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A COMPARISON OF UV SPECTROSCOPY AND ION SELECTIVE ELECTRODE IN THE DETERMINATION OF NITRATE IN *HEVEA* FOLIAGE

Nitrate (NO_3^-) is the predominant ionic form by which most plants take up nitrogen from the soil. For a better understanding of the dynamics of nitrate in soil-plant systems, monitoring nitrate concentration within the plant is essential. Foliage nutrient content is taken as an index of plant nutrient status in *Hevea*. Hence determination of nitrate becomes frequently necessary. There are several methods for determination of nitrate (Milham *et al.*, 1970) of which the most important are reduction to ammonia followed by titration or colorimetry, selective chemical or bio-chemical reduction and its diazotisation with a variety of reagents and nitration of active organic molecules and quantitative determination of nitrate product. Most of the methods are too cumbersome for routine analysis, but the ion selective method is comparatively easy (Keeney *et al.*, 1970; Milham *et al.*, 1970). An improved method employing UV spectrophotometer has been detailed by Heanes (1982). The present attempt is a comparison of ion selective and UV spectroscopic methods in nitrate determination with a view to find out whether the latter can be used for routine analysis.

Foliage samples of *Hevea brasiliensis*, the Para rubber tree, were collected as per the method followed by the Rubber Research Institute of India (Karthikakutty Amma, 1976). Based on earlier analytical values 16 foliage samples were selected with varying total nitrogen content. The samples were dried at 65°C, ground and sieved through 25 mesh. 0.4 g of the oven dried sample was extracted with 0.025 M $Al_2(SO_4)_3$ containing 0.1 per cent sulphamic acid and oxidized activated charcoal. The absorbance of the extractant after being made up was read at 225 nm in a Hitachi UV visible spectrophotometer (Model No. 200 – 20). For the determination of nitrate using ion selective electrode, the method outlined by Keeney *et al.*, (1970) was followed. The interferences due to other anions was controlled by buffering the solutions. Buffer concentration was adjusted to suit the requirements of precipitation, decomposition or removal of interfering anions and to provide uniform physical conditions for measurement of nitrate concentration. The buffer solution used for the study was 0.010 M aluminium sulphate, 0.010 M silver sulphate and 0.020 M sulphamic acid, adjusted to pH 3.0 with 0.10 M sulphuric acid. Ionic strength adjustor (ISA) was added to all nitrate standards and samples so that the background ionic strength is high and constant relative to variable concentrations of nitrate ions. For the nitrate electrode ammonium sulphate, the recommended ISA, was used. Nitrate was determined using specific ion selective electrode model 93–07 with double junction reference electrode in an Orion 901 microprocessor ion-analyser. Ion selective electrode senses the activity which is derived from the Nernst equation:

$$E = E^\circ \pm \frac{RT}{ZF} \text{ in } a_x$$

where, E° = standard potential of cell
a_x = activity of ion x, and
Z = number of charges on x.

Simple correlation was worked out as per the method described by Snedecor and Cochran (1967) and paired 't' test was done following the method of Nageswara Rao (1983).

The data on NO_3^- content determined employing UV visible spectroscopy and ion selective electrode are presented in Table 1.

Table 1. **Nitrogen concentration in leaf samples**

Serial number	Nitrate N (ppm)	
	UV spectroscopy	Ion selective electrode
1	226.30	225.00
2	203.25	227.00
3	239.10	226.50
4	211.00	235.00
5	234.20	234.50
6	230.50	248.00
7	236.40	238.00
8	210.25	216.50
9	221.90	216.50
10	193.05	200.00
11	279.95	299.00
12	218.00	218.00
13	160.00	166.00
14	181.20	168.00
15	209.30	215.00
16	216.95	240.50
Mean	216.96	223.34

The mean NO_3^- content was 216.96 ppm for the method employing UV visible spectroscopy and 223.34 ppm for the determination with ion selective electrode. A significant positive correlation (r = 0.92**) between the two methods and a non-significant paired 't' test value (t = 0.557) indicate that both these methods could be employed for the determination of NO_3^- in *Hevea* foliage Table 2. A regression relating the two methods was also worked out and it was noted that $y = 1.05\ x - 3.87$, where y is the value for ion selective electrode method and x that for the UV spectroscopy method. Since the procedure is not too cumbersome, the use of ion selective electrode is more rapid and the equipment (Ionalyser) has the advantage of being portable. It must however, be mentioned that the determination using ion selective electrode is susceptible to temperature and voltage fluctuations and hence routine nutrient nitrate assessment in mobile laboratories may be possible only if the laboratory is air conditioned. Though the method is rapid, the comparatively short shelf life of the electrode makes the determination costlier.

Table 2. **Comparison of UV spectroscopy and ion selective electrode methods**

Method	Paired 't' test	SD	CV (%)	Mean (ppm)
UV spectroscopy	0.557*	26.04	12.00	216.96
Ion-selective electrode		29.80	13.34	223.34

* Not significant

For research purpose, though both the methods could be employed for determination of nitrate in *Hevea* foliage, wherever UV spectrophotometer is available, this method would be a better choice, when the sample size is not large. When a large number of samples are to be handled, ion selective electrode could be preferred.

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