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A Simple Spectrophotometric Method for the Determination of Molybdenum in Industrial, Environmental, Biological and Soil Samples Using, 2 -Hydroxyacetophenonebenzoylhydrazone.

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Abstract

A very simple, ultra-sensitive and highly selective non-extractive spectrophotometric method for the determination of trace amounts of molybdenum(VI) using 2-hydroxyacetophenonebenzoylhydrazone (HAPBH) has been developed. 2-hydroxyacetophenonebenzoylhydrazone reacts in acidic (0.03-1 M H_2SO_4) and 30% 1, 4-dioxane media with molybdenum(VI) to give a redish-yellow chelate which has an absorption maxima at 443 nm. The average molar absorption coefficient and Sandell's sensitivity were found to be 2.59×10^4 L mol^{-1} cm^{-1} and 15 ng cm^{-2} of Mo^{VI}, respectively. Linear calibration graphs were obtained for 0.1-30 mgL^{-1}. The stoichiometric composition of the chelate is 1:2 (Mo:HAPBH). The absorbance intensity of the metal chelate reaches to a constant value within few seconds and remains unchanged for more than 48 hours. Large excess of over 60 cations, anions and complexing agents (like tartrate, oxalate, chloride, phosphate, EDTA, ascorbic acid, bromide, citrate, etc.) do not interfere in the molybdenum determination. The developed method was successfully used for assaying several standard reference materials (alloys and steels), environmental waters (inland and surface), biological samples (human blood and urine), soil samples, solutions containing both molybdenum(V) and molybdenum(VI) and complex synthetic mixtures. The method has high precision and accuracy (s = ±0.01 for 0.5 mgL^{-1}).

Introduction

Molybdenum in trace amounts is industrially important [1], as a: biological nutrient [2], toxicant [3], environmental pollutant [4] and occupational health hazard [5]. The industrial uses of this metal includes the manufacture of high temperature resistant engines and production of catalysts, lubricants and dyes. Molybdenum is a bio-essential element and its occurrence in water and soil samples is a matter of interest from both environmental and chemical points of view [2]. It is an essential metal as a cofactor for the enzymes xanthine oxidises and aldehyde oxidizes. In plants it is necessary for fixing of atmospheric nitrogen by bacteria during protein synthesis. Molybdenum is added in trace amounts to fertilizers to stimulate the plant growth. On the other hand excess exposure can result in toxicity to animals and humans [6]. Molybdenum poisoning causes severe gastrointestinal irritation with diarrhea, coma, ruminants and death from cardiac failure [6]. Pastures containing 20 to 1000 ppm molybdenum may produce a disease referred to as "teart" in cattle and sheep. All of these findings cause a great concern regarding public health, demanding an accurate determination of this metal at trace and ultra-trace levels.

Very low concentrations of molybdenum can found in plants, soils, natural and seawater. Therefore, it is important from an analytical point of view to find sensitive methods for its determination. Molybdenum can be determined by

atomic absorption spectrophotometry using either flame [7] or electro thermal atomization [8] as well as by plasma emission spectrometry [9]. Both methods have certain disadvantages in terms of cost and lack of sensitivity, and are affected by the matrix conditions of samples, such as salinity [10]. Catalytic solvent extractive methods are highly sensitive and less expensive, but generally lack simplicity [10].

The aim of this study was to develop a simpler direct spectrophotometric method for the trace determination of molybdenum. 2-hydroxyacetophenonebenzoylhydrazone (HAPBH) has not previously been used for the spectrophotometric determination of metals. This paper reports on its use in a very sensitive, highly specific spectrophotometric method for the trace determination of molybdenum. The method possesses distinct advantages over existing methods [11-19] with respect to sensitivity, selectivity, range of determination, simplicity, speed, pH/acidity range and ease of operation. The method is based on the reaction of HAPBH in acidic solution (0.03-1 M H_2SO_4) with molybdenum (VI) to produce a highly absorbent redish-yellow chelate product, followed by the measurement of absorbance in aqueous medium. With suitable masking the reaction can be made highly selective and the reagent blank solution does not show any absorbance.

Experimental

Apparatus

A Shimadzu (Kyoto, Japan) (Model-160) double-beam UV-VIS spectrophotometer and a Jenway (England, UK) (Model-3010) pH meter with combined electrodes were used for measurements of absorbance and pH, respectively. A Polarized Zeeman (Model-Z-5000) atomic absorption spectrometer equipped with a computer controlled nitrous oxide-acetylene flame was used to compare the results.

Reagents and Solutions

All chemicals used were of analytical-reagent grade or the highest purity available. Doubly distilled de-ionized water and HPLC-grade 1,4-dioxane, which is non-absorbent under ultraviolet radiation, were used throughout the studies. Solutions of a large number of inorganic ions and complexing agents were prepared from their AnalaR grade or equivalent grade water-soluble salts or from the oxides and carbonates in hydrochloric acid, according to the recommended procedures of Mukharjee [20]. Glass vessels were cleaned by soaking in acidified solutions of $KMnO_4$, followed by washing with concentrated HNO_3, and were rinsed several times with high-purity deionized water. Stock solutions and environmental water samples (1000 mL each) were kept in polypropylene bottles containing 1mL of concentrated HNO_3. More rigorous contamination control was used when the molybdenum levels in specimens were very low.

