

## ***Automated Determination of Sulphur in Hevea and Associated Cover Plants***

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*The automated turbidimetric method for the determination of sulphur in Hevea and associated cover plant materials was found to be free from phosphate and calcium interferences, but highly dependent on perchloric acid concentration of the 'wet-ash' solution. The results were found to be comparable with those obtained by the colorimetric procedure of using potassium chromate; good reproducibility and recoveries were obtained.*

Sulphur exists in the plant principally as a constituent of the amino acids—cystine, cysteine and methionine, and hence in the proteins containing these amino acids. It is absorbed by the plant mainly in the form of sulphate ions which are converted into organic compounds by reduction<sup>1,2</sup>. Although sulphur deficiency is not generally observed on rubber probably because of its regular application to *Hevea* in the form of ammonium sulphate, a principal nitrogen fertiliser, and/or magnesium sulphate, the importance of sulphur in relation to *Hevea* nutrition requires no emphasis. Symptoms of sulphur deficiency in *Hevea* and associated cover plants are well-documented<sup>3</sup>.

In the past, the method used for determining sulphur in *Hevea* and associated cover plant materials was based on the tedious and time-consuming colorimetric procedure of using potassium chromate to determine the excess of barium chloride used for the precipitation of sulphur from the 'wet-ash' solution<sup>4</sup>. The number of samples which can be analysed using this method is very limited. To cope with the increasing number of samples requiring sulphur determination in the analytical laboratory of the RRIM, an investigation was undertaken

to automate the procedures used for sulphur analysis. This can be done in a variety of ways, of which colorimetric<sup>5</sup> and turbidimetric<sup>6</sup> techniques are the most popular. This paper describes the use of the turbidimetric technique for determining sulphur in *Hevea* and associated cover plants on the Technicon autoanalyser.

### EXPERIMENTAL

#### *Apparatus*

A standard Technicon autoanalyser equipment was used. The phototube colorimeter was fitted with a 15 mm tubular flow cell and 490 nm filters. The flow is indicated in *Figure 1* with a double-probe system.

#### *Reagents*

All solutions were prepared from analytical-reagent grade materials.

*Barium chloride solution.* A 2.5% solution in 0.006M hydrochloric acid containing 0.25% gelatin was used.

*Buffer solution.* This consisted of 40 g EDTA (disodium salt), 7 g of ammonium chloride and 60 ml of concentrated ammonia solution, diluted to 1 litre with distilled water.

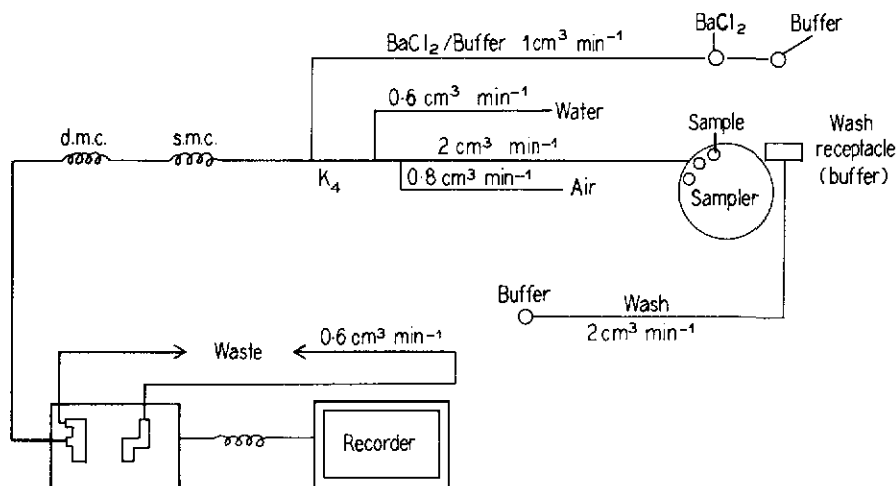


Figure 1. Flow diagram of automated sulphur analysis.

**Standard sulphate solutions.** Sulphate solutions containing 0, 5, 10, 20, 30, 40, 60, 80 and 100 p.p.m. of sulphur and 2 ml of concentrated perchloric acid were each made up to 100 millilitres.

#### Preparation of Sample

Two grammes ground plant material was digested with 15 ml concentrated nitric acid at medium heat ( $\sim 95^{\circ}\text{C}$ ) until no visible signs of solid matter remained. To the cooled digest 5 ml of 72% perchloric acid was slowly added. The solution was then heated until the volume was decreased to about 2 millilitres. Evaporation to dryness should be avoided. Distilled water, 20 ml, was added to the flask and the solution was filtered through Whatman No. 1 paper. The paper was rinsed thoroughly with hot water, adding the washings to the filtrate. The solution was allowed to cool and diluted to 100 millilitres.

