Spectrophotometric Determination of Trace Amounts of Molybdenum in Vegetal and Tobacco Tissues with a New Chromogenic Reagent, Dimethoxyhydroxyphenylflurone

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A simple method for the direct determination of trace molybdenum in vegetal and tobacco tissues with selectivity and sensitivity is reported. The advantages are the high sensitivity, ease of operation, good reproducibility, and simplicity of apparatus. In the presence of Triton X-100 and sulfuric−phosphoric acid medium, a new chromogenic reagent, dimethoxydroxyphenylflurone, forms a red complex with molybdenum(VI). The molar absorptivity is obeyed in the range of 0−8 μg/25 mL for molybdenum(VI). Most metal ions and 35000-fold amounts of iron do not interfere with the determination of molybdenum. The proposed method has been successfully used for the determination of trace amounts of molybdenum in vegetal and tobacco tissues.

Keywords: *Dimethoxyhydroxyphenylflurone; spectrophotometry; molybdenum; vegetal; tobacco*

INTRODUCTION

Molybdenum is an important element in nature, required by both plants and animals in very small amounts, food being the major source for man (Berman, 1980). In plant nutrition, its action is related to several biochemical functions in nitrate reduction, biosynthesis of nucleic acids, and biochemical processes related to the fixation of molecular nitrogen by microorganisms and bacteria. The Mo requirement of plants varies with species, so its routine control is highly recommended for normal growth and development (López et al., 1984). Hence, Mo is frequently determined in vegetal and tobacco tissues, and rapid and sensitive methods are required.

The method usually used for the determination of trace amounts of molybdenum in vegetal and tobacco tissues is extraction of the thiocyanate complex and spectrophotometric measurement after reduction (Sandell et al., 1978; Patil et al., 1973; Mitra et al., 1977; Gowda et al., 1985; Johnson et al., 1954; Ssekaalo, 1971; Salinas et al., 1985) . It suffers from interference by metal ions and requires fairly rigid control of conditions. Methods based on other reagents also have several drawbacks.

Derivatives of 2,3,7-trihydroxyfluorone are sensitive and selective reagents for molybdenum. Among these reagents, phenylfluorone (PF) (Feng et al., 1994; Warner et al., 1975), salicylfluorone (SAF) (Shen et al., 1982, 1993), dibromophenylfluorone (DBPF) (Wang et al., 1984), dibromohydroxyphenylfluorone (DBHPF) (Pan et al., 1991), methylfluorone (MF) (Majumdar et al., 1960), *o*-hydroxynaphthylfluorone (o-HNF) (Mo et al., 1990), *p*-fluorphenylfluorone (p-FPF) (Li et al., 1991), and *p*-amminophenylfluorone (p-APF) (Xi et al., 1987) have been used for the spectrophotometric determination of molybdenum in steel, but prior extraction or masking is required; no results for vegetal and tobacco tissues of low amounts of molybdenum have been reported.

One of the derivatives synthesized in our laboratory (Liu et al., 1999), such as dimethoxyhydroxyphenylflurone [2,3,7,-trihydroxy-9-(3,5-dimethoxy-4-hydroxy)-phenylflurone-3, DMHPF], reacts with molybdenum in acidic medium in the presence of Triton X-100 to form a red complex, which gives an absorption peak at 526 nm and can be used to determine molybdenum.

In this paper, a simple method for the spectrophotometric determination of trace amounts of molybdenum is developed. The proposed method offers the advantages of simplicity, rapidity, high sensitivity, and determination of molybdenum without the need for separation or heating.

In sulfuric−phosphoric acid medium, 35000-fold amounts of iron do not interfere with the determination, and the tolerance limits of other foreign ions are improved. This is an advantage over methods using other derivatives of 2,3,7-trihyydroxyflurone. The recommended method has been satisfactorily applied to the spectrophotometric determination of trace amounts of molybdenum in vegetal and tobacco tissues.