The reagent 2-hydroxyacetophenonebenzoylhydrazone (HAPBH) was synthesized according to the method of Ahmed *et al*. [21] and recrystallized from ethanol. The stock solution (3.94×10^{-3}M) was prepared by dissolving the requisite amount of HAPBH in a known volume of 1,4-dioxane, no appreciable absorbance was observed within seven days. More dilute solutions of the reagent were prepared as required. A 100 mL amount of stock solution (1 mg mL^{-1}) of hexavalent molybdenum was prepared by dissolving 184.0 mg of purified-grade (Merck pro-analysis grade) ammonium molybdate tetrahydrate $(NH_4)_6Mo_7O_{24}.4H_2O$ in doubly distilled de-ionized water, and subsequently standardized gravimetrically by the 8-quinolinol [22]. A 100 mL amount of stock solution (1 mg mL^{-1}) of pentavalent molybdenum was prepared by dissolving 284.7 mg of molybdenum (V) chloride (Aldrich A.C.S. grade) in doubly distilled deionized water containing 1-2 mL of nitric acid (1+1). More dilute standard solutions were prepared by appropriate dilution of aliquots from the stock solution with deionized water as and when required.

Procedure

A volume of 0.1-1.0 mL of a neutral aqueous solution containing 1-300 μg of molybdenum(VI) in a 10 mL calibrated flask was mixed with a 1:300-1:1000 fold molar excess of the HAPBH reagent solution (preferably 2.0 mL of 3.94×10^{-3} M) followed by the addition of 0.03-1.0 mL (preferably 0.5 mL) of 1 M sulfuric acid. After

a few seconds, 3 mL of 1,4-dioxane was added and the mixture was diluted to the mark with de-ionized water. The absorbance was measured at 443 nm against a corresponding reagent blank. The Molybdenum content in an unknown sample was determined using a concurrently prepared calibration graph.

Determination of molybdenum in synthetic mixtures

Several synthetic mixtures of varying compositions containing molybdenum and diverse ions of known concentrations were determined by the present method using tartrate as masking agent and the results were found to be highly reproducible. The results are shown in Table 3. Accurate recoveries were achieved in all solutions.

Determination of molybdenum in brass, alloys and steels (Certified reference materials)

A 0.1-g amount of a brass or alloy or steel sample containing 4.95-0.040% of molybdenum was weighed accurately and placed in a 50 mL Erlenmeyer flask following a method recommended by Parker *et al*. [22]. To it, 10 mL of concentrated HNO_3 and 1 mL of concentrated H_2SO_4 was added, carefully covering the flask with a watch glass until the brisk reaction subsided. The solution was heated and simmered gently after the addition of 5 mL of concentrated HNO_3 until all carbides were decomposed. The solution was evaporated carefully to drive off the oxides of nitrogen and then cooled to room temperature (25 + 5)°C. After suitable dilution with de-ionized water, the contents of the flask were warmed to dissolve the soluble salts. The solution was then cooled and neutralized with a dilute NH_4OH solution. The resulting solution was filtered, if necessary, through a Whatman No. 40 filter paper into a 25-mL calibrated flask. The residue was washed with a small volume of hot (1 + 99) H_2SO_4, followed by water and the volume was made up to the mark with de-ionized water.

A suitable aliquot (1-2 mL) of the above solution was taken into a 10-mL calibrated flask and the molybdenum content was determined as described under procedure, using tartrate or EDTA as masking agent. The proposed procedure for the spectrophotometric determination of molybdenum was applied to the analysis of seawater (NASS-2) and river water (SLRS-1). CRMs obtained from the National Research Council of Canada using tartrate or EDTA as masking agent following a method recommended by Sun *et al*.[23]. Based on five replicate analysis, the average molybdenum concentration determined by the spectrophotometric method was in close agreement with the certified values shown in Table 4.

Determination of molybdenum in environmental waters

Each filtered (through Whatman No. 40) environmental water sample (1000 mL) evaporated nearly to dryness with a mixture of 3 mL concentrated H_2SO_4 and 10 mL of concentrated HNO_3 in a fume cupboard, following the method recommended by Greenberg *et al*.[24]. The residue was heated with 10 mL of de-ionized water in order to dissolve the salts. The solution was then cooled and neutralized with dilute NH_4OH solution in the presence of 1–2 mL of 0.01 % (w/v) tartrate or EDTA solution. The resulting solution was then filtered and quantitatively transferred into a 25-mL calibrated flask and made up to the mark with de-ionized water.

An aliquot (1-2 mL) of this preconcentrated water sample was transferred into a 10-mL calibrated flask and the molybdenum content was determined as described under the Procedure, using tartrate or chloride as masking agent. The analyses of environmental water samples from various sources for molybdenum is highly reproducible.

Determination of molybdenum in biological samples

Human blood (2-5 mL) or urine (20-30 mL) was collected in polyethane bottles from the affected persons. Immediately after collection, they were stored in a salt-ice mixture and later were kept at -20°C. The samples were taken into a 100 mL micro-Kjeldahl flask. A glass bead and 10 mL of concentrated nitric acid were added and the flask was placed on the digester under gentle heating. When the initial brisk reaction was over, the solution was removed and cooled following a

method recommended by Stahr [25]. One mL volume of concentrated sulfuric acid was added carefully, followed by the addition of 0.5 mL of 70% $HClO_4$, and heating was continued to dense white fumes, while repeating HNO_3 addition if necessary. Heating was continued for at least half-an hour and then cooled. The content of the flask was filtered and neutralized with dilute ammonia in the presence of 1-2 mL of a 0.01 % (w/v) tartrate or EDTA solution. The resultant solution was then transferred quantitatively into a 10-mL calibrated flask and made up to the mark with de-ionized water.

