CRITICAL ABSORPTION LIMIT OF NITRATE AND OPTIMIZING ESTIMATION METHODOLOGY FOR SOIL SOLUTIONS AND EXTRACTS USING UV-VIS SPECTROSCOPY

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ABSTRACT

Absorption behavior of nitrate and estimation methodology has been studied using different types of samples and UV wavelengths in order to optimize the use of samples and wavelengths to ensure better results. Absorption was highest in 190-310 nm range scanning compared to the single wavelength (210) or combination wavelengths (210+301 nm). Linearity in absorption was up to 40 µg/ml nitrate nitrogen in case of 190-310 nm scan. The wavelengths identified for nitrate in soil leachate samples for maximum absorption was combination of 210+301 nm wavelengths, whereas, wavelength scan from 190 to 310 nm was found better for soil extracts and nutrient solutions. Molar absorption coefficient was highest in case of 190-310 nm wavelength scan region for aqueous solutions. The critical absorption value of nitrate was found as 3.35 at different treatment wavelengths. Using these combination of treatment wavelengths to suitable sample types could yield better absorption results and with a little cost under laboratory conditions.

Key words: Nitrate estimation, Critical absorption limit, Soil solution, UV-VIS Spectroscopy.

INTRODUCTION

Nitrate is the most oxidized form of nitrogen compounds commonly present in natural waters, because it is the product of the aerobic decomposition of organic nitrogenous matter. Significant sources of nitrate are chemical fertilizers, decayed vegetable and animal matter, domestic effluents, sewage sludge, industrial discharge, leachates from refuse dumps and atmospheric washout. This anion is to be quantified through different analytical methods in order to find out its extent in the soil or to know the water quality. There are good analytical methods ranging from colorimetric to spectrophotometric. However, these need wide range of chemicals and tedious procedural requirements.

A number of investigators have also used ultra violet ray absorption property of nitrate as a method for direct spectrometric quantification of nitrate under *in situ* conditions with portable photometric cells (Cawse, 1967; Wetters and Uglum, 1970; MacDonald and Haddad, 1970; Gambrell *et al.*, 1975; Langergraber *et al*, 2003). In the light of sophisticated instrumentation, the same principle is used in the present study, however, to optimize the procedure for laboratory and find out the critical concentrations (Linearity maxima) for different types of samples and suitable wavelength regions, which help in analyzing large samples without much cost and reasonable acceptance.

MATERIALS AND METHODS

*(Apparatus:*1) UV-VIS Spectrophotometer, for use at 210, 301nm with quartz cells of 1 cm. 2) Acid washed, ash less Whatman filter paper no.42.

Reagents: a) Double distilled water was used for the preparation of all solutions and dilutions. b) Stock nitrate solution: It was prepared dissolving 7.218g of potassium nitrate (KNO_3) in a liter double distilled water resulting in 1000 µg/ml nitrate stock

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solution c) Primary standard nitrate solution: 100.0 ml of stock nitrate N solution diluted to 1000 ml with distilled water d) 1 N Hydrochloric acid solution e) Aluminum hydroxide suspension.

Sample collection: Soil solution samples were collected from soil columns and by *in situ* sampling from porous cups installed at different depths in the rice fields treated with nitrogenous fertilizers (Zhu et al, 2003) and Kim Dea Wook *et al.* (2001). The samples were filtered through whatman no.42 filter paper to remove sediments.

Soil extract samples*:* Soil samples were taken from different depths from the fields as well as column and nitrate was extracted with the 2M KCl solutions (Bremner and Keeney, 1966) and filtered with Whatman filter paper No.42.

Sample preparation: The sample with high color or is known to contain organic interference, were treated with one drop (approx.0.5 ml) of Aluminum hydroxide suspension to sample, mixed thoroughly and kept it for settlement. The solution was filtered with a Whatman No.42 filter paper. One ml of 2% w/v sulfamic acid was added to the sample and swirled to dispel the NO_2. Samples were filtered after color removal and added 2-3 drops of 1N HCl.

Standards for calibration: Nitrate calibration standards were prepared in the range of 0 to100 μg/ml nitrate nitrogen using primary standard. The nitrate standards were also pre-treated similar to that of samples.

