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Separation and Determination of Chromium (III) Chromium (VI), Gold (III) and Arsenic (V) by Capillary Zone Electrophoresis Using 2-Acetylpyridine-4-phenylthiosemicarbazone as Complexing Reagent

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Summary: Capillary zone electrophoretic procedure has been developed for the speciation of Cr (III) and Cr (VI) along with separation from Au (III) and As (V) as chelate compounds of 2-acetylpyridine-4-phenylthiosemicarbazone (APPT). APPT reacts with Cr (III), Cr (VI), Au (III) and As (V) to form water-methanol soluble chelates which absorb maximally within 360 - 418 nm with molar absorptivity of 3.2×10^3 to $1.2{\times}10^4$ L $mol^{-1}cm^{-1}$. Off line pre-capillary complexes of the cations prepared were introduced onto uncoated fused silica capillary of 54 cm effective length with 75 μm id by auto-sampler and separated with background electrolyte (BGE) system of oxalate buffer at pH-4 at an applied voltage of +15 kV. Photodiode array detection was carried out at 241 nm. Linear calibrations were obtained within 1-80 μgmL^{-1} for all the cations. The separation and quantitation was repeatable in terms of migration time and peak height with relative standard deviation (RSD) within 2% (n=4). The limit of detection (LOD) measured were 0.125 μgmL^{-1}, 1.0 μgmL^{-1}, 0.2 μgmL^{-1} and 0.1 respectively for Au(III), As(V), Cr (III) and Cr(VI). The limits of quantitation (LOQ) were in the range of 0.375- 3.0 μgmL^{-1}. The method was applied for the determination of Cr (III) from tap water, Cr (VI) from industrial effluents and Au (III) and As (V) from pore water of coal mines with RSD within 4%. The results of analysis were supported by standard addition method and rechecked by atomic absorption spectrometry (AAS).

Key Words: Capillary zone electrophoresis, chromium speciation, gold and arsenic, pre-capillary derivatization, cations separation.

Introduction

Various environmental, medical, geological and biological applications require the accurate determination of different oxidation states of elements. The properties (chemical and physical) of an elemental species depend on its oxidation state and their determination in each species is important to estimate their possible risks. For chromium (Cr), Cr (VI) and Cr (III) are considered as biologically and environmentally important oxidation states [1]. Cr (VI) is a reported toxin even in very small concentration but Cr (III) is considered as crucial metal for insulin working in human if taken within safe concentration levels. Consumption of higher concentration of Cr (III) is reported to have toxic effect [2, 3]. The determination of gold (Au) and arsenic (As) from geological samples is significant, because Au is a precious element and As is commonly known as Au marker. The determination of Cr, Au and As is commonly carried out by spectroscopic techniques such as ICP-AES, AAS or ICP-MS [4-7], but ICP-AES and ICP-MS determine only total amount of an element and do not differentiate between oxidation states. They also involve expensive equipment with high running cost and are not available with many analytical laboratories.

There are number of methods available for the speciation of metal ions, but HPLC is the leading technique for speciation of metal ions [8, 9]. The speciation procedures often involve the formation of stable derivatives of metal species before their analysis is carried out [10, 11]. The treatment may result insensitive direct spectrophotometric detection and prevention of the original oxidation state [12].

Capillary electrophoresis (CE) has been developed parallel to HPLC and is an attractive alternate for the separation of metal species owing to its separation speed, high efficiency and low consumption of toxic and expensive organic solvents

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[13- 16]. The application of CE for elemental analysis is steadily increasing due to low operating cost, ease of operation and automation. Derivatization allows the conversion of positively charged elements into complexes bearing similar charge, neutral or negative charge, thus providing the possibility to determine all species in a single run. The complexation of species prior to CE analysis allows their direct UV detection. This approach resulted into an increase in selectivity and sensitivity and ease of successful analysis real samples [17- 24]. Padarauskas [11] reported CE speciation of Cr (VI/III), vanadium (V/IV) and iron (III/II) using aminopolycarboxylic acids as complexing reagent. Chen et al [25] carried out speciation of Cr by capillary zone electrophoresis (CZE) using 2,6-pyridinedicarboxylic acid as chelating reagent.