A suitable aliquot (1-2 mL) of the final solution was transferred into a 10-mL calibrated flask and the molybdenum content was determined as described under the procedure using tartrate or EDTA as masking agent.

Determination of molybdenum in biological samples by AAS

The absorption measurements were made with a Polarized Zeeman model –Z 5000 atomic absorption spectrophotometer, equipped with a hollow cathode lamp for molybdenum as well as a deuterium lamp for background correction, under the following conditions: wavelength: 313.3nm; slit width: 0.5nm; lamp current: 10 μA; flame: air/acetylene. The sample was digested and measured following the method recommended by Khan et al [7]. A suitable aliquot of the final biological sample solution was aspirated into an air-acetylene flame and the absorbance was measured against a similarly prepared reagent blank. The molybdenum concentration was calculated in mg L^{-1} or μg L^{-1} with the aid of a calibration graph. The results of biological analyses by the spectrophotometric method were found to be in excellent agreement with those obtained by AAS. The results are shown in Table 6.

Determination of molybdenum in soil samples

An air-dried homogenized soil sample (100 g) was weighed accurately and placed in a 100-mL micro-Kjeldahl flask. The sample was digested in the presence of an oxidizing agent ($KMnO_4$), following the method recommended by Hesse [27]. The contents of the flask were filtered through a Whatman No.40 filter paper into a 25-mL calibrated flask and neutralized with dilute NH_4OH solution in the presence of 1-2 mL of a 0.01 % (w/v) Na- tartrate or EDTA solution. It was then diluted up to the mark with de-ionized water.

Suitable aliquots (1-2 mL) were transferred into a 10-mL calibrated flask and a calculated amount of 0.05 M H_2SO_4 required to give a final acidity of 0.03-1.0 M H_2SO_4 was added, followed by 1-2 mL of 0.01% (w/v) Na-tartrate or EDTA solution as masking agent. The molybdenum content was then determined by the above procedure. The results are shown in Table 7.

Determination of molybdenum(V) and molybdenum(VI) in mixtures

Suitable aliquots (1-2 mL) of molybdenum (V) and molybdenum(VI) mixtures (preferably 1:1, 1:5, 1:10) were taken in a 25-mL conical flask. A few drops of 1 M sulfuric acid and 1-2 mL of 1% (w/v) potassium permanganate solution were added to oxidize the pentavalent molybdenum. A 5 mL volume of water was added to the mixtures, which were then heated on a steam bath for 10-15 min, with occasional gentle shaking, and then cooled to room temperature. Then, 3-4 drops of a freshly prepared sodium azide solution (2.5% w/v) was added and heated gently with the further addition of 2-3 mL of water, if necessary, for 5 min to drive off the azide. The contents were cooled to room temperature. The reaction mixture was neutralized with dilute NH_4OH and transferred quantitatively into a 10-mL volumetric flask. Then the total molybdenum (V/+/VI) content was determined according to the general procedure with the help of the calibration graph.

An equal aliquot of the above molybdenum (V/+/VI) mixture was taken into a 25-mL beaker. One mL of 0.01%(w/v) tatrate was added to mask molybdenum (V) and neutralize with dilute NH_4OH. After, the contents were transferred into a 10-mL volumetric flask and molybdenum(VI) content was determined according to the general procedure. The molybdenum(VI) concentration was calculated in μg L^{-1} or mg L^{-1} with the aid of a calibration graph. This gives a measure of molybdenum originally

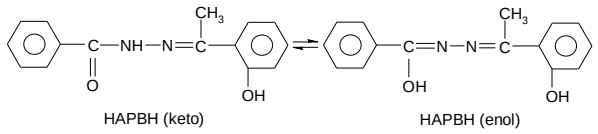
present in the mixture. This value was subtracted from that of the total molybdenum content to determine the molybdenum(V) concentration. The results of a set of determination are given in Table 8.

Results and Discussion

Factors Affecting the Absorbance

Absorption spectra

The absorption spectra of the Mo(VI)-HAPBH system in 1 M sulfuric acid medium was recorded in the range 200-800nm. The absorption spectra of the Mo(VI)-HAPBH is a symmetric curve showing the maximum absorbance at 443 nm and the average molar absorption coefficient of 2.59×10^4 L $mol^{-1}cm^{-1}$ is shown in Fig.1. The reagent HAPBH did not show any noticeable absorbance at the wave-length measured. Throughout the studies measurements were made at 443 nm against a reagent blank. The reaction mechanism of the present method is as reported earlier [21].



2-hydroxyacetophenonebenzoylhydrazone (HAPBH).

Effect of solvent

Because HAPBH is partially soluble in water, an organic solvent was used for the system. Of the various solvents (benzene, chloroform, acetone, carbon tetrachloride, nitrobenzene, isobutyl alcohol, 1-butanol, isobutyl methyl ketone, ethanol and 1, 4-dioxane) studied, 1, 4-dioxane was found to be the best solvent for the system. Different volumes (0 - 8mL) of 1,4-dioxane were added to fixed metal ion concentration, and the absorbance was measured according to the general procedure. It was observed that at 1 mgL^{-1} Mo-chelate metal, 2-6 mL of 1, 4-dioxane produced a constant absorbance of the Mo–chelate. For all subsequent measurements, 3 mL of 1,4-dioxane was added.

