

## Elimination of Phosphate Interference in EDTA Determinations of Calcium and Magnesium in Plant Ash

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Precipitation of calcium and magnesium from ammoniacal solutions by phosphate can be suppressed with ammonium molybdate, so permitting interference from phosphate to be prevented in the determinations of calcium and magnesium with ethylenediaminetetra-acetic acid. The use of ammonium molybdate with sodium tungstate to separate calcium also permits direct determination with ethylenediaminetetra-acetic acid of magnesium in mixtures of calcium, magnesium and phosphate. Because this has a useful application to the analysis of plant ash, a rapid procedure for determining calcium and magnesium in the leaf ash of *Hevea brasiliensis* has been devised and found to compare favourably with independent methods. The precision of the proposed procedure is 0.76 per cent. for magnesium and 0.41 per cent. for calcium, and the accuracy for both elements is to within about 1 per cent.

CHEMICAL analysis of plant ash in order to assess mineral-nutrient requirements is now well established practice. For tree crops, the leaf samples usually taken<sup>1</sup> always contain appreciable amounts of phosphate, which has been found to interfere in determinations of calcium and magnesium with ethylenediaminetetra-acetic acid (EDTA).<sup>2</sup> To prevent such interference, previous workers have either removed phosphate (by precipitation<sup>3</sup> or with ion-exchange resins<sup>4</sup>) or used dilute solutions for the EDTA titration, often with photometric determination of the end-point.<sup>5</sup> A rapid and simpler approach is described in this paper; it is based on masking the phosphate ion as the soluble molybdophosphate complex. Further, a procedure is described for determining calcium and magnesium in plant ash, magnesium being determined directly and calcium by difference. This is an improvement on existing procedures,<sup>3,6</sup> in which calcium is determined directly and magnesium by difference, because magnesium is normally present in much smaller amounts than is calcium; moreover, when the results are used to diagnose plant-nutrient requirements, magnesium is usually the more important element.<sup>7</sup>

### EXPERIMENTAL

Precipitates of ammonium calcium phosphate and ammonium magnesium phosphate are formed when weakly acidic solutions of plant ash are made alkaline with ammonia before titration with EDTA. The cloudiness produced interferes visually with the estimation of the end-point, which is drawn-out and also premature,<sup>2</sup> because of the slow interaction between EDTA and the insoluble phosphates. Precipitation of calcium and magnesium from ammoniacal solutions containing phosphate is suppressed by adding ammonium molybdate. This is shown in Fig. 1, in which the amounts of ammonium molybdate solution needed to prevent precipitation (experimentally determined by a repeated process of trial and error) can be seen to be proportional to the concentrations of phosphate and of calcium plus magnesium in solution. The points plotted in Fig. 1 represent the minimum amounts of 20 per cent. w/v ammonium molybdate solution needed to prevent precipitation of calcium and magnesium phosphates for at least 1 hour after the addition of 20 ml of an ammonia-ammonium chloride buffer solution to 50-ml portions of solutions containing calcium, magnesium and phosphate. Curve B corresponds to the normal amounts of these constituents found in the leaf ash of *Hevea brasiliensis* and curve A to the maximum amounts likely to be found.

Solutions stabilised in this manner eventually produce precipitates, usually after about 1 hour, and when they are heated, calcium molybdate is partly deposited. More complete precipitation of calcium under such conditions is attained by using sodium tungstate, as suggested by Shapiro and Brannock.<sup>8</sup> The efficiency of molybdate and tungstate as precipitants of calcium from ammoniacal solutions containing calcium, magnesium and phosphate ions can be determined by titrating with standard EDTA solution the cations remaining after the precipitated calcium has been separated by filtration. When 100-ml portions of

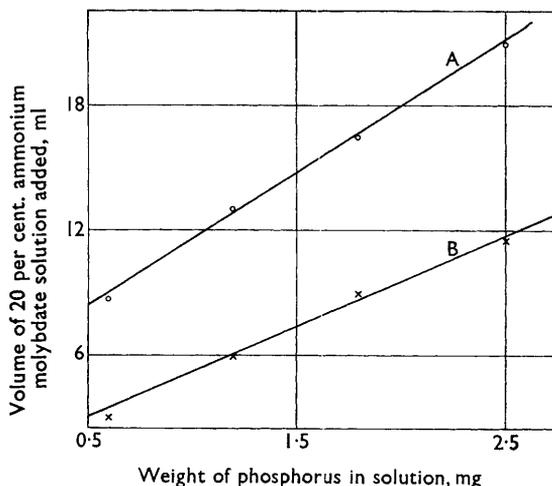


Fig. 1. Suppression by ammonium molybdate of precipitation of calcium and magnesium from ammoniacal solution by phosphate: curve A, solution containing 0.15 mg of calcium and 0.05 mg of magnesium per ml; curve B, solution containing 0.075 mg of calcium and 0.025 mg of magnesium per ml

hot ammoniacal solutions containing 7.5 mg of calcium, 2.5 mg of magnesium and 5 mg of phosphorus were treated with aqueous 20 per cent. w/v solutions of (a) ammonium molybdate and (b) sodium tungstate, the results were—

