JAAS

Accepted Manuscript

www.rsc.org/jaas

A sensitive speciation analysis of chromium in natural and industrial effluents by electrothermal atomic absorption spectrometry after a novel ligandless mixed-micelle dispersive liquid-liquid microextraction

Noorbasha N. Meeravali, K. Madhavi and Sunil Jai Kumar*

Bhabha Atomic Research Centre, ECIL Post, Hyderabad-500 062, India.

*Corresponding author. Fax: +91 40 27125463.

E-mail address: suniljaikumar@rediffmail.com (S. J. Kumar).

A simple, fast and sensitive method is developed for the determination of Cr (III), Cr (VI) and total Cr separately in natural river and lake waters and tannery effluents samples. In this method, a new strategy of micelle and mixed-micelles have been introduced in dispersive liquid-liquid microextraction (M-MM-DLLME) for the sequential and simultaneous separation and pre-concentration of Cr(VI) and Cr(III) at sampling site. Cationic, Aliquat-336 and anionic dioctylsulfosuccinate, DOSS surfactant aggregates are used as dispersive and chelating solvents, respectively for the extraction of Cr(VI) and Cr(III). In sequential speciation, the hydrophilic $HCrO_4^-$, first reacts electively with cationic head group solubilising sties of Aliquat-336 micelles through electrostatic interaction and form extractable charge neutral Cr(VI)-Aliquat-336 at room temperature, in presence of Cr(III). The extracting trichloroethylene pre-concentrates this extractable species from bulk aqueous phase and leaves the un-reacted cationic $Cr(H_2O)_6^{3+}$ in the supernatant. This supernatant is subjected to a similar extraction procedure after the addition of an anionic DOSS surfactant. In the simultaneous speciation, both the species are extracted by adapting a similar extraction procedure after addition of DOSS and Aliquat-336 mixed-micelles to fresh aliquots, to determine total Cr, avoiding the addition of chelating agent and oxidation/reduction steps. The surfactant stabilized chromium species in trichloroethylene phases are transferred to laboratory for quantification using electrothermal atomic absorption spectrometry (ETAAS) The parameters affecting the process are evaluated and optimized. Under the optimized conditions, the pre-concentration factors and limits of detection for Cr(III) and Cr(VI) in sequential and total Cr in simultaneous extractions are 100, 90 and 83; and 0.5, 0.6 and 0.7 pg mL^{-1}, respectively. The accuracy of the procedure is verified by comparing the sum of the concentrations of each individual species obtained from the sequential extraction with total Cr obtained in the simultaneous extraction procedures and applied to certified references materials such as BCR 714 industrial influent, BCR 715 industrial effluent and NIST 1640 natural water. This method has been applied to on-site extraction of individual and total Cr from natural waters and effluents.

Introduction

Chromium is widely used in various industries like steel, chromoplating, paint, leather, wood preservative and corrosion inhibitor.[1-3] The effluents from these industries contains chromium in the range of 50 to 25,000 µg mL^{-1}.[4] Toxic effects of chromium depends on its chemical form, Cr(III) is one of the essential trace elements for mammalians, needed for glucose and lipid metabolism, whereas Cr(VI) is carcinogenic, mutagenic and inducer of stomach and skin cancer and is water soluble, hence labile, therefore, leads to widespread contamination from the point of source.[5,6] The US Environmental Protection Agency (EPA) tolerance limit is 50 ng mL^{-1} for total Cr and recently introduced new norm of 10 ng mL^{-1} for Cr(VI), based on the toxicity data available presently.[7] At the same time, California EPA has set a public health goal of 0.02 ng mL^{-1} for Cr(VI) in drinking water to protect the health of non-concerned human beings at a lifetime risk level of "one-in-a-million". Hence, it is essential to monitor the levels of Cr(VI) and Cr(III) species and total Cr in industrial effluents and natural water bodies to understand their impact on the environment and human health.

Accurate speciation analysis of chromium at trace and ultra trace levels in tannery effluents and natural water bodies is a difficult task, even by using versatile hyphenated chromatographic techniques.[8-10] It is mainly due to the complexity of matrix and in-stability of chromium species during the time between the sample collection and its analysis.[11] Addition of any stabilizing agents during sampling causes oxidation or reduction of species even on storing at 4^{o}C. EPA 218.6 method indicates that the samples collected in alkali buffer stabilizes Cr (VI) only up to 24 h.[12] Therefore, an on-site method for the separation of chromium species will help to prevent inter-conversion of species and thus provide more reliable results.

Nowadays, dispersive liquid-liquid microextraction (DLLME) has emerged as an efficient extraction procedure for the separation and pre-concentration of a variety of uncharged hydrophobic species.[13-22] Recently, environmental friendly surfactant assisted-DLLME has been introduced to overcome the limitations of lower extraction efficiencies of hydrophilic organic and inorganic speices.[23-28] Over decades, chromatographic separation followed by diphenylcarbazide (DPC) spectrophotometric detection is the most widely used differential speciation approach for the determination of Cr(VI) and total Cr after oxidation of Cr(III). Recently, Yousefi et al. and Abadi et al, have used the same concept in surfactant assisted microextraction of anionic monomer stabilized Cr(VI)-DPC ion-associate into extracting organic solvents.[29,30] Our group has reported a solidified floating organic drop microextraction using the same concept and extracted the anionic surfactant stabilized Cr(VI)-DPC.[31] Sadeghi et al. reported a temperature controlled sequential microextraction using the differential reactivity of APDC with Cr species and the chelates formed are extracted into non-ionic monomer stabilized ionic liquid.[32] Irrespective of the extraction procedure, electrothermal atomic absorption spectrometry (ETAAS) is most extensively used techniques for the determination of chromium. Recently, Liu et al. reported carbon dots capped with polyethleneimine slurry disperser for selective extracton of Cr(VI).[33]

This paper describes a new method for the sequential and simultaneous extraction and separation of chromium species from natural river and lake waters and tannery effluents samples at sampling sites using micelles and mixed-micelles dispersive liquid-liquid microextraction (M-MM-DLLME). Determination is carried out in laboratory using ETAAS. The accuracy of the procedure is validated by the certified total Cr values in certified references materials such as BCR 714 industrial influent, BCR 715 industrial effluent, and NIST 1640 natural water and also by using alternative reported procedure. To best of our knowledge, the micelle and mixed-micelles concept is being used for the first time for on-site sequential and simultaneous microextraction of Cr(III) and Cr(VI) and total Cr, without using any chelating agent.

