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Article

Preconcentration Method on Modified Silica Fiber for Chromium Speciation

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A new method involving pre-concentration on modified silica fiber is described for the speciation of chromium(III) [Cr(III)] and chromium(VI) [Cr(VI)] in aqueous media. This method is based on the different chelating behavior of Cr(III) and Cr(VI) with morpholine-4-carbodithioate (MDTC). Both complexes are extracted on silica fiber modified by sol-gel technology by using 3-aminopropyltriethoxysilane (APS) as a precursor. All extracted samples are directly injected into an high-performance liquid chromatography injector for the simultaneous determination of Cr(III) and Cr(VI). Cr(VI) forms two different complexes, and Cr(III) forms a single complex with MDTC. Therefore, the concentration of Cr(VI) is determined directly from the peak area obtained at 5.4 min; whereas, the assay of Cr(III) is based on subtracting the peak area of Cr(VI) from the total peak area obtained at 4.3 min. Under the optimized conditions, the limits of detection for Cr(III) and Cr(VI) are found to be 0.7 ng/mL and 0.2 ng/mL, respectively.

Introduction

The deleterious effects of chromium depend on the species present in the environment. Chromium(III) [Cr(III)] is an essential nutrient, whereas chromium(VI) [Cr(VI)] is found to be very toxic (1, 2). Usually, chromium species come from industries due to their use in metallurgy processes (mainly as alloys) and as components in arc welding and leather tanning (where it is used to denature proteins in the skin). Consequently, there are many exposure routes to man, and the potential detrimental health effects of the chromium species are of concern (3). A common problem with chromium speciation is the well-known inter-conversion of Cr^{3+} and Cr^{6+}; therefore, it is very important to develop a sensitive and selective method, which truly represents the original concentration in the sample (4–7).

These days, solid phase pre-concentration techniques (SPE and SPME) have gained a wide acceptance for the determination of various analytes (8–10). These techniques involve the extraction of an analyte on some solid phases comprised of inorganic polymeric surfaces. Solid-phase microextraction (SPME), a time-efficient and solvent-free extraction technique, has already been applied for the extraction of trace levels of organic and inorganic pollutants (11, 12). A variety of different coating materials have been used to achieve a selective determination of various analytes by using SPME (13, 14). Commercially available extracting phases are comprised of nonpolar [poly(dimethylsiloxane) (PDMS) and carboxen–PDMS], semipolar (PDMS–divinylbenzene), and polar (polyacrylate; Carbowax–divinylbenzene and Carbowax–templated resin). SPME fibers possess some important drawbacks due to their instability at a higher temperature and swelling in organic solvents. Low thermal and chemical instability of the fiber may arise due to a lack of chemical bonding between the coating and fiber surface. To overcome this problem, sol-gel technology has been used for the incorporation of organic moieties in inorganic polymeric network, which improves the selectivity and stability of fiber. Sol-gel chemistry provides a simple and convenient pathway for the synthesis of inorganic polymers and advanced hybrid material systems, which can be used as surface coatings. It also offers efficient incorporation of the organic components into inorganic polymers under mild thermal conditions. It is advantageous to use sol-gel technology due to low costs, ability to achieve molecular level uniformity in the synthesis of organic-inorganic hybrids, and strong adhesion properties of the coating, which are responsible for its use in the modification of SPME fibers (15).

In the past, some sensitive and accurate methods have been developed for the determination of trace amounts of various elements, such as copper, nickel, cobalt, and palladium in various samples by using an SPME–high-performance liquid chromatography (HPLC)–UV method (16–18). In a recent approach, the speciation of Cr(III) and Cr(VI) were reported as morpholine-4-carboditioate complexes using liquid–liquid extraction (LLE) with an HPLC–UV system (19). The method involves a complex formation of chromium species with morpholine-4-carbodithioate at pH 4 with subsequent LLE using chloroform at 55°C. These pre-concentrated species were separated on a C_{18} column and analyzed by an HPLC–UV system. The analysis conditions were optimized by studying the effect of the ligand concentration, pH, temperature, composition of the mobile phase, and various metal ions. It is a traditional method for sample preparation and employs a multistep procedures, which makes it time consuming. It uses extensive amounts of organic solvents, and sometimes shows a high risk for loss of the analytes. Therefore, in the present method, an effort is made to reduce the use of the organic solvent by using modified silica fiber for the extraction of chromium species.

