Supercritical Fluid Extraction and Gas Chromatography or Electroanalysis of Metal Chelates from Different Sample Matrices

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Abstract

The supercritical fluid extraction of $Pb(DDC)_2$ and $MoO_2(acac)_2$ complexes is performed. The previously formed complexes are used in order to simplify the extraction process. In the extraction cell, 9.0 mg of $Pb(DDC)_2$ or 30.0 mg of $MoO_2(acac)_2$ is added. With these two complexes, a study of static and dynamic extraction as a function of pressure (1000–2500 psi), temperature (40–160°C), and presence of modifier (methanol) is performed. Under the best conditions, 5.6 mg of $Pb(DDC)_2$ (2.3 mg of Pb^{2+}) is recovered. The parameters are 2500 psi of pressure, 160°C of temperature, 0.5 mL methanol (placed in a 10-mL extraction cell), 60.0 min of static extraction, and 2.0 min of dynamic extraction. It is necessary to add 3.0 mL of methanol to enhance efficiency on the $MoO_2(acac)_2$ complex recovery. Quantitative extractions of $MoO_2(acac)_2$ (9.0 mg of Mo^{VI}) are obtained when the experiments are carried out under 1000–2500 psi of pressure, 140°C, and times no longer than 10.0 min. Then, the study is carried out forming the in situ complexes. For this purpose, metallic ion and ligand are added. Under these conditions, the Pb^{2+} recovery decreases from 2.3 to 1.9 mg, and the Mo^{VI} recovery decreases from 9.0 to 1.0 mg. When 1.9 mg of Pb^{2+} and 1.0 mg of Mo^{VI} or less is placed in the extraction cell, the recoveries are always 100%. The Pb^{2+} extracts are directly accomplished using gas chromatography–flame ionization detection (GC–FID), and the Mo^{VI} extracts are analyzed using GC–FID and catalytic adsorption voltammetry. The quantitation of pure extracts is carried out by constructing calibration curves with complex solutions and sample solutions using the standard addition method. This method is applied by determination of Pb^{2+} in sodium alginate extracted from algae and blood, urine, and human milk from patients with diagnosed plumbunemy. Mo^{VI} is determined in irrigation water and pasture of animal intake.

Introduction

In recent years, there has been great concern about the proliferation of heavy metals as pollutants. They can be highly toxic and dangerous, and the amount of pollution they produce is second only to pesticides. They are not biodegradable and begin to accumulate in the vital organs of humans, eventually reaching toxic levels in a short period of time. The most dangerous metal ions are lead, mercury, cadmium, arsenic, thallium, and selenium, which are related to cardiac diseases and even cancer. Others such as copper, zinc, or tin are essential but also toxic nutrients, depending on their level of concentration. Others such as molybdenum are nontoxic for humans but toxic for animals, particularly ruminants. Usually, these metal ions are strongly bound to biological ligands, and they change or inactivate their functions.

The largest emission source by far is lead from gasoline combustion, but because of the introduction of lower compression engines, lower octane fuel requirements, and the availability of low-lead or unleaded gasoline, these emissions have diminished in the last years. Lung absorption depends on the size distribution of lead-containing particles and the volume of air breathed per day. On the other hand, age and nutrition also have influence on the gastrointestinal absorption of lead. Elevated lead concentrations in human blood are associated with damage to the kidneys, liver, and gastrointestinal tract, as well as the central nervous system. In children, lower levels of lead can result in decreased intelligence, developmental disabilities, and behavioral disturbances (1–3).

Molybdenum must presently be considered as an essential element in humans. It occurs in some flavin-dependent metalloenzimes (e.g., xanthine oxidase). The xanthine oxidase, a molybdenum-containing enzyme, catalyzes the breakdown of purines into uric acid in vivo. This may be responsible for the goutlike diseases observed in molybdenum industry workers and in regions where a high incidence of gout along with high uric acid levels in blood has been observed. Molybdenum exhibits a relatively moderate toxicity in comparison with other heavy metals, and no cumulative effect has been noticed. However, "teart syndrome" is especially observed in ruminants (bovines) where pastures contain 20–100 mg/K Mo (dry weight). Normal pasture contains 3–5 mg/K Mo. General symp-

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toms of chronic intoxication in ruminants, especially in bovines, are loss of weight, anorexia, anemia, deficient lactation, male sterility, osteoporosis, and bone joint abnormalities (4–6).