Effect of acidity

Among the various acids (nitric, hydrochloric, sulfuric and phosphoric) studied, sulfuric acid was found to be the best acid for the system. Thus the maximum absorbance was found by using 0.01-2.5 mL of 1M sulfuric acid. The absorbance was at a maximum and constant when the 10 mL of solution (1 mg L^{-1}) contained 0.03-1.0 mL of 1M sulfuric acid at room temperature (25 ± 5°C). Outside this range of acidity, the absorbance decreased (Fig. 2). For all subsequent measurements, 0.5 mL of 1 M sulfuric acid was added.

Effect of temperature

The effect of different temperatures on absorbance was studied. The molybdenum(VI)-HAPBH system attained maximum and constant absorbance at room temperature (25 ± 5°C).

Effect of time

The reaction is very fast. Constant maximum absorbance was obtained within few seconds after the dilution to volume and remained strictly constant for over 48 h; a longer period of time was not studied.

Effect of reagent concentration

Different molar excesses (1:5 – 1:1000) of HAPBH were added to a fixed metal ion concentration and absorbencies were measured according to the standard procedure. It was observed that at 1 mg L^{-1} Mo^{VI} metal, the reagent molar ratios of 1:5 to 1:1000 was studied but of 1:300-1:1000 produced a constant absorbance of the Mo-chelate (Fig. 3). Greater excesses of reagent were not studied. For all subsequent measurements, 2 mL of 3.94×10^{-3} M HAPBH reagent was added.

Calibration graph (Beer's law and sensitivity:

The well-known equation for spectrophotometric analysis in very dilute solutions derived from Beer's law. The effect of metal concentration was studied over 0.01-0.1 mg L^{-1}, 0.1- 1.0 mg L^{-1}, 1.0-10.0 mg L^{-1} and 10.0-100 mg L^{-1} for convenience of measurement. The absorbance was linear for 0.1-30 mg L^{-1} of molybdenum at 443 nm. The average molar absorption coefficient (studied range 1.12 - 4.06×10^4 L $mol^{-1}cm^{-1}$) and Sandell's

sensitivity[33] were found to be 2.59×10^4 L $mol^{-1}cm^{-1}$ and 15 ng cm^{-2} of Mo^{VI}, respectively. Of the three calibration graphs, the one showing the limit of the linearity range is shown in Fig. 1; the next two were straight-line graphs passing through the origin (R^2= 0.9998). The selected analytical parameters obtained with the optimization experiments are summarized in Table-1.

Table 1. Selected analytical parameters obtained with the optimization experiments

Parameters	Studied range	Selected value
Wavelength / λ_{max} (nm)	200 - 800	443
Acidity H_2SO_4 / M	0.01-2.5	0.03 - 1.0 (preferably 0.5)
Time/h	0 – 48	1min – 48h (preferably 5min)
Temperature/°C	25 ± 5	25 ± 5
Reagent (fold molar excess, M:R)	1:5 -1: 1000	1:300 - 1:1000 (preferably 1:530)
Molar absorption Co-efficient / L mol^{-1} cm^{-1}	1.12×10^4 – 4.16×10^4	2.59×10^4
Linear range/mg L^{-1}	0.01-100	0.1 – 30
Detection limit/μg L^{-1}	1 – 100	10
Reproducibility (% RSD)	0 – 2	0 – 2
Solvent / mL	0.0 – 8.0	2.0 - 6.0 (preferably 3.0)

Effect of foreign ions

The effect of over 60 ions and complexing agents on the determination of only 1 mg L^{-1} of Mo(VI) was studied. The criterion for an interference[29] was an absorbance value varying by more than 5% from the expected value for Mo(VI) alone. The results are summarized in Table 2. As can be seen, a large number of ions have no significant effect on the determination of molybdenum. The some interferences were from V(V), Fe(III) and Mn(VII) ions. Interference from these ions are probably due to complex formation with HAPBH.

The greater tolerance limits for these ions can be achieved by using several masking methods. In order to eliminate the interference of V(V), Fe(III) and Mn(VII) ions, tartrate, EDTA, citrate or chloride can be used as masking agents [30]. A 10-fold excess of V(V) could be masked with tartrate or chloride, and a 50-fold excess of Fe(III) or Mn(VII) could be masked with EDTA or chloride. During the interference studies, if a precipitate was formed, it was removed by centrifugation. The amount mentioned is not the tolerance limit but the actual amount studied. Interference from these three metal ions have been effectively removed by a short single-step ion-exchange separation process, using an Amberlite XAD-8 resin (100-200 mesh) anion exchanger [30].