Herein, a simple and convenient method is reported for the speciation of Cr(III) and Cr(VI) as morpholine-4-carbodithioate complexes by using modified silica fiber as an extraction surface. Silica fiber is modified by incorporating 3-aminopropyltriethoxysilane in a three dimensional network formed by polydimethylsiloxane on the surface of silica by using the reported method (20). The speciation of metal ions by using modified silica fiber is not reported in literature; therefore, this method extends the potential of SPME in the field of

the analysis of metal ions. The method is found to be advantageous over classical methods as it is sufficiently sensitive and selective. It also provides a reduction in analysis time, lower cost of operation, as well as the elimination of a large amount of organic solvent during the extraction procedure. In addition, the fiber used during the process is more stable and selective than commercially available fibers.

Materials and Methods

Instruments and equipment

Waters HPLC system consisting of a Waters 515 solvent delivery HPLC pump, a sampling valve with a 20 μL sample loop, a Waters Spherisorb column of 250 mm × 4.6 mm (i.d.) filled with C_{18} material (5 μm ODS2) and a Waters 2996 Photodiode array detector was used for this project (Waters, Milford, MA). The signal from the detector was processed by an Empower 2 PDA software package and the chromatograms were monitored on an interfaced computer.

Chemicals and reagent

All the solvents used were of HPLC-grade purchased from Acros Organics and filtered by using nylon 6,6-membrane filters (Rankem, India) in a filtration assembly (from Perfit, India) before using in an HPLC system. Chromium(III) chloride, potassium dichromate, sodium acetate, acetic acid, sodium hydroxide, and sodium chloride were obtained from Merck (India). 3-(Triethoxysilyl)propylamine, hydroxy-terminated polydimethylsiloxane, poly(methylhydrosiloxane), dichloromethane, and trifluoroacetic acid were purchased from Sigma Aldrich (Darmstadt, Germany). All HPLC solvents were degassed with an ultrasonic bath prior to use. Fused-silica fiber, with protective polyimide coating, was obtained from Polymicro Technologies Inc. (Phoenix, AZ). Morpholine-4-carbodithioate (MDTC) reagent was prepared in the lab by using the method reported by Macrotrigiano et al. (21). It was purified by recrystallization with 2-propanol. The purity was checked by recording the melting point and the IR spectra on a Perkin-Elmer FTIR. A sodium acetate–acetic acid buffer of pH 4.0 was prepared by mixing 847 mL of 0.1 M acetic acid and 153 mL of 0.1 M sodium acetate solution, and the pH was checked by using a digital pH meter.

Pretreatment of the silica fiber

The silica fiber was activated to expose the maximum of the silanol groups before employing a coating procedure. It involves the removal of a segment of the protective polyimide layer from (about 1 cm out of 6 cm length) one end of the fiber by burning off the polyimide protective layer using a burner flame. The exposed silica fiber was cleaned with methanol for 1 h, dried, and immersed into NaOH solution (1 mol/L) for 1 h. Then, it was held inside HCl solution (0.1 mol/L) for ∼ 30 min for activation. It was rinsed with deionized water and dried at 120°C for ∼ 1 h.

Preparation of the sol solution

The sol solution was prepared as follows: 100 μL of 3-aminopropyltriethoxysilane (precursor), 200 mg of hydroxy-terminated poly(dimethylsiloxane) (coating polymer), 50 mg of poly(dimethylhydrosiloxane) (deactivation reagent), and 80 μL of 95% TFA (acid catalyst containing 5% water) were thoroughly mixed in a plastic vial at room temperature. The mixture was then transferred into an Eppendorf microcentrifuge tube and centrifuged at 9000 rpm for 15 min. After the phase separation, an upper clear layer was removed by syringe and used for coating purpose.