Volume of precipitant added, ml	..	..	..	10	20	30	40
Calcium precipitated by molybdate, %	..	..	..	75.9	85.7	94.4	95.0
Calcium precipitated by tungstate, %	..	..	..	113.0	119.9	110.3	106.1

Greater efficiency in precipitating calcium only can be attained by using ammonium molybdate and sodium tungstate together; results of experiments similar to those described above, but in which molybdate - tungstate mixtures were used as precipitant are shown in Table I and confirm this. The results above and in Table I were obtained from standard

TABLE I

## PRECIPITATION OF CALCIUM BY MIXTURES OF MOLYBDATE AND TUNGSTATE

Composition of precipitant		Calcium precipitated, %
Ammonium molybdate solution, 20 per cent. w/v, ml	Sodium tungstate solution, 20 per cent. w/v, ml	
5	10	82.5
10	10	84.1
10	20	97.6
20	10	96.8
20	15	100.8
20	20	100.8
30	1	100.0
30	3	100.0
30	5	100.4
30	10	100.4
30	15	100.0
30	20	99.7
40	10	100.0

solutions of calcium chloride, magnesium sulphate and diammonium hydrogen orthophosphate, which were mixed to correspond to the average amounts of calcium and magnesium and the maximum amount of phosphorus found in leaf samples from rubber trees. The way in which these results can be used to frame a satisfactory procedure for determining calcium and magnesium is discussed later. The procedure described below was developed specifically for the analysis of leaf samples from *Hevea brasiliensis*, but should be applicable to other leaf samples of broadly similar composition.

## METHOD

## REAGENTS—

All solutions should be prepared from analytical-reagent grade chemicals and distilled water.

*Ammonium molybdate solution, 20 per cent. w/v.*

*Ammonium chloride - ammonium hydroxide solution*—Dissolve 66 g of ammonium chloride and 600 ml of ammonia solution, sp.gr. 0.880, in 2.5 litres of water.

*Hydroxylamine solution, 3 per cent. w/v.*

*Potassium cyanide solution, 2 per cent. w/v.*

*Eriochrome black T - potassium chloride mixture*—Mix 0.2 g of Eriochrome black T with 50 g of potassium chloride until the indicator is evenly distributed.

*EDTA solution, 0.01 M*—Prepare from disodium ethylenediaminetetra-acetate dihydrate, and standardise against a solution of magnesium sulphate heptahydrate.

*Sodium tungstate solution, 20 per cent. w/v*—Prepare from the hydrated salt.

*Ammonia solution, 50 per cent. w/v.*

## PROCEDURE—

*Titration of calcium plus magnesium*—To a 25-ml aliquot containing about 4 mg of calcium and about 1.5 mg of magnesium add 20 ml of ammonium molybdate solution, mix thoroughly, and add 50 ml of ammonium chloride - ammonium hydroxide solution, 1 ml of hydroxylamine solution, 2 ml of potassium cyanide solution and 0.2 g of Eriochrome black T - potassium chloride mixture. Swirl thoroughly, and titrate with 0.01 M EDTA to the usual red to blue end-point.

*Titration of magnesium*—To a 50-ml aliquot of sample solution, *i.e.*, twice as much calcium and magnesium as in the previous titration, add 30 ml of ammonium molybdate solution and 10 ml of sodium tungstate solution. Swirl thoroughly, add 10 ml of 50 per cent. ammonia solution, and heat for 30 minutes at 80° to 90° C to precipitate calcium completely. Filter the solution immediately through a Whatman No. 2 filter-paper, and carefully wash the precipitate four times with 2 per cent. ammonia solution. Add the washings to the filtrate, then add 15 ml of 50 per cent. ammonia solution, 1 ml of hydroxylamine solution, 2 ml of potassium cyanide solution and 0.2 g of Eriochrome black T - potassium chloride mixture, and titrate as before with 0.01 M EDTA.

The second titre is a direct measure of the magnesium present in a 50-ml aliquot of the sample solution; the amount of calcium present is obtained from the difference between the second and twice the first titre.

