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Spectrophotometric Determination of Micro Amounts of Aluminium in Plant Material with 8-Hydroxyquinoline

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The 8-hydroxyquinoline method, previously found to be more satisfactory than the aluminon method for the analysis of soil extracts, has been examined for its suitability for determining aluminium in plant tissues. An initial extraction with chloroform of the diethyldithiocarbamate complexes of the large amounts of heavy metals found in plant material has been devised. The resulting method was tested on synthetic solutions and compared with emission-spectrochemical procedures in the analysis of plant materials.

The lower limit for reliable determination by the proposed method corresponds to 4 p.p.m. of aluminium in the dried plant tissue. At this lower limit, the following interfering elements in the tissue at the percentage concentrations indicated are tolerated in the method: copper, 0·005; zinc, 0·030; iron, 0·030; manganese, 0·10; phosphorus, 0·20; and calcium, 4·0. For tissue containing 20 p.p.m. of aluminium, or more, at least five times these amounts are tolerated.

The determination of small amounts of aluminium in plant tissue in the presence of high concentrations of interfering ions presents a considerable analytical challenge. Recently, several methods have been proposed; two are based on the triammonium aurin tricarboxylate (aluminon) lake reaction,[1,2] while two others involve solvent extraction of the complex formed between aluminium and 8-hydroxyquinoline.[3,4]

In our laboratory, aluminon methods have failed to yield reproducible standard curves, while the 8-hydroxyquinoline method has proved extremely reproducible.[5] Further, for analyses of plant digests, one of the aluminon procedures[1] requires a preliminary separation by elution through an ion-exchange column; the other[2] involves separation by precipitation with ammonia solution and centrifugation, followed by adjustment of sample acidity with a pH meter. For routine analyses, these procedures are time consuming and require the analyst's attention for each individual sample. In addition, the concentrations of interfering ions tested[2] were much lower than those observed in plant tissue in subsequent studies.[1]

Rubins and Hagstrom[4] have described a sensitive and accurate method based on the fluorescence of the extracted aluminium 8-hydroxyquinolinate complex. In addition to a fluorimeter being required, the high concentrations of interfering ions observed in some plant tissue do not appear to be tolerated with their method. Further, for the initial extraction of iron it involves the use of bathophenanthroline, which is a rather expensive reagent unless iron is to be determined.[4]

Extraction of the aluminium 8-hydroxyquinolinate complex into chloroform was used by Middleton[3] to determine aluminium in leaves of the rubber tree, but no precautions to remove interfering ions were described. It is clear from previous work[5] that several ions likely to be present in plant digests could cause severe interference in the 8-hydroxyquinoline method. Thus, to provide a spectrophotometric procedure in which high concentrations of interfering ions would be tolerated, without the need for tedious precipitation or ion-exchange procedures, we undertook the investigations described below. The resulting procedure, which involves an initial extraction of the diethyldithiocarbamate complexes of heavy metals with chloroform, permits spectrophotometric determination of aluminium in 18 samples in about 2 hours.

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For tissue containing as little as 4 p.p.m. of aluminium, the following elements, with their percentage concentrations in the tissue, are tolerated: copper, 0·005; zinc, 0·030; iron, 0·030; manganese, 0·10; phosphorus, 0·20; and calcium 4·0. These are at least 10 times the relative concentrations of interfering ions tested for other methods.[2,4]

Experimental

Reagents—

Concentrated hydrochloric acid, glacial acetic acid and concentrated ammonia solution were distilled in a glass still. As the resulting ammonia solution was about 10 N, it was not used in the concentrated buffer described below. Concentrated nitric, sulphuric and perchloric acids were not purified because their distillation is somewhat hazardous. Purification of the chloroform used was not found to be necessary. All aqueous reagent solutions were prepared with de-ionised distilled water.

Buffer solution—Mix 275 ml of glacial acetic acid and 310 ml of concentrated ammonia solution, cool, and dilute with water to 500 ml. The final pH should be adjusted to 6·2, if necessary.

8-*Hydroxyquinoline solution*—Dissolve 20 g of 8-hydroxyquinoline in 1 litre of chloroform and store in a dark glass bottle. This reagent is rather variable and some batches are highly coloured. We found the "Baker Analyzed" reagent to be satisfactory.

Diethyldithiocarbamate solution—Dissolve 3 g of sodium diethyldithiocarbamate in 100 ml of water, filter through Whatman No. 41 paper, and store in the cold. Prepare freshly every few days.

Thymol blue indicator—Dissolve 0·1 g of thymol blue in 10 ml of ethanol and dilute to 50 ml with water.