MATERIALS AND METHODS

Chemicals. All reagents were of analytical grade, and distilled water was used throughout. Standard solution of molybdenum(VI) was prepared from a stock solution (1 mL = 1.0 mg) of molybdenum(VI), which had been prepared from sodium molybdate dihydrate and standardized gravimetrically with 8-hydroxylquinoline (Vogel, 1968). The working solution (1 mL = 5 μg of Mo) was prepared by appropriate dilution just before use. A sulfuric−phosphoric acid solution was prepared by mixing 24 mL of concentrated sulfuric acid and 80 mL of concentrated phosphoric acid and carefully adding the mixture to 200 mL of water, cooling, and diluting to 500 mL. The Triton

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X-100 solution was a 5% aqueous solution. The DMHPF solution was prepared with 0.04 g of pure DMHPF dissolved in 20 mL of 95% alcohol; 7 mL of sulfuric acid of 3 mol/L was added, and the mixture was then diluted to 100 mL with 95% alcohol.

Instrument. Absorption spectra and absorbances were recorded and measured with a Beckman DU-7 spectrophotometer using a 1.0-cm cell.

General Procedure for the Determination of Mo. To a test solution containing not more than 8 μg of molybdenum in a 25 mL calibrated flask were added the following solutions successively: 2 mL of sulfuric–phosphoric acid solution, 5 mL of Triton X-100, and 2 mL of DMHPF; the mixture was diluted to the mark with water and mixed well. The absorbance was measured at 526 nm with a 1.0-cm cell against the reagent blank.

Determination of Mo in Vegetal Tissues. Three samples of vegetal (carrots, cabbages, and bean) were bought from the local market; their origin was unknown.

The whole bean, carrot (edible parts), and cabbage leaves were air-dried at room temperature. A 300 g portion of each vegetal sample was finely pulverized and dried at 60 °C.

Subsamples of 8.0 g were digested in a muffle furnace at 500 °C during 2 h. The resulting ashes were moistened with distilled water, treated with 3 mL of 1 + 1 hydrochloric acid, and heated at 70 °C on a hotplate. In each instance, the residue was washed with deionized water and filtered by using a common filter. The filtrate was neutralized and diluted to 25 mL. Aliquots of this solution of not more than 6 mL were used for the spectrophotometric determination of Mo.

Determination of Mo in Tobacco Tissues. The dry tobacco samples of 10 g were treated with 15 mL of nitric acid and heated. A mixture (10 mL) of nitric and perchloric acid (2 + 1) was added, and the solution was heated to almost dryness. The residue was dissolved in hot water, the solution was filtered, and filtrate was diluted to 25 mL with deionized water.

RESULTS AND DISCUSSION

Experimental Theory. The vegetal and tobacco solutions were digested by dry or wet method, in which Mo existed with the state of Mo(VI), not Mo^{6+}. The existence of acid medium and the Triton X-100 complex of Mo(VI)–DMHPF was shown by the reaction of Mo(VI) and chromogenic reagent DMHPF, which had the characteristic absorbance at 523 nm.

The experimental data show that the reaction of Mo(VI) and DMHPF was instantly completed in a molar proportion of 1:1. [The molar composition of the Mo–DMHPF complex was ascertained by the mole ratio method (Vogel, 1968) and by Job's method of continuous variations (Schläfer, 1961).] The red complex of Mo(VI)–DMHPF was stable for ~48 h under the experimental conditions (the absorbance change of the complex was <5% in the error range of the software).

In the experiment, it was necessary to digest the sample to release elemental Mo from plant tissues. In the dry method of digestion, the temperature should be controlled to the following: the initial temperature should be low and then incrementally raised to 500 °C and kept at that temperature for ~2 h. The reaction of Mo(VI) and the chromogenic reagent DMHPF should be processed in acid medium and effected by the acidity of the solution. The precipitate readily appeared, which made spectrophotometric determination difficult if the acidity of the solution was high, whereas it was difficult to produce the complex, which lowers the sensitivity of the method, if the acidity of the solution was low. The surfactant acted in a double role of increasing both solubility and sensitivity because adding surfactant in the solution could reduce the precipitation of the complex and increase the absorbance of the complex.