Atomic absorption spectroscopy is the most popular method for the routine determination of metallic ions. Furnace techniques provide high sensitivity and the ability to measure small samples. Anodic stripping voltammetry is one the most sensitive analytical methods for the determination of lead in biological matrices. A number of other technically feasible methods are available, including optical emission spectroscopy, neutron activation, isotope dilution, and mass spectrometric analysis (7–9).

In recent years, a technique that is broadly used on the treatment of complex samples is supercritical fluid extraction (SFE). One of the principal advantages this method presents is the reduction or elimination of the use of organic solvents. It minimizes waste disposal, flammability, and toxicity. Also, SFE reduces the number of steps for analysis. The organic compounds are directly extracted with an adequate solvent or solvent mixtures. However, with metallic ions, a neutral, stable, and rapid complexation kinetics complex must be previously formed (10–15).

The first objective of this study was to reach the optimum conditions for performing the extraction of Pb^{2+} and Mo^{VI} as complexes. To this aim, the "in situ" formation was carried out, and the determination method of these ions in real samples was applied. Pb^{2+} in sodium alginate from algae and biological samples such as blood, urine, and human milk; Mo^{VI} can be found in irrigation water and pastures of animal intake. The second objective was to search for a method that allowed the direct quantitation of the small methanolic extract volume obtained without further treatment. Gas chromatography–flame ionization detection (GC–FID) and catalytic adsorption voltammetry methods were adequate to complement offline SFE.

Experimental

Reagents and materials

Stock solutions of Pb^{2+} and Mo^{VI} (1000 µg/mL) were obtained from Merck (Darmstadt, Germany). Sodium diethyldithiocarbamate trihydrate ($NaDDC{\cdot}3H_2O$) and acetylacetone (Hacac) chelating agents were obtained from Aldrich (St. Louis, MO). The respective complexes $Pb(DDC)_2$ and $MoO_2(acac)_2$ were synthesized according to the procedures outlined in the literature. The solvents used in this study (methanol, carbon disulfide, diethylether) were of analytical grade (Merck or Aldrich). Deionized water from a Millipore (Milford, MA) Milli Q system was used in the preparation of the aqueous solutions. Wood glass (Aldrich) and Whatman (Haverhill, MA) 42 cellulose filter paper were used in solid and liquid samples, respectively. SFE-grade carbon dioxide (Indura, Santiago, Chile) was used in this study. Carbon dioxide modified with methanol is not commercially avalaible; therefore, the modifier was added directly to the extraction cell.

Apparatus

All experiments were performed with an SFE-300 extractor (Supelco, Bellefonte, PA). A 10-mL stainless steel vessel obtained from Supelco was used as an extraction cell. Fused-silica tubing (15 cm × 50-µm i.d.) was used as a restrictor. The flow rate of the extraction fluid was approximately 1.0 mL/min (as a liquid).

The chromatograms were run using a Perkin Elmer (Norwalk, CT) model Autosystem 9000 with a flame ionization detector. The signals were recorded and processed using a PE-Nelson model 1020 computer. A 1.8-m × 0.24-mm-i.d. methylpolisiloxane (SE-30) and 25-m × 0.53-mm-i.d. 8% phenylpolisiloxane (HT-8) capillary column were used in analytical determinations of lead and molybdenum complexes, respectively.

The voltammetric measurements were carried out using a potentiostat bank (model Wenking ST-72) coupled to a voltage scan generator (model USG-72) and a Graphtec recorder (model WX-1100). A conventional three-electrode system comprised of hanging mercury drop electrode (HMDE) (Beckman, Fullerton, CA), a platinum wire counter electrode, and a Hg/Hg_2Cl_2 (in saturated KCl) reference electrode was used. All experiments were performed in an argon atmosphere at room temperature (25°C) in a clean environment.