Aluminium standards—Prepare a stock solution containing 50 μg of aluminium per ml in 0·1 N hydrochloric acid, either as previously described,[5] or, as in the present study, from recrystallised aluminium chloride standardised independently by precipitation of aluminium 8-hydroxyquinolinate. Prepare a working standard containing 5 μg of aluminium per ml by appropriate dilution of the stock solution with 0·1 N hydrochloric acid.

Digestion of plant material—

Three different plant tissues were analysed: tomato tops, tobacco leaves and apple leaves. The materials were dried at 60° C in a forced-draught oven and ground in a Wiley mill to pass a 40-mesh sieve. Then, 1·0-g samples were treated with 5 ml of concentrated nitric acid and digested with 5 ml of a mixture of nitric, sulphuric and perchloric acids (10 + 1 + 3), following the procedure of Johnson and Ulrich.[6] After digestion, the salts were dissolved by boiling with water,[6] and the silica residue was washed once by centrifugation with 30 to 40 ml of 6 N hydrochloric acid. The acid digest was then diluted to 100 ml with water. For tissue containing less than 8 p.p.m. of aluminium, the final volume should not be more than 50 ml.

Determination of aluminium—

Transfer, by pipette, an appropriate aliquot (up to 25 ml) of the plant digest or the standard solution, containing 2 to 20 μg of aluminium, into a 120-ml Squibb pear-shaped separating funnel calibrated at about 50 ml. Add 1 ml of N acetic acid, 2 to 4 drops of thymol blue indicator, and neutralise carefully with 6 N ammonia solution until the red colour disappears. Add 5·0 ml of buffer solution, dilute to 50 ml with water, and mix well. The resulting solution should have a pH of between 5·1 and 5·2. Add 2 ml of the diethyldithiocarbamate solution, and rapidly make three consecutive extractions, each with 5-ml portions of chloroform, shaking for 5 minutes each time and discarding the chloroform phase. Details of the extraction method have been previously described.[5] After the third extraction, allow the solution to stand for 15 minutes to decompose any remaining diethyldithiocarbamate. Add 5·0 ml of the 8-hydroxyquinoline solution in chloroform, and extract for 5 minutes. Filter the chloroform phase through a cotton pledget into a 1-cm cell and measure the absorbance at 385 mμ.

The extracted chloroform phase from the reagent blank should be used as the reference solution to minimise the effect of an absorption peak at 372 mμ, as discussed below. Throughout the procedure care must be exercised to prevent contamination of the glassware, and also to avoid losses of aluminium by adsorption.[5]

EMISSION-SPECTROCHEMICAL ANALYSIS—

Plant materials were also analysed in two independent laboratories by emission-spectrochemical procedures. The methods used by laboratory No. 1 have been previously described.[7] In laboratory No. 2, the samples were dry ashed in quartz crucibles and dehydrated with concentrated hydrochloric acid. The residue was extracted with N hydrochloric acid containing 0·5 per cent. w/v of lithium as a radiation buffer, and 0·02 per cent. w/v of nickel as an internal standard. Analyses were performed by a rotating-disc solution technique, with parameters described by Baker and Greweling.[8]

DEVELOPMENT OF METHOD

The larger amounts of copper, zinc, iron and manganese found in plant materials require extensive modifications of procedures that have been developed for the determination of aluminium in soil extracts.[5] Complexing agents, which either prevent extraction of these metals or permit their extraction before the extraction of aluminium, have been used. We sought a single agent that would extract large amounts of all of the interfering ions and tested several, including dithizone, 8-hydroxyquinaldine and diethyldithiocarbamate, with chloroform or carbon tetrachloride as solvents, and adjusting the pH to various levels. The most satisfactory combination appeared to be extraction of the diethyldithiocarbamate complexes with chloroform at pH 6, but, for the reasons described below, extraction at pH 5 was found to be preferable. Extraction of the diethyldithiocarbamate complexes is usually accomplished in alkaline solution but, as magnesium is extracted from alkaline solution by 8-hydroxyquinoline, it must be acidified before extraction of aluminium. This latter procedure gave low recoveries of aluminium, apparently because of the precipitation of aluminium hydroxide, which failed to dissolve on acidification.[9] Hence, extraction of the diethyldithiocarbamate complexes from acidic solutions was required.