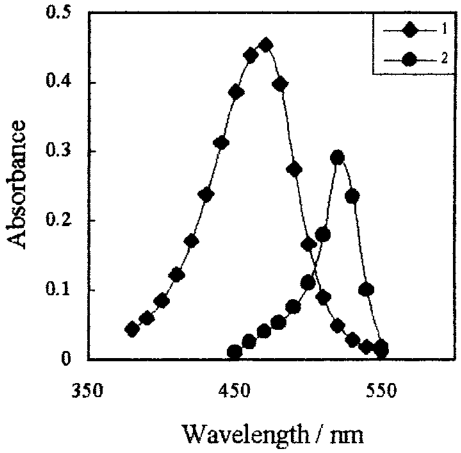

Figure 1. Absorption spectra of DMHPF (against water blank, curve 1) and Mo–DMHPF (complex against reagent blank, [Mo] = 2.5 × 10^{-6} mol/L, curve 2) with Beckman DU-7 spectrophotometer using a 1.0-cm cell.

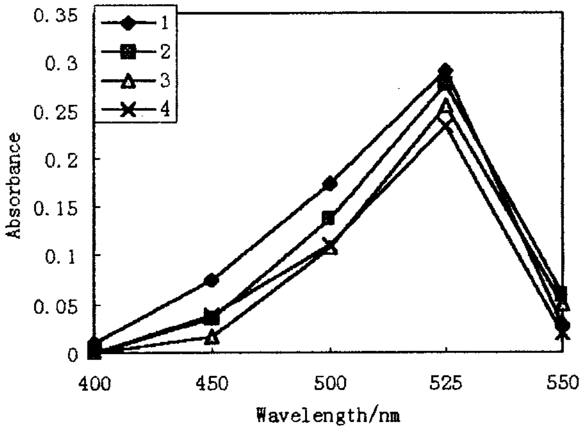

Figure 2. Influence of various acids on the absorbance of the Mo–DMHPF complex: (1) H_2SO_4; (2) HNO_3; (3) H_3PO_4; (4) HCl.

Absorption Spectra of DMHPF and the Complex of Mo—DMHPF. The DMHPF solution is yellow in acid medium and with a maximum absorption wavelength at 465 nm. The solution is red in neutral pH, weak alkali medium, with an absorption maximum at 506 nm. The reagent is stable for months in acid or neutral media.

DMHPF forms a red complex with molybdenum(VI). The complex has an absorption maximum at 526 nm. The spectra of the reagent and the complex are shown in Figure 1.

Optimal Conditions for the Formation of the Complex. Various acids were introduced to examine their effects on the Mo–DMHPF complex formation reaction. It was found that the absorbances of the complex in sulfuric, hydrochloric, nitric, and phosphoric acid media were almost unchanged (see Figure 2). However, the reaction revealed the highest sensitivity in sulfuric acid solution and good selectivity in phosphoric acid solution; therefore, sulfuric–phosphoric acid was chosen as the reaction medium. In 25 mL of solution, the presence of 1.2–2.5 mL of sulfuric–phosphoric acid solution gave a maximum and constant absorbance, and so 2 mL was selected for use in the experiments.

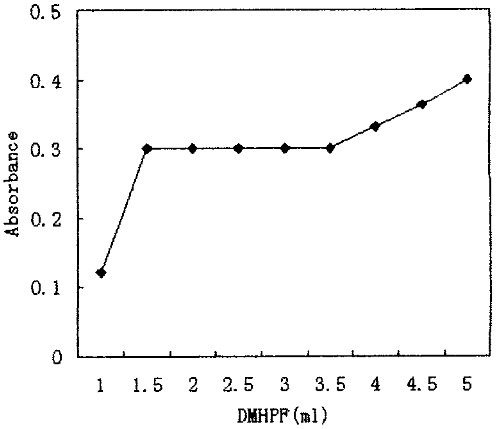

Figure 3. Influence of the DMHPF concentration on the absorbance of the Mo–DMHPF complex at $[H_2SO_4] = 6.9 \times 10^{-2}$mol/L and $[Mo] = 2.5 \times 10^{-6}$mol/L.