Experimental

Instrumentation

Chromium concentrations in the trichloroethylene phases were determined by using continuum source ETAAS (Contr AA-700, Analytik Jena AG, Jena, Germany). A transversely heated graphite tube, MPE 60 auto sampler and xenon short arc lamp in hot-spot mode operated at 300W as a continuum radiation source were used. A high resolution double monochromator consisting of a prism and an echelle grating monochromator, providing a spectral bandwidth per pixel of 2 pm at 200 nm were used. A linear charge coupled device (CCD) array detector total 588 pixels, out of which 200 pixels were used for the determination of dispersed radiation. An analytical line at 357.6687 nm was used to measure the integrated absorbance of chromium. The absorbance was measured using the central pixel (CP) ± 1 pixels. Argon with a purity of 99.99% was used as the purge gas. The optimized temperature program is given in Table 1.

Reagents and solutions

Ultra-pure water (18.3 MΩ cm) obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA) was used to prepare all the solutions. The analytical grade HCl and HNO_3 (Merck, Mumbai, India) were further purified in our laboratory using sub-boiling quartz stills distillation system kept in a laminar clean bench. Stock solutions of (0.1 mg mL^{-1}) Cr(III) and Cr(VI) were prepared using 1 mg mL^{-1} Cr(III) solution (Sigma-Aldrich, Steinheim, Germany) and respective amount of $K_2Cr_2O_7$ (Merck, Darmstadt, Germany) in 0.2% v/v nitric acid and ultra pure water, respectively. Working solutions were prepared daily by subsequent dilution of stock solutions. The solutions of 10% m/v sodium dodecylsulfate ($C_{12}H_{25}OSO_3^-Na^+$, SDS) Aliquat-336 ($C_{25}H_{54}N^+Cl^-$), and cetyltrimethylammonium bromide ($(C_{16}H_{33})N(CH_3)_3Br$, CTAB) from Sigma-Aldrich, Steinheim, Germany) and dioctylsulfosuccinate ($C_4H_9CH(C_2H_5)CH_2OCOCH(SO_3^-Na^+)CH_2COOCH_2CH\text{-}(C_2H_5)C_4H_9$, DOSS) from Chemsworth, Surat, India were prepared respectively in 1% v/v HCl and 50% methanol, water and 100% methanol. Sodium chloride (NaCl). trichloroethylene (C_2HCl_3, TCE) and chloroform ($CHCl_3$) from Merck, Mumbai, India were used without any further purification. Stock solutions, 1 mg mL^{-1} of Na^+, K^+, Mg^{2+}, Ca^{2+}, Cd^{2+}, Hg^{2+}, Pb^{2+}, Bi^{3+}, Al^{3+}, Ni^{2+}, Zn^{2+}, Cu^{2+}, Fe^{3+}, PO_4^{3-} and SO_4^{2-} were used for interference study. All the containers were cleaned by soaking in 20% v/v HNO_3 and then rinsing with ultra-pure water kept it in a class-10 clean bench. Urban lake water samples were collected in to clean plastic containers from a lake polluted with domestic as well as various industrial effluents. River waters were also collected in the same manner. Tannery effluent samples were collected from tannery industries, Kanpur, India. These water samples were filtered through 0.45 µm membrane filter and on-site sequential and simultaneous extractions were performed at sampling sites. The micelles and mixed-micelles stabilized Cr(III) and Cr(VI); and total Cr in trichloroethylene were transported to laboratory for quantification.

Micelle and mixed-micelles assisted DLLME procedure

Sequential extraction of chromium species

Extraction of Cr(VI)

Aliquots of 1-10 mL of lake water, tannery effluents, river water and certified reference materials were taken in pre-cleaned samples vials and pH was adjusted to 2 using HCl. To this solution, 1 mL of 10% m/v Aliquat-336 was added and mixture was made up to 50 mL using the ultra pure water. Then, 0.6 mL trichloroethylene (TCE) was injected rapidly into the sample solution by using a micropipette and the mixture was mixed well for 2 min to enhance the rate of the reaction between Cr(VI) and surfactant aggregates. The cloudy solutions were formed by dispersion of fine droplets of trichloroethylene in the sample solution. The solutions were left at room temperature, after 10 min gravitational phase separation was obtained. This phase was dissolved in 0.2 mL methanol containing 2% v/v HNO_3 and analysed for Cr(VI) by ETAAS after injecting 10 µL into graphite tube using peak area measurement of the absorption profile. Procedural blanks were also prepared in a similar manner.

Extraction of Cr(III)

The above supernatant consisting of cationic Cr(III) was treated with HCl to maintain pH 2. To this solution, 2 mL of 10% m/v sodium dioctyl sulfo succinate, DOSS was added and subjected to a similar extraction procedure. The trichloroethylene phase was analysed for Cr(III) by ETAAS as mentioned above.

Simultaneous extraction of Cr(III) and Cr(VI)

For the determination of total Cr, fresh aliquot of 1-10 mL urban wastewater and tannery effluent, river water and certified reference materials were taken in pre-cleaned samples vials and pH was adjusted to 2 using HCl. These solutions were subjected to a similar extraction procedure after addition of 2 mL of 10% m/v sodium dioctylsulfosuccinate, DOSS and 1 mL of 10% m/v

Aliquat-336. These mixed surfactants react simultaneously with both the species of chromium and pre-concentrated into 0.35 mL of trichloroethylene phase. This phase was dissolved in 0.25 mL methanol containing 2% v/v HNO_3 and analysed for total Cr by ETAAS as mention in sections above.

Results and discussion

The parameters affecting the micelle and mixed-micelles dispersive liquid-liquid microextraction (M-MM-DLLME) procedure for the sequential and simultaneous extraction of labile $HCrO_4^-$ and kinetically inert $Cr(H_2O)_6^{3+}$ species at room temperature using Aliquat-336 and dioctylsulfosuccinate were studied. Optimization was carried out by spiking 0.05 ng mL^{-1}each of chromium species and mixture to urban lake water and tannery effluent samples.

Optimization of M-MM-DLLME parameters

Effect of pH

Chromium exists in various forms and the relative concentration of each species depends on the pH of the aqueous solution. Hence, the effect of pH on the recovery of Cr(III) and Cr(VI) in wastewater samples were studied in the pH range of 1- 6.5, in presence of 0.2% m/v Aliquat-336 and 0.4% m/v DOSS sequentially and simultaneously. The results are shown in Fig. 1 (a-c). As shown in Fig. 1(a), in sequential extraction, between pH 1-2.8, 97-99% recovery of Cr(VI) was achieved. Further increase in the pH of solution to 6.5, decreased the recovery to 40%. This behaviour is due to existence of Cr(VI) as $HCrO_4^-$ in the pH range 1 to 3, which reacts efficiently with cationic Aliquat-336 micelles. In case of Cr(III), it was also observed that only 5-7% recovery of Cr(III) between pH 1-2.5, beyond which it increased gradually to 25% up to studied pH of 6.5. This behaviour is due to existences of Cr (III) as $Cr(H_2O)_6^{3+}$ in the pH range between 1-2.5 and shows strong repulsion with cationic Aliquat-336 micelles. At higher pH (>2.5), the behaviour of Cr(III) is due to its existence in various forms such as $Cr(H_2O)_5OH^{2+}$,

$Cr(H_2O)_4(OH)_2^+$ and $Cr(H_2O)_5(OH)_3$. Thus pH 2 was selected for the extraction of Cr(VI), in presence of Cr(III).

