

Agarose Based Magnetic Solid-Phase Extraction-Magnetic Field Agitation for Determination of Trace Amounts of Molybdenum in Beans

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A magnetic solid-phase extraction method based on agarose coated magnetic nanoparticles (ACMNPs) coupled to a magnetic field agitation (MFA) device was developed and used for the preconcentration and graphite furnace atomic absorption spectrometric determination of trace amounts of Mo^{VI} in beans. The formation of the nanoparticles and their encapsulation in agarose micro-flakes was conducted in a single step. The nanomagnetic agarose particles were activated by an epichlorohydrin method, functionalized by phenylephrine and used for selective preconcentration of Mo^{VI}. The influence of different analytical parameters such as pH, extraction time, type and volume of eluent and amount of adsorbent on the preconcentration of Mo^{VI} were investigated. No important interferences were observed for the determination of the analyte in presence of several other metal ions. The capacity of the adsorbent for Mo^{VI} at pH 4.0, was 13.2 $\mu\text{g per mL}$ of its packed volume. Eight replicated analyses at the optimized conditions resulted in a recovery of 95.3% with a relative standard deviation of 4.9%. The detection limit of the method (3σ) for Mo^{VI} was 49 ng L⁻¹. The method was successfully applied to the determination of Mo^{VI} in beans samples.

Keywords: agarose coated magnetic nanoparticles, magnetic solid-phase extraction, magnetic field agitation, molybdenum(VI), beans

Introduction

Molybdenum is an essential trace element for both animals and plants. In plants, this element is necessary for the fixation of atmospheric nitrogen by bacteria to begin the protein synthesis. Deficiency or excess of molybdenum can cause damage to plants, and hence its routine control is highly recommended for healthy plant growth.¹ Thus, monitoring of molybdenum in environmental and biological samples is necessary in order to know the exposure level of this element.² There are many analytical techniques available for the direct determination of molybdenum such as colorimetry,³ inductively coupled plasma-mass spectrometry (ICP-MS),⁴ inductively coupled plasma-atomic emission spectrometry (ICP-AES) and graphite furnace atomic absorption spectrometry (GFAAS).⁵ The two crucial parameters in all analytical chemical procedures are sensitivity and selectivity. Atomic absorption spectrometry is now probably the most used technique for determination of metals in different samples.³ A number of methods such as solvent extraction,⁶ cloud

point extraction,⁷ dispersive liquid-liquid microextraction,⁸ co-precipitation⁹ and solid phase extraction (SPE)^{10,11} have been used for the separation and preconcentration of molybdenum. Among different separation and preconcentration techniques, SPE is advantageous in terms of simpler and faster operation, higher enrichment factors with better recoveries, quicker phase separation, lower cost and reduced consumption of organic solvents.¹²

The use of magnetic materials in solid phase extraction has received considerable attention in recent years taking into account many advantages arising from the inherent characteristics of magnetic particles. Magnetic solid phase extraction (MSPE) methodology overcomes problems such as column packing and phase separation, which can be easily performed by applying an external magnetic field.^{13,14} The final size of the magnetic adsorbent particles may be in the micrometer or nanometer range. Generally, a magnetic solid phase is made from magnetic iron oxides to create magnetic properties for the adsorbent.¹⁵ The particles are coated by a polymer matrix which is in turn functionalized by different active groups. By applying an external magnetic field, separation of the adsorbent particles from the solution for its washing or elution before the final analysis is fulfilled.¹⁶

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Agarose-based adsorbents, due to their hydrophilicity and chemical resistance in a wide pH range of 0-14, have shown to be excellent support materials for the preconcentration of metal ions.¹⁷ In a recent work in our laboratory,¹⁸ a single step method was developed for the formation of nanomagnetic agarose micro-flakes to be used for the separation, preconcentration and determination of Pd^{II} in aqueous solutions after being functionalized by iminodiacetic acid.

In the present study, the agarose coated magnetic nanoparticles (ACMNPs) are functionalized by phenylephrine (PhP), a known medicine, to be coupled to a magnetic field agitation (MFA) system and used for preconcentration of molybdenum in some beans, before determination by GFAAS.