Procedures

Extraction parameters with synthetic solutions

In order to determine the optimum extraction parameters, 9.0 mg of $Pb(DDC)_2$ or 30.0 mg of $MoO_2(acac)_2$ were placed in the extraction cell and supported on glass wool to enhance the contact area. Methanol (0.0, 0.5, 1.0, 2.0, and 3.0 mL) was added as a modifier into the extraction cell, and the system was closed. The CO_2 inlet valve was opened, allowing the mixture of CO_2–methanol to homogenize. Conditions were as follows: pressure, 1000–2500 psi; oven temperature, 40–160°C; and restrictor temperature, 40–100°C. Extractions at different static and dynamic extraction times were performed. The extracts were received in empty volumetric flasks.

Formation of "in situ" complexes

Solutions (1.0–2.0 mL of Pb^{2+} or Mo^{VI}) were spiked onto filter paper and dried in an oven at 80°C. The filter paper that contained the sample was introduced into the extraction vessel. The diethyldithiocarbamate (150 mg) or acetylacetone (500 µL) ligands and the modifier (0.0, 0.5, 1.0, 2.0, or 3.0 mL of methanol) were added.

Extraction of metallic ions from samples

When collecting biological sample extracts, pollution must be avoided. Five urine samples, five blood samples, and one human milk sample of a family poisoned with lead were analyzed. The blood was collected using an anticoagulant (heparin) and stored at 4°C for 1–2 days. Then, 1.0 mL of each sample was added to filter paper, dried at 80°C, and then placed in the extraction vessel; subsequently, ligand and methanol were added. The urine, human milk, and water samples were treated in the same way, excluding the use of anticoagulant.

Five pasture samples were dried at 120°C for 3 h, commin-

uted, and homogenized. Portions (1.0000 g) of these samples were transferred to the extraction vessel containing glass wool, ligand, and methanol.

In order to evaluate the metal ion extraction in the real matrix, these ions were added to uncontaminated blood, urine, milk, water, and pasture samples. To eliminate the matrix effect, lead and molybdenum contents were quantitated using the standard addition method.

Determination of $Pb(DDC)_2$ and $MoO_2(acac)_2$ on extracts using GC–FID

A 0.5-µL amount of extract was injected into a chromatograph equipped with an SE-30 (1.8 m × 0.24-mm i.d.) capillary column for $Pb(DDC)_2$ complex and HT-8 (25 m × 0.53-mm i.d.) capillary column for $MoO_2(acac)_2$ complex, respectively. Studies as a function of injection volume, split relation, carrier gas flow rate, and oven, injector, and detector temperatures were carried out. Quantitative analysis was carried out with reference to standard calibration curves.

Determination of $MoO_2(acac)_2$ on extracts using catalytic-adsorptive stripping voltammetry

A 1.0-mL amount of methanol extract (with ligand excess) was added to an electrochemical cell containing a supporting electrolyte solution and buffer and then purged with argon for 10.0 min. The accumulation potential (0.00, –0.10, or –0.20 V versus sce) was applied to HMDE while the solution was stirred. A study was performed as a function of pH (2.2, 3.5, 4.0, 4.4, and 6.1), accumulation times (0, 30, 60, 120, 240, 300, and 600 s), methanol volumes (0, 0.5, 1.0, and 1.5 mL), and supporting electrolyte solution (potassium perchlorate, potassium chlorate, and potassium bromate, 0.02–0.12M). A cyclic scan was recorded from 0.00–0.70-0.00 V versus sce with a potential scan rate of 0.05 V/s. Next, aliquots of the Mo^{VI} standard solution were introduced into the electrochemical cell (standard addition method).

