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Synthesis of 2,3,7-trihydroxy-9-(3,4,5-trimethoxy)-phenylflurone-3 and Its Application to Spectrofluorescence Quenching Determination of Microamounts of Molybdenum in Tobacco

YUEQIN XIE†, XIANGYANG YAN, HONGWU TONG and SHAOMIN LIU*
Research Center of Tobacco and Health, University of Science & Technology of China
Hefei-230052, P.R. China
E-mail: liusm@ustc.edu.cn

A new fluorescence reagent, namely 2,3,7-trihydroxy-9-(3,4,5-trimethoxy)phenylflurone-3 was synthesized and sensitive method for the spectrofluorescence quenching determination of molybdenum with the fluorescence reagent, in presence of cetyltrimethyl ammonium bromide, is described. In H_3PO_4 medium, the fluorescence intensity of trimethoxyphenylfluorone is quenched due to formation of complex of molybdenum and trimethoxyphenylfluorone. The detection limit is 1.5 ng mL^{-1} and linear range is between 2-80 ng mL^{-1}. The proposed method has been applied to the determination of Mo in tobacco.

Key Words: Spectrofluorescence, Molybdenum, Tobacco, 2,3,7-Trihydroxy-9-(3,4,5-trimethoxy)phenylflurone-3.

INTRODUCTION

Among the methods described for the determination of molybdenum, the most commonly used methods are based, with different modification, on measuring the absorbance of the molybdenum-thiocyanate complex[1-3]. Great sensitivity is achieved when spectrofluorimetric methods based on use of carminic acid[4], on 8-hydroxyquinoline (in the presence of peroxide and tetraphenylborate)[5] and on morin[6] and when fluorescence-quenching methods based use of Rhodamine B plus thiocyanate[7] and 4,7-diphenyl-1,10-phenanthroline[8] are applied.

Derivatives of 2,3,7-trihydroxy fluorone reagents have been reported as a significant branch of organic chromogenic reagent which have high sensitivity, good selectivity and easy synthesis. Many researchers investigated the determination of molybdenum[9-19]. To continue improving the sensitivity and selectivity of this kind of reagent and its fluorescence analytical characteristics of the reagents, a new reagent, namely 2,3,7-trihydroxy-9-(3,4,5-trimethoxy)phenylflurone-3 (TMPF), was synthesized

†Department of Chemistry, Anhui Teachers College, Hefei-230061, P.R. China.

successfully in our laboratory. The reagent show a high fluorescence and the fluorescence of the reagent is quenched due to reaction with molybdenum in acidic medium in the presence of cetyltrimethylammonium bromide (CTMAB). In this paper, the TMPF has been used as a new spectrofluorescence-quenching reagent for estimation of Mo(VI) is reported. A sensitive spectrofluorescence-quenching method for the determination of molybdenum is proposed. In H_3PO_4 medium, Mo (VI) and TMPF react to form a complex, Its constituent is established as Mo (VI): TMPF = 1:2. Its maximum excitation wavelength is 488 nm and the maximum emission wavelength is 520 nm. In phosphoric acid medium, 6000-fold amounts of iron, 6000-fild amounts of aluminum and 1000-fold amounts of manganese do not interfere with the determination and the tolerance limits of other foreign ions are improved. The method has been satisfactorily applied to the determination of molybdenum in tobacco.

EXPERIMENTAL

Fluorescence measurements were performed with a 970-MC fluorescence spectrophotometer (Shanghai Third Analytical Instruments Factory, P.R. China) with standard 10 mm path length silica. Wavelengths used were 488 and 520 nm for fluorescence excitation and emission, respectively. A model PHS-2 pH meter (Shanghai Second Analytical Instruments Factory, P.R. China) was used for pH measurement.

Elemental analyses were performed on a Carlo Erba 1106 instrument. Infarared spectra were recorded on a Nicolet 60SXR spectrometer.

A standard stock solution of molybdenum (1.0 mg mL^{-1}) was prepared from sodium molybdenum dihydrate and standardized gravimetrically with 8-hydroxylquinoline. The working solution (1 μg mL^{-1}) was prepared by dilution with deionized water just before use.

