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A novel on-line preconcentration method for trace molybdenum determination by USN-ICP OES with bio-sorption on immobilized Yeasts

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ABSTRACT

A new system for on-line preconcentration of molybdenum by sorption on a mini-column associated to inductively coupled plasma – optical emission spectrometry with ultrasonic nebulization was studied. It is based on the sorption of molybdenum on a column packed with immobilized baker's yeasts on controlled pore glass without further complexing reagent. The molybdenum preconcentrated by biosorption was subsequently eluted with hydrochloric acid. Considering a sample flow rate of 5.0 mL min^{-1}, 10 mL of sample was preconcentrated in 2 min. achieving a sensitive total enhancement factor of 480-fold, and the detection limit (3s) obtained was 21 ng L^{-1}. Additionally, the calculated precisions expressed as percent relative standard deviation (RSD%) was 1.9%.

Satisfactory results were obtained for the determination of molybdenum in standard reference material NIST 1643e Trace Elements in Water and real water samples.

Keywords: on-line preconcentration; immobilized baker's yeasts on CPG; FI-USN-ICP OES; Molybdenum; Water samples.

1. Introducción

As early as 1953 [1] molybdenum was recognized to be an essential trace element for many species including man. It is a component of many enzymes responsible for the initial stages of nitrogen, carbon and sulphur metabolism of plants, animals and man [2, 3]. There is an absolute dependence by plants on Mo as it plays a vital role in the earth nitrogen cycle where it is involved both in nitrate reduction and nitrogen fixation. Although molybdenum is essential for animals it shows evidence of toxicity at high levels [4, 5]. Molybdenum intoxication depends on its speciation and is also influenced by the uptake of other elements such as S, W, Cu, Pb and Zn. It must be emphasized that studies of Mo roles in man or environment have often be hampered by the lack of sufficiently sensitive analytical methods for determining trace Mo levels. For this reason in many cases it is difficult to determine whether the symptoms attributed to Mo deficiency or excess are due to biological variations or simply to experimental error [6]. A better understanding of the role of Mo in human, plants or animal nutrition as well as in the environment depends on improving the sensitivity and accuracy of the analytical methods involved [7].

One of the major routes of incorporation of Mo is water. In this context, inductively coupled plasma mass spectrometry (ICP-MS) [8] has the analytical capability for the determination of Mo at trace and ultra-trace levels because of its high sensitivity, selectivity and sample throughput. However, the cost of the instrumentation is not affordable to many laboratories. Trace molybdenum determination in water samples has been carried out by atomic absorption with electrothermal atomization (ETAAS) [9], atomic emission spectrometry with inductively coupled plasma (ICP OES) [10]. The concentration of molybdenum is too low to be directly determined with these technique in well water, tap water [11], seawater samples [12], etc. Preconcentration is an

effective means for extending the detection limits of ICP OES technique. However, when practised manually in the batch mode, the operations are usually too tedious to be compatible with the ICP OES measurements.

Therefore, many preconcentration procedures for determination of molybdenum have been developed involving different analytical techniques. Preconcentration and determination of trace molybdenum had been developed by using different complexing agents in liquid-liquid extraction and several adsorbent materials for its on-line solid phase extraction (SPE) assisted by complexing reagents [10-16].

Metal preconcentration using living organisms, such as algae, fungi, bacteria, red cells, and yeasts [17–20], has been used as an attractive alternative to other adsorbent material for SPE because of its low cost, high accumulation capacity and the large variety of microorganisms available. One of the reasons why the adsorption ability of living organisms is higher than that of chemical adsorbents is due to the many functional groups available (amine, hydroxyl, carboxyl groups, phosphate, and sulfhydryl groups) to bind the metal without complexing reagents and the high apparent diffusion coefficients [21]. Yeast cells have been widely used because of its easy growth, non-hazardous nature, considerable tolerance towards metals and high cell-binding capacity. Saccharomyces cerevisiae is the specie of yeast most commonly used for metal accumulation [22–27]

In the present work, a method for on-line preconcentration and determination of inorganic Mo is proposed. Molybdenum has various oxidation states and ionic forms in aqueous solution. However, it exists in one oxidation state, Mo(VI), in well aerated water samples.

The coupling of flow injection (FI)-SPE and ICP OES with ultrasonic nebulization (USN) was used for Mo determination at trace levels. Molybdenum was retained by

sorption on a conical minicolumn packed with immobilised yeast cells in the absence of complexing reagent. The pH adjustment of the solution suffices to retain Mo.