The pretreated fiber was immersed in sol solution for ∼ 30 min. The process was repeated three times by preparing a fresh sol solution. The coated fiber was kept in a desiccator for ∼ 6 h, followed by conditioning at different temperatures.

Preparation of metal complexes

For the determination of chromium species, 1 mL sodium acetate–acetic acid buffer solution (pH 4) and 1 mL 0.01 % (w/v) MDTC solution were added into a 5-mL sample vial, then Cr(III)–Cr(VI) was added. The total volume was made to 5 mL with de-ionized water. Subsequently, the sample solution was heated with constant stirring on a magnetic stirrer. The contents were reduced to 4 mL and used for extraction. A similar procedure is followed for the mixture of Cr(III) and Cr(VI).

Extraction on the modified silica fiber

To the sample solution prepared as described, 10% (w/v) NaCl was added. Modified silica fiber was inserted into the sample tube, and the fiber was exposed to the sample solution for ∼ 25 min. The solution was stirred continuously so that the maximum number of analytes was exposed to the fiber for sorption. After extraction, the fiber was introduced into a sample tube containing 0.5 mL of acetonitrile for ∼ 15 min, which resulted in the complete desorption of the analytes. The sample solution containing metal complexes was injected into the HPLC for separation on a C_{18} column and detection at 254 nm. The fiber was washed with methanol, and it was ready to use for another sample.

Results and Discussion

Possible mechanism of the coating process

In sol-gel chemistry, a gel can be formed by the simultaneous hydrolysis and polycondensation of a silane precursor, followed by aging and drying under ambient atmospheres (20). The silica surface of the fiber consists of two types of functional groups [i.e., the siloxane (Si–O–Si) and the silanol (Si–OH) groups). The modification of silica fiber occurs mainly through the reaction of functional moiety with the Si–OH group of silica surface. Aminopropyltriethoxysilane (APS) is a very efficient precursor and undergoes polymerization at a neutral pH. It forms a stable coating on the surface of the silica fiber. APS contains $-NH_2$ functionality, which is capable of forming a hydrogen bond with various analytes. This characteristic of APS is responsible for its use as a component in the coating of a stationary phase. A schematic illustration of the steps involved from making sol-gel derived APS–SPME coating to the sorption of metal complexes is represented by the following steps:

Step 1: Hydrolysis of precursor

Hydrolysis of ethoxysilanes can be catalyzed by the presence of acids or bases. APS precursor used in this study was hydrolyzed by reacting with water in the presence of acid catalyst (Step 1).

Step 2: Condensation of hydrolyzed products

The hydrolyzed precursor undergoes condensation to form a three dimensional polymeric network (Step 2).

Step 3: Polycondensation with coating polymer

In this approach, hydroxyl terminated poly(dimethylsiloxane) is used as a coating polymer due to the presence of terminal hydroxyl groups. These groups can be polycondensed with the precursor to form a cross-linked polymer. The use of a coating polymer gives strength to the growing silica network, as well as provides an anchorage to the silanol groups of the silica fiber. The chemical reaction involved can be schematically represented by the following equation (Step 3).

Step 4: Sol-gel Coating

The growing network of the polymer is used for the coating of the silica fiber by simply dipping the fiber with exposed silanol groups into the obtained gel. The silanol groups of the silica fiber underwent condensation with −OH groups of a three-dimensional polymeric network to form a covalent bond (Step 4). This makes the coating formed by sol-gel technology more stable when compared with other techniques.