Results and Discussion

SFE parameters with complexes in the solid state

The extraction of the studied complexes using CO_2 alone is inefficient and requires the addition of methanol as a modifier. The effect of pressure and temperature on the extraction of $Pb(DDC)_2$ solid-state complex is shown in Figure 1. These values are related to the average of three measurements under the same conditions. These recoveries were obtained with 5.0 min of static extraction and 2.0 min of dynamic extraction. As a rule, increased temperature and pressure results in increased recovery.

The effect of the amount of methanol added to the extraction cell is shown in Figure 2. These results were also obtained after 5.0 min of static extraction and 2.0 min of dynamic extraction. As can be seen in this figure, the optimum volume of methanol for the extraction of the $Pb(DDC)_2$ complex is 0.5 mL. Due to the critical high temperature of methanol, it is not convenient to add it in large volumes. The modified

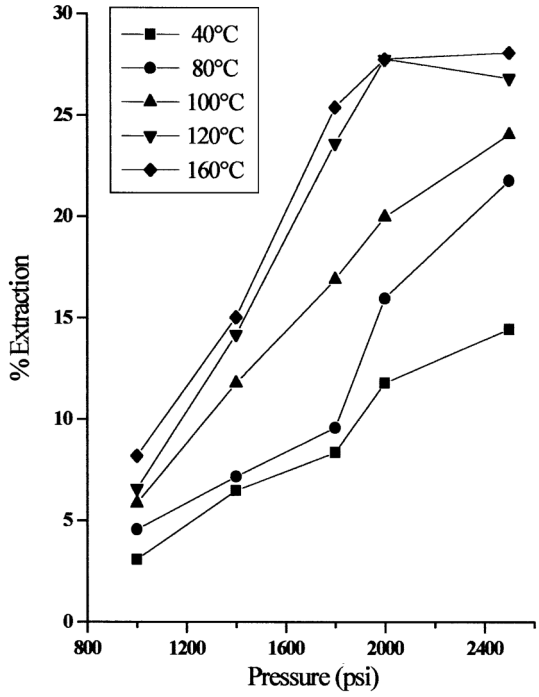

Figure 1. Recoveries of $Pb(DDC)_2$ as a function of pressure (1000–2500 psi) at different temperatures (40–160°C). Conditions: 1.0 mL of methanol (added in extraction vessel of 10-mL); 5.0 min in the static mode, followed

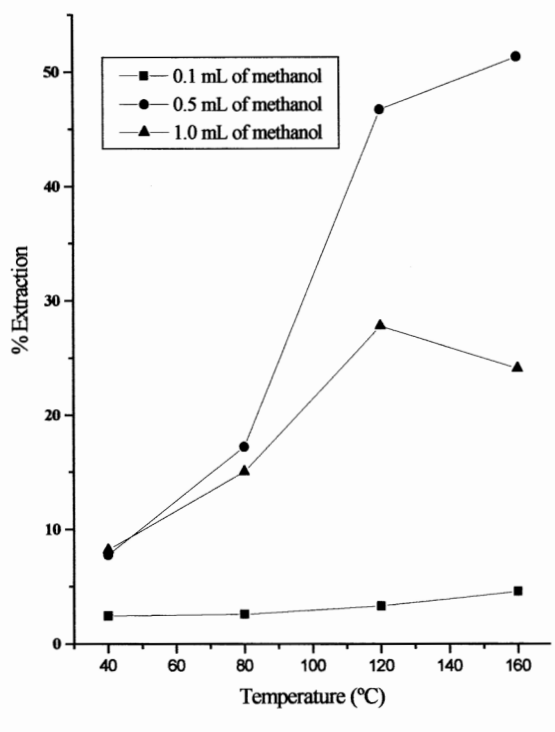

Figure 2. Recoveries of $Pb(DDC)_2$ as a function of temperature (40–160°C) with different amount of methanol (0.1–1.0 mL) in the extraction vessel. Conditions: pressure, 1000–2500 psi; 5.0 min in the static mode, followed by 2.0 min in the dynamic mode; 9.0-mg portions of $Pb(DDC)_2$.