Sulphur and hydrazine nitrogen atoms of Thiosemicarbazones and phenylthiosemicarbazone are able to bond with a number of metal ions to formhighly stable and intensely colored complexes. . The formed metal chelates have adequate sensitivity for spectrophotometric detection [26-29]. APPT has been reported for HPLC determination of copper (II), cobalt (II) and iron (II) in pharmaceutical preparation [30], separation of plantinum (II), selenium (IV), ruthenium (IV) and determination of platinum (II) in serum and urine of cancer patients and also from pharmaceutical preparation, [31], and separation and determination of vanadium (V), palladium (II), tin (II) and iron (III) [32]. The present work reports a procedure for the separation and determination of Cr (III), Cr (VI), As (V) and Au (III) by CZE after pre-capillary chelation with APPT. The condition for chelation and CZE separation are optimized for real samples analysis.

Results and Discussion

Spectrophotometric Studies

APPT is reported to react with various cations to form stable colored chelate compounds suitable for spectrophotometric detection [30-34]. The reaction of reagent APPT towards Cr (III), Cr (VI), As (V) and Au (III) were initially observed qualitatively in acidic medium. The reactions were examined spectrophotometrically to optimize the complexation reaction for quantitative determinations. The reactions were found to be rapid and the color of the complexes formed was observed to be stable. The maximum color development with highest values of absorbance was considered as optimum. The effect of pH was examined within 1-10 and maximum absorbance was observed within pH 1-6. Au(III) APPT complex showed good absorbance with in pH 1-3, As(V) within 4-5, Cr(III) within 4-5 and Cr(VI) within 3-6. For simultaneous determination of all four cations, pH 3 was selected (Fig. 1). Metal, chelate composition at optimized pH was examined by changing mole ratios of metal andligand. Au (III) indicated the formation of the complexes with 1:2 metal- ligand ratio, whereas Cr (III), Cr (VI) and As (V) showed the formation of the complexes with 1:3 metal- ligand ratio (Table-1). The reagent APPT is less soluble in methanol and 0.02 % (W/V) solution of the reagent was prepared by sonication for 30 min. The amount of the reagent added was varied from 1-5 mL with an interval of 0.5 mL for each cation. Maximum absorbance was observed with 2.0-2.5 mL. Therefore 3 mL was selected to achieve complete complexation. . The complexes absorbed maximally within 360 nm to 418 nm and obeyed Beers-Lamberts law within 0.2- 15 μg mL^{-1}. The solution stability of the complexes was examined by measuring the absorbance of solution after formation of complexes and after different intervals of the time. A change in the absorbance was not observed up to 18 h indicating stable complex formation with the ligand. The spectrophotometric study showed good sensitivity and selectivity for the formed metal chelates of APPT, but overlapping bands within 360-418 nm were also observed. Therefore, CE was considered for their separation and possible simultaneous determination. Qiam and Fritz have reported that thiosemicarbazones form water soluble cationic complexes [35], thus capillary zone electrophoresis (CZE) was examined as promising technique for the separation of the cationic species of Cr (III), Cr (VI), As (V) and Au (III) formed with APPT.

Table-1: Spectrophotometric data for metal complexes of 2-acetylpyridine-4-phnyle-3-thisemicarbazone (APPT).

Metal Chelates	λ_{max} (nm)	pH	Metal:Ligand Ratio	□ (L.mol^{-1}cm^{-1})	Calibration range μg/mL	R^2
Cr (III)	360	5	1:3	3.4×10^3	1-15	0.996
Cr (VI)	390	6	1:3	1.4×10^4	2-10	0.999
As (V)	385	5	1:3	$3.2\ \times10^3$	50-250	0.990
Au (III)	418	1	1:2	$1.2\ \times10^4$	0.2-10	0.998