2. Experimental

2.1. Instrumentation

All measurements were performed with a sequential ICP spectrometer [Baird (Bedford, MA, USA) ICP2070]. The 1 m Czerny–Turner monochromator had a holographic grating with 1800 grooves mm^{-1}. A U-5000 AT ultrasonic nebulizer (CETAC Technologies, Omaha, NE, USA), involving a desolvation system, was used.

The ICP OES and NUS conditions are listed in Table 1. Minipuls 3 peristaltic pumps [Gilson (Villiers-Le-Bel, France)] were used. Sample injection was achieved using a Rheodyne (Cotati, CA, USA), Model 50, four-way rotary valve. A conical minicolumn (40 mm length, 4.5 mm internal upper diameter and 1.5 mm internal lower diameter) was used as the Saccharomyces cerevisiae holder. Pump tubes, Tygon type (Ismatec, Cole-Parmer Instrument Company, Niles. IL, USA), were employed to propel the sample, reagent and eluent. The FI system used is shown in Fig. 1. The 202.030 nm spectral line was used and FI system measurements were expressed as peak height emission, which was corrected against the reagent blank.

2.2. Reagents

A molybdenum stock solution (1000 mg L^{-1}) was prepared by dissolving 920.3 mg $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ in water and the volume was filled up to 500 mL with water and a few drops of concentrated nitric acid (Fluka).

Ultra-pure water (18 MO cm^{-1}) was obtained from an EASY pure RF (Barnstedt, Dubuque, IA, USA).

All other solvents and reagents were of analytical-reagent grade or better, and the presence of Mo was not detected in the working range.

2.3. Biosorbent material

The organisms used in this study as biosorbent material were from commercial dry bakers' yeast. The yeast cells were microbiologically identified by the CNEA Radiobiology Laboratory as a pure culture of Saccharomyces cerevisiae. Identification of the biomass was performed by their morphological characteristics and other properties. These included the ability to ferment sucrose, maltose and galactose and an inability to assimilate potassium nitrate, to degrade urea and to grow in creatinine. The immobilisation of microorganisms on controlled pore glass (CPG) is performed by covalent attachment. CPG is an effective insoluble substrate that is fairly inert, and therefore not subject to microbiological degradation or swelling due to varying ionic strengths. It exhibits good mechanical properties in flowing streams and has been widely used as a solid support for the immobilisation of enzymes, microorganisms and other reagents. The immobilisation procedure followed was described previously [28]

2.4. Column preparation

The conical minicolumn was filled with about 100 mg of biomass immobilised on CPG into an empty column using the dry packing method. Small amounts of quartz wool were placed on both sides of the conical minicolumn, which was connected to a peristaltic pump with PTFE tubing to form the preconcentration system.

2.5. Sample preparation

Water samples were filtered through 0.45 mm pore size membrane filters immediately after sampling, and the pH was adjusted to pH 7.0 with hydrochloric acid or sodium hydroxide solution and stored at 4.0 ºC in polyethylene bottles (Nalgene; Nalge, Rochester, NY, USA). All the glass materials used were washed with 10% (v/v) HNO_3 and with ultra-pure water before use.

2.6. Preconcentration step

Before loading, a pH 7.0 solution was passed through the conical minicolumn through valve V_1 in position B (Fig. 1). The Mo solution was then loaded on the bakers' yeast immobilised on CPG at the desired flow rate with valve V_1 in position S and valve V_2 in the load position (a). After the loading time, the sample still present in the lines and the conical minicolumn was removed with a further washing with an aqueous solution (at working pH), with valve V_1 again in position B. Finally, peristaltic pump P was stopped and the injection valve V_2 was switched on to the injection position (b) and the retained metal was eluted with HCl at a flow rate of 1.8 mL min^{-1} directly into the USN and later into the plasma. Measurements were expressed as peak-height emission, and were corrected against the reagent blank.

3. Results and discussion

3.1. Design of the column

The column design strongly influences the performance of preconcentration systems [29]. The proposed method was applied to a classical column (3.0 mm internal diameter) and to a conical minicolumn (4.5 mm internal upper diameter and 1.5 mm internal lower diameter), both columns being packed with Saccharomyces cerevisiae inmobilised on CPG. From our previous studies [30], we could verify that the performance of the conical minicolumn was much better than that of the classical column due to a lower dispersion effect [29], the improvement attained being 90%. In this work, comparable results were obtained.

