

UV-spectrophotometric Flow Injection Determination of Nitrate Using On-line Charcoal Column

M. YAQOOB AND M. MASOOM

Institute of Biochemistry, University of Baluchistan, Quetta, Pakistan

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Summary: A method is proposed for the determination of nitrate using ultraviolet spectrophotometry. The use of oxidized activated charcoal minicolumn on-line at an elevated pH eliminates organic and inorganic interferences. The absorbance is measured at 225 nm. The procedure has a limit of detection down to 1 mg l^{-1} with a relative standard deviation less than 1.0%.

Introduction

The greatest asset of the single-line manifold in FIA is its simplicity, and rapidity permitting the use of simple means of propelling the carrier stream. Therefore, even a constant head or a gas pressure reservoir, which both yield a pulse-free stream allowing excellent reproducibility of measurement, may be employed [1,2].

Various methods have been reported for the separate and simultaneous determination of nitrate including direct spectrophotometry [3,4], ion-selective electrode methods [5,6], and reduction to nitrite [7,8]. In these techniques, one of the most reliable, simple and rapid is direct spectrophotometry in the ultraviolet (UV) region, but its range is limited by interferences. In the proposed method nitrate is determined by direct spectrophotometry using oxidized activated charcoal column to obtain maximum adsorption of interfering constituents. The method can easily be applied to plant extracts for nitrate-N determination.

Results and Discussion

Activated charcoal (AC) has been found to flocculate colloidal material [10] and absorb large amount of organic and inorganic constituents present in solution [11]. The adsorption of organic ions by AC is increased in acidic solution and the inorganic acid and base adsorbing properties of activated charcoal can be modified by preliminary heating in air or oxygen [12]. Several researchers recommend aluminium sulphate buffered at pH 3.0, because it provides specific advantages for the

determination of plant nitrate-N: colloidal constituents are flocculated [13,14], the ionization of weak acids is depressed and the chelation of organic anions with aluminium is enhanced [15].

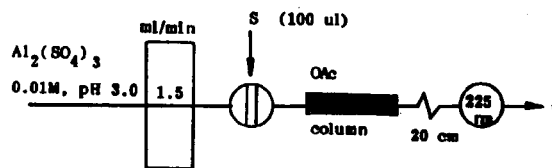


Fig. 1: Single channel FIA manifold for the determination of nitrate. OAC = oxidized activated charcoal column.

Various experimental parameters including reagent concentrations, oxidized activated charcoal column, flow rate and sample volume were optimized on the basis of univariate process using $1 \text{ M } 10^{-4}$ nitrate solution prepared in aluminium sulphate (0.01 M). The same concentration of aluminium sulphate solution was used as a carrier stream for propelling the sample towards the detector. No overall difference in the absorbance was observed using aluminium sulphate solution in the range of 0.01 - 0.025 M. However a slight effect of pH was observed as shown in Fig. 2. In place of aluminium sulphate, deionized water was also used as a carrier stream, injected nitrate standard ($1 \times 10^{-4} \text{ M}$ prepared in water). The absorbance was decreased 10 - 15% than that of aluminium sulphate and therefore, aluminium sulphate 0.01M, pH 3.0 was selected and used subsequently.

The effect of flow rate and mixing coil length on sensitivity, sampling rate and reagent consump-

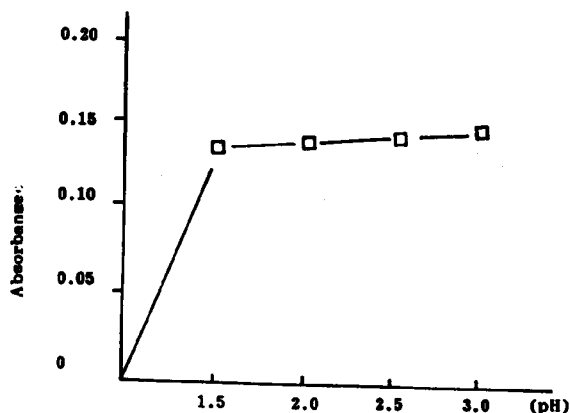


Fig. 2: Effect of pH of aluminium sulphate, on absorbance.

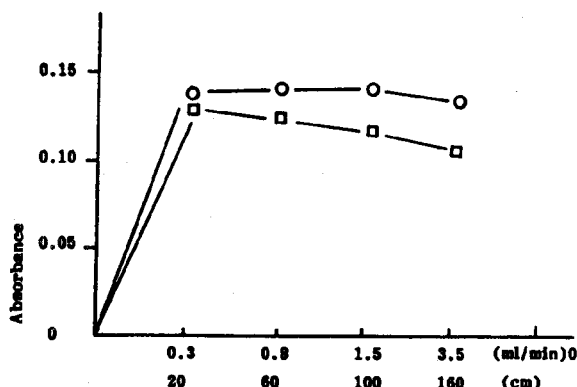


Fig. 3: Effect of flow rate (ϕ) and mixing coil length (ξ) on the peak height absorbance.

tion was calibrated shown in Fig. 3. At lower flow rates peak broadening was observed due to sample dilution with the carrier and therefore a flow rate of 1.5 ml min^{-1} was chosen. A mixing coil length of 20 cm was used at which highest signals were obtained, above this length the absorbance was decreased slightly. The sample volume of $100 \mu\text{l}$ was used throughout the experiment.