Experimental

Reagents and materials

Agarose, ethanol, hydrochloric acid, nitric acid and other chemicals used in the experiments were purchased from Merck (Darmstadt, Germany) and used without additional purification. Ammonium molybdate tetrahydrate, (NH₄)₆Mo₇O₂₄·4H₂O, and phenylephrine hydrochloride were purchased from Sigma-Aldrich. Mo^{VI} stock solution (100 mg L⁻¹) was prepared by dissolving appropriate amount of the salt in 5 mL of 2% nitric acid and diluted to 100 mL volume by double-distilled water.

Apparatus

A GFAAS instrument with pyrolytic coated graphite tubes and deuterium background correction (Shimadzu, AA 6650, Japan) equipped with an automatic sample injection system was used for determination of Mo^{VI} under optimal conditions. A hollow-cathode lamp operated at 7 mA was used and absorbances were measured at 313.3 nm with a slit width of 0.5 nm. The analytical conditions used for the determination of Mo^{VI} is shown in Table 1.

A totally glass Hamilton (model WSC/4D) double distiller was used for preparation of doubly distilled water. A Shimadzu (Japan) model 1650PC double-beam spectrophotometer was used for running absorption spectra. Fourier transform infrared (FTIR) spectra were recorded by a Shimadzu, model 8400 (Japan), spectrometer in the transmittance mode. A Philips transmission electron microscope (TEM) (model EM208, Philips) was used in order to investigate the topology and size of nanoparticles. A Memmert type (SV 1422) shaker water bath (Germany) was used for shaking the solutions

and mixtures. ACMNPs were stirred with a home-made magnetic field agitation (MFA) device, designed by Safdarian *et al.*¹⁸ in the Department of Chemistry, Lorestan University (Figure 1).

Table 1. GFAAS analytical conditions for Mo determination

Stage	Temperature / °C	Heat mode	time / s	Gas flow rate / (mL min ⁻¹)
Drying	150	ramp	20	100
Drying	250	ramp	10	100
Pyrolysis	1000	ramp	10	1000
Pyrolysis	1000	step	10	1000
Pyrolysis	1000	step	3	0
Atomization	2600	step	2	0
Cleaning	2700	step	2	1000

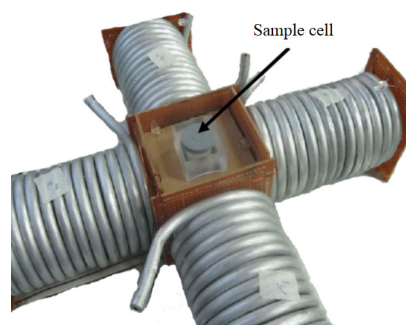


Figure 1. A photograph of the electrical magnets placed around the sample cell at the MFA device.

Preparation of ACMNPs

Preparation of ACMNPs was performed by a one-pot process reported elsewhere.¹⁸ In this procedure, preparation of iron oxide nanoparticles and their coating by agarose is performed in one step. Briefly, 0.2 g FeCl₃ and 0.073 g FeCl₂ were dissolved in 1 mol L⁻¹ HCl (12 mL). Then, 0.05 g agarose was dissolved in 2 mol L⁻¹ NaOH (25 mL) under heating to 80 °C and 2 mL of the Fe³⁺/Fe²⁺ solution was added to it, dropwise. The addition was made under nitrogen atmosphere at reflux conditions (90 °C) and magnetic stirring (800 rpm). The color of the bulk solution changed to black immediately, indicating the production of Fe₃O₄ nanoparticles. By this method, magnetic micro-flake agarose particles were produced. The final mixture was cooled to ambient temperature and the prepared ACMNPs were separated by a permanent magnet and washed with water and 50% (v/v) water/ethanol. To obtain more uniform particles and remove coarse particles, the particles were passed through a sieve of 120 μm before being stored under 20% (v/v) ethanol at 4 °C.

Activation and functionalization of ACMNPs

For partial crosslinking of the agarose particles and their epoxy activation, an epichlorohydrin activation method was used as reported elsewhere.¹⁹ After activation, ACMNPs were functionalized by the PhP ligand. For this purpose, 1 mL of a 20% v/v suspension of ACMNPs was added to 10 mL of 0.1 mol L⁻¹ ligand solution (adjusted on pH 10 by 2 mol L⁻¹ NaOH solution). The mixture was then shaken for 24 h, washed with water and stored under 20% (v/v) ethanol at 4 °C (as a 20% suspension) for later uses. Figure 2 shows a schematic representation of the preparation procedure for ACMNPs and their activation and functionalization by phenylephrine.