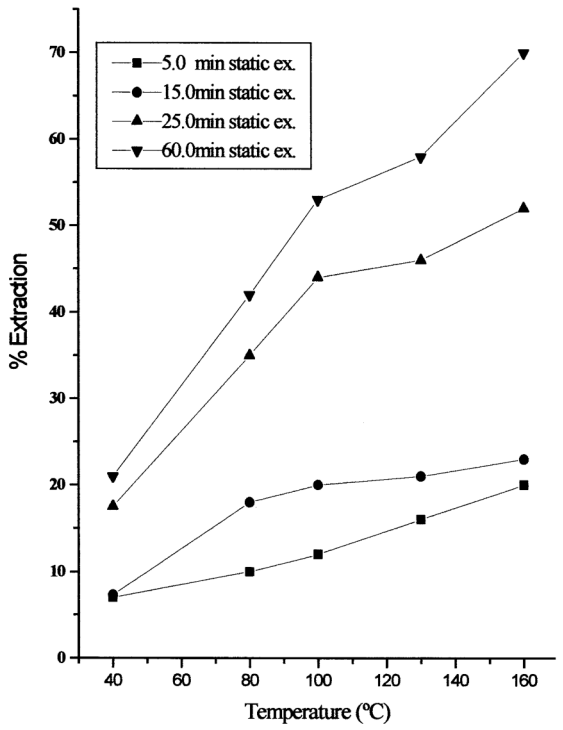

Figure 3. Recoveries of $Pb(DDC)_2$ as a function of temperature (40–160°C) at different static extraction times (5.0–60.0 min). Pressure, 2500 psi; 0.5 mL of methanol (extraction vessel, 10 mL); dynamic extraction, 2.0 min; 9.0-mg portions of $Pb(DDC)_2$.

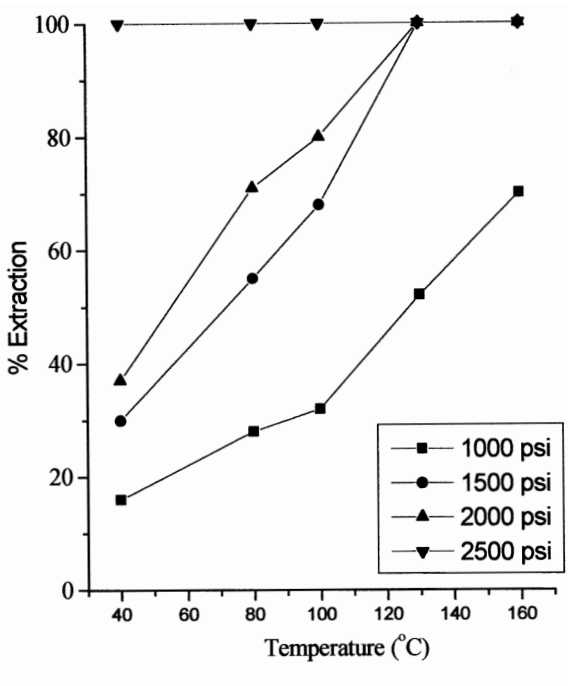

Figure 4. Recoveries of Pb^{2+} as a function of temperatures (40–160°C) at different pressures (1000–2500 psi). Conditions: 0.5 mL of methanol (extraction vessel, 10 mL); 60.0 min in the static mode, followed by 2.0 min in the dynamic mode; filter paper with 1.9 mg of Pb^{2+} and 150 mg of sodium diethyldithiocarbamate placed in the cell.

Handinson–Brobst–Thomson equation (16) allows the calculation of the critical temperature and pressure of a supercritical fluid modified by an organic solvent. The critical temperature for a CO_2–methanol (7:3) binary mixture ratio is approximately 121°C.

Figure 3 shows the effect of static extraction time on $Pb(DDC)_2$ performance. As can be seen in this figure, the performances do not increase much after increasing the static extraction time from 25.0 to 60.0 min. The dynamic extraction must be stopped when methanol is completely exhausted.

