Simplified Determination of Molybdenum in Plant Material by 4-Methyl-1,2-Dimercaptobenzene, Dithiol

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Dithiol has been applied to the determination of microgram quantities of molybdenum in plant material, without the need for preliminary separation. The effect of acidity has been assessed. A more detailed study of the influence of iron shows that the presence of iron is of significance in the development of maximum absorbance of the molybdenum-dithiol complex. Copper in quantities normally present in plant material does not interfere. The method is sensitive, simple, and capable of determining from 0.1 to 5.0 γ of molybdenum.

THE ACCURATE determination of molybdenum has assumed greater importance in recent years, because of the demonstration of the specific biological functions of this element (*4*, *11*). The analytical methods used for the determination of molybdenum have, for many years, been based principally on the reaction with thiocyanate (*5*, *10*). This method has been made more sensitive and accurate following the demonstration that an iron-molybdenum-thiocyanate complex is more stable and of greater color intensity (*3*, *5*, *6*). Piper and Beckwith (*9*) described a sensitive method in which they used dithiol (4-methyl-1,2-dimercaptobenzene), but they found no function of iron in the production of the molybdenum complex. Others have considered the preliminary separation of molybdenum to be necessary (*1*, *12*). Clark and Axley (*2*) applied dithiol directly to the determination of molybdenum in soils and rocks, but encountered serious interference from iron, which they overcame by reduction with potassium iodide.

The present paper describes the application of a procedure, similar to that of Clark and Axley, to the acid digest of plant material. The effects of sulfuric acid concentration, time of standing, and of copper and iron on the reaction have been examined.

Reagents

Water. Glass-distilled water is used throughout.

Dithiol, 0.2% solution. A 1-gram vial of analytical grade dithiol is warmed to 38° C. and the contents are then poured into 500 ml. of a 1% sodium hydroxide solution also warmed to 38° C. The dithiol dissolves readily and a few minutes later thioglycolic acid is added, until a faint turbidity persists. The reagent is then stored in a refrigerator and may be used for at least 4 weeks.

Potassium iodide, 50% w./v. solution in glass-distilled water.

Sodium thiosulfate, 10% w./v. solution in glass-distilled water.

Tartaric acid, 50% w./v. solution in glass-distilled water.

Ferric ammonium sulfate, 100 mg. per ml. Dissolve 9.1 grams of ferric ammonium sulfate dodecahydrate in 100 ml. of 2% sulfuric acid.

Isoamyl Acetate. The fraction boiling between 136° and 142° C. is employed. The used material can be recovered by steam distillation over basic lead acetate.

Standard Molybdenum Solution. Ammonium molybdate is of variable composition and unsuitable for the preparation of standard solutions. Dry molybdenum metal is employed; 100 mg. are dissolved in sulfuric acid, containing a trace of nitric acid, diluted with water, then boiled, and a slight excess of ammonia is added. The solution is then made up to 100 ml. By further dilution a solution containing 1 γ per ml. is obtained.

Apparatus

The Beckman Model B spectrophotometer was used in all measurements of absorbance.

Method

A 1-gram sample of dried and ground plant material is weighed into an 8 × 1 inch Q & Q borosilicate glass digestion tube. The organic matter is destroyed by wet digestion with sulfuric, perchloric, and nitric acids (*7*). When decomposition of the sample is complete and fumes of sulfuric acid are being evolved, the tube is allowed to cool, the mouth and sides are washed down with 2 ml. of water and a further 0.5 ml. of perchloric acid is added. The water is boiled off and the contents are again taken to fumes of sulfuric acid. After the tube has cooled, 4 to 5 ml. of water are added and the contents are boiled vigorously for 15 seconds; 0.25 ml. of the iron solution is added and the volume is adjusted to 25 ml. with water. After the addition of 0.25 ml. of a 50% potassium iodide solution the mixture is allowed to stand for 10 minutes, while the tube is swirled from time to time. The iodine color is discharged by the dropwise addition of sodium thiosulfate, which is followed by 0.25 ml. of tartaric acid solution and 2 ml. of dithiol reagent. The tube is then stoppered and shaken vigorously for 30 seconds. After standing for 30 minutes, 5.0 ml. of isoamyl acetate are added, and vigorously shaken for 30 seconds. When the phases have separated, the isoamyl acetate layer is drawn off and centrifuged to remove droplets of water. The absorbance of this solution is then measured and the amount of molybdenum determined by reference to a calibration curve prepared similarly.

Experimental Results

Effect of Acid Concentration. Concentrations of sulfuric acid between 1.25N and 5.0N in the mixture were without influence on the color intensity of a given amount of molybdenum. At acid concentrations above 5.0N, the iodide is readily reoxidized and may be extracted with the isoamyl acetate. A concentration of 4N was chosen as most convenient.

Effect of Iron on Absorbance of Molybdenum - Dithiol Complex. Graded amounts of pure iron from 0 to 10 mg. were added to systems containing 5 γ of molybdenum. The iron was then reduced with potassium iodide, and after addition of the dithiol reagent, the colored complex was extracted with isoamyl acetate. A very large increase in absorbance was observed when 2 mg. of iron were added; maximum and constant absorbance values were obtained only when 2 to 10 mg. of iron were added. This effect is shown in Table I.