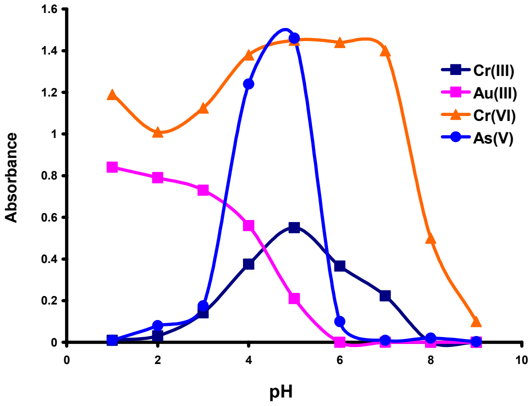


Fig. 1 pH influence on the formation of Cr (III), Au (III), Cr (VI) and As (V)-APPT complexes

CZE Studies

Cr exists in multiple oxidation states but Cr (III) and Cr (VI) ere most important species forseparation and speciation studies [3,25]. Their separation along with cations As (V) and Au (III) is also environmentally and analytically important.

Choice of Background Electrolyte

Separation efficiency was evaluated using various buffer systems such as borate, phosphate, carbonate and acetate. Borate buffer of pH 7 gave some mobility to the chelate compounds, but indicated poor resolution and sensitivity. As the metal-APPT complexes absorbed maximally in acidic buffer system, acetate buffer of pH 3-6 was tested as background electrolyte (BGE). The metal chelates did not indicate mobility and capillary current was also high. Alternatively oxalic acid-sodium oxalate (100 mM) buffer was investigated within pH 3 to 5. Mobility of all the cations were observed but with poor resolution. It was observed that change in the composition of BGE from pH 4 to 5 had significant effect on mobility and resolution. Various concentration ratios of oxalic acid and sodium oxalate within pH 4 to 5 were examined at the interval 10 mM. It was observed that increase in ratio of sodium oxalate buffer had adverse effect on mobility trends and hence separation of analyte was effected (Fig. 2). Separation was good at 10:10 to 10:20 but as the ratio was increased to 10:30 chromium (VI) and arsenic (V) get merged, the similar trend was observed up to the ratio of 30:100. Similarly at higher pH, not only resolution was decreased, but migration time also increased to 10 min. Therefore, pH 4 was preferred over pH 4.5 (Fig. 3) where the cations showed an acceptable separation with resolution >1.5 within 6 min using 10 mM oxalate buffer.

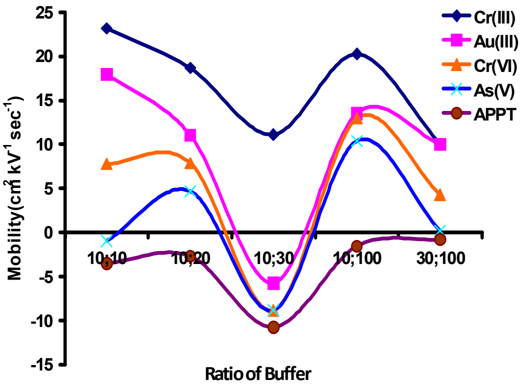


Fig. 2: Effect of variation of sodium oxalate and oxalic acid concentrations ratio in BGE on the mobility of Au (III), As (V), Cr (III), and Cr (VI) APPT chelates at pH 4.0.

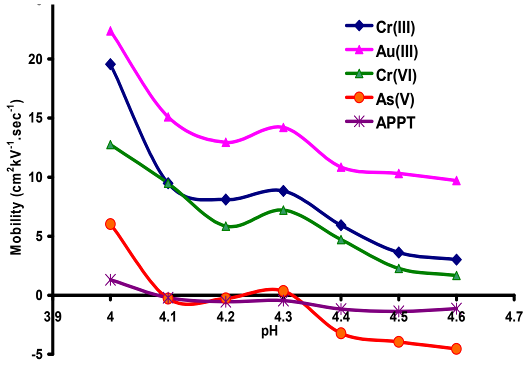


Fig. 3: Migration of Au (III), As (V), Cr (III) and Cr (VI) APPT chelates as a function of pH

Selection of Separating Voltage

The variation in the mobility of chelates was investigated within 10-20 kV with an interval of 5 kV. An increase in migration time was recorded with decrease in the voltage (Fig. 4) without effecting resolution. In order to avoid the Joule heating effect, attributed to high voltage, +15 kV was selected. Final separation of all four species along with reagent is given in Fig 5. The analytes indicated symmetrical peak and electropherogram did not show a significant difference when recorded after 8h from the formation of the chelate compounds and supports the formation of stable complexes.