Step 5: Deactivation of reaction

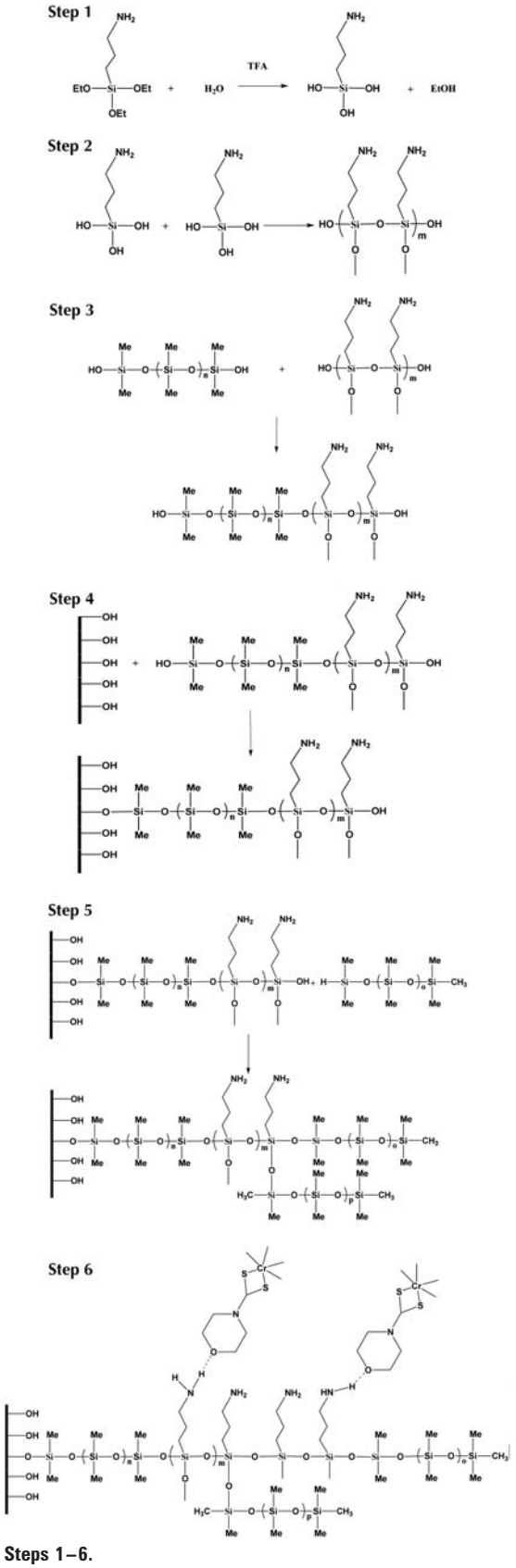
The final step of this method, deactivation of the growing silica network, can be achieved by adding poly(methylhydrosiloxane) (PMHS). The addition of PMHS to the reaction mixture deactivates to the free −OH groups. PMHS is considered as a suitable deactivating reagent because of its similar structure with PDMS (Step 5).

Mechanism of sorption of metal complexes

Metal complexes were prepared by adding a suitable amount of MDTC to the aqueous solution of the metal ions at pH 4. All the optimized parameters for the complex formation, separation, and detection of complexes are already reported in a previous work (19). Metal complexes of Cr(III) and Cr(VI) with morpholine-4-carbodithioate are not soluble in water. Therefore, these can be extracted directly from the aqueous solutions by sorption on solid supports.

In this project, sorption of metal complexes is achieved on a lab-made SPME fiber. The extent of sorption can be increased by using sodium chloride, which reduces the solubility of metal complexes due to salting out effect. Metal complexes get sorbed on the surface of APS–SPME fiber from aqueous medium.

The most probable interaction between the metal complexes and the amino functionality is a hydrogen bond, which leads to an easy sorption of metal complexes on the modified silica fiber (Step 6). The silanol mediated polar interactions are other possible contacts for the sorption of complexes. Modified fiber coating possesses high surface area due to the porous nature of



Steps 1–6.

polymeric surface, which results in complete sorption of complexes in about 25 min.