Tests on aluminium standards, however, indicated that the extraction of aluminium was still incomplete after extraction of the diethyldithiocarbamate complexes with chloroform at pH 6. Aluminium was not extracted by diethyldithiocarbamate, contrary to the suggestion of Goldstein, Manning and Menis,[10] because a second extraction with 8-hydroxyquinoline removed the remaining aluminium from the aqueous phase. Further tests established that diethyldithiocarbamate was not a factor; merely by shaking the aqueous phase with either chloroform or carbon tetrachloride before extraction of aluminium 8-hydroxyquinolinate in this method, or that of Frink and Peech,[5] low recoveries of aluminium were caused. Lowering of the pH gave higher recoveries[10]; similarly, an increase in the acetate concentration at pH 6 gave higher recoveries. No ready explanation of this phenomenon is apparent. Okura, Goto and Yotuyanagi[11] reported that only monomeric aluminium ions are extracted by 8-hydroxyquinoline. However, as these solutions can be shown to be supersaturated with respect to crystalline aluminium hydroxide (gibbsite) at pH 6, it seems likely that by shaking such solutions with chloroform or carbon tetrachloride the precipitation of the hydroxide is initiated or promoted.

Further studies were required of this modified method at pH 5 in the presence of the concentrated acetate buffer. Despite the relatively short half-life of diethyldithiocarbamate in acidic solutions,[9] we found that the heavy metals could be successfully extracted at pH 5: the time of contact of diethyldithiocarbamate with the aqueous phase, however, should be kept to a minimum. In addition, at pH 5, considerable amounts of acetate enter the chloroform phase, thus causing a change in the dissociation of 8-hydroxyquinoline, and creating an absorbance peak at 372 mμ.[12] The effect of this peak is to increase considerably the absorbance of the chloroform phase extracted from the reagent blank. Therefore, pH and acetate concentration must be carefully controlled. To avoid the tedious adjustment of acidity with a pH meter, which many procedures require at this point, several indicator and buffer combinations were tested. We found that the acidity could be conveniently and reliably adjusted to between pH 5·1 and 5·2 by the recommended procedure. The resulting yellow colour of the indicator at this pH does not interfere because it is removed by the initial extraction with chloroform. Other procedural details, such as time of shaking, the drying of the chloroform phase and stability of the aluminium 8-hydroxyquinolinate complex were previously investigated[5]; the lowering of the pH from 6 to 5 for extraction should not affect these findings and, therefore, will not be discussed here.

Results and discussion

Several interfering ions were tested, both singly and in various combinations, as shown in Table I. The amounts chosen represent the upper limits of these elements likely to be found in 1 g of plant material grown in acidic soils. As the aliquot necessary for the determination of aluminium at concentrations in the tissue of 20 p.p.m., or more, represents only 0·1 g of plant material, it is obvious that, in most instances, ten times the expected amounts of the individual interfering ions can be tolerated with this method. In the event of iron and manganese interference, the tolerance is about five times the anticipated amount. The synthetic plant digest (Table I) contains five times the expected total amount of all interfering ions, and in no instance was the amount of aluminium recovered significantly different from that taken. In addition, recovery tests were made with 2 and 4-ml aliquots of actual plant digests from five different samples. An analysis of variance showed no significant differences between aliquot sizes, thus giving no evidence for the presence of negative errors.

Table I

Determination of aluminium by the proposed method in the presence of various interfering ions

Interfering ion added, μg						Amount of aluminium taken, μg		
						0	10	20
Copper	Zinc	Iron	Manganese	Phosphorus	Calcium	Aluminium found, μg		
—	—	—	—	—	—	0·0	10·0	20·2
50	—	—	—	—	—	0·2	10·5	20·7
—	300	—	—	—	—	0·2	10·3	21·5
—	—	150	—	—	—	0·4	10·0	19·9
—	—	300	—	—	—	2·0	11·5	21·3
—	—	—	500	—	—	0·6	10·4	20·2
—	—	—	1000	—	—	1·2	10·3	22·0
50	300	150	500	—	—	0·8	10·8	20·2
50	300	300	1000	—	—	3·5	13·8	23·5
—	—	—	—	2000	—	0·0	9·8	19·9
—	—	—	—	—	40,000	0·2	8·7	20·6
—	—	—	—	2000	40,000	0·2	9·8	19·7
Synthetic plant digest*						0·4	10·1	20·0

* Contains one half of the maximum amounts of each of the interferences tested singly above.