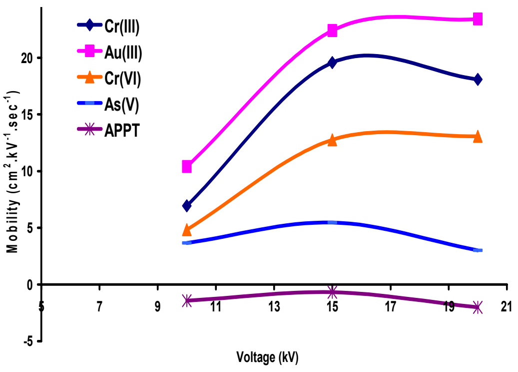
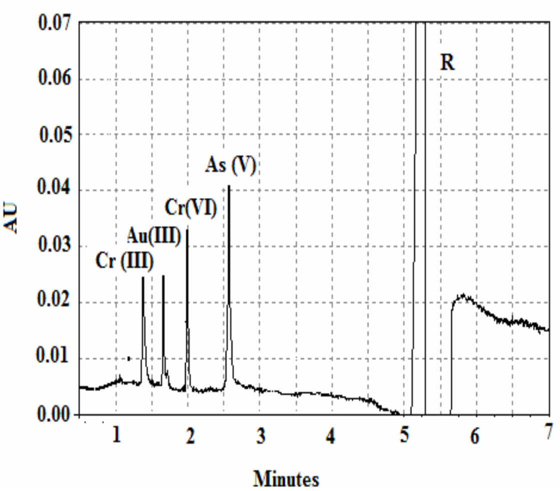


Fig. 4: Effect of voltage on mobility of Cr (III), Cr (VI), Au (III) and As (V) APPT complexes.



Conditions: capillary effective length 40 cm, 75 mm i.d and 375 µm o.d; applied voltage +15 kV; hydrostatic injection 6 s. UV-Vis detection at 241nm.

Fig. 5: Electropherogram of metal ions at pH 4.0 using 10 mM oxalic acid-sodium oxalate.

Quantitation

The linear calibration curves were obtained by recording average peak area (n=3) versus concentration and were obtained in concentration range 0.95 -11.4 µgmL^{-1} for Au (III), 10-200 µgmL^{-1} As (V), 3-12 µgmL^{-1} for Cr (III) and 10-50 µgmL^{-1} for Cr (VI) with coefficient of determination (r^2) 0.9979 to 0.9988. The limit of detection (LOD) measured as three times the background noise (S/N) (3:1) was lowest for Au 0.125 µgmL^{-1} and highest 1.0 µgmL for As (V). Signal to noise ratio (S/N) of 10:1 was used to calculate LOQ values. Range of values obtained were 0.375-3.0 µgmL^{-1}. The separation and quantitation were repeatable in terms of peak height and migration time (n=6) with relative standard deviation RSD within the range 0.7 - 3.2 % and 1.3-3.8 % respectively (Table-2). The reagent APPT reacts with a number of cations to form complexes in acidic medium, therefore the possibility of interfering effect of Cu (II) and Fe (III) on the determinations was investigated. The copper (II) and iron (III) indicated poor mobility in the background electrolyte employed and did not interfere. The interfering effects of cations (Ca(II), Mg(II), Na(I) and K(I) and anions (Cl^-, CO_3, SO_4, NO_3, NO_2) commonly present in water were investigated at ten times the concentration of the analyte and they did not absorb at the wavelength selected (241 nm) and did not interfere.