Determination of Cr(III) and Cr(VI)

It is well known that Cr(III) forms a 1:3 complex, $Cr(MDTC)_3$, with morpholine-4-carbodithioate, whereas Cr(VI) forms a different product $Cr(MDTC)_2(OMDTC)$ in addition to $Cr(MDTC)_3$, in which an oxygen atom is introduced between one of the Cr–S bonds (20, 22, 23). This different behavior of both metal ions towards dithiocarbamates makes their determination easier. The complexes were prepared in excess for their characterization by IR and UV–vis spectroscopy. The IR spectrum of Cr(VI) showed a band at 878 cm^{-1} due to S–O stretching vibrations.

In the case of Cr(III), only the single peak at 5.4 min (Peak B) is observed due to the $Cr(MDTC)_3$ complex, as shown in

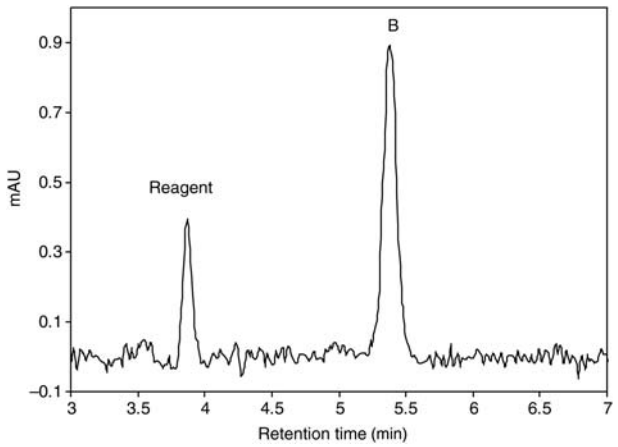


Figure 1. HPLC–PDA chromatogram for Cr(III) (25 ng/mL). The extraction conditions on the modified silica fiber were as follows: sorption time, 25 min; NaCl, 10% w/v; desorption time, 15 min in acetonitrile; column, C_{18}; mobile phase, 70:30 (acetonitrile–water); detection, 254 nm.

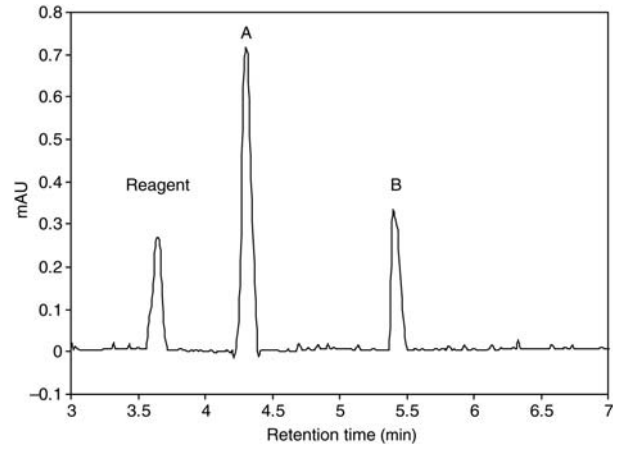


Figure 2. HPLC–PDA chromatogram for Cr(VI) (25 ng/mL). The extraction conditions on the modified silica fiber were as follows: sorption time, 25 min; NaCl, 10% w/v; desorption time, 15 min in acetonitrile; column, C_{18}; mobile phase, 70:30 (acetonitrile–water); detection, 254 nm.

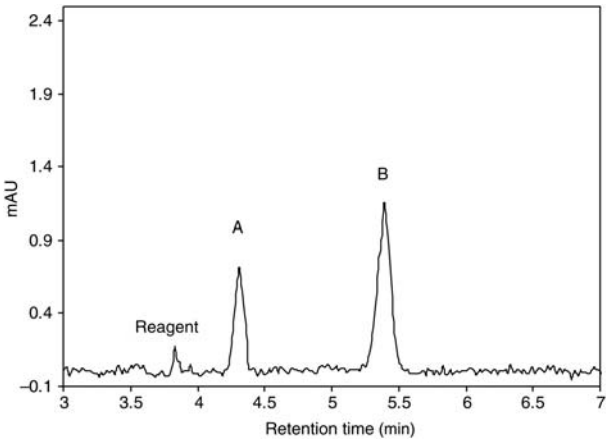


Figure 3. HPLC–PDA chromatogram for the mixture of Cr(III) (40 ng/mL) and Cr(VI) (40 ng/mL). The extraction conditions on the modified silica fiber were as follows: sorption time, 25 min; NaCl, 10% w/v, desorption time, 15 min in acetonitrile; column, C_{18}; mobile phase, 70:30 (acetonitrile–water); detection: 254 nm.