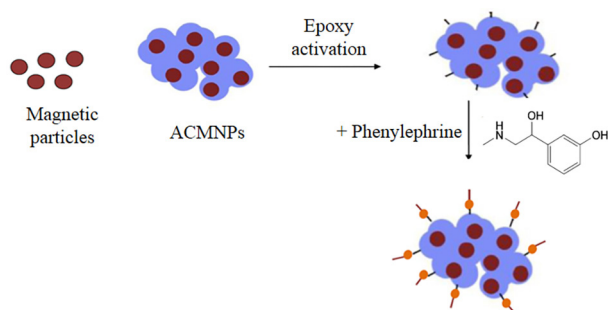


Figure 2. Schematic representation of ACMNPs and their activation and functionalization by phenylephrine.

Preparation of beans samples

Pea, corn, bean and lentil samples were purchased from local markets. The beans samples were ground to a fine powder using a porcelain mortar. To 1.00 g of each powdered sample, 6 mL of concentrated HNO₃ (65%) and 2 mL hydrogen peroxide (30%) were added and the mixture was gently heated until the sample was completely digested. After digestion, the volume of the sample was made up to 100 mL with distilled water.²⁰

The MSPE-MFA procedure

In the MSPE-MFA procedure, 5 mL of the sample or Mo^{VI} test solution were transferred into the sample cell of the MFA device, surrounded by four electric magnets, and 200 μL of the 20% suspension of the ACMNPs was added to this solution. The MFA device was programmed to change the magnetic field in a circular manner around the sample cell, in 500 and 1000 ms periods, so that the adsorbent particles were rotated in the solution.¹⁸ After 20 min of agitation, the magnetic particles were collected on a wall of the vessel, by keeping one of the electrical magnets on, and the solution was withdrawn by a syringe.

Then, the particles were washed with water and finally desorbed by 500 μL of a 1 mol L⁻¹ HCl solution for the determination by GFAAS method. The steps have been summarized in Figure 3.

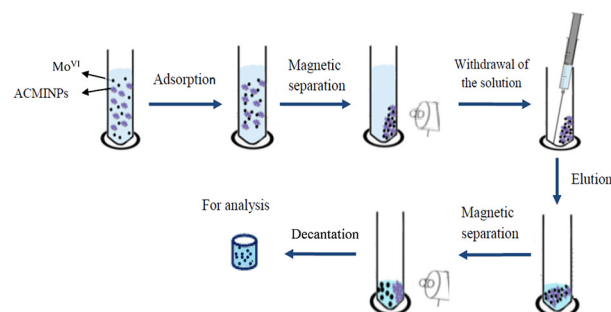


Figure 3. The steps of the MSPE-MFA method.

Results and Discussion

Spectrophotometric studies

To study complex formation between Mo^{VI} and PhP ligand, UV-Vis absorption spectra at constant concentration of the ligand and increasing concentrations of the metal ion were recorded. As shown in Figure 4, PhP shows a minimum absorbance at 244 nm and a maximum at 266 nm. By addition of Mo^{VI}, the minimum is increased and the maximum is decreased and a shoulder appears at about 290 nm. Appearance of two clear isobestic points indicates the formation of a complex between the metal ion and the ligand. Study of the absorbance changes at 266 nm as a function of metal ion to ligand concentration ratios shows that Mo^{VI}-PhP complex is formed in a 1:2 ratio in low metal concentrations (Figure 5). However, this ratio is

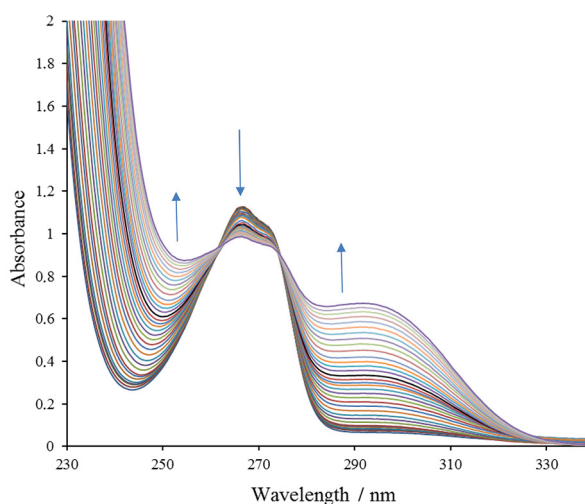


Figure 4. Absorption spectra of Mo^{VI}-PhP solutions. PhP concentration: 5×10^{-4} mol L⁻¹; and Mo^{VI} concentration: 2.53×10^{-5} - 2.18×10^{-3} mol L⁻¹. The arrows show the direction of increasing concentration of the metal ion.

