Preconcentration and determination of vanadium and molybdenum in milk, vegetables and foodstuffs by ultrasonic-thermostatic-assisted cloud point extraction coupled to flame atomic absorption spectrometry

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ARTICLE INFO

ABSTRACT

A new ultrasonic-thermostatic-assisted cloud point extraction procedure (UTA-CPE) was developed for preconcentration at the trace levels of vanadium (V) and molybdenum (Mo) in milk, vegetables and foodstuffs prior to determination via flame atomic absorption spectrometry (FAAS). The method is based on the ion-association of stable anionic oxalate complexes of V(V) and Mo(VI) with [9-(diethylamino) benzo[a]phenoxazin-5-ylidene]azanium; sulfate (Nile blue A) at pH 4.5, and then extraction of the formed ion-association complexes into micellar phase of polyoxyethylene(7.5)nonylphenyl ether (PONPE 7.5). The UTA-CPE is greatly simplified and accelerated compared to traditional cloud point extraction (CPE). The analytical parameters optimized are solution pH, the concentrations of complexing reagents (oxalate and Nile blue A), the PONPE 7.5 concentration, electrolyte concentration, sample volume, temperature and ultrasonic power. Under the optimum conditions, the calibration curves for Mo(VI) and V(V) are obtained in the concentration range of 3–340 $\mu g\,L^{-1}$ and 5–250 $\mu g\,L^{-1}$ with high sensitivity enhancement factors (EFs) of 145 and 115, respectively. The limits of detection (LODs) for Mo(VI) and V (V) are 0.86 and 1.55 $\mu g\,L^{-1}$, respectively. The proposed method demonstrated good performances such as relative standard deviations (as RSD %) ($\leq 3.5\%$) and spiked recoveries (95.7–102.3%). The accuracy of the method was assessed by analysis of two standard reference materials (SRMs) and recoveries of spiked solutions. The method was successfully applied into the determination of trace amounts of Mo(VI) and V (V) in milk, vegetables and foodstuffs with satisfactory results.

1. Introduction

Although molybdenum (Mo) and vanadium (V) are essential elements for cell growth at $\mu g\,L^{-1}$ levels, the high concentrations of both elements have toxic effects on human health [1]. The importance of Mo for biological systems is that it has active role in the act of some enzymes such as xanthine oxidase (XO), aldehyde oxidase (AO), and sulfite oxidase (SO) situated in carbohydrate metabolisms [2]. On the contrary, some health problems like severe gastrointestinal irritation, cardiac failure, bone deformation, teeth vulnerable to caries and disturbances are appeared depending on the intake of high concentrations of Mo [3]. According to report of the Food & Nutrition Board of the Institute of Medicine (FNB), daily intake limit of Mo should be 45 $\mu g\,day^{-1}$ body weight for people ages > 19 years [4]. The V is essential for some

biological events like insulin receptivity, prevention of vascular diseases, glucose metabolism and carbohydrate metabolism [5]. The vanadium species, V(V), V(IV) and V(III) can especially reduce growth of cancer cells in low concentrations [6]. Despite these features, it causes toxic effects on the hematological, biochemical, reproductive at high concentrations [7]. In addition to, its inorganic forms are the most toxic, and the V(V) more toxic than V (IV) and V(III). Because of these reasons, the simultaneous detection and determination of the V and Mo in the real time samples with rapid, economical and suitable method is an important topic for analytical chemists.

Because there is usually present at low concentrations of V and Mo in the selected samples (milk, vegetables and foodstuffs), using the preconcentration method is necessary prior to their determination by flame atomic absorption spectrometry (FAAS). In this study, ultrasonic-thermostatic-assisted cloud point extraction procedure (UTA-CPE) was used as preconcentration tool. The UTA-CPE is greatly simplified and accelerated compared to traditional cloud point extraction (CPE), because the use of ultrasonic power

has improved the conditions (low temperature, less time and low reagent usage) that is required for preconcentration. Furthermore, the UAT-CPE method has many advantages such as high preconcentration factor, cheap, highly efficient, lower toxicity and simple compared to other separation/preconcentration methods (liquid–liquid extraction and solid phase extraction) [8].

Many analytical methods such as adsorptive cathodic stripping voltammetry (AdCSV) in water and foodstuff samples [9], spectrophotometry in plant foodstuff samples [3], inductively coupled plasma–optical emission spectrometry (ICP-OES) in gasoline samples [10], electrothermal atomic absorption spectrometry (ETAAS) in milks and environmental samples [11,12], graphite furnace atomic absorption spectrometry (GF-AAS) in waters and solids [13,14], inductively coupled plasma mass spectrometry (ICP-MS) in chicken, duck, goose, and pigeon eggs [15], inductively coupled plasma-atomic emission spectrometry (ICP-AES) in environmental water samples [16], high performance liquid chromatography inductively coupled plasma mass spectrometry (HPLC-ICP-MS) in ground waters and solids [17] and flame atomic absorption spectrometry (FAAS) in foods/beverages and corn samples [18,19] in the last decade have been developed for the preconcentration and determination of the Mo and V in the various samples. Some of the instrumental techniques for simultaneous determination of V and Mo in the various samples have disadvantages such as expensive, time consuming due to long furnace program, poor precision at especially low concentrations, spectral background interferences as well as memory effect (ICP-OES, ICP-MS and GF-AAS) and few others require specific electrodes (AdCSV) [20]. Among these methods for the analysis of metals, the FAAS has a great attraction for its simplicity, low cost, sensitivity and availability in almost every analytical research laboratory [21]. Due to use of the hollow-cathode lamp as radiant source at a specific resonance wavelength, the FAAS also provides advantages such as low interference effect and element selectivity.