The TMPF working solution (0.001 %) was prepared by dissolving 0.001 g of TMPF in 20 mL of 95 % alcohol and 1 mL of sulphuric acid of 1 mol L^{-1} and then diluted to 100 mL with 95 % alcohol. An aqueous solution of phosphoric acid of 0.1 mol L^{-1} concentration and 0.2 % aqueous solution of CTMAB were prepared. All other reagents used were of analytical grade and deionized water was used to make up the solution.

Benzotriphenolester [$(CH_3COO)_3C_6H_3$, 8.4 g] was dissolved in the solution containing 50 mL 95 % alcohol and 25 mL water, cooled to room temperature. 3.5 mL of concentrated sulphuric acid was added slowly with stirring, then 3.0 g 3, 4,5-trimethoxybenzaldehyde was dissolved in the above solution with heat. The mixture was allowed to stand for 10 d. The red-brown precipitate formed was filtered off and washed with water several times, and dried at 70-80ºC. The yield was about 52%. Elemental analysis: Calculation for $C_{22}H_{18}O_8$, C, 64.3 %; H, 4.40 %. Found: C, 64.12%;

H, 4.45%. IR (KBr, cm^{-1}): 1582,1583,1467,1414 (Benz. ring); 1642 ν(C=O); 1392, 1298 ν(OH); 1237, 1205,1127,999 ν(C-H).

Dissolution of samples: The dry tobacco samples of 10 g were treated with 15mL 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.

General procedure for the determination of Mo (VI): To a test solution containing *ca.* 2 μg of molybdenum in a 25 mL calibrated flask were added the following solution successively: 1 mL of phosphoric acid solution, 4.5 mL of TMPF solution, 1.5 mL of CTMAB solution and 1 mL of 0.2 % EDTA solution. The mixture solution diluted to the mark with deionized water and mixed well. The fluorescence of this solution was detected and was compared with that of an equivalent TMPF solution in blank solution, then a quenching factor calculated ($\Delta F = F_{TMPF}-F_{Mo(VI)-TMPF}$). This factor was applied to all subsequent samples of the same tobacco.

RESULTS AND DISCUSSION

Physical and chemical properties of the reagent: 2,3,7-Trihydroxy-9-(3,4,5-trimethoxy)phenyflurone-3 (TMPF) is a red-brown solid. It is soluble in alcohol, acetone and chloroform. TMPF solution is yellow in acid medium and with a maxnum absorption wavelength at 468 nm. The solution is red in neutral pH and weak alkaline medium, with maximum absorption wavelength at 502 nm. The reagent is stable for months in acid or neutral media. TMPF has fluorescence in acid medium and the fluorescence remains for at least 4 h. It exhibits fluorescence excitation and emission maxima at 488 and 520 nm, respectively.

Effect of acidity and media: Various acids were introduced to examine their effects on the fluorescence quenching. It was found that the ΔF of Mo-TMPF in sulphuric, hydrochloric and phosphoric acid media was almost unchanged. However, the fluorescence quenching revealed the highest sensitivity in phosphoric acid solution, therefore, phosphoric acid was chosen as the reaction medium. In 25 mL of solution, the presence of 0.5-3.0 mL of phosphoric acid solution gave a maximum and constant fluorescence quenching intensity. 1 mL of phosphoric acid solution was selected use in the experiments.

Effect of surfactants: Generally speaking, introduction of surfactant can improve sensitivity and/or selectivity the fluorescence method. Here, effect of surfactant was tested various surfactant were tried for this purpose. The experiments showed that the nonionic surfactants such as Tween-40, Triton X-305, Triton X-100, PVC and Tween-80 did not give maximum enhancement for measurement signal. The use of cationic

surfactants such as TCMAB, CPB and CPC markedly effects the fluorescence quenching intensity. But CTMAB was found to be the best and was chosen for all subsequent works. The optimum concentration of CTMAB ranged 1.1×10^{-4} to 4.4×10^{-4} mol L^{-1}; a concentration of 3.3×10^{-4} mol L^{-1} is recommended.