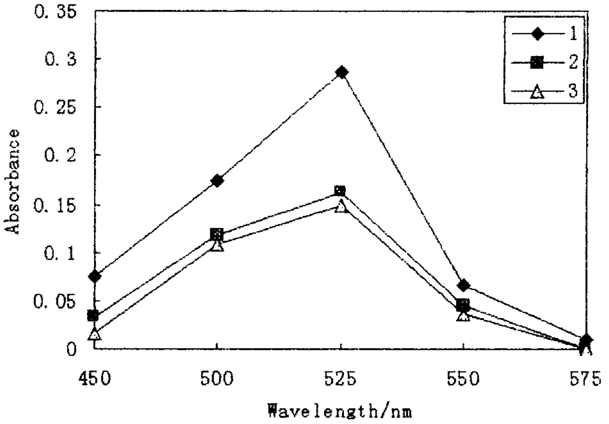

Figure 4. Influence of the various surfactants on the absorbance of the Mo–DMHPF complex at $[Mo] = 2.6 \times 10^{-6}$ mol/L.

The absorbance was maximal and constant using 1.5–3.5 mL of 0.04% DMHPF solution (see Figure 3). Hence, 2.0 mL of reagent was recommended. Color development is complete instantly at room temperature (10–35 °C), and the complex is stable for at least 48 h.

Because a surfactant should be used as a solubilizing agent, various surfactants were tried for this purpose. The experiments showed that cationic surfactants, CTMAB, CPB, and CPC, did not give any enhancement for the formation of the complex, but nonionic surfactants such as Tween 40, Triton X-305, and Triton X-100 gave a remarkable increase in sensitivity of the Mo–DMHPF complex (see Figure 4) and improved its solubility; the latter was found to be the best, and so Triton X-100 was chosen for all subsequent work. The optimum concentration of Triton X-100 ranged from 6×10^{-5} to 1.2×10^{-4} mol/L. Therefore, a concentration of 8×10^{-5} mol/L Triton X-100 is recommended.

The order of addition of the reagents was found to have no effect on the absorbance. Despite this, it is advisable to add the sulfuric–phosphoric acid and Triton X-100 before the DMHPF reagent, to avoid precipitation. However, any precipitate formed upon addition of the DMHPF reagent will redissolve upon addition of the Triton X-100 solution, and there is no adverse effect on the results.

Composition of the Complex. The molar composition of the Mo–DMHPF complex was ascertained by Job's method of continuous variations (Schläfer, 1961) and the mole ratio method (Schläfer, 1961). Both methods proved that the metal-to-ligand ratio was 1:1.

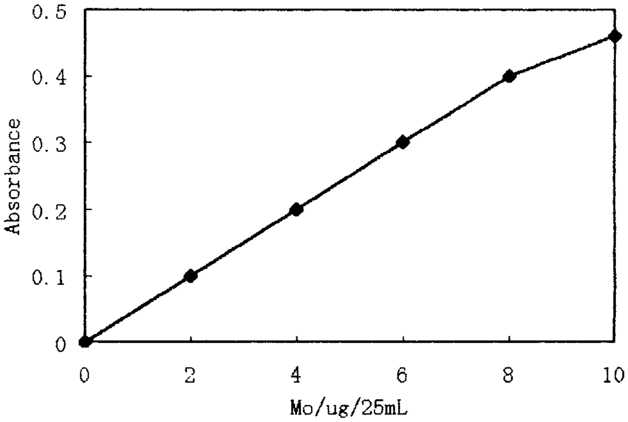

Figure 5. Calibration graph.