In general, the interferences observed were in agreement with those previously reported.[9] Copper, iron and manganese are all extracted as the 8-hydroxyquinolinate complexes and, if not removed by the proposed diethyldithiocarbamate extraction, will lead to apparently high results. Although Rubins and Hagstrom[4] cite previous work that indicates that zinc 8-hydroxyquinolinate is not extracted, we found that zinc forms a white precipitate in the aqueous phase, which subsequently dissolves in the chloroform phase and causes apparently high results. As Rubins and Hagstrom[4] found that zinc did not interfere in their procedure, we assume that zinc 8-hydroxyquinolinate does not fluoresce. As previously reported,[5] both calcium and phosphorus (as orthophosphate) may lead to apparently low results, presumably because of the formation of insoluble aluminium phosphates in the one instance and of a calcium 8-hydroxyquinolinate complex, insoluble in chloroform, in the other. Magnesium was previously reported[5] to interfere in a similar manner; however, the interference was less severe than with calcium. As the amounts of magnesium in plant digests are generally less than the amounts of calcium, this source of interference was not investigated further.

From these tests, we can conclude that the proposed method should be free from interferences. However, we also wished to test the method in the actual analysis of plant material. Because published procedures[1,2,3,4] have lower tolerances for interfering ions, or lower sensitivity, or both, it seemed of little consequence to use these methods as a basis for comparison. Therefore, we chose emission-spectrochemical analysis as an independent analytical procedure. As this method may lack precision and accuracy for tissue containing low concentrations of aluminium, we selected tissue containing higher amounts, when both spectrochemical and spectrophotometric methods could be used.

Results of the comparative analyses of 18 samples are shown in Table II, and indicate considerable variability between the two spectrochemical methods, as might be expected. The results obtained by laboratory No. 1 are generally lower than those obtained by the proposed method at low concentrations of aluminium in the tissue, and the same or higher at high concentrations. The results obtained by laboratory No. 2 are generally the reverse. Some of these discrepancies are undoubtedly caused by different methods of extracting aluminium from the plant ash. However, the agreement between the proposed method and the average spectrochemical analyses is considered quite good; indeed, the correlation coefficient between the two is 0·986. As the differences shown in Table II reflect sampling and ashing errors, as well as analytical errors, it seems unlikely that the results suggest any consistent bias in aluminium analyses by the proposed procedure.

The precision of the proposed method was tested on standard solutions and plant digests. The mean coefficient of variation was 5·44 per cent. for six replicate analyses of five different standard solutions containing from 0 to 20 μg of aluminium. This is higher than the coefficient of variation of 2·89 per cent. observed for thirty similar analyses conducted during initial studies of the method when aluminium was extracted at pH 6. The greater variability at pH 5 apparently arises from the increased absorbance caused by the larger amounts of acetate in the chloroform phase, as previously discussed. Duplicate analyses were made of the digests of the 18 samples shown in Table II. An analysis of variance of the results for each kind of tissue gave a mean coefficient of variation of 6·63 per cent., which is only slightly larger than that observed for standard solutions. It should be emphasised that these estimates of the precision of the proposed method were not obtained from special replicate analyses run side by side, but rather from analyses run over a considerable period of time under ordinary laboratory conditions. Thus, they are somewhat larger than those frequently reported, but reflect more accurately the reliability of the method in routine analysis.

TABLE II

DETERMINATION OF ALUMINIUM IN PLANT TISSUE BY THE PROPOSED METHOD AND BY EMISSION-SPECTROCHEMICAL METHODS

Sample		Aluminium found, p.p.m.			
			Emission-spectrochemical method		
		Proposed method	Laboratory 1	Laboratory 2	Mean
Tomato tops	1	67	40	79	60
	2	68	30	79	54
	3	68	40	74	57
	4	87	60	84	72
	5	88	50	87	68
	6	90	60	100	80
	7	100	60	124	92
	8	114	80	124	102
Tobacco leaves	1	156	102	157	130
	2	162	137	176	156
	3	217	172	209	190
	4	228	195	221	208
	5	314	280	258	269
Apple leaves	1	249	230	200	215
	2	289	320	264	292
	3	448	440	441	440
	4	546	610	546	578
	5	562	550	500	525

CONCLUSIONS

A survey of existing spectrophotometric methods for the determination of aluminium in plant material indicated that none was entirely satisfactory for tissue containing small amounts of aluminium and large amounts of interfering ions. The 8-hydroxyquinoline method, previously found to be more reliable than the aluminon method for the analysis of soil extracts, was therefore chosen for further study. An initial extraction with chloroform of the diethyldithiocarbamate complexes of the large amounts of heavy metals found in plant

material was devised. The resulting method was tested on synthetic solutions and then compared with emission-spectrochemical procedures. These tests showed that the proposed method will enable aluminium to be determined in the presence of a 5-fold excess of the interfering ions expected in plant digests, has good precision and high sensitivity, and is particularly suitable for plant material containing less than 100 p.p.m. of aluminium.

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