Nitrate measurement: Nitrate nitrogen stock solution was used for preparing different molar concentrations. Different measurements for i) Aqueous solution, ii) Soil solution and extract separately using 210 nm, 210+301 nm, 190-310 nm (scan) were made with double beam Uv-Vis spectrophotometer. Single and range wavelength scanning measurements were done. Absorption trends were studied by plotting absorption against concentrations. Similarly, differential absorption behavior between wavelengths at a nitrate concentration was also studied. Linearity coefficients were identified using linear mathematical equations. Molar absorption coefficients were calculated using equation:

$$A = \Sigma \, cl$$

A= Absorption

Σ = Molar absorption coefficient; c = Concentration (mol L^{-1}); l = Cell thickness (cm).

Behavior of molar absorption coefficients was observed against concentration in different sample types and respective wavelength curves were plotted.

Critical absorption limit: Critical limit of nitrate was obtained by using scatter plot technique by plotting absorption against concentration and dividing scatter plot in to four quarters such that left upper and right lower quarters should have maximum numbers of points. The intersection point on horizontal line of y axis and vertical line on x axis will give the critical concentration and critical absorption limit (Cate and Nelson, 1971).

RESULTS AND DISCUSSION

Aqueous solutions: Absorption behavior of nitrate has been studied, absorption was better in case of 190-310 nm range scanning compared to the single wavelength or combination of wavelengths (210+301 nm). Linearity in absorption was observed up to 40 μg/ml of nitrate concentration in case of 190-310 nm scan, whereas, it was less than 40 μg/ml for single as well as combination of wavelengths. The highest absorption value obtained was 3.90.

Differential absorption curves of different treatments showed that combined wavelength measurements had larger difference of absorption compared to single wavelength measurement (Fig.1&2). Fig.4 illustrates that molar absorption coefficient was highest in case of scan of 190-310 nm wavelength region for aqueous solutions and it maintained with increase in molar concentrations.

In case of leachate samples from soil columns, it was found that combination wavelength (201+310 nm) measurement had better absorption than single wavelength absorption, where better linearity in absorption would be obtained (Fig.3) i.e. above 40 μg/ml. Whereas, in soil extracts, higher absorption was found with wavelength range scanning (190-310 nm) compared to other wavelengths (Fig.5). However, differential absorption was less between single and combination of wavelengths.
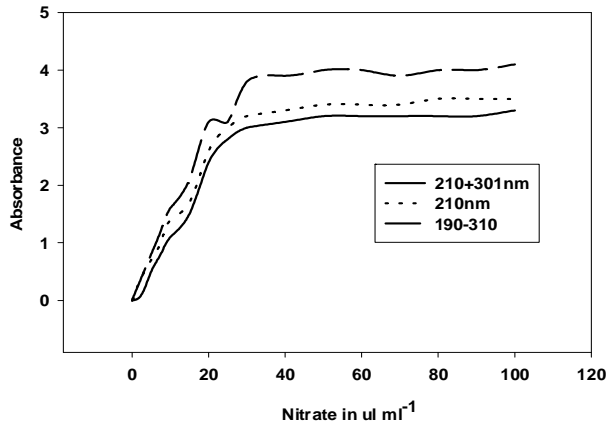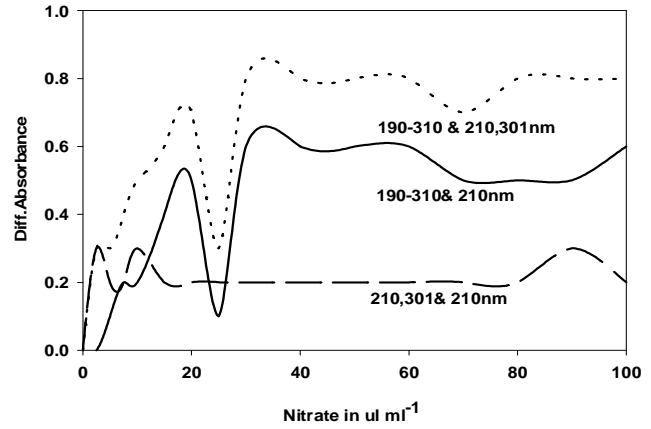
FIG 1: UV absorption behavior of nitrate under different ionic strengths.