As shown in Fig. 1(b), 96-99% recovery of Cr (III) was observed in sequential extraction of supernatant with anionic DOSS micelles, in between pH 1 and 2.5, and then decreased to 40% at pH 6.5. The quantitative recovery of Cr (III) is due to strong electrostatic interaction between $Cr(H_2O)_6^{3+}$ and anionic DOSS micelles in the pH range 1-2.5 beyond this pH low recoveries of Cr(III) due to existence of species with lower charge. In case of Cr(VI), it was also observed that up to pH 1, the recovery of Cr(VI) was 25%, beyond which it decreased gradually to 4-6% up to studied pH of 6.5. This behaviour is due to existence of Cr(VI) as neutral H_2CrO_4, which could be adsorbed on the micelles of DOSS at below pH 1, beyond this pH Cr(VI) exists as $HCrO_4^-$ and showed low recoveries due to strong repulsion with anionic DOSS micelles. Thus pH 2 was selected for the extraction of Cr(III), in presence of Cr(VI). These experiments indicated that either of Cr (VI) or Cr (III) could be recovered first in the sequential extraction process. However, the extraction of toxic Cr (VI) was carried out first to prevent its likely inter-conversion to Cr (III).

In simultaneous extraction, DOSS and Aliquat-336 dispersive mixed-micelles were added to a fresh aliquot of sample showed 97-99% and 93-96% recoveries for Cr(III) and Cr(VI), respectively in the pH range between 1-2 and the results are presented in Fig. 1(c). It is mainly because of existence of cationic $Cr(H_2O)_6^{3+}$ and anionic $HCrO_4^-$ species reacting simultaneously with anionic and cationic head group of mixed-micelles. Thus, pH 1.5 was selected for the simultaneous extraction of total Cr.

Effect of type and volume of the disperser surfactants

The success of sequentially and simultaneous extraction of chromium species depends on the formation of strong ternary complex between chromium species, disperser and extracting solvents. The role of ionic dispersive surfactants is very important in the extraction of hydrophilic ionic chromium species by utilizing its head group sites of aggregates by forming charge neutral hydrophobic species, which avoids the need of chelating agents. Therefore, the effects of anionic (dioctylsulfosuccinate DOSS and sodium dodecyl sulfate, SDS) and cationic (Aliquat-336 and cetyltrimethylammonium bromide, CTAB) surfactants concentration in the range of 0-0.6% m/v on the sequential and simultaneous recovery of Cr(III) and Cr(VI) from tannery effluent samples were studied for selecting appropriate surfactants. The results are given in Fig. 2 (a, b and c). As can be seen in Fig. 2(a), the anionic Cr(VI) shows a very low recovery of 10% below the critical micelles concentration (CMC) of Aliquat-336 (0.006% CMC) and CTAB (0.04% CMC). Above CMC's, the micelles of the CTAB does not show any influence on the recovery of Cr(VI) due to its strong hydrophilic nature. The hydrophobic Aiquat-336 micelles show quantitative recovery of 94-96% by forming hydrophobic charge neutral Cr(VI)-Aliquat-336. Hence, 0.2% m/v Aliquat-336 was selected for the extraction of Cr(VI). As can be seen in Fig. 2(b), below CMC of DOSS (0.17% CMC) and SDS (0.23% CMC), the recovery of Cr (III) was between 5-50% and 5-18%, respectively. These recoveries increased to 98% and 35%, respectively by increase in the concentration of DOSS and SDS above CMC up to 0.3% and then reached a plateau by further increase in the concentration. These results indicated that above CMC, micelles shows strong electrostatic interactions and are responsible for extraction of Cr (III), however DOSS forms strong hydrophobic charge neutral species compared to SDS. Hence, a 0.4% m/v DOSS surfactant micelles was selected for the extraction of Cr (III).

As can be seen in Fig. 2(c), simultaneous extraction of both Cr(III) and Cr(VI) were carried out by taking a fresh aliquot of sample in presence of mixed surfactant (DOSS and Aliauqt-336). These results indicates that above 0.4% m/v DOSS and 0.2% m/v Aliquat-336 showed quantitative recoveries between 94-96% and 96-98% respectively for Cr(III) and Cr(VI) in the effluent samples. Hence, the concentrations of 0.4% m/v DOSS and 0.2% m/v Aliquat-336 mixed surfactants were selected for the simultaneous extraction.

Effect of type and volume of the extracting solvent

The selection of an appropriate extraction solvent is important because, its physicochemical properties affect the formation of homogenous cloudy solution and extraction efficiency of targeted species. Hence, chloroform and trichloroethylene were studied as extracting solvents for the sequential and simultaneous extraction of Cr (III)-DOSS and Cr (VI)-Aliquat-336 formed in aqueous solution sequentially and simultaneously. The results showed that the extraction efficiency of chloroform and trichloroethylene were 88% and 75%; and 96% and 98% respectively for sequential extraction of Cr(III) and Cr(VI). The high extraction efficiency of trichloroethylene may be due to the high hydrophobic nature and lower volatility compared to chloroform. Therefore, trichloroethylene was selected and optimized its volume in the range of 0.1 to 1 mL for the individual and simultaneous extraction of Cr(III) and Cr(VI). The results showed that extraction efficiency of 96, 98 and 97% were obtained respectively for sequential and simultaneous extraction of Cr(III) and Cr(VI) above 0.6 mL trichloroethylene. Therefore, a concentration of 0.6 mL trichloroethylene was selected.