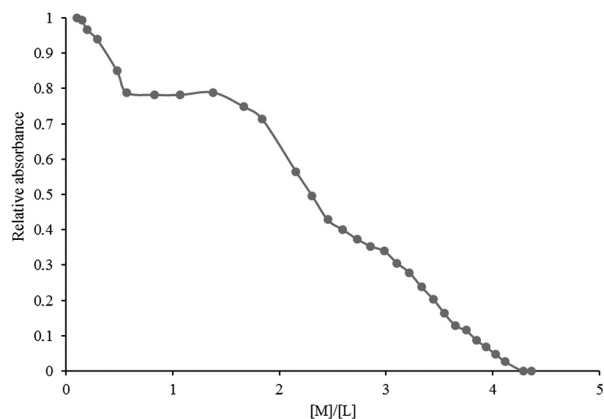


Figure 5. Relative absorbance as a function of metal to PhP ligand concentrations ratios for Mo^{VI} at 266 nm.

eventually changed in the higher metal concentrations and a 2:1 complex is formed as shown in the mole ratio curve.

Characterization of the adsorbent

Characterization of the ACMNPs was performed by optical microscopy, TEM and FTIR spectrometry. The optical microscopy images showed that the ACMNPs are in the shape of micro-flakes with a relatively narrow size distribution in the range of 90-130 μm . Iron oxide nanoparticles usually have a high affinity to aggregate together. However, polysaccharide matrices can avoid this and act as stabilizers for their synthesis.²¹ Agarose is also a polysaccharide and as the TEM image (Figure 6) shows, the nanoparticles have been fairly dispersed in the agarose matrix in the ACMNPs. By the study of the TEM images, the average sizes of the Fe_3O_4 nanoparticles were estimated to be in the range of 10-14 nm. Figure 7 compares the FTIR spectra of the Fe_3O_4 MNPs, agarose micro-flakes and ACMNPs. An OH stretching band has shifted from 3445 cm^{-1} (in agarose) to 3418 cm^{-1} (in ACMNPs) suggesting the formation of hydrogen bonds between agarose and hydroxyl functional groups onto the surface of the magnetic nanoparticle.¹⁵

Optimization of the MSPE-MFA procedure

Preliminary experiments showed that the phenylephrine-ACMNPs adsorbent can efficiently adsorb Mo^{VI} and can probably be used for the MSPE of this metal ion in combination with GFAAS. In order to avoid grinding of the agarose micro-flakes during stirring by magnetic bars, the home-made MFA system was used for efficient agitation of the magnetic agarose particles during the adsorption/desorption procedures.¹⁸ Effects of several experimental parameters on the preconcentration of Mo^{VI} by the MSPE-MFA method were carefully studied and optimized.

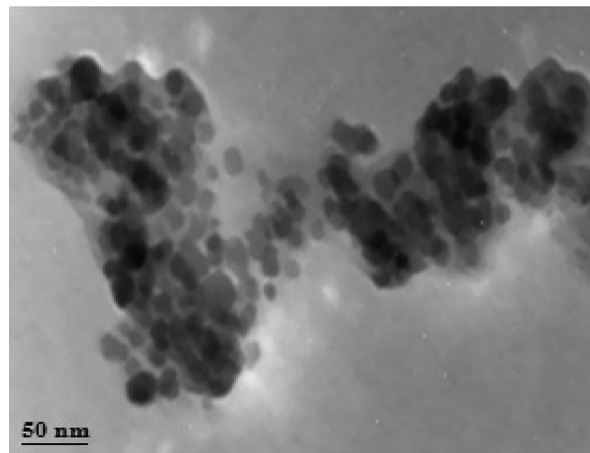


Figure 6. The TEM image of ACMNPs.

