Introduction

Chromium in the Earth’s crust occurs in various associated forms.1 Its concentration in natural water is often found to be very low, but it may be discharged into water as effluents from industries such as steel, alloys, pigments, electroplating and tanneries.2 Contamination of drinking water with chromium can occur due to the leaching of corrosion inhibitors used in municipal water pipes.3 WHO recommended the maximum limit for total chromium in drinking water to be 0.05 mg L–1.4 The two most stable forms of chromium, +3 and +6, have entirely contrasting biological and toxicological effects. While Cr(III) is considered to be an essential micronutrient for humans, exposure to Cr(VI) compounds can cause damage to skin, respiratory tract and kidneys, and also increases risk of lung cancer.5

Spectro-analytical methods such as FAAS,6 ET-AAS,7 ICP-OES8 and ICP-MS9 can detect the total chromium in water samples. However, these singular detection techniques suffer from low sensitivity and precision due to interferences in the sample matrix. Thus, a pretreatment step is required prior to detection, which can preconcentrate chromium from the matrix selectively. In recent years, several chemical enrichment methods have been employed for the preconcentration of chromium species, such as fractionation,10 liquid-liquid extraction,11 dispersive liquid-liquid micro-extraction,12 solid-phase extraction13 and coprecipitation.14 Solid-phase extraction (SPE) studies have been widely used for the preconcentration of chromium, even in the presence of interferences, while reducing the usage of toxic organic solvents. Moreover, the regeneration of the solid extractant is effortless, and can be regenerated repeatedly. Several materials have been used in the past as solid supports for chromium speciation, such as activated carbon,15 alumina,16 chelating resins,17,18 Amberlite XAD series resins such as Amberlite XAD-4,19 Amberlite XAD-16,20 etc., silica gels,21,22 nano-materials,23 etc. However, SPE-FAAS in the batch mode has several disadvantages, such as: high reagent consumption, time consuming, prone to contamination, poor result reproducibility and risk of analyte loss. On-line flow-injection SPE has the advantages of being quick, relatively more sensitive, less sample and reagent consuming and gives high-precision results. Moreover, analyte loss is minimized, and thus, the preconcentration factor is high. Flow injection-solid phase extraction with FAAS (FI-SPE-FAAS) has been applied for the preconcentration of several other heavy metal ions, such as cadmium,24 copper,25 lead,26 zinc,27 etc.

The aim of the reported work is to use Amberlite XAD-16 chemically modified with xylenet orange chelating resin as an on-line preconcentration method to achieve the speciation of Cr(III) and Cr(VI), and for determination in industrial water samples using FIA interfaced to FAAS. Xylenol orange is one of the few agents that has been shown to form a complex with Cr(III) spectrophotometrically,28 and hence has been chosen as the chelating agent for chromium preconcentration.
Experimental

Instrumentation
A PerkinElmer AAnalyst400 flame atomic absorption spectrometer with an air-acetylene flame was used. A chromium hollow cathode lamp (PerkinElmer Lumina) was used as the light source with the wavelength and operating current set at 357.9 nm and 10 mA, respectively. A PerkinElmer FIAS 400 flow-injection system was coupled with the AAnalyst400. This on-line system was controlled by Winlab32 TM (Ver. 6.5.0.0266) application software through a personal computer. The flow-injection preconcentration system consisted of two peristaltic pumps, a 5-port injection valve and a mini-column. The pH of the sample solutions was adjusted with an ELICO pH meter Model LI614 (ELICO Ltd., India).

Reagents and materials
All analytical reagents used were of analytical reagent grade. The polymeric support used was an Amberlite XAD-16 (Sigma-Aldrich), which has a surface area of approximately 800 m² g⁻¹ and a moisture holding capacity of 62 – 72%. Xylenol orange (Thomas Baker) was used as the chelating agent. Certified standard solutions of Cr(III) and Cr(VI) (Fluka) having concentrations 1000 mg L⁻¹ traceable to National Institute of Standards and Technology (NIST) were used for preparing standard working solutions of Cr(III) and Cr(VI) by dilution. Ultrapure 18.2 MΩ water from a DirectQ3 purification system (Merck Millipore) was used for dilutions. For pH adjustments, 0.1 M HCl, phosphate buffer (pH 6 – 8) and trisodium phosphate buffer (pH 11.7) were used. Polypropylene apparatus (POLYLAB) was used; it was washed with 10%(v/v) nitric acid, rinsed with 18.2 MΩ water and dried thoroughly before use.