Sample Analysis

Cr (III) and Cr (VI) determination from tape water and industrial effluents

Cr (VI) was determined in industrial waste water, collected from Kasur Punjab. While tap water samples were collected from University of Sindh Campus. The samples were analyzed by CZE procedure after necessary preconcentration. The amount of Cr (VI) in industrial effluents was found to be 2.08 and 2.17 µgL^{-1} with RSD of 0.80% and 3.10% respectively. The amount of Cr (III) in tap water was found to be 6.60 µgL^{-1} with RSD of 1.20%. The samples were also analyzed by spiking the preconcnetrated aliquots of industrial effluent and tap water samples with Cr (VI) and Cr (III) standard solutions up to the final concentration of 5.0 µgmL^{-1} followed by CZE determination. The amounts were calculated from external calibration and increase in the response with added standards. The result of analysis agreed with direct calibration (Table-3). The results were confirmed by AAS and found to be in agreement with CZE method with RSD 1.2-2.3%.

Determination of As (V) and Au (III) from coal mine water samples

Two water samples from coal mine were analyzed by CZE after preconcentration. The amount of As (V) and Au (III) were calculated as 0.23 and 0.25 µgL^{-1} and 0.15-0.20 µgL^{-1} with RSD 3.1-3.4% and 2.9-4.3% respectively. The samples were also analyzed by standard addition method. An aliquot of preconcentrated samples was spiked with As (V) 2.0 µgmL^{-1} and Au (III) 1 µg mL^{-1} and CZE procedure was carried out. The amount found agreed with direct calibration with quantitative recovery. The results obtained were further confirmed by AAS (Table-4).

Table-2: CZE data for analysis of metal-APPT complexes.

Metal ion	Calibration range (µg/mL)	LOQ (µg/mL)	LOD (µg/mL)	R^2	Linear regression equation
Au (III)	0.95-11.4	0.375	0.125	0.998	Y=0.0002x+0.0012
As (V)	10-200	3.0	1.0	0.998	Y=0.0252+2.0932
Cr (III)	3-12	0.6	0.2	0.999	Y=0.0001x+0.0025
Cr (VI)	10-50	0.3	0.1	0.998	Y=0.1124x+3.05

Table-3: CZE data for analysis of Cr (III) and Cr (VI) from tape water and industrial waste water samples.

Samples	Metal Ions	Amount of metal found (µg/L) with % RSD (n=3)	Amount of metal found with St. Add (µg/L) with %RSD(n=3)	Amount of metal found by AAS (µg/L) with %RSD (n=3)
Tape Water	Cr (III)	6.60± 1.2	6.90 ±4.2	6.20 ±1.1
Industrial Waste water 01	Cr (VI)	2.08± 0.8	2.11±1.2	1.91 ± 1.9
Industrial Waste water 02	Cr (VI)	2.167± 3.1	2.14 ±4.2	2.01 ±2.3

Table-4: Quantitative CZE determination of Au(III) and As(V) from coal mine water samples.

Samples	Metal Ions	Amount of metal found by CE(µg/L) with %RSD	Amount of metal with St.Add (µg/L) with %RSD	Amount of metal found by AAS(µg/L) with %RSD
Coal mine sample (S_1)	Au (III)	0.25 ± 3.4	0.234 ± 3.1	0.21 ± 3.5
Coal Mine sample (S_2)	As (V)	0.15 ± 2.9	0.20 ± 4.3	0.21 ±1.5

Analysis of Au (III) in certified reference material

The developed method was applied for the determination of Au in a certified reference material after necessary acid digestion. The amount of Au found was 1.2 µgg^{-1} with RSD 3.1 % (Table-5). The sample was spiked with Au (III) standard at the concentration of 5 µgmL^{-1} and corresponding increase in response was observed with ~94% recovery with RDS 1.7% and also agreed with certified value.

Comparing the results of separation of Cr (III) and Cr (VI) by CZE using 2,6-pyridinedicarboxalic acid as precolumn complexing agent reported by Z. Chen [25], with the present method showed that present method indicated better peak shape with shorter preparation time than reported method. The present method also indicated a comparable sensitivity for quantitation, but the use of simple background electrolyte and possible simultaneous determination of As (V) and Au (II) are added advantages of the present method.