Table 2. Table of tolerance limits of foreign ions*, Tolerance ratio [species(x) / Mo^{VI} (w/w)]

Species x	Tolerance ratio x / Mo^{VI} (w/w)
NH_4^+, As(V), Bi(III), Al, Cd, Co(II), Cr(III), Mg, Zn, Mn(II), Ag(I), Na, K, Cs, azide, EDTA, citrate, iodide, oxalate,	100
As(III), Ba, Pb(II), Sb(III), Ni(II), Se(IV), Se(VI), SCN^-	50
Fluoride, chloride, bromide,	500
Tartrate, phosphate	1000
Ascorbic acid	200
Ca, Co(III)	100[a]
Cr(VI), W(VI), Cu(II), Mo(V), Ce(III), Sn(IV)	50[a]
Mn(VII), Tl(I)	50[b]
Fe(III), Hg(II)	50[c]
V(V)	10[a+c]

*Tolerance limit was defined as ratio that causes less than 5 percent interference
[a]with 10 mg L^{-1} tartrate.
[b]with 10 mg L^{-1} EDTA.
[c]with 10 mg L^{-1} chloride.

Composition of the absorbent complex

Job's method [31] of continuous variation and the molar ratio [32] method were applied to ascertain the stoichiometric composition of the complex. A Mo-HAPBH (1:2) complex was indicated by both methods.

Precision and accuracy

The precision of the present method was evaluated by determining different concentrations of molybdenum (each analyzed at least five times).

The relative standard deviation (n = 5) was 2-0% for 1-300 µg of Mo(VI) in 10.0 mL, indicating that this method is highly precise and reproducible. The detection limit (3s of the blank) and Sandell's sensitivity (concentration for 0.001 absorbance unit) for molybdenum(VI) were found to be 10 ng mL^{-1} and 15 ng cm^{-2}, respectively. The results for total Mo were in good agreement with certified values (Table 4). The reliability of our Mo-chelate procedure was tested by recovery studies. The average percentage recovery obtained for addition of molybdenum(VI) spike to some environmental water samples was quantitative (Table 5. The method was also tested by analyzing several synthetic mixtures containing molybdenum(VI) and diverse ions (Table 3). The results of biological analyses by the spectrophotometric method were in excellent agreement with those obtained by AAS (Table 6). The results of speciation of molybdenum(V) and molybdenum(VI) in mixtures were highly reproducible (Table 8). Hence, the precision and accuracy of the method were excellent.

Applications

The present method was successfully applied to the determination of molybdenum (VI) in a series of synthetic mixtures of various compositions (Table 3) and also in a number of real samples e.g. several Certified Reference Materials (CRM) (Table 4). The method was also extended to the determination of molybdenum in a number of environmental, biological and soil samples. In view of the unknown composition of environmental water samples, the same equivalent portions of each such sample was analyzed for molybdenum content (Table 5). The results of biological analyses by spectrophotometric method were found to be in excellent agreement with those obtained by AAS (Table 6). The results of soil sample analysis by the spectrophotometric method are shown in Table 7. The results of speciation of molybdenum(V) and molybdenum(VI) in mixtures are shown in Table 8.

The results of synthetic mixture analyses were found to be highly reproducible. The average percentage recovery obtained for addition of molybdenum(VI) added to some synthetic mixtures was quantitative as shown in Table 3.

Table 3 Determination of molybdenum in some synthetic mixtures

Sample	Composition of mixtures (μgmL^{-1})	Molybdenum(VI) (μgmL^{-1})		
		Added	Found[a]	Recovery ± SD[b] (%)
A	Mo^{VI}	0.50	0.50±0.0	100 ± 0.0
		1.00	0.99±0.005	99 ± 0.5
B	As in A + Ca^{2+}(25) + Mg(25)	0.50	0.49±0.006	98 ± 0.6
		1.00	1.02±0.008	102 ± 0.8
C	As in B + Cr^{3+}(25) + Cl^-(25)	0.50	0.49±0.005	98 ± 0.5
		1.00	0.98±0.005	98 ± 0.8
D	As in C + Zn(25) + Ni^{2+}(25) + Tartrate (10)	0.50	0.52±0.012	104 ± 1.2
		1.00	1.03±0.15	103 ± 1.5
E	As in D + Mn^{2+}(25) + Hg^{2+}(25)	0.50	0.55±0.016	110 ± 1.6
		1.00	1.09±0.018	109 ± 1.8

[a] Average of five analyses of each sample
[b] The measure of precision is the SD.

Table 4. Determination of molybdenum in certified reference materials

Certified Reference Materials	Molybdenum		
	Certified value	Found (n=5)	R.S.D. (%)
BAS-CRM 64b high-speed steel (Cr, Mo,V and Te)	4.95[a]	4.94[a]	1.50
BCS-CRM 200/2 high-speed steel (Mo, W, Mn, C, Si, S. P, V, Cr and Ni).	4.92[a]	4.90[a]	1.58
BCS-CRM 220/1 high-speed steel (C, Si, S, P, Mn, Mo, V, Ni, Co, Cr, W and Cu)	0.05[a]	0.049[a]	1.21
NBS-CRM 336 Alloy cast iron (Mn, Cr, Mo, Ni, Si and P)	0.04[a]	0.042[a]	1.85
NASS-2, Sea water[c]	11.5[b] ±1.9	11.6[b]±1.5	1.5
SLRS-1, River water[c]	0.78[b]±0.04	0.75[b]±0.06	1.9

[*]Average of five determinations.