Synthesis and characterization of Amberlite XAD-16 functionalized with xylenol orange (AXAD16-XO) resin
The synthesis of Amberlite XAD-16 (styrene-divinylbenzene copolymer) functionalized with xylenol orange was done as specified before.²⁹

Packing of minicolumn
The minicolumn was prepared by packing ~80 mg of the synthesized AXAD16-XO resin in a glass column (3.0 cm × 3.0 mm). The resin was injected into the minicolumn with the help of a syringe; the ends were sealed with cotton wool. The column was then washed with 2.0 mol L⁻¹ HNO₃, and after that with 18.2 MΩ water.

On-line preconcentration procedure for determination of Cr(III)
The flow injection-flame atomic absorption spectrometry (FI-FAAS) manifold was operated by a computer controlled program, which involves pre-filling, filling, loading and elution steps. The schematic diagram (Fig. 1) and computer program of the on-line preconcentration system using flow injection analysis system (FIAS) were as described before.³⁰ During the final elution step, the eluent containing the desorbed Cr(III) was transported directly to the nebulizer of the FAAS, since the output of the FI system was directly connected to the nebulizer via a PTFE tube of suitable length. The peak height (absorbance) was taken as the analytical signal. Each measurement was preceded by a blank measurement. Three replicate measurements for all standard and sample solutions were recorded.

Total chromium determination
For the preconcentration of Cr(VI), it was reduced to Cr(III) by the addition of 0.1 mL of 5% hydroxylamine hydrochloride to a 25 mL solution of 50 μg L⁻¹ Cr(VI). The solution was kept at room temperature for 45 min, and was analyzed according to the optimized preconcentration procedure. After the reduction of Cr(VI) to Cr(III), the total chromium concentration was determined. The concentration of Cr(VI) was calculated by subtracting the concentration of Cr(III) from the total chromium concentration.

General procedure for the analysis of industrial water samples
Water samples from three industrial areas in Delhi (Anand Parbat, Seelampur and Wazirpur) were collected, acidified and stored in polypropylene bottles. Then, 25 mL of each of the three water samples were first filtered through a 0.45-μm pore size filter paper (Merck Millipore) to remove suspended particulates. The water samples were analyzed for Cr(III), and then spiked with a certified Cr(III) solution traceable to NIST. Cr(VI) was determined by the reduction of Cr(III); its concentration in water samples was obtained by subtracting the Cr(III) concentration from the total chromium concentration. Spiking of the water samples with a certified Cr(VI) solution traceable to NIST was done.

Results and Discussion

Characterization of AXAD16-XO
The characterization of Amberlite XAD-16 (styrene-divinylbenzene copolymer) functionalized with xylenol orange was done as given before.²⁹

Fig. 1 Schematic diagram of the FI-FAAS system.
Effect of the pH. The retention of Cr(III) on the minicolumn was studied in the pH range of 2 – 10 with a 50 μg L⁻¹ of Cr(III) solution, while keeping the other parameters constant. As shown in Fig. 2a, the maximum retention of Cr(III) on the minicolumn was achieved at pH 5. Thus, all subsequent studies were carried out at pH 5.

Effect of the eluent concentration. Two potential eluents were studied for the desorption of Cr(III) from the sorbent: HNO₃ and HCl. The absorbance signals achieved with HNO₃ as eluent were sharper and better than those with HCl as the eluent. Thus, HNO₃ was chosen as the eluent, and effect of its concentration on the Cr(III) desorption was studied in the range of 0.01 – 2.0 mol L⁻¹. Thus, 0.5 mol L⁻¹ HNO₃ was chosen as the eluent for the maximum recovery of Cr(III). Figure 2b shows the dependence of absorbance on the eluent concentration.