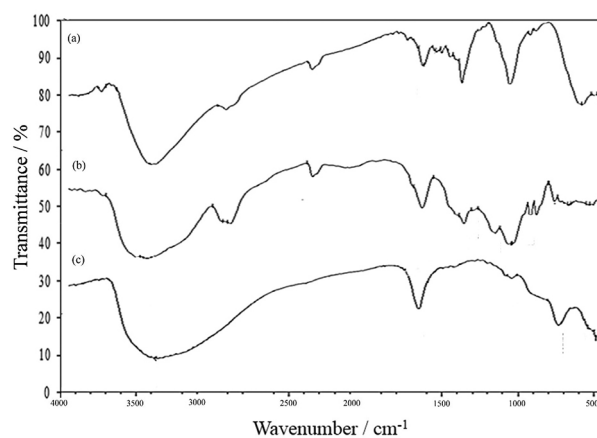


Figure 7. Comparison of the FTIR spectra of (a) ACMNPs; (b) agarose micro-flakes and (c) Fe_3O_4 MNPs.

Effect of pH

The solubility of metal ions in water is pH-sensitive, because many of these ions will precipitate in high pH values. In the low pH region, due to competition of protons with metal ions, stability of metal complexes will be greatly decreased. Therefore, optimization of pH is a priority in SPE experiments. The samples were buffered by 0.02 mol L^{-1} phosphate buffer for stabilization of pH during this study. As shown in Figure 8, the maximum recovery for Mo^{VI} was obtained at pH 4.

Effect of the amount of the sorbent

The amount of sorbent is a significant parameter in MSPE. Increasing the amount of sorbent requires higher volumes of the eluent to wash out metal ions that in turn reduces the preconcentration factor. On the other hand, reducing the amount of adsorbent may reduce the extraction efficiency. Given these issues to achieve the best results, the effect of the PhP-ACMNPs adsorbent amount was

studied in a constant eluent volume of 500 μL . As shown in Figure 9, an increase of the sorbent amount up to 200 μL increased the recovery. Then, the recovery gradually decreased in higher sorbent volumes probably due to the inefficient desorption by the aforementioned volume of the eluent. Since increasing the eluent volume was not suitable, 200 μL was selected as the optimum volume of the sorbent in subsequent experiments.

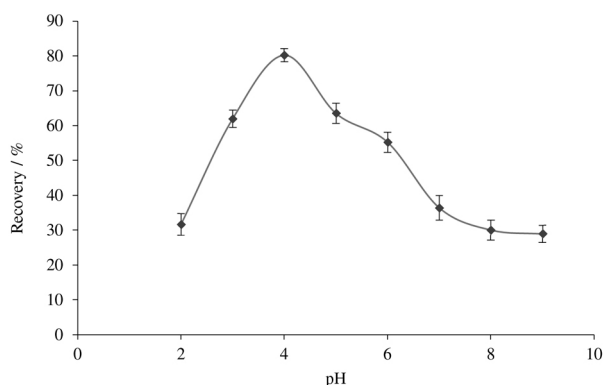


Figure 8. Effect of pH of test solutions on the recovery of Mo^{VI} by the MSPE-MFA method. Mo^{VI} concentration, $10 \mu\text{g L}^{-1}$; amount of adsorbent, 100 μL ; eluent, 500 μL of 1 mol L^{-1} HCl.

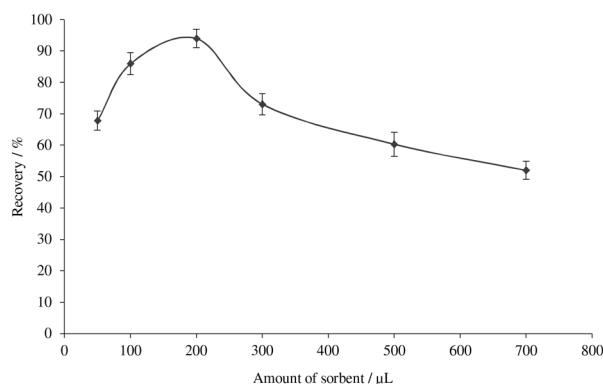


Figure 9. Effect of the amount of the sorbent on the recovery of Mo^{VI} by the MSPE-MFA method. The sample pH was 4.0 and other conditions were as in Figure 8.