Based on five replicate analyses the average molybdenum concentration determination by the spectrophotometeric method was in good agreement with the certified values as shown in Table 4. The method was also extended to the determination of molybdenum in a number of environmental water samples. In view of the unknown composition of environmental water samples, the same equivalent portions of each such

samples were analyzed for molybdenum content; the recoveries in both the "spiked" (added to the samples before the mineralization or dissolution) and the "unspiked" samples are in good agreement. The average percentage recovery obtained for addition of molybdenum(VI) spike to some environmental water samples was quantitative (Table 5). The results of environmental water samples from various sources indicate that very low amount of molybdenum contains in natural and sea water, so pre-concentration method was applied during the measurements. Most spectrophotometric methods for determination of molybdenum in natural and sea water require preconcentration of molybdenum [22]. The concentration of molybdenum in natural and sea water is a few nanograms per milliliter in Taiwan [23]. The mean concentration of molybdenum found [24] in US drinking water is <10 ng mL^{-1}. Therefore, the results of environmental analyses by the spectrophotometric method were found to be in good agreement with those obtained by literature.

The abnormally high value for the gastrointestinal disturbance and cardiovascular patient are probably due to the involvement of a high molybdenum concentration with Zn and As. The occurrence of such high molybdenum contents is also reported in gastrointestinal disturbance and cardiovascular patients from some developed countries [22]. The different types soil samples were analyzed. The high value of molybdenum was found in marine soil. The occurrence of such high value is also reported for marine soil of molybdenum [27]. The results of molybdenum (V) and molybdenum (VI) speciation were found to be highly reproducible. The occurrence of such reproducible results is also reported for different oxidation states of molybdenum.

Table 5. Determination of molybdenum in some environmental water samples

Sample		Molybdenum/µg L^{-1} Added	Molybdenum/µg L^{-1} Found [a]	Recovery ± s (%)	s_r [b] (%)
Tap water		0	6.5		
		100	105.0	99.0 ± 0.1	0.31
		500	506.0	100 ± 0.2	0.35
Well water		0	8.5		
		100	109.0	100.5± 0.5	0.39
		500	510.0	99.7 ± 0.4	0.29
Rain water		0	2.5		
		100	103.0	100.5± 0.5	0.39
		500	504.0	99.7 ± 0.6	0.42
River water	Karnaphuly (upper)	0	12.0		
		100	114.0	101.8 ± 0.4	0.10
		500	512.0	100.0 ± 0.0	0.00
	Karnaphuly (lower)	0	15.0		
		100	116.5	100.8 ± 0.7	0.37
		500	514.0	100.0 ± 0.5	0.25
Sea water	Bay of Bengal (upper)	0	7.5		
		100	108.0	100.5 ± 0.5	0.21
		500	508.5	99.9 ± 0.4	0.16
	Bay of Bengal (lower)	0	9.0		
		100	110.0	100.9 ± 0.7	0.18
		500	510.0	99.8 ± 0.6	0.15
Drain water	Elite Paint	0	93.0		
		100	190.0	98.5 ± 0.6	0.27
		500	595.0	99.6 ± 0.5	0.21
	Karnaphuly paper Mill[d]	0	75.0		
		100	175.0	100 ± 0.0	0.00
		500	578.0	99.6 ± 0.7	0.35
	Kafco water	0	145.0		
		100	148.0	98 ± 0.8	0.45
		500	650.0	99.2 ± 0.9	0.58

[a]average of the five replicate determination . [b]The measure precision is the relative standard deviation(s_r).
[c]T. S. P. complex, Patenga, Chitagong . [d]Karnaphuly Paper Mill, Chandraghona, Chittagong. [e]Eastern Refinery, Chittagong.

Table 6. Concentration of molybdenum in blood urine samples

S.No.	Sample	Molybdenum / µg L^{-1}				Sample Source*
		AAS (n = 5)		Proposed method n = 5		
		Found	RSD,%	*Found*	RSD,%	
1	Blood	165.0	1.2	168.0	1.5	Gastrointestinal disturbance patient (male)
	Urine	38.0	0.8	40.0	1.0	
2	Blood	128.0	1.8	129.5	1.6	Cardiovascular diseases (male)
	Urine	32.5	1.5	35.5	1.0	
3	Blood	13.5	1.7	14.8	1.2	Normal adult (Male)
	Urine	3.55	1.2	4.0	1.0	

[a]Samples were from Chittagong Medical College Hospital.

Table 7. Determination of molybdenum in some surface soil.

S.No	Molybdenum[a] / µg g^{-1}	Sample source
S_1	2.25 ± 0.5[b]	Agriculture soil (Chittagong University Campus)
S_2	2.90 ± 0.8	Industrial soil (T. S. P. Complex, Chittagong)
S_3	5.51 ± 1.2	Marine Soil (Bay of Bengal)
S_4	2.53 ± 1.0	Traffic soil (Kadamtali bus terminal, Chittagong)
S_5	2.65 ± 1.5	Paint soil (Elite Paint, Chittagong)

[a]Average of five analyses of each sample
[b]Measure of precision is the standard deviation
[c]Composition of the soil samples: C, N, P, K, Na, Ca, Mg, Cu, Fe, Pb, NO_3, NO_2, Zn, SO_4, Mn, Mo, Co, etc.