3.2. Effect of pH

Molybdenum has various oxidation states and ionic forms in aqueous solution. It exists in one oxidation state, Mo(VI), in well aerated natural and industrial waters. Accordingly, only this specie was studied.

The influence of the solution pH on the retention of Mo(VI) onto the column has been investigated separately. The pH of the solution was adjusted in a range of 2–12 using HCl or NaOH, and solutions containing Mo were passed through the column. The retained ions were eluted with HCl (1.5 mol L^{-1}) and Mo was determined in the eluate by USN-ICP OES. As can be seen in Fig. 2, the highest recoveries for Mo were obtained within the pH range from 6.0 to 8.0. Taking into consideration these results, the selected pH was 7.0.

3.3. Effect of flow rate on analyte retention

As the retention of analytes on adsorbent depends upon the flow rate of the sample solution, it was examined under the optimum pH (pH 7.0) by passing 10 mL of sample solution through the minicolumn with the flow rate varying in the range 1.0–12.0 mL min^{-1}. The results indicated that with flow rates up to 5.0 mL min^{-1}, a constant analyte recovery (close to 100%) is obtained. At higher flow-rates, recovery decreases. Thus, a flow rate of 5 mL min^{-1} is employed in this work.

3.4 Effect of the eluent

A satisfactory eluent should effectively elute the analyte in a discrete volume in order to obtain the best analyte recovery. Nitric and hydrochloric acid have turned out to be good eluents in many on-line preconcentration systems. Both acids were tested at different concentrations flowing at 1.8 mL min^{-1} through the column in order to evaluate and compare analyte recovery. Similar results were obtained with both acids; however, due to the oxidant nature of the nitric acid which could damage the package material of

the minicolumn, HCl was employed as eluent. In this way, we verified that 1.0 mol L^{-1} was the minimum concentration necessary to obtain best response.

Eluent flow rate was established at the NUS optimal intake flow rate; this was at 1.8 mL min^{-1}.

3.5. Performance of the pre-concentration system with immobilised yeasts

The overall time required for pre-concentration of 10 mL of sample, 2 min at a flow rate of 5 mL min^{-1}; elution, approx. 0.5 min at a flow rate of 1.8 mL min^{-1} and washing, 0.2 min at a flow rate of 2.0 mL min^{-1}, was approximately 2.7 min. Thus, sample throughput was approximately 22 samples h^{-1}. A sensitivity enhancement factor of 480 (30 for USN and 16 for yeasts-minicolumn) was obtained with respect to ICP OES using pneumatic nebulization.

3.6. Precision of the method

The reproducibility of the preconcentration method was evaluated by passing 10 mL of standard solution of molybdenum (5.0 µg L^{-1}) through the minicolumn and repeating this procedure 10 times. The relative standard deviation (RSD) was 2.2%, calculated with the peak height values.

3.7. Detection limit

The LOD was calculated as the amount of Mo required to yield a net peak that was equal to three times the standard deviation of the background signal (3*s*). The value of LOD obtained for the preconcentration of 10 mL of aqueous solutions of Mo was 20.8 ng L^{-1}. Better LODs are to be expected with larger sample volume, but this would increase the time of analysis and the efficiency of concentration. A comparison between the analytical performances of the proposed method at several pre-concentration times is shown in table 2.

3.8. Effect of potential interfering ions

The proposed system can tolerate the presence of ions at the concentration levels that may by found in natural water samples. Thus, Cu^{2+}, Zn^{2+}, Cd^{2+}, Ni^{2+}, Co^{2+}, Cr^{3+}, Mn^{2+} and Al^{3+} could be tolerated up to at least 2500 µg L^{-1}. Fe^{2+} and Fe^{3+} could be tolerated up to at least 5000 µg L^{-1}. Commonly encountered matrix components, such as alkaline and alkaline earth elements, are not retained on the mini-column.

3.9. Recovery Study and Application to Real Samples and Reference Materials

In order to evaluate the Mo recovery of this method, 60 mL of water sample was collected and divided in six portions of 10 mL each. The proposed method was applied to three portions and the average quantity of the molybdenum obtained was taken as base value. Then, increasing quantities of molybdenum were added to the other aliquots of sample determined later by the same method. The recoveries were in the range of 96.0-101.0 %.

Additionally, the accuracy of the proposed method was evaluated by molybdenum determination in a certified reference material NIST CRM 1643e, with a certified value for Mo of 121.4 ± 1.3 µg L^{-1} and a density of 1.016 g mL^{-1} at 22ºC .