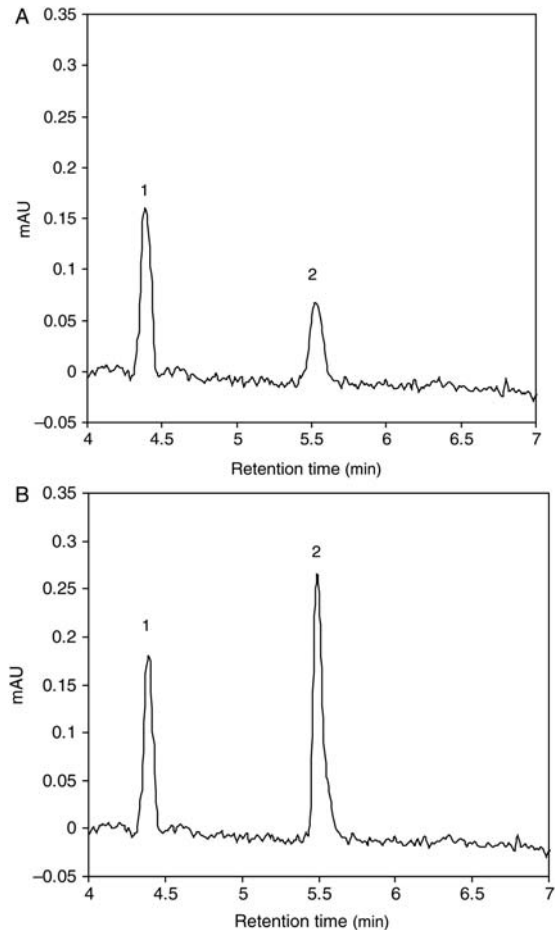


Figure 4. HPLC–PDA chromatogram (A) of the water sample spiked with Cr(VI) (5 ng/mL), and (B) the water sample spiked with Cr(III) (5 ng/mL) and Cr(VI) (5 ng/mL). The extraction conditions on the modified silica fiber were as follows: sorption time, 25 min; NaCl, 10% w/v; desorption time, 15 min in acetonitrile; column, C_{18}; the mobile phase, 70:30 (acetonitrile–water); detection, 254 nm.

the chromatogram (Figure 1). Whereas both the complexes, $Cr(MDTC)_3$ and $Cr(MDTC)_2(OMDTC)$, formed by Cr(VI), are eluted at different retention times [i.e., 4.3 (Peak A) and 5.4 min (Peak B), respectively (Figure 2)]. The calibration curves were prepared by measuring the area of Peak A for Cr(VI) and Peak B for Cr(III). A chromatogram for the mixture of Cr(III) and Cr(VI) is shown in Figure 3. In the mixture, the concentration of Cr(VI) can be calculated directly from the regression equation by measuring the area of Peak A, while Cr(III) can be determined by subtracting the value for Cr(VI).

The calibration curves were prepared under the optimized conditions for the analytes spiked with 2.0, 10.0, 20.0, 30.0, and 40.0 ng/mL. The peak areas were used to prepare the calibration curve. The calibration curves were linear over this range of 2.0–80.0 ng/mL and 5.0–90.0 ng/mL for Cr(VI) and Cr(III), respectively. The linearity was calculated, and the coefficient of determination was found to be in the range 0.990–0.993 for both species. The analytical characteristics of the method (i.e., the detection limit, precision, and linearity) were evaluated for these metal complexes based on 3σ of baseline noise. The relative standard deviation was found to be 1.2% and 1.7% for Cr(III) (25 ng/mL) and Cr(VI) (25 ng/mL), respectively.

