# Automated Colorimetric Determination of Magnesium in Foliage of Hevea and Other Tropical Crops

# L. A. CHOONG LANCASTER

An automated procedure for determining magnesium in plant tissues using the Technicon Auto-Analyzer is described. Magnesium is complexed with eriochrome black T (EBT) and the interfering free calcium ions are removed as the stable calcium chelate of 1, 2-bis-2-aminoethoxyethane-NNN'N'-tetra-acetic acid (EGTA). Interferences from other ions normally occurring in plant tissues were small. The method has been tested on tissues of Hevea, oil palm, rice, groundnuts, sugarcane, coconut and cocoa. At present the method is unsuitable for samples with more than 250 p.p.m. manganese (e.g. cocoa leaves).

Magnesium is an essential element for plant growth and its determination in plants is an established practice in assessing nutritional requirements. For advisory leaf analyses a rapid and reasonably accurate procedure is desirable. Nowadays atomic absorption (DAWSON AND HEATON, 1961; WILLIS, 1961) is commonly used for magnesium determination in view of its sensitivity, but in this laboratory it has been found that at least a 250-fold dilution of the dry ashing solution is required, which largely removes the time saved by instrumentation. Hence, a quicker alternative technique has been sought.

Colorimetric procedures have been reported for determining magnesium in water using EBT (HARVEY *et al.*, 1953; YOUNG AND SWEET, 1955). LEVINE AND CUMMINGS (1956) stated that the complex forms a true solution in which the magnesium concentration is proportional to the colour intensity up to  $80 \mu g/ml$ . The serious disadvantage of all these methods is that Ca complexes almost as readily as Mg, and at pH 7.75 the absorption curves are identical (HARVEY *et al.*, 1953). However, GITELMAN *et al.* (1966) eliminated interference by adding EGTA which forms a more stable chelate with Ca than EBT. Their automated method for determining magnesium content in metabolic products seemed suitable to adapt for determining magnesium in plant material of Hevea and other tropical crops. A rapid and simpler method is described in this paper, together with the interfering effects of several ions which normally occur in plant material.

# EXPERIMENTAL

# Reagents

- Strontium nitrate solution (15 000 p.p.m.). Dissolve 36 g Sr(NO<sub>3</sub>)<sub>2</sub> and make up to 1 litre.
- Calcium solution (1000 p.p.m.). Dissolve
   2.4973 g dried CaCO<sub>3</sub> by adding concentrated HCl drop by drop (about 5 ml) and make up to 1 litre.
- Standards. Dissolve 10.1308 g magnesium sulphate in 1 litre of distilled water so that 1 ml of solution contains 100 μg Mg. From this solution prepare a range of standards containing 0-14 μg per millilitre. Each magnesium standard also contains 750 μg Sr and 10 μg Ca per millilitre of solution.

# L.A. CHOONG LANCASTER: Automated Colorimetric Determination of Magnesium in Foliage

- 4. Ethylenediamine solution. Add 132 ml of anhydrous ethylenediamine with constant stirring to 400 ml of distilled water in a Pyrex beaker in an ice bucket, keeping the temperature of the solution below 10°C. In a separate beaker add 75 ml concentrated HCl to 300 ml distilled water; cool to room temperature before adding slowly to the ethylenediamine solution. Mix and, when cool, dilute to 1 litre with distilled water. Store in the refrigerator.
- 5. Eriochrome black T stock solution. The dye solution is prepared by dissolving 0.4 g EBT in 100 ml absolute methanol. The solution is filtered through glass wool into a polyethylene bottle and stored in the refrigerator. The solution is stable for several months.
- 6. Eriochrome black T buffer solution. Dissolve 5 g hydroxylamine hydrochloride in 125 ml ethylenediamine solution. Add 10 ml anhydrous ethylenediamine and 2.5 g potassium cyanide and mix well. Add 15 ml eriochrome black T stock solution and dilute to 250 ml with ethylenediamine solution and store in the refrigerator. This buffer is unstable and should be used within three days of preparation.
- 7. EGTA stock solution. Dissolve 9.5 g EGTA in 30 ml distilled water, add 3 g NaOH and make up to 50 ml with distilled water.
- 8. EGTA reagent solution (0.005 M). Dilute 2 ml 0.5 EGTA stock solution to 200 ml with ethylenediamine solution.
- 9. 0.1 N sodium hydroxide solution.
- 10. Wash. A solution of 0.1 N HCl.

# Sample Preparation

Dry ground material (2 g) was weighed into a silica dish and ashed as described by MIDDLETON *et al.* (1964). The ashed residue was taken up in 8 ml of 20% HNO<sub>3</sub>, filtered into a 100 ml volumetric flask, washed with warm water and made up to the required mark. An aliquot (2 ml) of the dry ashing solution was pipetted into a 50 ml flask containing 2.5 ml of strontium nitrate solution and 0.5 ml of calcium solution and the contents made up to the mark. Each sample was digested and analysed in triplicate.