## DISCUSSION OF RESULTS

The curves in Fig. 1 show the conditions under which precipitation of calcium and magnesium from ammoniacal solutions containing phosphate may be prevented by adding ammonium molybdate to form a soluble molybdophosphate complex. In titrations with EDTA, interference from phosphate can be prevented in this way, but the amount of molybdate needed depends on the concentrations of calcium, magnesium and phosphate in solution. For example, when the proposed procedure is used, 20 ml of the ammonium molybdate solution may be interpolated from Fig. 1 as the amount needed for the maximum concentrations of calcium, magnesium and phosphate likely to be present in ash from the leaves of *Hevea brasiliensis*.

The ammonium molybdate solution, together with the ammonium chloride - ammonium hydroxide solution, buffers the solutions, which are titrated at a pH of about 9.5; this is lower than the value recommended by Schwarzenbach,<sup>2</sup> but has nevertheless been found to give satisfactory end-points. The recommended pH of 10 cannot be used, as, above pH 9.7, excessively large amounts of ammonium molybdate are needed to prevent precipitation of calcium phosphate.

## DIRECT DETERMINATION OF MAGNESIUM—

The direct determination of magnesium in mixtures of calcium and magnesium depends on an initial quantitative removal of calcium. This is made difficult by the presence of phosphate, which forms precipitates with both calcium and magnesium in alkaline solutions. Precipitation of calcium as its oxalate from acid solution has been suggested, but the large excess of oxalate ion needed to avoid simultaneous precipitation of magnesium<sup>9</sup> interferes

in the subsequent titration of magnesium with EDTA.<sup>2</sup> Ammonium molybdate will precipitate calcium from hot ammoniacal solutions; the results on p. 112 show that, in the presence of phosphate, precipitation is incomplete (between 75 and 95 per cent., depending on the amount of molybdate used). Cheng and Bray<sup>6</sup> used sodium tungstate to precipitate calcium from ammoniacal solutions in an EDTA procedure adapted for soil extracts. In the presence of appreciable amounts of phosphate, however, magnesium is also precipitated as its phosphate, and the results on p. 112 show that magnesium equivalent to more than 10 per cent. of the calcium present may be lost in this way from solutions containing phosphate.

The results in Table I show that suitable mixtures of ammonium molybdate and sodium tungstate solutions will effectively precipitate calcium from hot ammoniacal solutions also containing magnesium and phosphate. Calcium tungstate is deposited, but the large excess of molybdate forms a complex ion with phosphate and prevents simultaneous precipitation of magnesium phosphate. Extrapolation from curve B in Fig. 1 suggests that, for the titration of magnesium only by the proposed procedure in an aliquot containing 7.5 mg of calcium, 2.5 mg of magnesium and 5 mg of phosphorus, about 25 ml of 20 per cent. ammonium molybdate solution should be needed to prevent interference from phosphate. This is confirmed by the figures in Table I, which indicate that a mixture of 30 ml of the ammonium molybdate solution and 1 ml of the sodium tungstate solution is adequate to precipitate calcium without loss of magnesium from hot solutions. The precipitation of calcium by so small an amount of tungstate is slow, and in practice the excess provided by 10 ml of the sodium tungstate solution was found to be most suitable for leaf samples.

#### CATIONIC INTERFERENCE—

Cheng and Kurtz<sup>10</sup> have suggested the use of mixtures of potassium cyanide, triethanolamine and potassium ferrocyanide to prevent interference from heavy metals, iron, aluminium and manganese in EDTA titrations of magnesium and calcium in soil extracts. These metals are present in smaller amounts in leaf samples of *Hevea brasiliensis*, and it was found in practice that the slight interference from them could be overcome by using potassium cyanide only; this reagent has therefore been included in the proposed procedure.

#### COMPARISON WITH OTHER METHODS

A comparison was made between the proposed procedure and two standard methods used in these laboratories for routine analyses of leaf samples; these methods were Hunter's Titan yellow method for magnesium<sup>11</sup> and a flame-photometric method for calcium, which was applied after calcium had been precipitated as its oxalate in order to avoid interference from phosphate.<sup>12</sup> Solutions in dilute nitric acid of two dry ashes from each of five samples of leaves were analysed in duplicate by the three methods, and the results (see Table II) were expressed as percentages of oven-dry material.