#### Determination of Sulphur

The double-probe system was designed such that sample and barium chloride

solutions were sampled simultaneously and buffer solutions were taken in during the wash cycle. The addition of barium chloride was therefore alternated with the addition of EDTA-buffer solution. The length of the pump tubes was adjusted using coloured solution so that the barium chloride droplets superimposed completely with the same of the diluted sample at the K4 joints.

The recorder was set at 100% transmission with the buffer reagents going through the sample and the barium chloride lines; and with water and air flowing through its respective lines as shown in Figure 1. The sampling rate was set at 40 per hour with a 2:1 wash ratio between samples. A standard curve was prepared by plotting percentage transmission of the standards against concentration. The sample peaks were then read and their percentage transmissions were plotted on the standard curve to determine their respective concentrations.

## RESULTS AND DISCUSSION

*Calibration Graph*

The relationship between concentration of sulphur and percentage transmission plotted on semi-log paper was found to be linear up to 70 p.p.m. of sulphur. Beyond this the relationship was a curve and the change in percentage transmission per unit change of concentration decreased.

*Interference Studies*

Of the various elements capable of forming precipitates from the 'wet-ash' solution together with barium sulphate, phosphorous which forms insoluble barium phosphate and calcium which forms sparingly soluble calcium sulphate occur in appreciable amounts in plant materials. Tests were therefore performed to check whether phosphorous and calcium caused any interference.

Results obtained (Figure 2, Curve A) showed that the presence of phosphates ( $\text{PO}_4^{3-}$ ) and calcium, ( $\text{Ca}^{2+}$ ) at the levels

normally present in *Hevea* and associated cover plants did not cause any interference. It is, however, interesting to note that perchloric acid produced a marked effect on the results (compare Curves A, B and C of Figure 2). It is therefore important to ensure that the amount of perchloric acid in all the digests be the same, and that in calibrating the procedure against solutions of pure sodium sulphate, perchloric acid equal to the average amount present in the 'wet-ash' solution be added.

*Recovery Studies*

Recovery tests were performed by adding a known amount of sulphur to the various plant materials after digestion. Excellent recoveries of 93.3% – 101.5% with an average of 97.5% and a coefficient of variation of 2.7% was obtained (Table 1).

*Reproducibility of the Method*

The recorder tracing shown in Figure 3 showed that the reproducibility of the method was excellent. The coefficient

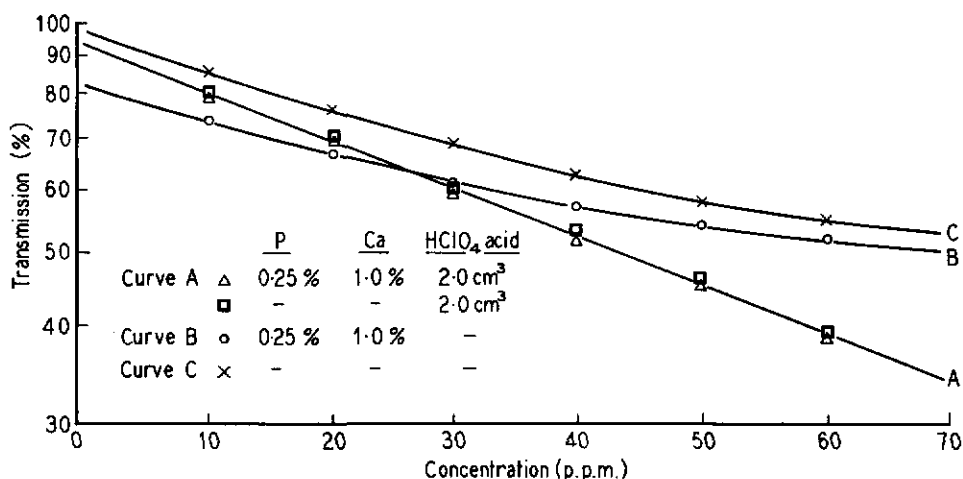


Figure 2. Calibration graphs for automated turbidimetric determination of sulphur.

TABLE 1. RECOVERY OF SULPHUR ADDED TO PLANT MATERIALS AFTER DIGESTION

Sample	S present in sample ( $\mu\text{g}$ )	Amount of S added ( $\mu\text{g}$ )	Amount of S recovered ( $\mu\text{g}$ )	Recovery (%)
<i>Hevea</i> leaf	620	4 000	3 980	99.5
	582	5 000	4 868	97.4
Petioles	440	2 000	1 975	98.7
<i>Hevea</i> stem	435	2 000	1 865	93.3
Legume covers	520	3 000	2 980	99.3
Kernels ( <i>Hevea</i> seeds)	520	5 000	4 930	98.6
	470	2 000	2 030	101.5
Shells ( <i>Hevea</i> seeds)	450	2 000	1 896	94.8

of variation of the percentage transmission values was found to be 0.93%.