Table 1. Effect of Foreign Ions on the Determination of 5.0 μg of Molybdenum

ion	tolerance limit (mg)	ion	tolerance limit (mg)
Al^{3+}	7.00	Ba^{2+}	15.0
Bi^{2+}	0.10	Ca^{2+}	15.0
Cd^{2+}	15.0	Co^{2+}	1.50
Cr^{3+}	0.10	Cu^{2+}	30.0
Fe^{3+}	175	Hg^{2+}	6.00
K^+	55.0	La^{3+}	5.00
Mg^{2+}	15.0	Na^+	50.0
NH_4^+	50.0	Ni^{2+}	10.0
Pb^{2+}	5.00	Sn(IV)	0.05
Th(IV)	0.20	Ti(IV)	0.04
U(IV)	0.50	V(V)	0.50
W(IV)	0.015	Y^{3+}	4.00
Zr(IV)	0.10	Cl^-	150
Br^-	150	F^-	150
PO_4^{3-}	230	SO_4^{2-}	250
tartrate	20.0	citrate	25.0
oxalate	0.50		

Calibration Graph and Sensitivity. The calibration graph was constructed in the usual way according to standard procedure. Linearity range was obeyed in the range of 0–8 μg of molybdenum in 25 mL of solution (see Figure 5). The apparent molar absorptivity was calculated from the slope of the calibration graph to be 1.36×10^5 L mol^{-1} cm^{-1}.

Effects of Interfering Ions. The effects of various cations and anions on the determination of 5 μg of Mo(VI) were examined by a general procedure. As shown in Table 1, most of the ions have no influence, and the tolerance limits of V(V), Ti(IV), W(VI), Zr(IV), and Sn(IV) were improved. Thirty-five thousand-fold amounts of iron do not interfere with the determination of 5 μg of molybdenum, which is an advantage over published methods.

Determination of Mo of Vegetal and Tobacco with This Method. To check the usefulness of the proposed spectrophotometric method, three vegetal and two tobacco samples were analyzed. The results obtained for the determination of Mo are summarized in Table 2. Direct determinations are possible for the Mo contents in all samples; the standard additions method was used to validate the analytical procedure. As can be seen in Table 2, recoveries very close to 100% were obtained for the different samples tested. The proposed method might find use as a rapid determination method for Mo in routine analysis.

Table 2. Determination of Molybdenum in Several Samples

sample	added ($\mu g \cdot g^{-1}$)	found[a] ($\mu g \cdot g^{-1}$)	recovery (%)	RSD[b] (%)
Vegetal Tissues				
carrot		0.564		2.83
	0.50	1.042	95.6	
	1.00	1.550	98.6	
	1.50	2.01	96.4	
cabbage		0.349		3.68
	0.50	0.838	97.8	
	1.00	1.342	99.3	
bean		2.43		1.52
	1.00	3.39	96.0	
	2.00	4.42	99.5	
	2.50	4.95	100.8	
Tobacco[c]				
HenanB$_2$F		1.08		2.68
	1.00	2.03	95.0	
	1.50	2.54	97.3	
	2.00	3.07	99.5	
YunnanC$_3$F		0.84		3.02
	1.00	1.80	96.0	
	1.50	2.32	98.7	
	2.00	2.97	97.5	

[a] Found, mean of three determination. [b] RSD, relative standard deviation (a sample is used in each plant). [c] Tobacco, samples are from Hefei Cigarette Factory Anhui, People's Republic of China.

LITERATURE CITED

Berman, E. *Toxic Metals and Their Analysis*; Heyden: London, U.K., 1980; pp 161−191.

Feng, Y. L. Spectrophotometric determination of the molybdenum with the phenylfluorone. *Lihua Jianyan (Phys. Testing Chem. Anal. China, Part B: Chem. Anal.)* **1994**, *30* (3), 168 (in Chinese).

Gowda, A. T.; Gowda, N. M. M. Extraction spectrophotometric determination of trace amount of molybdenum using thiocyanate and ethylisobutrazine hydrochloride. *Analyst* **1985**, *110*, 743−745.

Johnson, C. M.; Arkley, T. H. Determination of molybdenum in plant tissue. *Anal. Chem.* **1954**, *26*, 572−574.

Li, X. Y.; Zhang, H. S.; Yao, J. Synthesis and analytical application of the *p*-fluorophenylfluorone—Spectrophotometric determination of molybdenum in the steels. *Yeijing Fenxi (Metallurg. Anal. China)* **1991**, *11* (3), 9 (in Chinese).