Table 8. Determination of molybdenum(V) and molybdenum(VI) in mixtures

S. No.	Mo(VI) : Mo(V)	Mo, taken (µgmL^{-1})		Mo, found (µgmL^{-1})		Error (µgmL^{-1})	
		Mo(VI)	Mo(V)	Mo(VI)	Mo(V)	Mo(VI)	Mo(V)
1	1:1	1.00	1.00	0.99	0.98	0.01	0.02
1	1:1	1.00	1.00	0.99	0.99	0.01	0.01
1	1:1	1.00	1.00	1.00	0.98	0.00	0.02
		Mean error : Mo(VI) = ±0.007; Mo(V) = ±0.0017 Standard deviation : Mo(VI) = ±0.006; Mo(V) = ±0.0058					
1	1:5	1.00	5.00	0.99	4.99	0.01	0.01
1	1:5	1.00	5.00	0.98	4.98	0.02	0.02
1	1:5	1.00	5.00	0.99	4.98	0.01	0.02
		Mean error : Mo(VI) = ±0.0013; Mo(V) = ±0.017 Standard deviation : Mo(VI) = ±0.0058; Mo(V) = ±0.006					
1	1:10	1.00	10.00	0.98	9.99	0.02	0.01
1	1:10	1.00	10.00	0.97	9.98	0.03	0.02
1	1:10	1.00	10.00	0.99	9.97	0.01	0.03
		Mean error : Mo(VI)= ±0.02; Mo(V) = ±0.02 Standard deviation : Mo(VI) = ±0.01; Mo(V) = ±0.011					

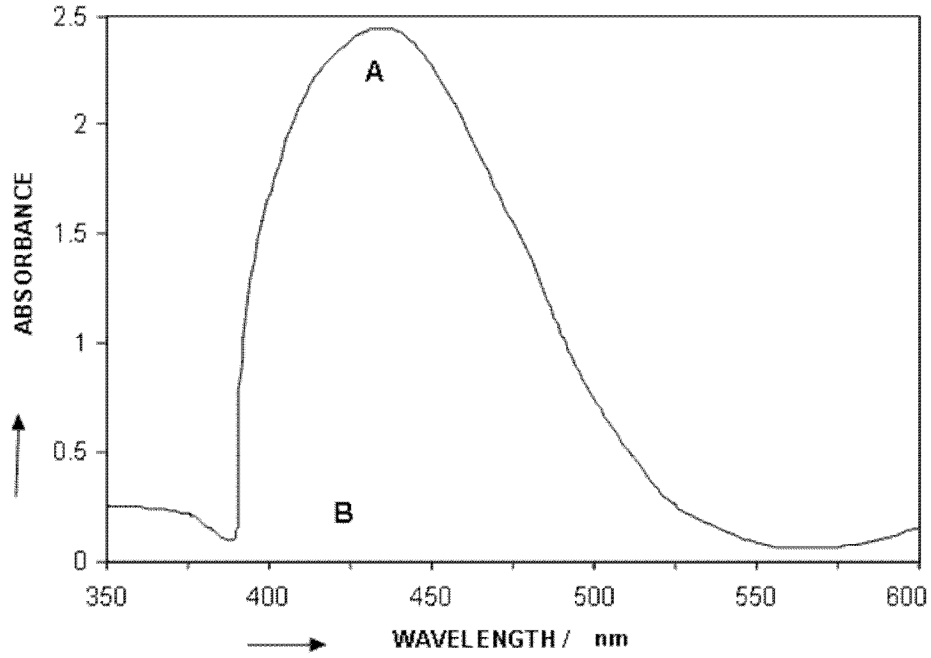


Fig. 1. A and B absorption spectra of molybdenum-HAPBH and the reagent blank (λ_{max} = 443 nm) in aqueous solution

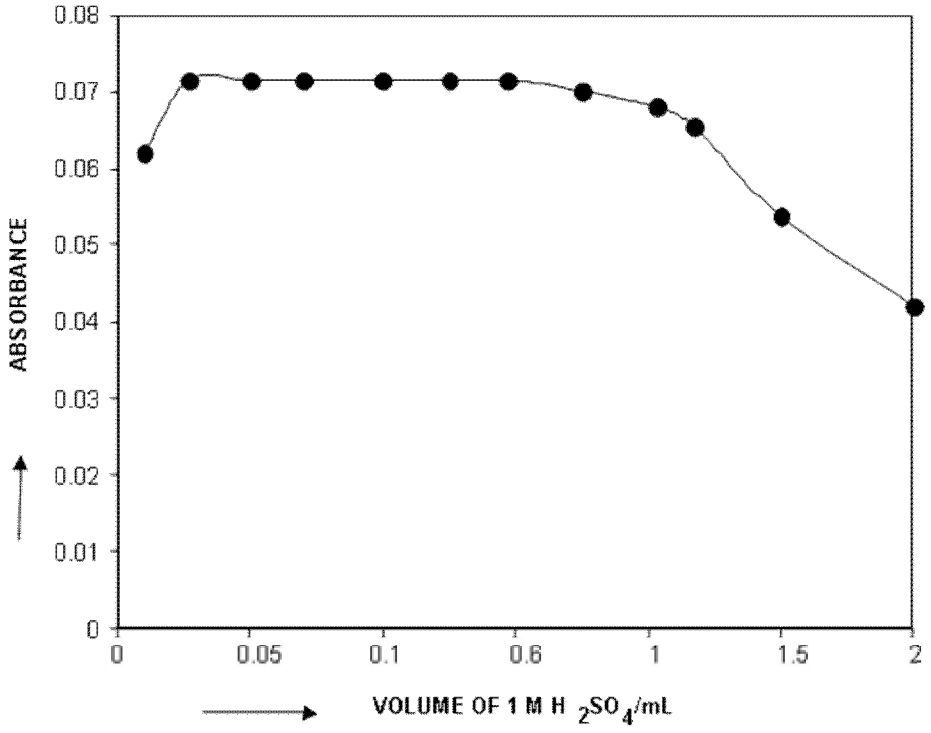


Fig. 2. Effect of the acidity on the absorbance of Mo(VI)-HAPBH system.