These results are showed in table 2.

4. Conclusions

The main difficulty in the determination of Mo in tap and pond waters is its low concentration level. The FI-USN-ICP OES procedure developed using an on-line preconcentration system has shown adequate sensitivity and simplicity, as only inexpensive immobilised bakers' yeast is used as biosorbent for the preconcentration of Mo without further complexing agent. The selectivity of biosorption of Mo by Saccharomyces cerevisiae in these working dynamic conditions permits its application for the analysis of different kinds of waters and other complex environmental matrices.

On the other hand, CPG has demonstrated to be an effective substrate as it exhibits good mechanical properties in flowing systems.

The almost instantaneous release of adsorbed metal during the elution phase and the stability of bakers' yeast exhibited during all the study are ideal properties for its exploitation in on-line SPE-USN-ICP OES systems. In addition, the coupling of an on-line preconcentration system with FI-USN-ICP OES increases the speed of the overall process, and reduces sample consumption and contamination risks.

This system of preconcentration associated with USN-ICP OES allowed the determination of Mo in tap and pond water samples with good reproducibility and accuracy at concentrations of $\mu g\ L^{-1}$. The method is simple and can be easily adapted to any laboratories and is suitable for routine analysis competing with modern powerful techniques.

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Table 1

ICP and ultrasonic nebulizer instrumental parameters

ICP conditions	
RF generator power Plasma	0.8 kW
Frequency of RF generator	40.68 MHz
gas flow rate	8.5 L min^{-1}
Auxiliary gas flow rate	1 L min^{-1}
Observation height-above load coil.	15 mm
Analytical line: Mo	202.030 nm
Ultrasonic nebulizer conditions	
Heater temperature	140ºC
Condenser temperature	4.0ºC
Carrier gas flow rate	1 L min^{-1}

Table 2

Analytical performance of the proposed method at four loading times (sample flow rate = 5.0 mL min^{-1})

Preconcentration time (s)	120	240
sample consumption (mL)	10	20
sample throughput (f) (h^{-1})	22	12
relative standard deviation %	2.2	2.9
detection limit (ng L^{-1})	21	12
enrichment factor (EF)	16	29
Total enhancement factor	480	870

Table 3

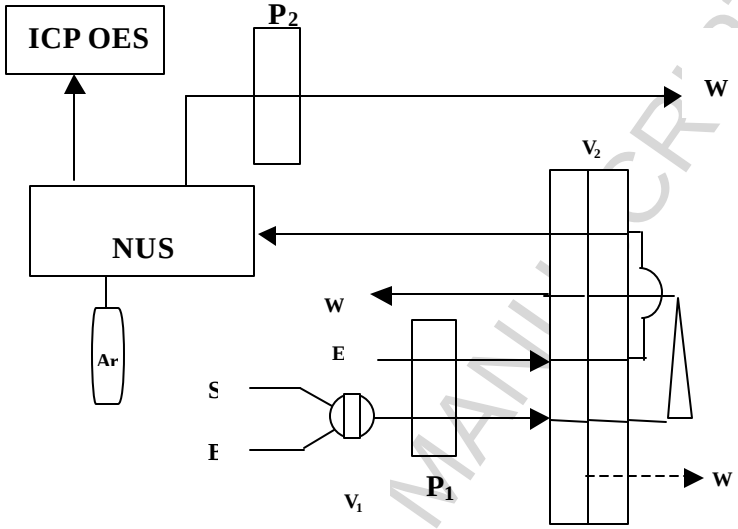
Concentrations of Mo in natural water samples (95% confidence level; $n = 3$)

Sample	Mo Added (µg L^{-1})	Mo Found (µg L^{-1})	Recovery (%)[a]
1[b]	0.0	0.84 ± 0.02	—
	0.5	1.32 ± 0.02	96.0
	1.0	1.84± 0.02	100.0
	1.5	2.33± 0.04	99.3
2[c]	0.0	0.28 ± 0.02	—
	0.5	0.77± 0.02	98.0
	1.0	1.29± 0.04	101.0
	1.5	1.77± 0.03	99.3
3[d]	0.0	121.4 ± 2.6	—

[a] [(Found - base)/added] x 100.
[b] Tap water
[c] Pond Water
[d] CRM (Trace Elements in Water - 1643e). Certified value: 121.4 ± 1.3 µg L^{-1} and a density of 1.016 g mL^{-1} at 22ºC

FIGURE 1

a)



b)