Liu, S. M.; Pan, J. M. Study on the simultameous spectrophotometric determination of aluminum and iron by dual-wavelength method with new chromogenic reagent dimethoxyhydroxyphenylfluorone. *J. Anal. Sci. China* **1999**, *15* (4), 68−72 (in Chinese).

Lopez Ritas, J.; Alopez Melida, J. *El Diagnostico de Suelosy Plantas*; Mundi-Prensa: Madrid, Spain, 1984; p 252.

Majumdar, A. K.; Savarial, C. P. Spectrophotometric determination of molybdenum with 9-methyl-2,3,7-trihydroxy-6-fluorene. *Anal. Chem. Acta* **1960**, *22*, 158−161.

Mitra, M.; Mitra, B. K. Liquid−liquid extraction of molybdenum thiocyanate with hexamethyl phosphortriamide and direct spectrophotometric determination in the organic phase. *Talanta* **1977**, *24*, 698−699.

Mo, W. M.; Li, S. Y.; Yu, X. M. Spectrophotometric determination of microamount molybdenum with o-hydroxynaphthyl fluorine and cetyltrimethylammonium bromide. *Yeijing Fenxi (Metallurg. Anal. China)* **1990**, *10* (2), 5 (in Chinese).

Pan, J. M.; Tian, W.; Yian, H. T.; Muo, W. M. Spectrophotometric determination trace amounts of molybdenum in steel with 2,3,7-trihydroxy-9-dibromo-hydroxyphenylfluorene (DBH-PF). *Lihua Jianyan (Phys. Testing Chem. Anal. China, Part B: Chem. Anal.)* **1991**, *27* (2), 70−71, 74 (in Chinese).

Patil, S. P.; Shinde, V. M. Solvent extraction and spectrophotometric determination of molybdenum in iron. *Anal. Chim. Acta* **1973**, *67*, 473.

Salinas, F.; Berzas, J. J.; Acedo, M. I. Extractive-spectrophotometric determination of molybdenum as ion-association complex with thiocyanate and adogen. *Talanta* **1985**, *32*, 63−65.

Sandell, E. B.; Onishi, H. *Photometric Determination of Trace of Metals*, 4th ed.; Wiley: New York, 1978; Part I.

Schläfer, H. L. *Kemplexbilolung in Lösung*; Springer: Berlin, Germany, 1961; pp 233, 253.

Shen, H. X.; Wuang, Z. Q. A study on the colour reaction of molybdenum with salicylfluorene and cetyl trimethylammonium bromide. *Gaodeng Xuexiao Huaxue Xuebo (Chem. J. Chinese Univ.)* **1982**, *3* (3), 300−304 (in Chinese).

Shen, H. X.; Liu, Y. G.; Wuang, Y. X.; Liu, L. Z. Flow injection spectrophotometric determination of micro molybdenum by using of salicylfluorone. *Yeijing Fenxi (Metallurg. Anal. China)* **1993**, *13* (5), 1 (in Chinese).

Ssekaalo, H. A rapid and accurate method for the determination of molybdenum in plant materials with toluene-3,4-dithiol. *Analyst* **1971**, *96*, 346−348.

Vogel, A. I. *Quantitative Inorganic Analysis*, 3rd ed.; Longmans: London, U.K., 1968; p 506.

Wang, D. J.; Bai, J. Y.; Chen, J. H.; Pan, Q. H. Spectrophotometric determination of in alloy steels and ores with 4,5-dibromophenylfluorene and cetyltrimethylammonium bromide. *Fenxi Huaxue (Anal. Chem. China)* **1984** , 12 (2), 140−142 (in Chinese).

Warner, D. A.; Paul, J. Simultaneous spectrophotometric determination of molybdenum and selenium, and of molybdenum and tellurium. *Microchem. J.* **1975**, *20*, 292−298.

Xi, G. Q.; Wang, F. K.; Liang, D. X. Study on the Mo−PAF−CTMAB system for the determination of molybdenum. *Yeijing Fenxi (Metallurg. Anal. China)* **1987**, *7* (6), 16 (in Chinese).

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