## Apparatus and Procedure

Technicon Auto-Analyzer equipment was used and arranged as in *Figure 1*. Each unit comprises a sampler, proportioning pump, delay coils, colorimeter with 15 mm tubular flow cell and pen recorder. To make full use of the proportioning pump and accessories already available, two sets of reagent lines were constructed on a common manifold tray.





A 40 h rate per sampler was used, but where the range of the magnesium content from sample to sample is narrow a faster speed may be employed. Cam settings were for a wash-out of 40 s and a sampling period of 20 seconds.

> The magnesium in solution was determined as the Mg-EBT complex at a buffered pH of 10.5 at which only one complex exists (HARVEY *et al.*, 1953). The absorption curve in *Figure 2* shows that indirect colorimetry



Figure 2. Absorption curve of the Mg-EBT complex.

at 625 nm is more sensitive than direct measurement at 550 nm. Indirect colorimetry requires the pen to be adjusted to the range 0-100% transmission with all the reagents, except the EBT dye passing through the unit. The line for the EBT is then dropped into its reagent bottle, whereupon the pen moves towards the lower readings — usually to about 7% transmission. The base-line is set five divisions above this reading.

# Other Tests

Recoveries and interferences from metallic ions which might be present in the ashed solutions were also investigated. Magnesium found in plant ash solutions was also compared with the results from atomic absorption using a Techtron Atomic Absorption Spectrophotometer AA-4.

#### **RESULTS AND DISCUSSION**

## Reproducibility

In twenty out of thirty-five samples, the coefficients of variation of the colorimetric procedure were higher than that given by atomic absorption, though in both methods the coefficients of variation were small (Table 1). The highest coefficient of variation of the colorimetric readings was 5.25% for one rubber sample, but most values were less than 2.0%. The mean coefficient of variation of the two procedures were not significantly different. The colorimetric procedure thus has a satisfactory degree of reproducibility.

Except for cocoa, the mean magnesium values obtained by colorimetry were within 6% of those given by atomic absorption, with the majority of results being within 2%. The colorimetric procedure gave appreciably higher results for cocoa leaves (*Table 1*). Copper, manganese, iron, aluminium, cobalt and nickel interfere in the colorimetric determination of magnesium using EBT (DIEHL et al., 1950). *Table 2* shows the concentration of some of these ions in the plant materials. The high content of manganese in cocoa leaves is notable.

## Interferences

Interferences from calcium (II), manganese (II), aluminium (III), copper (II), iron (III) and zinc (II) were tested. Of these only manganese, iron, copper and aluminium affected the determination, manganese having the greatest effect (*Figure 3*). The addition of potassium cyanide eliminated interference from iron



Figure 3. Interfering effects of aluminium, copper, iron and manganese on a standard solution containing 4 p.p.m. magnesium.

	Auto-A	Atomic absorption		
Material	Mean (p.p.m.)	c.v. (%)	Mean (p.p.m.)	c.v. (%)
Dubber leaves	0 390	3 23	0 390	4 36
KUUDEI ICAVES	0.258	1.55	0.242	1.65
	0.131	0.00	0.129	0.08
	0.238	5.25	0.241	4.56
	0.354	1.30	0.349	0.06
Oil palm leaves	0.498	1.81	0.490	0.00
-	0.467	1.50	0.463	1.73
	0.554	0.07	0.533	1.50
	0.539	1.86	0.533	1.50
	0.745	0.00	0.735	1.09
Sugarcane – sheath	0.296	1.35	0.291	0.08
root	0.050	0.00	0.048	0.00
leaf	0.304	3.29	0.318	1.26
leaf	0.236	1.70	0.239	2.09
leaf	0.204	1.96	0.201	1.49
Groundnut shoots	0.225	0.00	0.237	1.27
	0.191	1.57	0.194	2.06
	0.208	1.92	0.211	1.42
	0.216	3.70	0.211	1.42
	0.161	2.48	0.153	1.97
Rice shoots	0.342	1.17	0.340	0.00
	0.359	2.23	0.380	0.00
	0.373	2.41	0.377	1.06
	0.361	1.11	0.363	0.08
	0.367	1.91	0.367	0.08
Coconut leaves	0.340	1.18	0.330	1.82
	0.383	1.04	0.380	2.63
	0.442	0.09	0.430	0.00
	0.352	1.13	0.341	0.09
	0.344	0.00	0.340	0.00
Cocoa leaves	0.865	2.60	0.760	1.84
	0.865	2.60	0.710	0.00
	0./19	0.00	0.620	0.00
	0.819	1.00	0.700	0.00
	0.768	0,00	0.068	0.00
	c.v.	(%)	c.v.	(%)
	Mean	s.đ.	Mean	s.d.
	1.52	.1.1.34	1.06	.1.1.10