Influence of reagent of TMPF concentration: The effect of reagent concentration on the fluorescence quenching intensity for solution containing 1.92×10^{-6} mol L^{-1} of TMPF was studied. The intensity of fluorescence quenching signal increased with increasing TMPF concentration to 3.36×10^{-6} mol L^{-1}, then remained constant between 3.36×10^{-6} mol L^{-1} and 4.80×10^{-6} mol L^{-1} and decreased slowly thereafter.

A 4.32×10^{-6} mol L^{-1} TMPF concentration was selected to ensure a sufficient excess of the reagent throughout experimental work.

The stoichiometry of complex was studied under the established conditions by the Yoe & Jones and Job methods. From this study, it was concluded that the composition of the complex was 1 : 2 (Mo : TMPF).

Stability, effect of order of addition of reagents: The fluorescence quenching intensity of the complex reached maximum in a few seconds and remained constant for 4 h.

The order in which reagent surfactant, metal ion and acid solution were mixed had no influence the fluorescence quenching intensity, thus, the other metal ion, acid solution, reagent and surfactant was chosen for remaining of the experimental work.

Analytical characteristics: Calibration graphs with three replicates for each concentration value including, the blank as an additional value to calculate the regression-line equation[20] were established in the ranges 2-80 ng mL^{-1} ($r = 0.9956$). The precision, expressed by relative standard deviation calculated from the date set of the calibration experiment is 0.629 % for 20 ng mL^{-1} Mo(VI) and 1.02 % for 60 ng mL^{-1} Mo(VI).

Interferences: The effect of several ions on the fluorescence intensity of the Mo-TMPF complex was investigated. In the determination of 60 ng mL^{-1} of molybdenum, foreign ions can be tolerated at the levels reported in Table-1.

Application

To check the usefulness of the proposed spectrofluori-metic method, four tobacco samples were analyzed. The results obtained for the determination of molybdenum are summarized in Table-2. The standard additions method was used to validate the analytical procedure. To detect any losses of Mo(VI), known amounts were added before the sample treatment. As can be seen in Table-2, recoveries very close to 100 % were obtained for the different sample tested.

TABLE-1
TOLERANCE LIMITS FOR FOREIGN IONS IN THE DETERMINATION OF 60 ng mL^{-1} OF MOLYBDENUM

Ion	Tolerance limit [Ratio of ion to Mo(VI)]	Ion	Tolerance limit [Ratio of ion to Mo(VI)]
Li^+	20000	Co^{2+}	20000
Na^+	20000	EDTA	50000
K^+	20000	NO_3^-	10000
Ca^{2+}	20000	SO_4^{2-}	12000
Sr^{2+}	10000	I^-	10000
Mn^{2+}	1000	Br^-	10000
Zn^{2+}	1500	Cl^-	10000
Ni^{2+}	1500	Cu^{2+}	2000
Cd^{2+}	1000	Fe^{3+}	6000
Mg^{2+}	10000	Al^{3+}	6000
Cr(VI)*	150	W(VI)*	10
Ba^{2+}	10000	V(V)*	30

*Masked with 1 mL EDTA (0.2 %).

TABLE-2
DETERMINATION OF Mo IN SEVERAL SAMPLES

Samples[a]	AAS (μg g^{-1})	Added (μg)	Found[b] (μg g^{-1})	Recovery (%)
Yu xi C_2F	1.54	-	1.51	-
	-	1.0	2.53	102.0
	-	2.0	3.47	98.0
Yu xi C_3F	1.07	-	1.11	-
	-	1.0	2.08	97.0
	-	1.5	2.66	103.0
	-	2.0	3.10	99.5
Li jiang C_1F	1.86	-	1.85	-
	-	0.5	2.35	100.0
	-	1.0	2.82	97
	-	1.5	3.32	98
Yun nan C_3F	0.88	-	0.84	-
	-	1.0	1.80	96
	-	1.5	2.32	98.7
	-	2.0	2.97	97.5

[a]Tobacco sample are from Hefei Cigarette Factory Anhui, P.R. China
[b]Mean value of six determination

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