Effects of volume and concentration of elution solvent

Hydrochloric acid was found to be an efficient eluent for desorption of Mo^{VI} from the PhP-ACMNP. Using various volumes of a 1 mol L^{-1} solution of HCl, indicated that a minimum volume of 500 μL was necessary for the complete desorption of the analyte.

Elution solvent concentration was another important factor to be studied. Acid concentration should be high enough to break down the metal-ligand bound but should not be so high to destroy the agarose based adsorbent. Due to the destructive effects of acid concentrations above 1 mol L^{-1} on agarose, the highest possible concentrations of

hydrochloric acid, i.e., 1 mol L^{-1} , was chosen as the optimal acid concentration. Using lower acid concentrations reduced the recovery of the analyte.

Effect of extraction time

The required time for an efficient extraction depends on the kinetics of adsorption and the mixing efficiency of the solution. In a previous work in our laboratory,¹⁸ it was shown that the MFA device, due to its efficient and direct agitation of the ACMNPs, is more efficient than magnetic stirring and shaking methods for mixing of the solution. Using the same MFA device in this work, after 20 min the interaction between the adsorbent and the Mo^{VI} sample solution reached to a plateau as shown in Figure 10. This indicates that the extraction time in this work is even shorter than the previous work (30 min). This should be due to a faster sorption kinetics of Mo^{VI} on the PhP functionalized sorbent compared to the sorption of Pd^{II} on the iminodiacetic acid functionalized sorbent.¹⁸

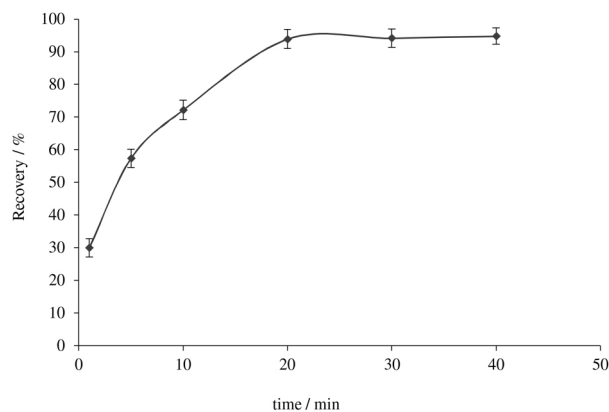


Figure 10. Effect of the extraction time on the recovery of Mo^{VI} by the MSPE-MFA method. Mo^{VI} concentration, $10 \mu\text{g L}^{-1}$; amount of adsorbent, 200 μL ; eluent, 500 μL of 1 mol L^{-1} HCl; pH, 4.0.

The analytical performances of the MSPE-MFA method

Eight replicated analysis at the optimized conditions for a sample containing $10 \mu\text{g L}^{-1}$ of Mo^{VI} (eluent volume, 500 μL ; eluent concentration, 1 mol L^{-1} , adsorbent amount, 200 μL ; extraction time, 20 min; pH, 4.0) resulted in a recovery of 95.3% with an relative standard deviation (RSD) of 4.9%. The calibration curve of the method (6 points) was linear over a range from 1 to $150 \mu\text{g L}^{-1}$ with a slope of $0.0051 (\pm 0.0001) \text{ L } \mu\text{g}^{-1}$, an intercept of $0.1375 (\pm 0.007)$ and a correlation coefficient (r) of 0.9990. The limit of detection (LOD) of the MSPE-MFA method was calculated from three times of the standard deviation of blank ($n = 20$) to be 49 ng L^{-1} for the analyte.

Table 2. Comparison of the proposed method with some other reported procedures for molybdenum determination

Determination method	LOD / (ng L ⁻¹)	RSD / %	Linear range / (μg L ⁻¹)	Enrichment factor	Reference
Reflectance spectroscopy	200000	3.3	500-12000	–	3
GFAAS and ICP-AES	50	1-3	–	100	5
Fiber optic spectrophotometry	1430	2.8	< 100	72.6	8
GFAAS	100	2.8-3.1	0.2-20	–	9
GFAAS	80	2-3	0.2-4	10	10
GFAAS	49	4.9	1-150	10	this work

LOD: limit of detection; RSD: relative standard deviation; GFAAS: graphite furnace atomic absorption spectrometry; ICP-AES: inductively coupled plasma-atomic emission spectrometry.