The aim of this study was to develop a new and simple UTA-CPE method for separation/preconcentration of these essential elements from different matrices such as milks, vegetables and foodstuffs prior to determinations of Mo(VI) and V(V) using the FAAS. The Nile Blue A reagent *(9-(diethylamino)benzo[a]phenox-azin-5ylidene]azanium sulfate)* was selected as an ion-pairing reagent, and sensitive and stable ternary complexes of Mo(VI) and V(V) ions were obtained in the presence of oxalate as primary chelating ligand and the PONPE 7.5 as extracting agent at pH 4.5. The effect on analytical signal of the parameters such as pH, surfactant type and concentration, concentrations of complexing agents, sample volume, the effect of foreign ions, equilibration temperature and ultrasonic power were carefully investigated to obtain maximum analytical signal for each analyte. Analysis of two standard reference materials (SRMs) was also performed to test the validity of the proposed method.

2. Experimental

2.1. Instrumentation and reagents

A Shimadzu AAS-6300 model atomic absorption spectrophotometer with a D_2 background correction was used for determination of trace levels of the mentioned analytes using their hallow cathode lamps. All measurements for each analyte were carried out in an N_2O/acetylene flame. Flow rates for N_2O/acetylene for vanadium and molybdenum are 7.0 L min^{-1}/11 L min^{-1} and 6.5 L min^{-1} and 13 L min^{-1}, respectively. An ultrasonic cleaner (UCS-10 model, Seoul, Korea) maintained at the desired temperature was used for preconcentration experiments and digestion of the samples. A centrifuge (Universal-320, Hettich Centrifuges,

DJB Labcare Ltd., Newport Pagnell, UK) was used for separation of the aqueous and surfactant-rich phases, and VM-96B model vortex mixer (Seoul, Korea) was used to fasten the extraction process and minimize the reagent consumption. The pH measurements were carried out with a pH meter (pH 2005, JP Selecta, and Barcelona, Spain).

All reagents used were of analytical grade, and ultra-pure water obtained from a Labconco water purification system (Kansas City, USA) was used throughout the experiments. The stock solutions of 1000 mg L^{-1} Mo(VI) and V(V) were prepared using $Na_2MoO_4 \times 2H_2O$ and NH_4VO_3 (Sigma-Aldrich Co., St. Louis, MO, USA) with ultra-pure water, respectively, and were diluted daily for obtaining reference and working solutions. Acetate buffer solutions at pH 4.5 were prepared by mixing appropriate volumes of 1.0 mol L^{-1} acetic acid and 1.0 mol L^{-1} sodium acetate solutions. The ion-pairing reagent solution, Nile blue A (3.0×10^{-3} mol L^{-1}) was prepared by dissolving an appropriate amount of reagent (NBH$^+$, Sigma) in the water. A 2.0×10^{-2} mol L^{-1} solution of oxalate ($C_2O_4^{-2}$) (Sigma) was prepared by dissolving appropriate amounts of sodium oxalate. A 2.0% (w/v) the PONPE 7.5 (sigma) was prepared by dissolving 2.0 g of the PONPE 7.5 and 20 mL ethanol and then completed to 100 mL volumetric flask stirring with the water. Before starting of experiment, all pipettes, glassware and PET bottles used for trace analysis were treated firstly with 10 (v/v)% HNO$_3$ solutions, and with diluted HCl solution, then rinsed in ultra-pure water and dried in an oven at 50 °C. Lastly, all the materials were washed five times with the water.

2.2. The UTA-CPE procedure

First, an aliquot of calibration standards and pretreated samples containing Mo(VI) and V(V) in the range of 3–340 µg L^{-1} and 5–250 µg L^{-1}, respectively, were placed in a 50 mL conical tube for construction of analytical curve. After that, 2.5×10^{-4} mol L^{-1} $C_2O_4^{2-}$, 2.0×10^{-3} mol L^{-1} NBH$^+$ and 0.45% (w/v) PONPE 7.5 were added, and the pH of the mixtures was adjusted to 4.5 using 0.1 mol L^{-1} acetate buffer solutions. The mixtures were then diluted with the water to 50 mL. The mixture for complete reaction was allowed to stand for 5 min, and incubated in an ultrasonic water bath performed with ultrasound frequency of 40 kHz at 300 W and 50 °C for 15 min. In order to separate into two separate phases, the mixture was centrifuged at 3500 rpm for 5 min. After keeping the resulting mixture for 10 min in ice bath, the aqueous phase was easily separated by using a syringe with a long needle that passed through the surfactant-rich phase. The surfactant-rich phase obtained was diluted with 1.0 mL of 1 mol L^{-1} HNO$_3$ in ethanol prior to determination of V and Mo via the FAAS.

2.3. Sampling

Milk samples (goat's milk, sheep's milk, full-fat cow's milk, semi-skimmed cows' milk) and foodstuff samples (cereals, and whole meal biscuit) were collected from local supermarkets in Sivas. Vegetable samples (mint, green beans, lentils, carrots, cabbage, green tea, tomato and mushroom) were collected from local greengrocers in Sivas, Turkey. The vegetables were placed in plastic bags. The samples were brought to the analytical chemistry research laboratory in which they were stored at -10 °C until sample prepared. The blank of the reagents were carried out following the same procedure without milk, vegetable and foodstuff samples. All measurements and processing were performed at least in five times.

2.3.1. Sample preparation for milks

A wet digestion procedure for the preparation of milk samples was used. Sample preparation steps are as follows; a 15 mL portion