According to these results, it can be concluded that the best extraction parameters for $Pb(DDC)_2$ complex were as follows: temperature, 160°C; pressure, 2500 psi; methanol, 0.5 mL (added to 10-mL extraction cell); 60.0 min of static extraction; and 2.0 min of dynamic extraction. When 9.0 mg of $Pb(DDC)_2$ complex was placed on the extraction cell, 5.6 mg was recovered as maximum (2.3 mg of Pb^{2+}). If 5.6 mg or lower amounts of $Pb(DDC)_2$ complex are placed, the performance is always 100%.

The same study was then carried out, but this time a metallic ion and a ligand were added to form the "in situ" complex. The same parameters (such as temperature, pressure, and extracting times) were used, and the Pb^{2+} recovery decreased from 2.3 to 1.9 mg. Problems that occur during the complex formation but not in the extraction procedure explain this difference. Figure 4 shows the results of the recovery of $Pb(DDC)_2$ complex obtained when 1.9 mg of Pb^{2+} and 150 mg of sodium dietyldithiocarbamate are added to the extraction cell. The applied pressure was varied from 1000–2500 psi, and the temperature was varied from 40–160°C.

Chromatographic determination

The $Pb(DDC)_2$ complex was injected into many columns of different lengths, materials, and stationary phases. In most of them, it was only possible to observe a peak under very limited temperature conditions and for concentrations higher than 100.0 mg/L of complex. The temperature range between volatility and decomposition is small. The peak areas depend on retention time; if this complex remains approximately 8–9 min in the column, the peak areas decrease notoriously. Due to this fact, short columns and high flow rates were used. A 1.8-m fused-silica short column with polydimethyl siloxane (SE-30) as a stationary phase (0.24-mm i.d.) was used, which proved to be adequate for the analysis of the complex. Oven temperature programming was utilized (80 to 220°C at 2°C/min), and 200°C was applied for the temperature injector and detector. Under these conditions, the $Pb(DDC)_2$ complex had a retention time of 5.3 min. It was possible to quantitate 0.5 mg/L of Pb^{2+}. Fe^{3+} does not interfere; it can be found in biological samples, and it is also extracted as a complex with diethyldithiocarbamate. Calibration curves were performed with $Pb(DDC)_2$ solutions.

Extraction of Pb^{2+} from sodium alginate

In order to prove the efficiency of the method, a sodium alginate sample extracted from algae was analyzed. For this purpose, 100.0-mg portions of sodium alginate were placed in the extraction cell. They were analyzed 25 times under dif-

ferent conditions. These extractions were performed in duplicate. These studies were conducted at pressures of 1000–2500 psi and temperatures of 40–160°C for 25.0 min in the static mode followed by 2.0 min in the dynamic mode. The results were 17.0 ± 0.3 mg/K Pb dry weight. All the accomplished extractions were 100%.

Extraction of Pb^{2+} from blood, urine, and human milk

The biological extracts were injected into the GC. Figure 5 shows the chromatograms for blood and milk samples. The result obtained for the milk sample was 15.9 mg/L, and for the 5 blood samples, the values were as follows: 13.4, 9.6, 5.4, 4.9, and 8.4 mg/L. The results for urine samples were 2.2, 3.8, 7.7, 3.1, and 8.9 mg/L. All the values obtained exceeded the normal level. It can be noticed that the Pb^{2+} content is higher in human milk than in blood (15.9 versus 13.4 mg/L Pb in the same individual), which represents a high risk for milk-fed babies. As these people are under treatment, they are eliminating lead through urine; this is not of normal occurrence. Each blood sample was analyzed in duplicate, and the urine and milk samples were studied in triplicate.

Extraction of Mo^{VI}

The results obtained with molybdenum complex were different. The main problem was in the formation of the "in situ" complex. The recovery decreased from 9.0 to 1.0 mg of Mo, replacing solid $MoO_2(acac)_2$ for filter paper, with Mo^{VI} and ligand added separately. The formation of this dioxo complex is slow; in order to synthesize it, reflux heating must be carried out for several hours. Having formed the "in situ" complex, studies based on pressure (1000–2500 psi), temperature (60–140°C), amount of methanol, and extraction times were carried out.

