentrated hydrochloric acid to 1 500 ml distilled water. Dissolve 20 g sulphanilamide in this dilute acid. Then add 1 g N-1-naphthylethylenediamine dihydrochloride and when this is dissolved, dilute to 2 litres with distilled water. Add 2 ml of Brij 35. Prepare weekly as a base line drift has been experienced when older colour reagent solutions have been used.

WASH SOLUTION. Add 5 ml Brij 35 to 5 litres distilled water and mix.

NOTE. If other wetting agents are to be used, it is strongly recommended that they be first tested with a little colour reagent. Wetting agents which gave colloidal precipitates with the colour reagent were Levor IV, Tween 20, Tween 40, Tween 60 and Tween 80.

Standard solutions

STOCK NITRATE-NITROGEN. Dissolve 0.7218 g oven dried potassium nitrate A.R. and 150 g potassium chloride A.R. in 400 ml distilled water. Dilute to 1 litre with distilled water.

WORKING STANDARDS. Prepare working standards in the range 0 to 4.0 p.p.m. by diluting stock nitrate solution with 2N KCl solution.

IV. RESULTS**Comparison with standard method**

A series of 107 soil extracts was prepared and the nitrate contents were determined by the AutoAnalyser method and by the distillation method of Bremner and Keeney (1966).

The linear regression relating these two sets of data is given by—

$$T=0.98 AA-0.003$$

$$r^2=0.98$$

where T=Titration result (p.p.m. NO₃-N)

AA—AutoAnalyser result (p.p.m. NO₃-N)

mean of titration results	1.25 p.p.m.	} range 0.04 to 5.2 p.p.m.
mean of AutoAnalyser results	1.27 p.p.m.	

Although the equation indicates that the AutoAnalyser results are slightly higher than those determined by distillation, a comparison of the means of the two sets of data shows a difference of only 0.02 p.p.m. In fact, this small difference is equivalent to less than one drop of acid in the titration method and is of the order of error associated with reading the graph in the AutoAnalyser method. Thus it can be seen that there is excellent agreement between the proposed method and an accepted one.

Recovery

The recovery capability of the method was evaluated by adding a known amount of nitrate-nitrogen to six soil extracts containing various nitrate levels and determining the amount of nitrate recovered. The data are presented in Table 1. Recoveries ranged from 93 to 106% with a mean recovery of 99%.

TABLE 1
NITRATE RECOVERY DATA FOR SIX SOIL EXTRACTS

Extract No.	µg NO ₃ -N/ml				% Recovery
	Present	Added	Found	Recovered	
183	1.24	0	1.24	0	..
183	1.24	0.4	1.64	0.4	100
183	1.24	1.0	2.24	1.0	100
718	0.53	1.6	2.04	1.51	94
728	0.53	1.6	2.06	1.53	96
730	3.75	1.6	5.24	1.49	93
739	2.84	1.6	4.54	1.68	105
796	0.82	1.6	2.52	1.70	106

Sensitivity

With the manifold presented in figure 1, nitrate can be determined in the range 0.04 to 4.0 p.p.m. nitrate-nitrogen in solution. Since the optimum reduction of nitrate to nitrite occurs at pH 12.2 (Kamphake, Hannah and Cohen 1967) an improvement in sensitivity could be obtained by incorporating a continuous filter assembly in the analytical train. This filter would enable interfering cations to be precipitated and removed prior to the reduction stage. A scale expander could also be used in conjunction with the recorder.

TABLE 2
PRECISION DATA FOR MEASUREMENTS OF NO₃-N IN 3 SAMPLES

Precision Index	Sample identification		
	A	B	C
Number of determinations	24	24	24
Mean (p.p.m.)	0.68	1.61	3.63
Range (p.p.m.)	0.64-0.72	1.59-1.64	3.60-3.68
Standard deviation	0.019	0.011	0.025
95% Confidence limits	0.68 ± 0.04	1.61 ± 0.02	3.63 ± 0.05
Co-efficient of variation	2.8	0.7	0.7

Precision

Three samples representing different levels of nitrate nitrogen most likely to be encountered in samples were re-analysed several times. The results are presented in Table 2.

A standard deviation of 0.011 p.p.m. for a sample containing 1.61 p.p.m. is comparable with those for other published results. Although standard deviations tend to become higher with higher nitrate samples, the co-efficients of variation are very low (0.7%) and well within acceptable limits for the type of sample submitted.

Rate of analysis

In routine work at the Queensland Wheat Research Institute, it has been found that some 160 extracts of a wide range of soil types can be analysed each day. This compares with 50 samples per day previously analysed by distillation procedures.

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