TABLE II  
CALCIUM AND MAGNESIUM FOUND IN LEAF SAMPLES FROM *Hevea brasiliensis*

• Results are expressed as percentages of oven-dry material

Leaf sample	Ash No.	Magnesium found by—		Calcium found by—	
		proposed method, %	Titan yellow method, %	proposed method, %	flame-photometric method, %
A	1	0.279, 0.280	0.283, 0.283	0.472, 0.466	0.460, 0.463
	2	0.274, 0.276	0.289, 0.286	0.481, 0.479	0.470, 0.470
B	3	0.195, 0.191	0.187, 0.196	1.246, 1.250	1.275, 1.267
	4	0.190, 0.191	0.190, 0.196	1.256, 1.250	1.267, 1.267
C	5	0.193, 0.195*	0.188, 0.188	0.769, 0.761	0.752, 0.755
	6	0.193, 0.195	0.188, 0.184	0.765, 0.757	0.758, 0.755
D	7	0.195, 0.198	0.188, 0.188	0.757, 0.757	0.765, 0.769
	8	0.198, 0.198	0.188, 0.188	0.757, 0.753	0.769, 0.765
E	9	0.201, 0.203	0.198, 0.198	1.428, 1.422	1.430, 1.447
	10	0.201, 0.204	0.201, 0.196	1.428, 1.424	1.438, 1.438
Mean . . . . .	..	0.212	0.210	0.934	0.939
Standard error . . . . .	..	± 0.002	± 0.002	± 0.004	± 0.004

A statistical examination of these results is summarised in Table III, from which the standard error of a single determination of magnesium by the proposed method can be calculated as  $\pm 0.00161$  (coefficient of variation 0.76 per cent.); the corresponding values for the Titan yellow method are  $\pm 0.00289$  and 1.37 per cent. For a single determination of calcium by the proposed method, the standard error is  $\pm 0.00380$  (coefficient of variation 0.41 per cent.), and for the flame-photometric method the corresponding figures are  $\pm 0.00454$  and 0.48 per cent. All these values are based on the variations between duplicate determinations on a given ash; differences in precision between the proposed and the other two methods are therefore not established, although a lower precision for the Titan yellow method is indicated at the 10 per cent. level of significance.

TABLE III  
ANALYSIS OF VARIANCE OF RESULTS IN TABLE II

Source of variation	Degrees of freedom	Mean square $\times 10^8$	
		Magnesium	Calcium
Methods .. .. .	1	5523 (N.S.)	26,010 (N.S.)
Samples .. .. .	4	1,233,798***	125,904,766***
Samples $\times$ methods .. .. .	4	9360***	31,816***
Ashes within samples .. .. .	5	83 (N.S.)	3865 (N.S.)
Ashes $\times$ methods within samples .. .. .	5	1033 (N.S.)	1425 (N.S.)
Duplicates { proposed method .. .. .	10	260	1440
{ standard method .. .. .	10	835	2060
	20	548	1750

\*\*\* Significant at the 0.1 per cent. level. N.S. = Not significant.

The analysis of the results also shows no significant difference between ashes on the same sample, whereas a highly significant interaction is shown between samples and methods. As a result, no significant differences between the means for the methods (see Table II) are established; this is because the standard errors for these means must be derived from the interaction mean square. These points can be confirmed by inspection of Table II, *e.g.*, it is evident that, for samples A and B, the Titan yellow method gave larger average values than did the proposed method, although for samples C, D and E the position was reversed.

#### ACCURACY—

The fact that the proposed method is sufficiently accurate for practical purposes is indicated by the good agreement with other methods. However, an independent check was made on prepared solutions containing calcium, magnesium and phosphate in combinations corresponding to the high and low amounts found in plant ash. The results are shown in Table IV, from which it can be seen that, on average, the recovery of magnesium is 1 per

TABLE IV  
RECOVERY OF CALCIUM AND MAGNESIUM FROM PREPARED SOLUTIONS  
BY PROPOSED METHOD

Composition of corresponding plant material			Recovery	
Calcium, %	Magnesium, %	Phosphorus, %	Magnesium, %	Calcium, %
1.0	0.5	0.4	98.2	101.7
1.0	0.5	0.1	98.6	101.3
0.3	0.5	0.4	99.6	102.9
0.3	0.5	0.1	99.7	102.4
1.0	0.1	0.4	99.5	99.9
1.0	0.1	0.1	99.1	99.7
0.3	0.1	0.4	99.1	100.9
0.3	0.1	0.1	99.1	100.9
		Average .. .. .	99.1	101.2

cent. low and that of calcium 1 per cent. high; the fact that the calcium figure depends upon the magnesium determination may explain this relationship. In general, the errors in recovery are acceptable since they are well within the error associated with preparing leaves for analysis.<sup>13</sup>

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