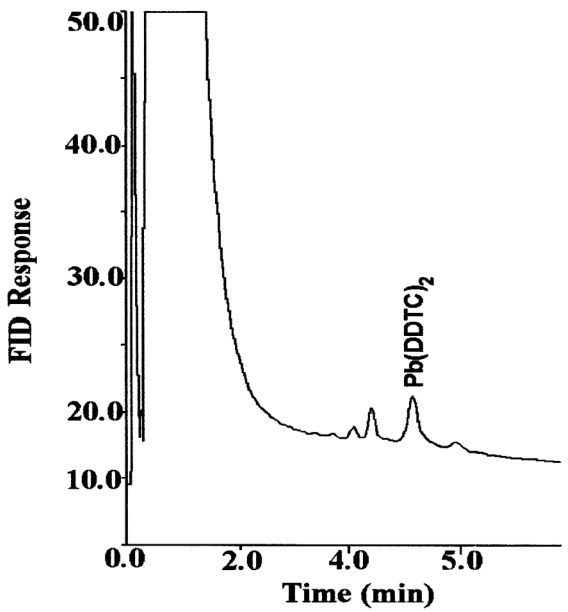

Figure 5. Chromatogram of human milk sample. column, SE-30 (1.8 m × 0.24-mm i.d.); injector, detector temperature 220°C; oven, 80 to 220°C at

Figure 6 shows the effect of temperature and pressure on the first 5.0 min of extraction. As can be seen in this figure, yield is increased at higher temperature (140°C). However, when pressure is increased, the performance decreases notoriously, obtaining the highest value at 1000 psi. This is because when pressure is increased, fluid diffusivity decreases, which does not help its penetration into the matrix; therefore, the metal–ligand interaction is less effective. These results are the average of two measurements. From the obtained results and studies as a function of the methanol volumes, compound weight added to the extraction cell, and static–dynamic extractions times, we concluded that the best conditions for extracting Mo^{VI} forming the "in situ" complex are as follows: pressure, 1000 psi; temperature, 140°C; methanol, 3.0 mL; 30.0 min of static extraction; and 3.0 min of dynamic extraction. Under these conditions, it was possible to extract 1.0 mg of Mo^{VI}.

Quantitation of MoVI using catalytic adsorption voltammetry

The extracts with $MoO_2(acac)_2$ complex were quantitated using GC and catalytic adsorption voltammetry. Of these two methods, the electroanalytical technique was the more sensitive method. From the studies as a function of the concentration and type of support electrolyte, pH solution, and accumulation time, the optimum conditions were determined as follows: chlorate, 0.1M (pH 4.5); 5.0 min of accumulation time; and 0.10 V versus sce of accumulation potential. The