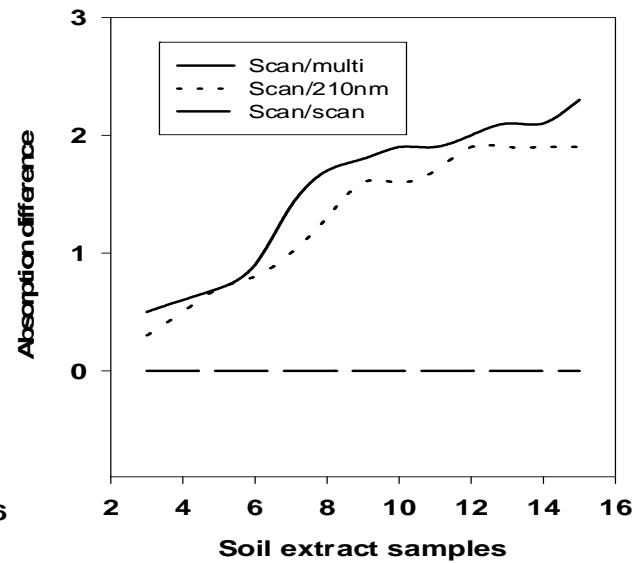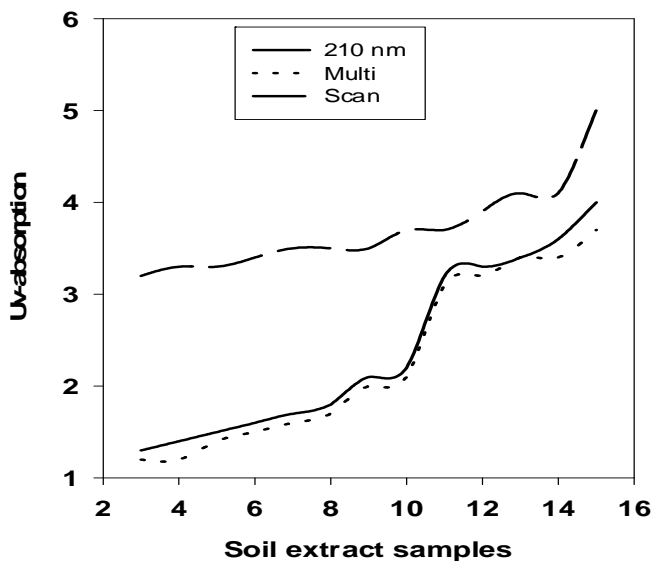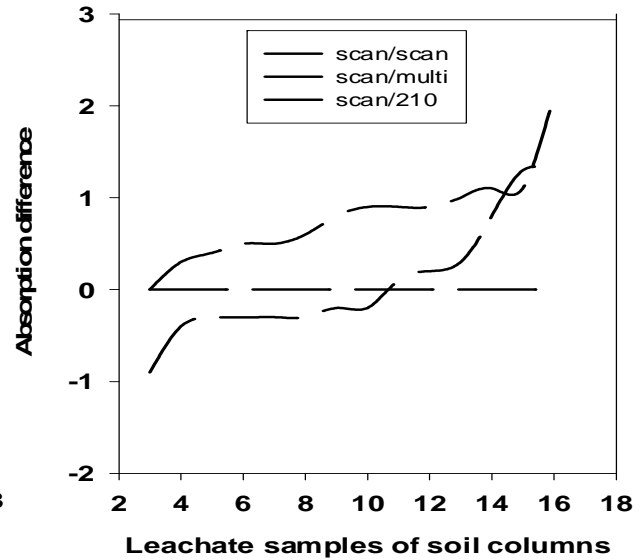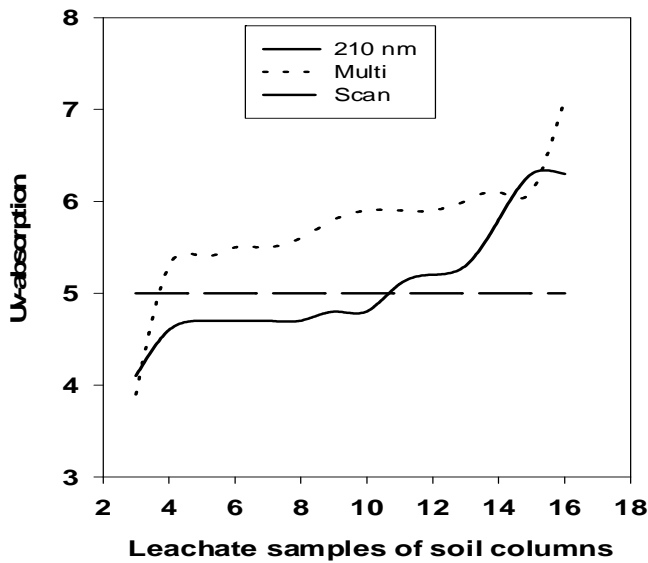FIG 2: Different absorption curves of nitrate.