Effect of extraction temperature

Optimization of extraction temperature is necessary for efficient interaction of dispersive surfactant aggregates with kinetically inert Cr(III) and thermally labile Cr(VI) species for quantitative sequential and simultaneous extraction. Hence, the effect of extraction temperature on sequential and simultaneous recovery of Cr(III) and Cr(VI) in spiked samples were studied in the range of 10 to 60^{o}C by keeping the other parameters constant. These results indicate that the extraction of reactive anionic Cr(VI) and even inert Cr(III) was independent of temperature in the studied temperature range. This process even works in the low temperature of 10^{o}C, it is one of the advantage of this method over solidified floating organic drop microextraction method[31], which does not work at this temperature. Therefore, an extraction temperature of 25^{o}C (room temperature) was selected for both sequential and simultaneous extraction, which provides an energy efficient process applicable to extraction of species in the field, where no heating and power facilities are available.

Effect of the extraction time

The extraction time is defined as the time required for the complete extraction of analytes i.e., time interval between the injection of extraction solvent and it separation from bottom of conical vial. Hence, the effect of extraction time was studied in the time intervals of 0-15 min on the recovery of both Cr(III) and Cr(VI) individual and total Cr. The results indicated that the extraction time of 10 min is sufficient to quantitatively extract the chromium species. Therefore, a 10 min extraction time was selected for both sequential and simulations extraction.

Effect of salt

The presence of electrolytes can improve the extraction efficiencies of analytes in the conventional microextraction because of the salting out effect. Hence, the effect of the concentration of NaCl, a common electrolyte, on the sequential and simultaneous recovery of chromium spices in spiked lake water samples were studied in the range of 0-4% m/v. These results indicated that no significant effect on the extraction efficiency of the both Cr(III) and Cr(VI) species up to studied concentration of NaCl. Further these experiments showed that high tolerability of salt can be used for extraction of these species from, high saline samples like underground water and seawater samples.

Stability of chromium species in the extracting solvents with time

The stability of both the chromium species were compared for 24 days after collection of the sample in the extracting solvents. The integrated absorbance signals of Cr(VI) and Cr(III) extracted under these conditions are compared in Fig. 3. The result indicate that in the extracted DOSS and Aliquat-336 the Cr(III) and Cr(VI) integrated absorbance signal was quite stable up to

24 days of observation. The stability of the species were found to deteriorate, if lake water samples collected and stored at 4^{o}C and analyzed every day by the proposed method. The value of Cr(III) is found to increase by 135% and Cr(VI), reduce to 75% if extraction was done in the laboratory after one day. Hence, extraction of chromium species at sampling site ensures good stability for chromium species compared to when samples are not extracted onsite, instead stored at 4^{o}C and extracted in the laboratory. Therefore, once the extraction is done at site the samples can be analysed at ease when required.

Interference studies

In order to evaluate the performance of this procedure to various environmental matrices, the highest tolerability of various common interfering ions were studied. Interference may occur due to interaction of cationic and anionic species present in the natural and wastewaters with DOSS and Aliquat-336 micelles. The sequential extraction was performed by analyzing 50 mL of lake water samples spiked with interfering ions at different concentrations. The tolerance limits, defined as the concentration of added ion that causes less than ± 5% relative error in the determination of 10 ng mL^{-1} Cr(III) in presence of 500 ng mL^{-1} Cr(VI) and vice-versa were 15 and 20 g L^{-1} of Na^{+} and K^{+}, 100 mg L^{-1} of Ca^{2+} and Mg^{2+}, 60 mg L^{-1} of Pb^{2+}, 50 mg L^{-1} of Cd^{2+} and Hg^{2+}, 30 mg L^{-1} of Al^{3+}, Bi^{3+}, Ni^{2+} and Zn^{2+} and 20 mg L^{-1} of Cu^{2+} and Fe^{3+}, 2 mg L^{-1} of PO$_4^{3-}$ and 200 mg L^{-1}SO$_4^{2-}$; and 15 and 20 g L^{-1} of Na^{+} and K+, 150 mg L^{-1} of Ca^{2+} and Mg^{2+}, 50 mg L^{-1} of Pb^{2+}, 40 mg L^{-1} of Cd^{2+} and Hg^{2+}, 20 mg L^{-1} of Al^{3+}, Bi^{3+}, Ni^{2+} and Zn^{2+} and 10 mg L^{-1} of Cu^{2+} and Fe^{3+}, 1 mg L^{-1} of PO$_4^{3-}$ and 100 mg L^{-1} SO$_4^{2-}$ respectively. Approximately the same tolerability of these foreign ions was obtained for the simultaneous extraction also. These results indicated that the major ions in the water samples had no obvious effect on the recovery of the target species; hence, it can be applied to various types of water samples.

Analytical recovery

The recoveries studied were performed by spiking the individual and mixture of Cr(III) and Cr(VI) in the range of 0.05-30 ng mL^{-1} to river waters, lake water and tannery effluent samples in different combinations and determined the sequential and simultaneous recovery of these species. The recoveries obtained are given in Table 2. The recoveries were between 95 and 101% in all studied matrices with relative standard deviation between 1 and 10%, which indicated that the studied matrices have no significant effect on the recovery of individual and total chromium species in the proposed extraction procedure. Therefore, the present procedure can be used for real water samples for the determination of individual and total chromium species.

The amount of sample volume, volumes of dispersive surfactants, initial and final volumes of the extraction solvent and also the extraction time will decide the pre-concentration factor (PCF) in the DLLME process. The PCF is the ratio of the analyte concentration in the final volume of the trichloroethylene phase to that of the initial aqueous phase. Under the optimized conditions, the PCF was found to be 100 and 90; and 83, respectively for sequential and simultaneous extraction of Cr(III) and Cr(VI); and total Cr.

Figures of merit

Under the optimized experimental conditions, the calibration curve was obtained by pre-concentrating the successively spiked standards of Cr(III) and Cr(VI) in the range of 0.02-1 ng mL^{-1}. The correlation coefficients were between 0.9953 -0.9994. Quantifications were performed by using aqueous standard calibration graph. The limit of detections (LOD's) calculated based on three times the standard deviation of ten measurements of procedural blanks were 0.5, 0.6 and 0.7 pg mL^{-1}, respectively for sequential and simultaneous extraction of Cr(III) and Cr(VI). The relative standard deviations of procedural blanks were in between 3-6%.

Comparison with other methods

A comparison of the proposed procedures with other reported pre-concentration methods for speciation analysis of chromium is presented in Table 3. As shown in Table 4, the analytical characteristic of the proposed procedure is better than the reported methods. The strong electrostatic interaction of Aliquat-336 and DOSS micelles replaced the strong hydrated sphere of Cr(III), and reacting strongly with labile Cr(VI), at room temperature, which provides the fast extraction and avoids the use of chelating agent and oxidation/reduction steps in the process.