#### *Comparison with the Standard Laboratory Method*

The reliability of the automated procedure was checked by comparing the results obtained by this method with the standard laboratory method on several representative *Hevea* and associated cover plant materials — leaf, petioles, stem,

legume covers, kernels and shells of *Hevea* seeds. The comparisons, shown in *Table 2*, indicated that the results obtained by the two methods were in good agreement with each other. The maximum deviation noted between the two methods was 26%; the overall average deviation was however 7.5%. Of the different plant materials, petioles showed the largest deviation, the values by the automated method being lower by 6% – 26% (mean 14.5%) than

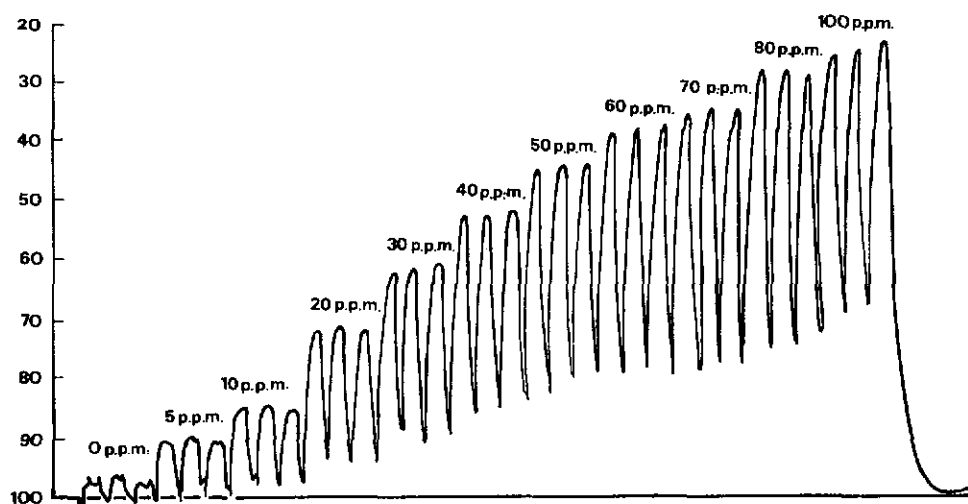


Figure 3. Recorder tracing showing reproducibility of turbidimetric method.

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TABLE 2. COMPARISON OF THE DETERMINATION OF SULPHUR IN *HEVEA* AND ASSOCIATED COVER PLANT MATERIALS BY THE AUTOMATED METHOD AND STANDARD LABORATORY METHOD

Sample		Sulphur (%)		Deviation (%)
		Automated method	Standard Laboratory method	
Hevea leaf	1	0.228	0.213	7.0
	2	0.238	0.236	0.85
	3	0.248	0.262	-5.3
	4	0.298	0.308	-3.2
	5	0.338	0.310	9.0
	6	0.362	0.393	-7.9
Petioles	1	0.156	0.166	-6.0
	2	0.130	0.138	-5.8
	3	0.112	0.138	-18.8
	4	0.104	0.141	-26.2
	5	0.137	0.160	-14.3
	6	0.111	0.132	-15.9
Stem	1	0.070	0.075	-6.7
	2	0.091	0.109	-16.5
	3	0.148	0.126	17.5
	4	0.150	0.154	-2.6
	5	0.154	0.169	-8.9
	6	0.162	0.162	0
Legume covers	1	0.122	0.140	-12.9
	2	0.168	0.142	18.3
	3	0.182	0.183	-0.6
	4	0.225	0.210	7.1
	5	0.230	0.221	4.1
	6	0.310	0.334	-7.2
Kernels ( <i>Hevea</i> seeds)	1	0.205	0.203	1.0
	2	0.205	0.208	-1.4
	3	0.218	0.233	-6.4
	4	0.232	0.237	-2.1
	5	0.235	0.223	5.4
	6	0.235	0.225	4.4
Shells ( <i>Hevea</i> seeds)	1	0.210	0.225	-6.7
	2	0.215	0.208	3.4
	3	0.222	0.240	-7.5
	4	0.225	0.216	4.2
	5	0.225	0.240	-6.3
	6	0.238	0.238	0

Results are expressed as percentages of oven-dried material.

the Standard Laboratory Method. Kernels gave the closest agreement, the deviations being from 1% to 6% (mean 3.5%). The order of deviations with the different

plant materials was as follows (with mean deviations shown in brackets): Petioles (14.5) > stems (8.7) > covers (8.4) leaves (5.6) > shells (4.7) > kernels (3.5).

#### CONCLUSION

The automated turbidimetric method is suitable for providing rapid and accurate analysis of sulphur in *Hevea* and associated cover plant materials. The method is more convenient than the colorimetric method (potassium chromate) as it is possible with the autoanalyser to treat every sample in exactly the same manner maintaining constant reaction conditions throughout the analysis.

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