Other Factors. Up to 30 γ of copper

Table I. Effect of Iron on Absorbance of Molybdenum-Dithiol Complex Developed from 5 γ of Molybdenum

Iron Added, Mg.	Absorbance (1 Cm. = 680 Mμ)	
0	0.115	0.135
0.25	0.198	0.208
1.0	0.210	0.216
2.0	0.225	0.229
5.0	0.228	0.228
10.0	0.230	0.230

are without effect on absorbance, when the molybdenum-dithiol complex is developed in the presence of optimum iron concentrations. It is unlikely that amounts in excess of this will often be encountered in pasture material.

Full color development occurs in the isoamyl acetate layer, whether 1 minute, 2 hours, or 16 hours elapse after addition of the dithiol reagent and before extraction of the complex. The complex once extracted with isoamyl acetate is unchanged in absorbance for periods of up to 72 hours. *n*-Amyl alcohol was not a satisfactory solvent for the complex. Isoamyl acetate has the added advantage of low water solubility and the colored complex can be extracted with small volumes of this solvent without introducing appreciable errors (*8*). Table II records absorbance of a replicated series of standard molybdenum solutions, covering the range of values encountered in pastures using 5 ml. of isoamyl acetate for extraction. The same order of accuracy was given when only 1 ml. of solvent was used for extraction from the same volume of aqueous phase. The method was then applied to some typical pasture samples, which were analyzed with and without added molybdenum. The results of these analyses are reported in Table III.

Table II. Absorbance of Standard Molybdenum Solutions, Extracted into 5 Ml. of Isoamyl Acetate

Molybdenum Taken, γ	Absorbance[a]				Mean	Confidence Limits, 95%
0	0.001	0.002	0.002	0.002		
	0.002	0.002	0.002		0.002	0.001–0.002
0.1	0.004	0.004	0.004	0.005		
	0.005				0.005	0.003–0.006
0.25	0.012	0.012	0.012	0.012		
	0.013				0.012	0.012–0.013
0.50	0.024	0.024	0.024	0.025		
	0.025	0.025	0.025	0.025	0.025	0.023–0.025
1.0	0.048	0.049	0.049	0.049		
	0.051				0.049	0.048–0.050
2.0	0.097	0.098	0.098	0.099	0.098	0.096–0.099

[a] Absorbance at 680 mμ, 1-cm. light path, 25° C. All values, except those for 0 γ Molybdenum corrected for blank.

Table III. Results of Analysis of Field Material with Recovery of Added Molybdenum

Sample	Mo in Sample,[a] P.P.M.	Mo in Sample Taken, γ	Mo Added, γ	Mo Found, γ	Added Mo Recovered, γ	Recovery Added Mo, %
Subterranean clover	0.19	0.20	0.50	0.76	0.56	112
		0.08	0.50	0.61	0.53	106
Lucerne hay	0.23	0.23	0.50	0.74	0.51	102
		0.24	0.50	0.78	0.54	108
Oaten hay	0.40	0.35	0.50	0.81	0.46	92
		0.32	0.50	0.83	0.51	102
Subterranean clover	0.89	1.02	1.00	1.95	0.93	93
		0.81	1.00	1.79	0.98	98
	0.89	0.83	2.00	2.77	1.94	97
		0.73	2.00	2.63	1.90	95
Natural pasture	2.87	1.83	2.00	3.63	1.80	90
		2.61	2.00	4.55	1.94	97
Natural grasses	3.19	1.67	2.00	3.52	1.85	93
		2.03	2.00	4.00	1.97	98

[a] Means of replicates.

Discussion

With the thiocyanate method for the determination of molybdenum in biological material (*5*) difficulties have been encountered with the use of stannous chloride as a reducing agent. The difficulties arise from the variability from batch to batch of the commercially available reagent, from the inability to purify it from traces of molybdenum, and from the persistent films which it tends to form, on the cuvettes, with continued use. None of the other reducing agents tested was satisfactory. The dithiol method was adopted, because it does not require preliminary separations and is rapid, sensitive, and accurate.

Johnson and Arkley (*8*) used isoamyl alcohol saturated with carbon tetrachloride, to reduce the volume of the solvent used for the extraction of the colored complex in samples of low molybdenum content, without involving appreciable error arising from the solubility of the extractant in the aqueous phase. However, in the present method as little as 1 part of isoamyl acetate to 25 parts of the aqueous phase can be employed, without appreciable error, thus enabling the accurate determination of 0.1-γ amounts of molybdenum. Because of the low solubility of isoamyl acetate in water, temperature equilibration of the sample extracts is not necessary.

Full and reproducible color production can, however, be attained only in the presence of adequate amounts of iron, and in this respect the behavior of the molybdenum-dithiol complex resembles the iron-molybdenum thiocyanate complex (*3, 6*).

The effect of iron, observed here, is much more pronounced than that observed by Piper and Beckwith (*9*), who found only a small increase in color intensity for an added 2 mg. of iron in standard molybdenum solutions before color development. Clark and Axley (*2*) found that larger amounts of ferric ion interfered seriously by oxidative destruction of the molybdenum-dithiol complex. This interference was overcome by reduction of the ferric iron to the ferrous state.

Because pastures rarely contain as much as 0.2% iron, addition of 2.0 mg. of iron to the sample is essential if an accurate result is to be secured.

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Received for review May 16, 1958. Accepted February 2, 1959.