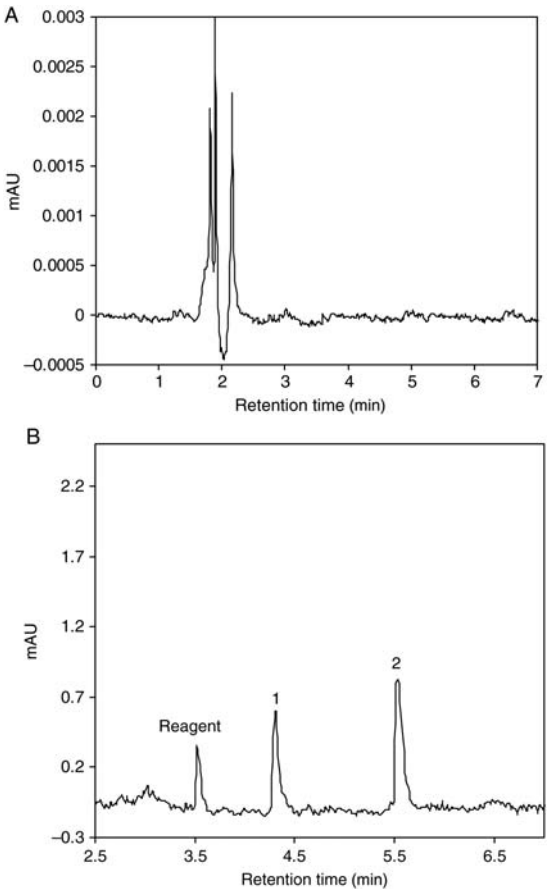


Figure 5. HPLC–PDA chromatogram for the (A) unspiked water sample, and (B) the water sample spiked with a mixture of Cr(III) (20 ng/mL) and Cr(VI) (20 ng/mL). The extraction conditions on the modified silica fiber were as follows: sorption time, 25 min; NaCl, 10% w/v; desorption time, 15 min in acetonitrile; column, C_{18}; the mobile phase, 70:30 (acetonitrile–water); detection, 254 nm.

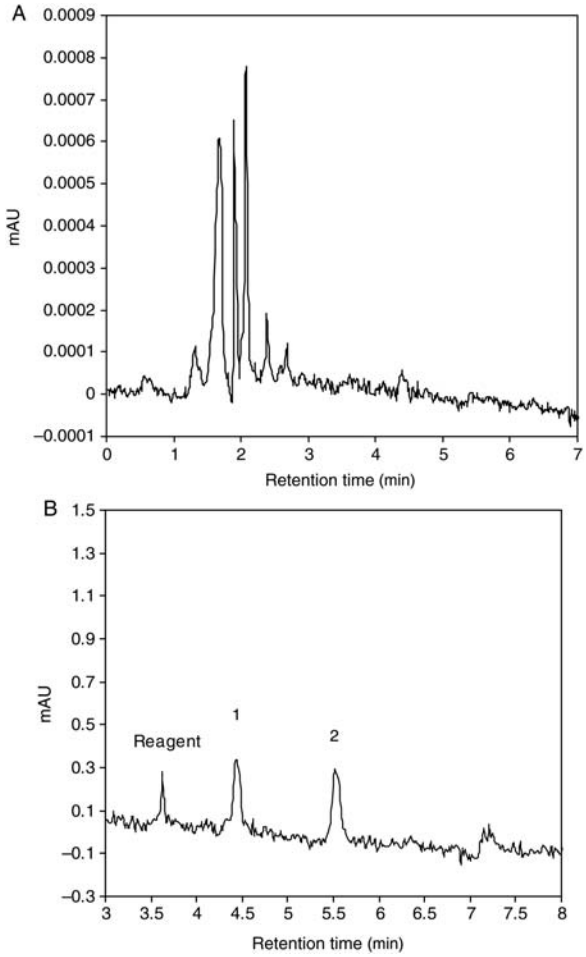


Figure 6. HPLC–PDA chromatogram (A) the unspiked water sample, and (B) the water sample spiked with Cr(III) (5 ng/mL) and Cr(VI) (15 ng/mL). The extraction conditions on the modified silica fiber were as follows: sorption time, 25 min; NaCl, 10% w/v; desorption time, 15 min in acetonitrile; column, C_{18}; mobile phase, 70:30 (acetonitrile–water); detection, 254 nm.