Experimental

Chemicals

APPT was prepared by reported [30-33] method as; equimolar (0.01M) solution of 2-acetylpyridine and 4- phenyl- 3 -thiosemicarbazide in methanol were heated together for 30 min. . The chemicals; 2 -acetylpyridine (Fluka, Switzerland), 4-phenyl-3-thiosemicarbazide (Sigma-Aldrich, USA) and methanol (RDH, Germany) were used. Reagent grade CH_3COOH, HCl (37%) and NH_4CH_3COO, KCl , $NaCH_3COO$, sodium tetraborate, sodium bicarbonate, sodium carbonate, NH_4Cl, ammonium solution, oxalic acid, sodium oxalate, citiric acid sodium citrate were purchased from E-Merck, Germany. Metal salts like nickel (II) acetate, cobalt (II) acetate, copper (II) acetate, Au (III) chloride, iron (III) chloride, Cr (III) chloride, potassium dichromate, As (V) oxide were also of E-Merck, Germany.

The stock solutions were prepared by dissolving the salts of the analyte in water with addition of few drops of appropriate acid. As (V) oxide was dissolved in water by adding few drops of NaOH. The pH of the solution was made to 5 before finally adjusting the volume.

The buffer solutions at were prepared from; HCl (0.1 M), KCl (1.0 M, pH 1-2), CH_3COOH (1.0 M) and $NaCH_3COO$ (1 .0 M, pH 3-6), oxalic acid and sodium oxalate (0.1M, pH 2-5), citric acid (1.0 M) and sodium citrate (1.0 M, pH 2-5), boric acid (0.1 M) and sodium tetraborate (0.1M, pH 4 -9) and sodium bicarbonate (0.1M) and sodium carbonate (0.1M, pH 8-10).

Table-5: CZE analysis of Au (III) in certified reference material (SH24).

Sample	Amount present in certified reference material (µg/L) with %RSD	% Recovery	Amount found by CE (µg/L) with %RSD
SH-24 (n=3)	1.291± 3.1	93.1%	1.20 ± 1.8

Sample Collection

Two ground water samples from coal mine rock field (pore water) from district Tharparker, Sindh, Pakistan were collected from deep boring in clean plastic bottles, after allowing water to run for 2-5 min. Tap water samples were collected from Sindh University campus area. One liter water sample from each location was collected in clean plastic bottle after allowing tap water to flow for 5 min. The samples were mixed in a bucket and a composite sample of 5 L was collected and further processed for analysis. A sample (5 L) from the effluents of a tannery from Kasur, District Kasur, Punjab, Pakistan in clean plastic bottle was collected after rinsing the bottle several times with the sample. The samples were quickly transferred to the laboratory and were analyzed as received. Certified reference material for gold (SH-24) was obtained from Rock labs Ltd., Auckland, New Zealand.

Equipment

Double beam spectrophotometer (Perkin Elmer, Singapore) with dual 1 cm silica cuvettes was used for spectrophotometric studies. Thermo Nicolet, USA was used to record IR spectrum of reagent within the range of 4000-660 cm^{-1}. The determination of Cr, As and Au was carried out with AAS (Analyst 800, Perkin Elmer, Singapore) using air acetylene flame, connected to AA accessory cooling system, AS-800 auto sampler and FIAS100 flow injection system with hydride manifold. The equipment was controlled by Win Lab 32 AA flame software. The analysis was carried out in triplicate (n=3) with integration and delay time of 3 s.