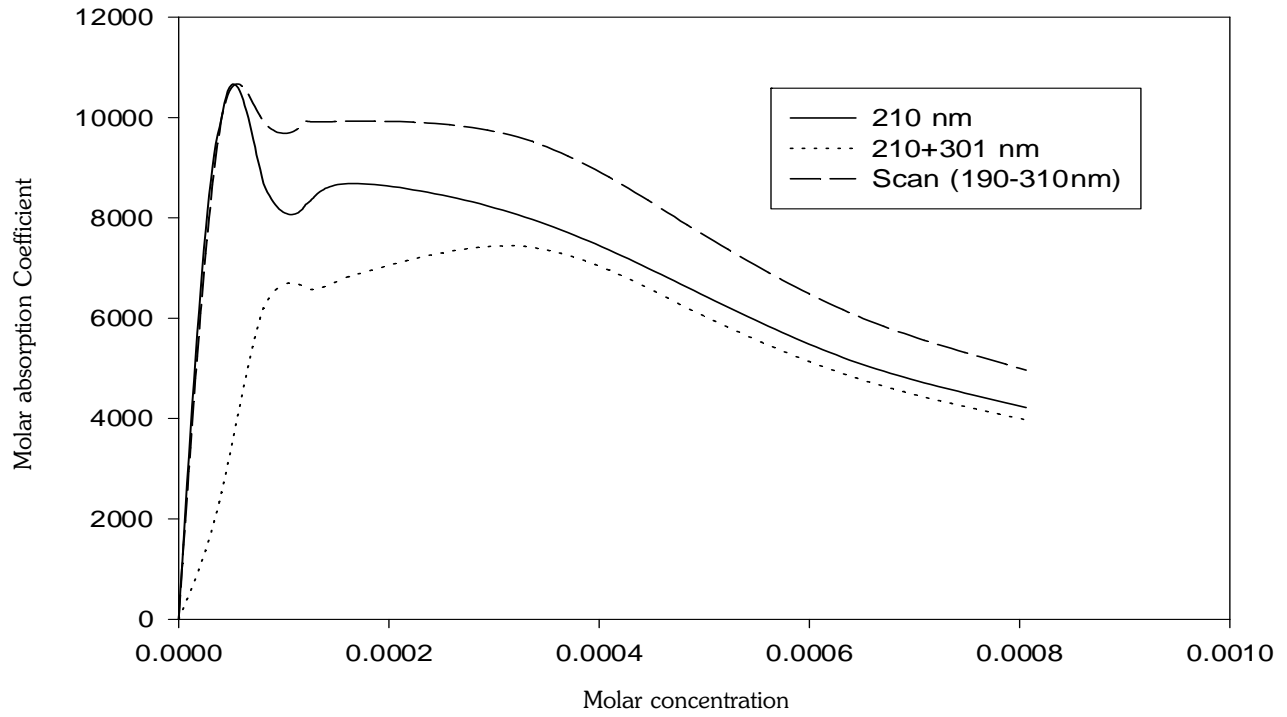FIG 3: Uv light absorption by nitrate in different type sample solution.