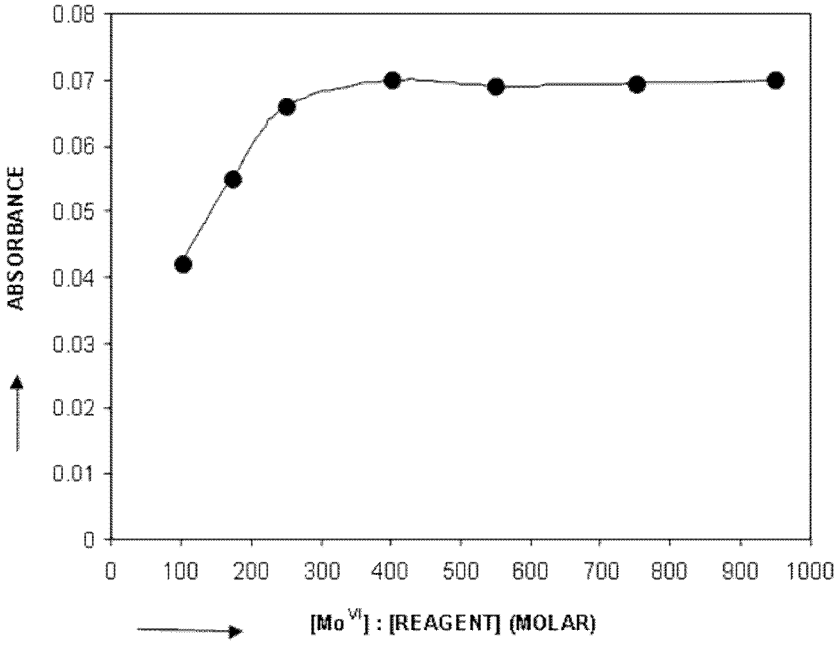


Fig. 3. Effect of reagent on the absorbance of Mo^{VI}-HAPBH system.

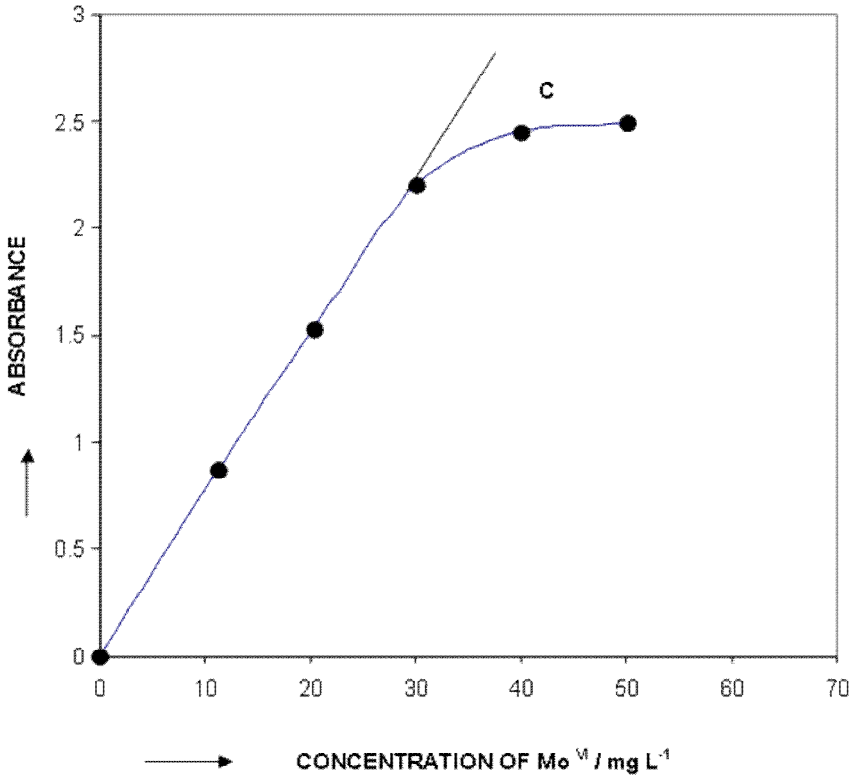


Fig. 4. Calibration graph C:10-30 mg L^{-1} of molybdenum(VI)

Conclusion

In this paper, a new, simple, sensitive, selective and inexpensive method with the Mo^{VI}-HAPBH complex was developed for the determination of molybdenum in industrial, biological, soil and environmental samples for continuous monitoring to establish the trace levels of molybdenum in difficult sample materials. Although many sophisticated techniques such as pulse polarography, HPLC, AAS, ICP-AES and ICP-MS are available for the determination of molybdenum at trace levels in numerous complex materials, factors such as the low cost of the instrument, easy handling, lack of requirement for consumable and almost no maintenance have caused spectrophotometry to remain a popular technique, particularly in laboratories of developing countries with limited budgets. The sensitivity in terms of molar absorptivity and precision in terms of relative standard deviation of the present method are very reliable for the determination of molybdenum in real samples down to ng g^{-1} levels in aqueous medium at room temperature (25 ± 5°C).

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