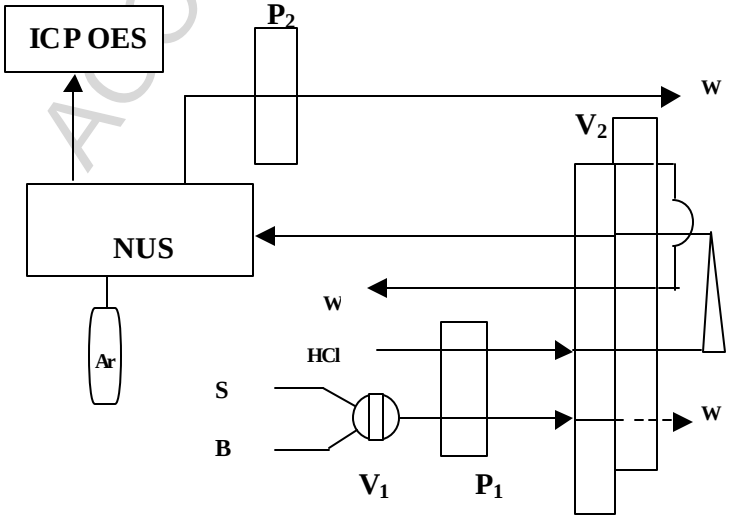
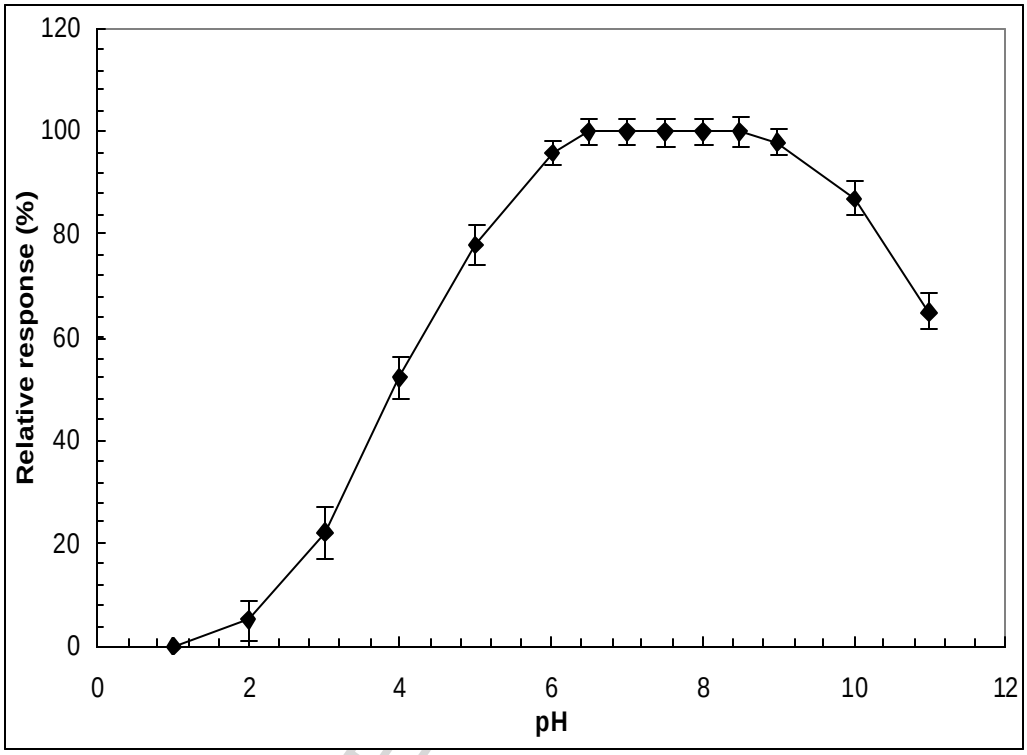


FIGURE 2



Captions of the figures

Figure 1

Schematic diagram of the instrumental setup. **B** , conditioning line; **S**, sampling line; **W**, waste; $\mathbf{V_1}$, two way rotary valve, $\mathbf{V_2}$**,** load injection valve (**a**, load position; **b,** injection position); **M**, conical minicolumn; **NUS**, ultrasonic nebulizer, **Ar**, argon gas suplyeither for plasma and for NUS.

Figure 2

Analytical signal (expressed as percent relative response) as a function of the sample pH. Mo concentration: 5.0 μg L^{-1}. Sample flow rate: 5.0 mL min^{-1}, HCl concentration: 1.5 mol L^{-1}.