Analysis of standard reference material and analytical application

The accuracy of the proposed procedure developed for sequential and simultaneous extraction and analysis of chromium was verified by analyzing certified reference materials such as NIST 1640 natural water, BCR 714 industrial influent and BCR 715 industrial effluent. The results are given in Table 4. The results showed that the sum of the concentrations of individual chromium species obtained by sequential extraction procedure were found to be in good agreement with the values obtained from reported surfactant assisted dispersive liquid-liquid microextraction based on the solidification of floating organic drop procedure and also agreed with the certified concentration for total chromium as well as values obtained by simultaneous extraction procedure.[31] It indicated the accuracy of the both sequential and simultaneous extraction procedures. This procedure has also been applied to the determination of Cr(III) and Cr(VI) species and total chromium in real samples such as lake water, tannery effluents and river water samples. These results indicated that the value of Cr(VI) and total Cr found in lake water and river water samples were below the values of 10 ng mL^{-1} specified by EPA. In the case of tannery effluent, Cr(VI) and total chromium were more than the EPA specified levels, which indicates the necessity of treatment of these effluents before discharged into the environmental water bodies.

Conclusions

The combination of ligandless micelles and mixed-micelles strategy with dispersive liquid-liquid microextraction (M-MM-DLLME) followed by ETAAS determination provides a fast and sensitive method for the sequential and simultaneous chromium speciation in lake water, tannery effluent and river water samples. This method can be applied for on-site separation and stabilization of species. It is easy to transport the low volumes of extracted phase to laboratory at room temperature for quantification. This process reduces the chemicals used such as organic solvents disperser, chelating agent and oxidation/reduction reagents There is no need to store and transport the samples at 4^{o}C. Therefore the method is cost effective and can be easily used by laboratories involved in water quality monitoring. This process works on different varieties of water samples and effluent containing sub ppb to ppm levels of chromium species.

References

1. J. O. Nriagu, E. Nieboer, Chromium in Natural and Human Environment, Wiley Interscience, New York, 1988.
2. M. I. N. Ahameda and P.M. Kashif, *Int. J. Pharm Sci. Res.,* 2014, **5,** 733-736.
3. A. A. Belay, *J. Environ. Prot.,* 2010, **1,** 53-58.
4. S.Vijayanand and J. Hemapriya, *Int. J. Curr. Mirobio. App. Sci.,* 2014, **3(9),** 971-982.
5. F. Baruthio, *Biol. Trace Elem. Res.,* 1992, **32,** 145-153.
6. S. A. Katz and H. Salem, *J. Appl. Toxicol.,* 1993, **13(3),** 217-224.
7. http://www.ct.gov/dph/lib/dph/drinking_water/pdf/Hexavalent_Chromium_in_Drinking_Water.pdf.

8. J. Wang, K. Ashley and D. Marlow, *Anal. Chem.,* 1999, **71,** 1027-1032.
9. https://www.agilent.com/cs/library/applications/5989-2481EN.pdf
10. E. Leese, J. Morton, P. H. E. Gardiner and V. A. Carolan, *J. Anal. At. Spectrom.*, 2016, **31,** 924-933.
11. S. Beaubien, J. Nriagu, D. Blowes and G. Lawson, *Environ. Sci. Technol.*, 1994, **28 (4),** 730-736.
12. www.epa.gov/sites/production/files/2015-08/documents/method_218-6_rev_3-3_1994.pdf
13. J. Ma, W. Lu and L. Chen, *Current Anal. Chem.,* 2012, **8,** 78-90.
14. H. M. A. Saidi and A. A. A. Emara, *J. Saudi, Chem. Soc.,* 2014, **18,** 745-761.
15. P. Viñas, N. Campillo, I. López-García and M. Hernández-Córdoba, *Anal. Bioanal. Chem.,* 2014, **406(8),** 2067-2099.
16. P. Hemmatkhan, A. Bidari, S. Jafarvand, M. H. M. Reza, Y. Assadi, *Microchim. Acta,* 2009, **166,** 69-75.
17. H. Chen, P. Du, J. Chem, S. Hu, S. Li and H. Liu, Talanta, 2010, **81,** 176-179.
18. J. A. Baig, A. Hol, A. Akdogan, A. A. Kartal, U. Divrikli, T. G. Kazi and L. Elci, *J. Anal. At. Spectrom.,* 2012, **27,** 1509-1517.
19. I. Lopez-García, M. Briceño, Y. Vicente-Martínez and M. Hernández-Córdoba, *Talanta,* 2013, **115,** 166-171.
20. I. Lopez-García , Y. Vicente-Martínez and M. Hernández-Córdoba, *J. Anal. At. Spectrom.,* 2012, **27,** 874-880.
21. I. Gaubeur, M. Á. Aguirre, N. Kovachev, M. Hidalgo and A. Canals,
22. M. R. Moghadam, S. Dadfarnia and A. M. H. Shabani, *J. Hazard. Mater.,* 2011 **186,** 169-174.

23. M. R. K. Zanjani, Y. Yamini, S. Shariati and J. A. Jonsson, *Anal. Chim. Acta,* 2007, **595,** 286-293.

24. M. Rezaee, Y. Yamini, A. Khanchi, M. Faraji and A. Saleh, *J. Hazard. Mater.,* 2010, **78,** 766-770.

25. M. Moradi, Y. Yamini, A. Esrafili and S. Seidi, *Talanta,* 2010, **82,** 1864-1869.

26. K. Shrivas and K. Dewangan, *J. Surfact. Deterg.,* 2015, **18,** 1137-1144.

27. Q. Deng, M. Chen, L. Kong, X. Zhao, J. Guo and X. Wen, *Spectrochim. Acta, Part A,* 2013, **104,** 64-69.

28. M. Behbahani, F. Najafi, S. Bagheri, M. K. Bojdi, M. Salarian and A. Bagheri, *J. Chromatogr. A*, 2013, **1308,** 25-31.

29. S. M. Yousefi and F. Shemirani, *J. Hazard. Mater.,* 2013, **254-255,** 134-140.

30. M. D. M. Abadi, M. Chamsaz, M. H. A. Zavara and F. Shemirani, *Anal. Methods,* 2013, **5,** 2971-2977.

31. N. N. Meeravali, R. Manjusha and S. J. Kumar, *J. Anal. At. Spectrom.,* 2014, **29,** 2168-2175.

32. S. Sadeghi and A. Z. Moghaddam, *Talanta,* 2012, **99,** 758-766.

33. Y. Liu, J. Hu, Y. Li, W. Hai-Ping, L. Xiao-Shuang, Z. Xi-Hua, C. Shi-Min and C. Xiao-Qin, *Talanta,* 2015, **134,** 16-23.

Table 1 Optimized temperature program used for the determination of chromium using ETAAS.