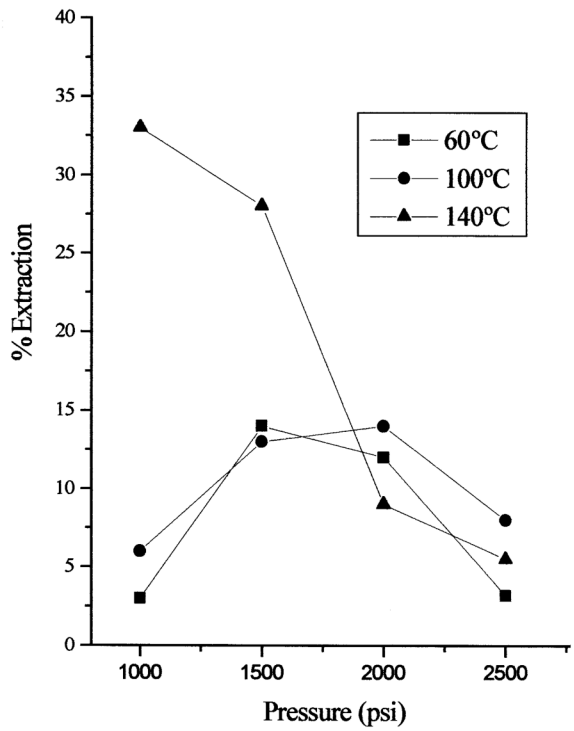

Figure 6. Recoveries of Mo^{VI} as a function of pressures (1000–2500 psi) at different temperatures (40–140°C). Coditions: 3.0 mL of methanol (extraction vessel, 10 mL); 5.0 min in the static mode, followed by 3.0 min in the dynamic mode; filter paper with 1.0 mg of Mo^{VI} and 250 µL of acetylacetone, placed in the extraction cell.

quantitation was made using the standard addition method.

The molybdenum complex is adsorbed in the hanging mercury electrode. The reduction of the Mo^{VI} complex to Mo^{V} occurs by scanning to negative potential, which in the presence of bromate or chlorate (supporting electrolyte) oxidizes to Mo^{VI}. This catalytic process produces an increasing current, and the method is more sensitive.

Extraction of Mo^{VI} from water and pasture samples

Figure 7 shows the cyclic voltammograms of the $MoO_2(acac)_2$ extracted from a pasture sample and the posterior addition of Mo^{VI} standard solution. Mo was quantitated using the standard addition method. The pasture samples contained 21.8, 23.5, 23.4, 20.3, and 22.6 mg/K (dry weight) Mo. The water samples contained 8.3, 8.9, 7.7, 10.3, and 8.8 mg/L Mo.

Conclusion

SFE has become a very simple technique for the treatment of biological samples such as blood, milk, and urine. Repeated measurements were made, and it was possible to prove that by applying the parameters previously mentioned (pressure, temperature, times, etc.), up to 1.9 mg of Pb^{2+} and 1.0 mg Mo^{VI} could be extracted, forming $Pb(DDC)_2$ and $MoO_2(acac)_2$ complexes, respectively. For the extraction of smaller amounts, the measurement times could be shorter. Also, this technique provided a reaction method to obtain the $MoO_2(acac)_2$ complex in methanol solution, because this reaction is not possible under standard conditions.

On the other hand, the sensitivity of the GC detector (FID) is generally not good for the quantitation of metallic complexes, but just a few microliters are needed for analysis. Therefore, when the solvent is evaporated, an important concentration factor is obtained. The addition of metallic ions to real samples that do not have them is a good method to check the efficiency of the extraction.

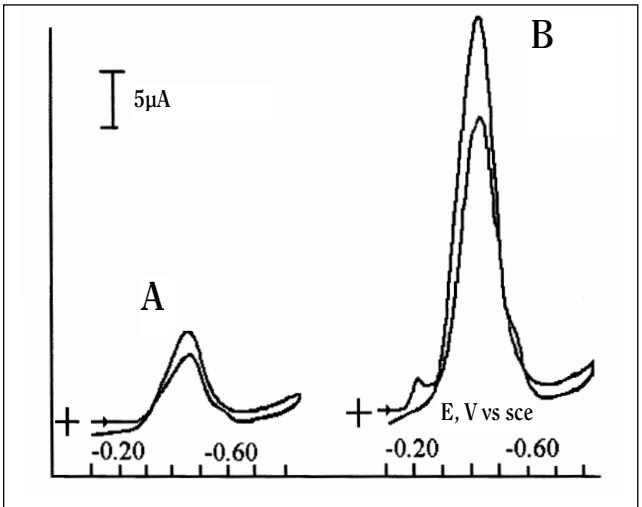

Figure 7. Cyclic voltammograms of the $MoO_2(acac)_2$ complex solution metanol–water (1:9) (A) and added to 50 µL of Mo^{VI} standard solution (aqueous 10.0 mg/L) (B). The support electrolyte is 0.1M potassium chlorate (pH 4.5) sodium acetate–acetic acid. Scan rate, 0.05 V/s; adsorption potential, –0.10 V versus sce; adsorption time, 5.0 min (1.0 mL of Mo complex extracted from a pasture sample).

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