TABLE 1. DE	FERMINATION OF	MAGNESIUM BY	AUTO-ANALYZER	AND ATOMIC	ABSORPTION
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and copper (Figure 4). Manganese increased the percentage transmission and hence the apparent magnesium content; aluminium had the reverse effect. However, up to 250 p.p.m. Mn and 1500 p.p.m. Al in dry matter could be tolerated, and, therefore, only manganese poses practical problem in analysis. No interference was observed from sulphate, phosphate or silicate.

Calcium ions was effectively removed by EGTA (*Table 3*). The addition of calcium (10 p.p.m.) to the standard solutions of mag-

Material	Ca	Al	Mn	Zn	Fe	Cu
	(%)	(p.p.m.)	(p.p.m.)	(p.p.m.)	(p.p.m.)	(p.p.m.)
Rubber leaves	1.02	87	75	35	91	6
	0.23	51	80	43	81	18
	0.92	36	195	29	93	9
	0.88	13	115	17	97	8
	0.29	27	40	40	80	11
Oil palm leaves	0.31	12	38	8	87	4
	0.37	15	18	10	53	7
	0.52	12	32	11	80	3
	0.34	21	48	9	67	3
	0.67	20	32	10	133	3
Sugarcane – sheath root leaf leaf leaf	0.19 0.08 0.21 0.37 0.27	220 3 250 366 366 328	158 37 140 106 82	17 220 24 24 24 20	149 571 167 136 123	5 8 4 9 6
Groundnut shoots <sup>a</sup>	1.52	1 140	69	46	320	9
	1.48	1 300	120	43	340	10
	1.31	1 330	90	57	293	10
	1.31	1 640	158	67	586	9
	1.39	1 420	97	23	347	7
Rice shoots <sup>a</sup>	0.36	118	75	36	83	6
	0.39	129	81	40	93	7
	0.36	91	77	39	87	6
	0.37	89	75	66	83	7
	0.37	91	77	39	67	6
Coconut leaves	0.14 0.17 0.21 0.17 0.20	18 20 14 9 16	77 97 101 59 65	15 15 17 14 15	51 56 56 49 43	4 3 4 3
Cocoa leaves	1.06	135	1 206	113	67	8
	0.73	85	1 020	102	56	9
	0.51	50	820	86	47	10
	0.61	62	800	81	47	9
	0.63	50	832	81	40	11

TABLE 2.	CONCENTRATIONS	OF	SOME ELEMENTS	IN	VARIOUS	PLANT	MATERIALS
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\*Whole plants with the roots removed

nesium improved the reproducibility of the results.

## Recovery of Magnesium

Table 4 shows the percentage recovery of known amounts of magnesium added to fifteen plant ash solutions of different origin (excluding cocoa). The final concentrations of magnesium ranged from 2-9 p.p.m.

## Removal of Manganese from Cocoa Samples

An attempt was made to extract manganese with sodium diethyldithiocarbamate and carbon tetrachloride after the manner of GJEMS (1960), who used this procedure in the titration of magnesium, manganese and calcium with EDTA, using EBT as indicator. However, when magnesium was determined by the automated procedure, residual car

(p.p.m.)	0	1.88	5.0	8.13
2.00	2.00	2.05	2.15	2.00
4.00	4.00	4.02	4.00	4.10
8.00	8.00	8.00	8.01	8.00
10.00	10.00	9.65	9.65	9.65

Calcium added in addition to the 10 p.p.m. Ca (or 1.25% Ca of dry matter) present.



Figure 4. Effect of potassium cyanide on the interference effects of aluminium, copper, iron and manganese in a solution containing 4 p.p.m. of magnesium. Points below 500 p.p.m. Fe, Zn and Cu are not shown.

Method	Mg added (μg)						
	50		100		150		
	Mean	s.d.	Mean	s.d.	Mean	s.d.	
Auto-Analyzer	101.7	±5.16	100.8	±3.31	99.9	±2.3	
Atomic absorption	102.8	±5.63	102.1	$\pm 2.53$	101.8	±1.8	

 TABLE 4. PERCENTAGE RECOVERY OF KNOWN AMOUNTS OF MAGNESIUM

 ADDED TO SOLUTIONS OF PLANT ASH

bamate in the aqueous phase caused the magnesium readings to be 10% higher than given by atomic absorption. In coccoa samples magnesium is best determined by atomic absorption.

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