Step	Temperature/^{o}C	Ramp/^{o}C s^{-1}	Hold/s	Ar flow/ mL min^{-1}
Drying-1	60	6	10	250
Drying-2	90	5	10	250
Pyrolysis	1000	300	10	250
Gas adaption	1000	0	5	250
Atomization	2300	2000	4	0 (read)
Cleaning	2450	1500	3	250

Table 2 Sequential and simultaneous recovery of Cr(III) and Cr(VI) in lake water, tannery effluents and river water samples (n=4)[a].

Matrices	Spiked/ng mL^{-1}		Recovery (%)		
			Sequential extraction		Simultaneous extraction
	Cr(VI)	Cr(III)	Cr(VI)	Cr(III)	Cr(III + VI)
River water	0	0	25 ± 9[b]	35 ± 10[b]	70 ± 8 [b]
	0.05	0	98 ± 4	-	101 ± 4
	0	0.05	-	97 ± 3	98 ± 3
	0.05	0.1	99 ± 5	99 ± 5	99 ± 4
	0.2	0.05	99 ± 3	97 ± 5	100 ± 3
Lake water	0	0	0.8 ± 0.4	7.4 ± 1	8.3 ± 0.7
	0.4	0	97 ± 5	-	98 ± 4
	0	0.8	-	95 ± 6	97 ± 5
	5	1	96 ± 4	98 ± 5	98 ± 6
Tannery effluent	0	0	26 ± 3	95 ± 8	127 ± 7
	20	0	98 ± 3	-	99 ± 3
	0	15	-	97 ± 3	98 ± 4
	15	30	99 ± 2	98 ± 1	100 ± 3

[a] Values are mean of four measurements ± standard deviation. [b] Values are in pg mL^{-1}.

Table 3 Comparison of the proposed ligandless micelle and mixed-micelle assisted DLLME with other reported microextraction procedures for the speciation of chromium.

Extraction scheme and instrument	Experimental conditions	LOD/pg mL^{-1}	Ref.
DLLME- LIBS[a]	Complexing agent: DDTC[f] Dispersive solvent: Ethanol Extraction solvent: 1-undecanol Oxidizing reagent: $KMnO_4$ Speciation process: Differential	3.1/Cr(VI)	21
SFODME[b]-ETAAS	Complexing agent: TTA[g] Extraction solvent: 1-undecanol Reducing reagent: Hydroxylamine HCl Speciation process: Differential	6/Cr(III)	22
DLLME-SFO-ETAAS	Complexing agent: DPC Dispersive solvent: SDBS micelles[h] Ox./reducing agent: No Extraction solvent: 1-undecanol Speciation process: Sequential & simultaneous	3/Cr(III) 1/Cr(VI) 4/Total Cr	31
SM-DLLME-SFO-SP[c]	Complexing agent: DPC and SDS monomer Dispersive solvent: THA[i] Extraction solvent: Decanoic acid Oxidizing reagent: $KMnO_4$ Speciation process: Differential	230/Cr(VI)	30
IP-SA-DLLME-SP[d]	Complexing agent: DPC Dispersive solvent: SDS monomers Extraction solvent: 1-octanol Oxidizing reagent: $KMnO_4$ Speciation process: Differential	50/Cr(VI)	29
TCME[e]-ETAAS	Complexing agent: APDC[j] Temperature/time: 45^{0}C for 25 min Ox./reducing agent: No Extraction solvent: [HMIM][PF_6] [k] Speciation process: Sequential	5.4/Cr(III) 2.45/Cr(VI)	32
M-MM-DLLME-ETAAS	Complexing agent: No Dispersive solvent: DOSS & Aliquat-336 micelles Oxd./reducing agent: No Extraction solvent: Trichloroethlene Speciation process: Sequential & simultaneous	0.5/Cr(III) 0.6/Cr(VI) 0.7/Total Cr	This work

[a] Laser-induced breakdown spectrometry, [b] Solidified floating organic drop microextraction, [c] Supra molecular dispersive liquid–liquid microextraction solidification of floating organic drops followed by UV-Vis spectrophotometery, [d] Ion pair based-surfactant assisted dispersive liquid-liquid microextraction followed by fiber optic linear array detection spectrophotometry, [e] Temperature-controlled microextraction, [f] diethyldithiocarbamate, [g] 2-thenoyltrifluoroacetone, , [h] sodium dodecyl benzene sulfonate, [i] tetrahydrofuran, [j] Ammonium pyrrolidine dithiocarbamate, [k] 1-hexy, l-3-methyli-midazoliumhexafluorophosphate ([HMIM][PF_6]) ionic liquid.

Table 4 The determined values of Cr(III), Cr(VI) and total Cr in certified and water samples using the proposed ligandless micelle and mixed-micelle assisted DLLME (M-MM-DLLME) procedure and compared with SFO-ME.[31]

Matrices	Measured values [a] (ng mL^{-1})				
	Sequential extraction				Simu. extraction
	Cr(VI)		Cr(III)		Total Cr
	M-MM-DLLME	SFO-ME[b]	M-MM-DLLME	SFO-ME[b]	M-MM-DLLME
BCR 715 Industrial effluent	265 ± 22	268 ± 28	740 ± 55	750 ± 60	1010 ± 75 (1000 ± 90)[c]
BCR 714 Industrial influent	50 ± 5	51 ± 6	80 ± 5	77 ± 5	131 ± 8 (123 ±10)[c]
NIST 1640 Natural water	4 ± 1	5 ± 1	35 ± 1	34 ± 1	39 ± 0.8 (38.6 ± 1.6)[c]
Analysis of real samples					
River water-1	0.030±0.008	0.036±0.012	0.040±0.009	0.038±0.013	0.072±0.007
River water -2	0.046±0.008	0.050±0.010	0.076±0.009	0.070±0.009	0.125±0.007
Lake water-1	0.8 ± 0.2	0.7 ± 1.1	7.2 ± 0.9	7.0 ± 1.1	8.2 ± 0.8
Lake water-2	2.3 ± 0.5	2.6 ± 1.1	6.5 ± 1.0	6.6 ± 1.1	8.6 ± 1.6
Tannery effluent-1	135 ± 12	120 ± 15	4900 ± 200	4845± 225	5000 ± 150
Tannery effluent-2	150 ± 15	145 ± 18	530 ±25	520 ± 35	680 ± 40
Tannery effluent-3	28 ± 3	32 ± 4	105 ± 12	110 ± 14	135 ± 22