The capillary electrophoresis system (Beckman Coulter P/ACE MDQ ,Fullerton, CA) equipped with an auto sampler, photodiode array detector and a P/ACE MDQ (32 Karat) software was used. Uncoated fused silica capillary of effective length 54 cm, 75 µm i.d. and 375 µm o.d was obtained from Beckman Instruments Inc. The capillary and sample temperature was adjusted at 25^0C. The capillary was regenerated and conditioned with methanol, water, hydrochloride acid (0.1 M), water, sodium hydroxide (0.1 M), water and BGS for 1 min, 0.5 min, 2 min, 0.5 min, 2 min, 0.5 min and 2 min respectively as reported [34] prior to each run or if any distortion in the peak shape in electropherograms during the day is observed. Before each sample injection the capillary was washed with sodium hydroxide (0.1 M) followed by water and run buffer respectively for 2 min, 1 min and 2 min. An auto sampler with a pressure of 0.5 Psi was used to inject the sample for 6 s. Injection volume was calculated using CE expertilite software provided by Beckman. The electrophoretic migration was optimized with buffer system; oxalic acid-sodium oxalate (10 mM) pH 4.0 at the applied voltage of +15 kV at UV detection of 241 nm. Identification of each analyte was done by comparing migration time with that of standard and verified by spiking sample with known standards of each species. Electro-osmotic flow (EOF), and electrophoretic mobilities were calculated from migration times at different pH by the equation as reported using acetone as a neutral marker [34].

Analytical Procedures

Spectrophotometric Studies

1-3 mL solution containing Cr (III) (2.5-125 µg), Cr (VI) (5-100 µg), As (V) (10-50 µg) or Au (2-100 µg) was transferred to 10 mL volumetric flask separately, and added sodium acetate acetic acid buffer pH 3 and 3 mL APPT solution (0.2% w/v in methanol). The final volume was adjusted with methanol and absorption spectra were recorded against within 250-600 nm.

CZE Procedure

An aqueous solution (1-2 mL) containing Cr (III) (2-120 µg), Cr (VI) (5-100 µg), As (V) (100-800 µg) and Au (III) (10-120 µg) were transferred to 10-mL volumetric flask and were added reagents as spectrophotometric procedure. The volume was adjusted to mark with methanol. The solution (2 mL) was transferred to septum sealed sample vial for electrophoretic analysis. The sample was injected hydrostatically by auto sampler on fused silica capillary at the optimized condition and electropherogram was recorded.

Determination of Au (III) and As (V) in coal mine water sample

Ground water sample (5 L) collected from coal mine field was transferred to a beaker and was heated gently on hot plate below the boiling point of water. The water was evaporated to about 10 mL and was filtered through filter paper (Whatman No.42). Final volume was adjusted and analysis was carried by CZE procedure. The quantitation was carried out from linear regression equation prepared from external calibration from standard Au (III) and As (V) solutions.

Determination of Cr (III) in tap water

Composite sample (5 L) collected from different taps within Sindh University campus was slowly preconcenetrated by evaporation at hot plate. The total volume was reduced to about 20 mL and the solution was filtered. The final volume was adjusted to 20 mL and 2 mL was analyzed by CZE procedure. The Cr (III) contents in tap water were evaluated from linear external calibration curve.

Determination of Cr (VI) in Industrial Effluent

Industrial effluent collected from tannery plant (5 L) was acidified with 1 mL HCl (1 M) and evaporated gently on hot plate upto 15 mL and filtered. The filtrate was made upto 25 mL in the volumetric flask and the solution (1 and 2 mL) was analyzed by CZE procedure. The Cr (VI) contents in the samples were evaluated from external calibration curve.

Determination of Au in Certified Reference Material (SH-24)

A 25 g sample of CRM was digested with 50 mL of aqua regia (HCl:HNO_3, 3:1 v/v) by refluxing for 6 h. Liquid phase was dried and neutralized with ammonia and volume was made up to the mark with deionized water.. The solution (2 mL) was taken and CZE procedure was followed. External calibration curve was used to quantify this solution by CZE.

Conclusion

APPT complexes of Au (III), As (V), Cr (III) and Cr (VI) are prepared and reported for the first time. A simple and easy method for separation and simultaneous determination of all four cations was developed using capillary zone electrophoresis. A fairly good separation of Au (III), As (V), Cr (III) and Cr (VI) along with reagent was achieved within 6 min. The method showed great applicability for the determination of the cations from water and reference material samples in both industrial effluents and from tape water.

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