Table I
Determination of Cr(III) and Cr(VI) in Drinking Water Samples

	Cr(III)			Cr(VI)		
Sample No.	Spiked conc. (ng/mL)	Found conc. (ng/mL)	Recovery (%)	Spiked conc. (ng/mL)	Found conc. (ng/mL)	Recovery (%)
1	5.00	4.58	91.60	15.00	14.25	95.00
2	20.00	19.14	95.70	5.00	4.66	93.20
3	15.00	13.80	92.00	10.00	9.31	93.10
4	10.00	9.57	95.70	20.00	18.56	92.80

To check the validity of the developed method, it was applied to drinking water by spiking different amounts of Cr(III) and Cr(VI) (Figure 4–6). An appropriate aliquot of the solution was used for the determination of chromium species by the procedure given earlier. The amounts of metal ions were calculated from the regression equation obtained from the calibration curve. The results are shown in Table I, and the calculated recoveries were found to be more than 90%.

Table II
Comparison of the Proposed Method with Different Methods for Speciation of Cr(III) and Cr(VI)

Method	Matrix	Reagents and surfaces used for extraction	Detection limit (ng/mL) Cr(III)	Cr(VI)	Ref.
CPE–HPLC	Sediment samples	1-(2-thiazolylazo)-2-naphthol and octylphenoxypolyethoxyethanol	7.5	3.5	1
CPE–GFAAS	Natural water samples	1-phenyl-3-methyl-4-benzoylpyrazol-5-one	0.2	–	2
LLE–GF–AAS	Water sample	Ethyl acetate	0.2	–	5
Micellar media	–	α-Benzoin oxime, triton X-100	0.8	–	6
SPE–RP–LC–UV	Standard reference water sample	Ammonium pyrrolidinedithiocarbamate, (a) Licrospher 100 RP-8 (R)	0.2	0.1	7
		(b) Lichrospher 60 RP-18 (R)	0.2	0.06	
SPE–FAAS	Certified reference materials	Nb_2O_5-SiO_2	0.34	4.6	8
SDME–DRS–FTIR	Human biological fluid	N^1-hydroxy-N^1,N^2-diphenylbenzamidine	–	0.5	3
SPME–GC–FPD	–	1,1,1-trifluoroacetylacetone, Silica fiber	2	–	4
SPME–HPLC–PDA	Water sample	Morpholine-4-carbodithioate, modified silica fiber	0.7	0.2	Presented method

Comparison with existing methods of extraction

A comparison of the developed method with the existing extraction methods is summarized in Table II. The limit of detection for the presented method is found to be better than some other methods. Classical extraction techniques like LLE (24–27) involve extensive use of organic solvents, which has become less desirable, as these solvents are expensive and harmful to the environment. A solvent-free or low-solvent consumption method used for the speciation of chromium such as SPE (28–29) overcomes the limitations of LLE, but it is still time consuming. SPME (30–31) has been proposed as a promising alternative to LLE due to its simplicity of use, high pre-concentration power and ability to extract volatile species. It was used in hyphenation with GC for the determination of chromium species but the presented method showed improved detection limits, which may be attributed to more selectivity of the modified silica fiber.

Conclusion

This study demonstrated that the developed method is a simple, rapid, precise and sensitive method for the speciation of chromium species from aqueous samples in the form of their complexes with morpholine-4-carbodithioate. Complete separation of both species was achieved in about 6 min. The detection limits for the method were found to be in the range of ppb levels for these metal ions, being better than other LLE methods. Modification of silica fiber by using sol-gel method was found to be simple, which resulted in the formation of more stable and selective fiber.

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