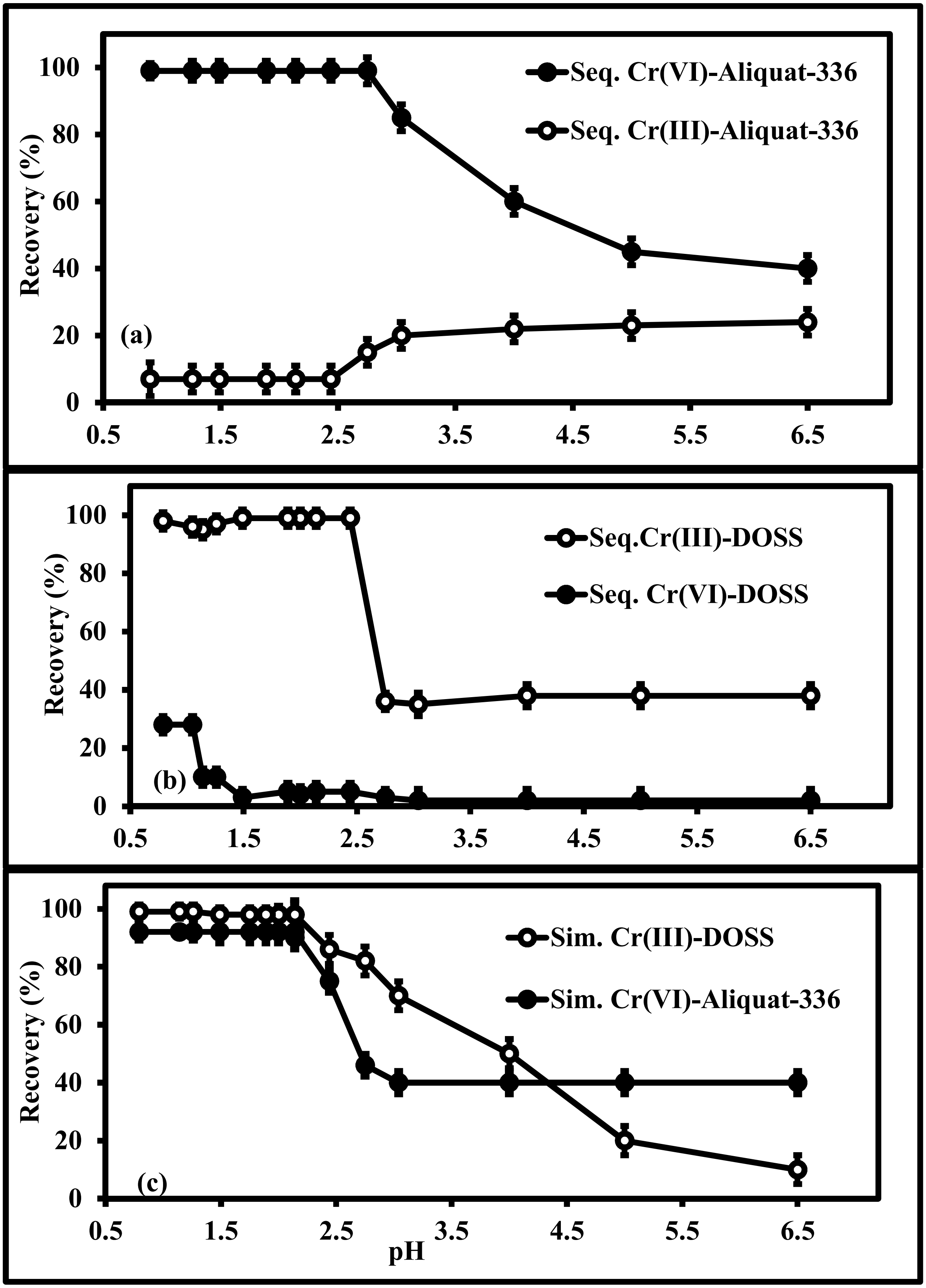
[a] Mean of four determinations ± standard deviation. [b] Values are obtained using surfactant assisted dispersive liquid–liquid microextraction based on the solidification of floating organic drop procedure.[31] [c] Certified values.

Figure captions:

Fig. 1. Effect of pH on the recovery of Cr(III) and Cr(VI). Sequential extraction: (a) extraction of Cr(III) and Cr(VI) in presence of 0.4% m/v DOSS and 0.6 mL of trichloroethylene and (b) extraction of Cr(VI) in presence of 0.2% m/v Aliquat-336 and 0.6 mL of trichloroethylene. Simultaneous extraction: (c) extraction of Cr(III) and Cr(VI) in presence of 0.4% m/v DOSS, 0.2% m/v Aliquat-336 and 0.6 mL of trichloroethylene. The error bars indicates the relative standard deviation (%) at each measurements ($n = 4$).

Fig. 2 Effect of surfactants on the sequential and simultaneous M-MM-DLLME of chromium species. (a) Recovery of Cr(III) in presence of pH 2 and 0.6 mL of trichloroethylene; (b) Recovery of Cr(VI) in presence of pH 2 and 0.6 mL of trichloroethylene and (c) Recovery of Cr(III) and Cr(VI) in presence of pH 1.5 and 0.6 mL of trichloroethylene. All the extraction were performed at room temperature and the error bars indicate the standard deviation at each measurement (n=3). The arrow indicates the critical micelles concentration (CMC) of surfactants.

Fig. 3 Stability of Cr(III) and Cr(VI) extracted at sampling site in to extracting phase and lake water samples analysed in laboratory using sequential extraction with time.



Seq. Cr(VI)-Aliquat-336
Seq. Cr(III)-Aliquat-336
Recovery (%)
(a)
0
20
40
60
80
100
0.5
1.5
2.5
3.5
4.5
5.5
6.5
Seq.Cr(III)-DOSS
Seq. Cr(VI)-DOSS
(b)
Sim. Cr(III)-DOSS
Sim. Cr(VI)-Aliquat-336
(c)
pH

Fig. 1.