The effect of oxidized activated charcoal (OAC) column was also examined using column length of 2.0, 3.0, 4.0 and 5.0 cm, all with an internal diameter of 2.0 mm. The results obtained are shown in Fig. 4. Optimum response was achieved using a 2.0 cm long column and was therefore, selected for further studies.

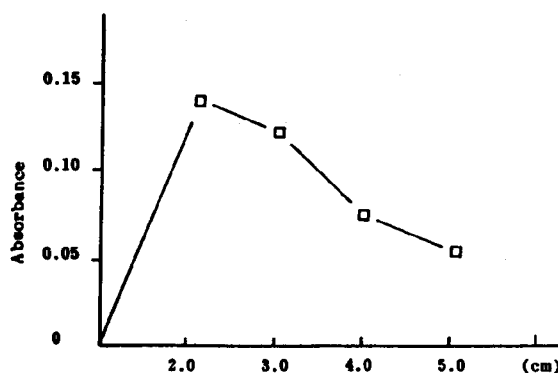


Fig. 4: Effect of oxidized activated charcoal column length.

Calibration graph

From a stock solution of nitrate (0.1 M), a series of standard solutions in the range of $1.00 - 9.00 \times 10^{-4} \text{ M}$ were analyzed in laboratory by the optimized manifold. The calibration graph had a correlation coefficient of 0.999 with a regression equation of $y = 1.34x + 0.001$. The sampling rate was 90 hr^{-1} with a relative standard deviation (r.s.d) less than 1.0% ($n = 6$) and the detection limit ($2 \times$ blank noise) was down to $1 \times 10^{-6} \text{ M}$ nitrate-N.

Effect of foreign ions

The effect of some anions and cations on the determination of nitrate was studied by addition method. The results obtained are summarized in Table 1.

Table 1: Effect of some anions and cations (10 folds) on the determination of nitrate $1 \times 10^{-4} \text{ M}$.

Ion	Absorbance
None	0.140
Ammonium	0.130
Chloride	0.136
Nitrite	0.150
Sodium	0.146
Sulphate	0.128

*Mean of three readings

Negligible interference was found for organic and inorganic constituents at concentration likely to be found in plant extracts.

Experimental

All chemicals were analytical reagent grade. Distilled/deionized water was used in the preparation of all solution.

Aluminium sulphate solution 0.01 M, pH 3.0

1.57 g of aluminium sulphate ($\text{Al}_2(\text{SO}_4)_3 \cdot 16\text{H}_2\text{O}$) was dissolved in 250 ml of water and pH 3.0 was adjusted with hydrochloric acid (0.1 M).

Nitrate stock solution 0.1 M

This solution was prepared by dissolving 2.1 g of sodium nitrate (oven dried) in 250 ml of water, treated with a few drops of chloroform and kept in refrigerator. Working standards were prepared by appropriate dilution.

Preparation of oxidized activated charcoal columns

About 25 g of activated charcoal powder, acid washed was taken and placed in a crucible which was then heated in an electric furnace at 325°C for 2 hrs. in the presence of oxygen. The powder was mixed thoroughly after every 30 min during heating [9]. After cooling the oxidised activated charcoal (OAC) was added to glass columns of different lengths (2 - 6 cm long, 2 mm i.d.) and inserted in an on-line stream of aluminium sulphate (0.01 M) to get the uniform low pressure packing of charcoal in columns. This insertion later on of the column on-line (Fig. 1) resulted in a stable base-line. A thin layer of glass wool was plugged at both ends of the glass column to prevent movement of the OAC by the carrier stream. A small piece of silicone rubber tubing was pushed into each of the glass tube so as to a tight connection.

Apparatus

For direct spectrophotometric (UV) determination of nitrate, a single channel flow through manifold was used shown in Fig. 1. The carrier stream was pumped at a rate of 1.5 ml min^{-1} using teflon tubing (0.8 mm, i.d) and nitrate solutions

were introduced by an injection valve (Rheodyne 5020) with a 100 μl sample loop. A uv/vis spectrophotometer (Perkin Elmer Lc. 55) equipped with a flow through cell (18 μl) was used for absorbance measurement at 225 nm.

Conclusion

The method developed in the present study is simple, and reliable for the determination of nitrate and can easily be tested to plant extracts. The negligible interference by foreign ions shows the suitability of this method for determinations. In addition to the advantage of high sampling rates, the automated operation in the flow injection systems results in a high reproducibility of determination.

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