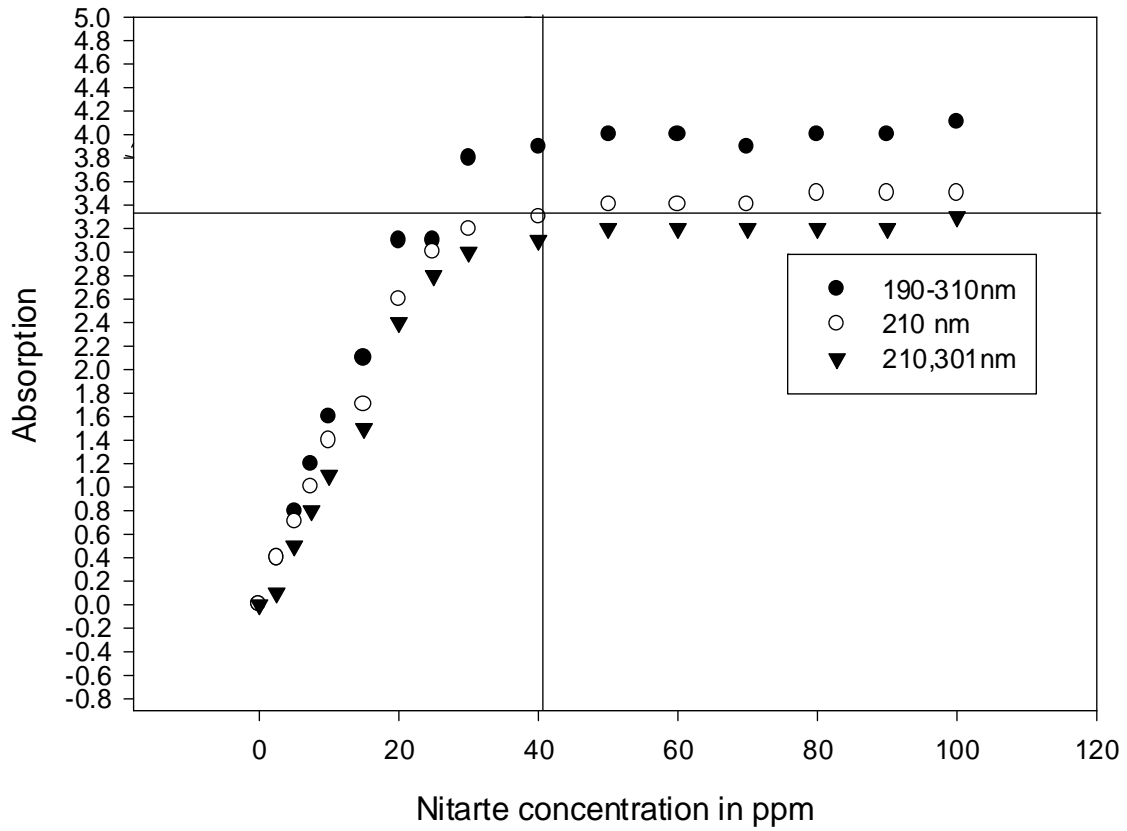FIG 4: Molar absorption of pure aqaous solutions.

FIG.5: Critical absorption limit of nitrate in UV range.

The results showed that there was a variation in absorption values of nitrate among different types of samples studied. It was due to the presence of interfering ions like nitrite (NO2) in the sample medium, which interfere in absorption of respective wavelength. These ions come from the extractant and soils to the medium as reported by Agarwal, and Mehd, (1983). Similarly, interference also comes from some organic materials/compounds present in the medium.

Highest detectable sample concentration found was 40 μg/ml nitrate nitrogen up to which linearity in absorption was observed. After that absorption became constant and reached a plateau. Similar observations were also made by Norman and Stucki (1981).

The critical absorption value of nitrate with combination wavelengths (210+301 nm) with scatter plot technique is 3.35 and is corresponding to 40 μg/ml of nitrate concentration (Fig.5). This is the point after which absorption by the respective wavelengths remain non-linear and became plateau. As discussed above this procedure is simple, flexible for measuring large number of samples with lower nitrate concentrations and economical as it does not require laboratory reagents for measurement.

CONCLUSIONS

Highest detectable sample concentration found was 40 μg/ml nitrate nitrogen up to which maximum linearity in absorption and highest molar absorption coefficient was observed under 190-310 nm scan. The critical absorption value of nitrate was 3.35. Use of combination of wavelengths (201+301nm) and 190-310nm scan than single wavelength (201nm) while estimating NO-3 –N in leachate or in extract of soil would yield in better results.

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