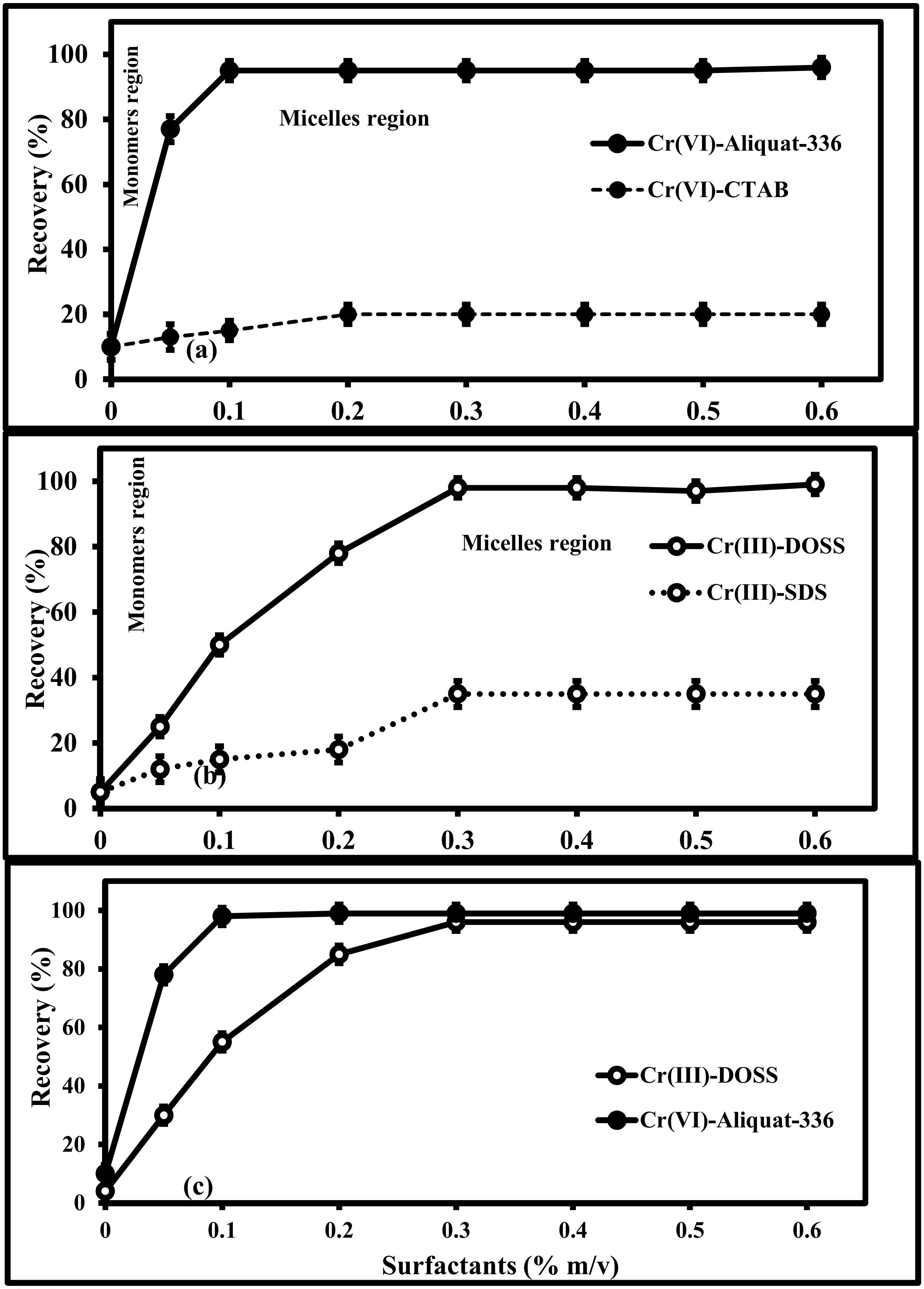

Recovery (%)
100
80
60
40
20
0
Monomers region
Micelles region
Cr(VI)-Aliquat-336
Cr(VI)-CTAB
(a)
0 0.1 0.2 0.3 0.4 0.5 0.6
Recovery (%)
Monomers region
Micelles region
Cr(III)-DOSS
Cr(III)-SDS
(b)
Recovery (%)
Cr(III)-DOSS
Cr(VI)-Aliquat-336
(c)
Surfactants (% m/v)

Fig. 2.

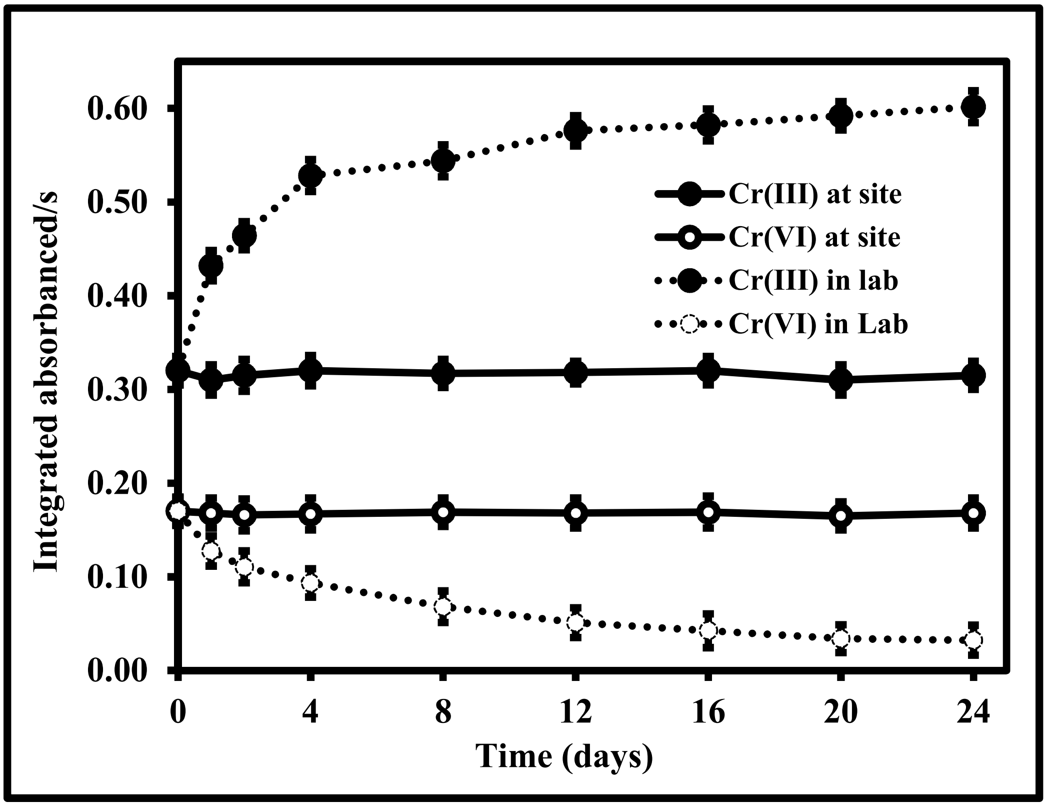

0.60
0.50
0.40
0.30
0.20
0.10
0.00
Integrated absorbanced/s
0
4
8
12
16
20
24
Time (days)
Cr(III) at site
Cr(VI) at site
Cr(III) in lab
Cr(VI) in Lab

Fig. 3.

Graphical abstract for Cr-M-MM-DLLME