An important characteristic of a sorbent is its adsorption capacity. The capacity of a sorbent indicates the number of active sites on it or the ability of a certain amount of the sorbent to form complex with a metal ion. The capacity of the PhP-ACMNPs sorbent was calculated with a method described elsewhere.¹⁸ The calculated capacity of the sorbent for Mo^{VI} at pH 4.0 was calculated to be 13.2 μg *per* mL of its packed volume. The capacity and magnetic properties of the sorbent was not significantly changed within at least 30 times of uses during a period of three months.

Table 2 compares the performance of the proposed method for the determination of molybdenum with some other reported methods. As shown in the table, the performances of the MSPE-MFA technique is comparable or better than many of the other reported methods.

Interference effect of metal ions

Preconcentration procedures for small quantities of metal ions can be influenced by other ions present in the sample. One of the important characteristics of an SPE method is its selectivity, which is the ability of the method to preconcentrate and measure the analyte without interference from other species present in the sample solution. The effects of potentially interfering ions such as Cu²⁺, Ni²⁺, Pb²⁺, Fe²⁺, Cr³⁺, Co²⁺, Zn²⁺, Mg²⁺, Sr²⁺ on the preconcentration/separation and determination of Mo⁶⁺ were examined under the optimum conditions described above. For this purpose, 5 mL of binary mixtures of Mo⁶⁺ (10 μg L⁻¹) and various metal ions with a concentration equivalent to 100 times the concentration of Mo⁶⁺ (1000 μg L⁻¹) were studied by the proposed method. Relative errors caused by the interfering ions on the extraction of Mo⁶⁺, were calculated using known relations.²² As shown in Figure 11, only Fe³⁺ caused an error of about 7% and the error produced by the other metal ions was less than 3%. Therefore, the method showed a good selectivity

towards Mo^{VI}, with little or no interference from common metal ions.

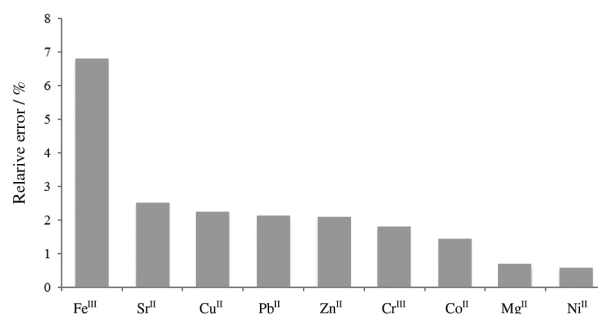


Figure 11. Effect of interfering ions on the relative error of Mo^{VI} determination by the proposed method. Concentration of each target ion was 1000 μg L⁻¹ and other conditions were as in Figure 10.

Application of the method

To test the applicability of the proposed method to real samples, Mo^{VI} concentration in some beans samples were determined after preconcentration on the PhP-ACMNPs by the proposed method. The analytical results have been summarized in Table 3. As the results show, the recoveries for the spiked beans samples are well comparable with the

Table 3. Analytical results for the determination of Mo^{VI} in real beans samples by the proposed method, under the optimized conditions (mean ± s, n = 3)

Sample	Added / (μg g ⁻¹)	Found / (μg g ⁻¹)	Recovery / %
Pea	–	1.91 ± 0.01	–
	1	3.04 ± 0.06	104.4
Corn	–	1.62 ± 0.06	–
	1	2.81 ± 0.02	107.6
Bean	–	1.11 ± 0.04	–
	1	2.33 ± 0.01	110.4
Lentil	–	1.03 ± 0.04	–
	1	2.14 ± 0.02	105.4

mean recovery of the method for standard Mo^{VI} solutions. Therefore, the method can be used for Mo^{VI} determinations in beans samples.

Conclusions

One-step method for the synthesis of nanomagnetic agarose micro-flake particles is simple, fast and without the need to any organic solvent. MFA device is efficient for increasing the rate of adsorption in MSPE and attainment of equilibrium in a reasonable time. The functionalized ACMNPs with PhP is relatively selective to Mo^{VI} and its application in the MSPE-MFA system for the preconcentration and GFAAS determination of Mo^{VI} in beans samples was successful. The proposed method is simple, sensitive and selective and can be used for the preconcentration of Mo^{VI} in different samples.

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