Effect of the sample and eluent flow rate. The effect of the sample flow rate was studied in the range of 4.0 – 10.0 mL min⁻¹. The results are shown in Fig. 2c. The maximum absorbance was obtained at a flow rate of 5.0 mL min⁻¹. Thus, the sample flow rate was kept at 5.0 mL min⁻¹ for all subsequent studies. The effect of the eluent flow rate on the recovery of Cr(III) was investigated in the range of 2.0 – 7.0 mL min⁻¹. The absorbance increased steadily until 6.0 mL min⁻¹, and then decreased at a higher flow rate. Thus, the eluent flow rate of 6.0 mL min⁻¹ was kept constant for all following analysis. The results are shown in Fig. 2d.

Effect of interfering ions. The selectivity for the preconcentration of 100 μg L⁻¹ of Cr(III) by AXAD16-XO resin under the optimized conditions was carried out in the presence of large concentrations of certain commonly present organic and inorganic interfering ions in water. The effect of these ions is considered to be interfering when the absorbance obtained in their presence is deviated by more than ±5%. In the presence of high amounts of these ions, the variation in the absorbance observed was within the limits. Table 1 lists the tolerance limit of the chelating resin towards large amounts of specific ions. It was found that AXAD16-XO tolerates high amounts of nitrate, sodium, chloride, etc. and achieves a high recovery of Cr(III) even under such conditions.

Analytical figures of merit for the Cr(III) preconcentration. The on-line preconcentration of Cr(III) using AXAD16-XO...
chelating resin under the optimized conditions was studied. The results are summarized in Table 2. The preconcentration factor is defined as the ratio of the slopes of the calibration curves with and without preconcentration. The proposed method gives preconcentration factors of 33 and 73 for preconcentration times of 60 and 120 s. The limit of detection (LOD) is defined as three-times the standard deviation of the blank (3σ), and was found to be 0.25 and 0.11 μg L⁻¹ for seven replicates and preconcentration times of 60 and 120 s, respectively. The limit of quantification (LOQ) is defined as ten-times the standard deviation of the blank (10σ), and was found to be 0.84 and 0.36 μg L⁻¹. The relative standard deviation (%RSD) is a measure of the precision of the observations. It was found to be 1.57 and 1.08% for 50 and 100 μg L⁻¹ of Cr(III), respectively.

**Adsorption capacity**

The adsorption capacity of the AXAD16-XO resin was studied using a 100 mg L⁻¹ of Cr(III) solution according to the reported procedure. The proposed method gives a sorption capacity of 26.7 mg g⁻¹, which is higher in comparison to other reported systems.

**Reusability of resin**

The loading of Cr(III) and its elution in a single program constituted one sorption-desorption cycle. After many such cycles, the sorption capacity of the used resin was compared with that of the untreated resin. It was found that the sorption capacity varied by <5% and the reproducibility of the measurements was unaffected even after ~300 cycles.

**Applications to industrial water samples**

The applicability of the FI-SPE-FAAS method was studied by employing the method described above on three different water samples. It was found that the system has good recovery for Cr(III). The detailed results of these studies are given in Table 3.

**Accuracy of the on-line preconcentration system**

The accuracy of the proposed system was studied by determining the concentration of Cr(III) in a standard reference material, 1643e (Trace Elements in Water), supplied by NIST, Gaithersburg, USA. It was found that there is no significant difference in the Cr(III) concentration obtained from the proposed methodology (19.81 ± 0.24) and the certified results (19.81 ± 0.24). The recovery of Cr(III) was found to be 97.1%.

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### Table 1

Effect of interfering ions on the recovery of Cr(III) by AXAD16-XO resin

| Interfering ion | Tolerance limit/ mg L⁻¹ |
|-----------------|------------------------|
| NO₃⁻            | 12300                  |
| Na⁺             | 6500                   |
| Cl⁻             | 5000                   |
| CH₃COO⁻         | 1600                   |
| I⁻              | 1150                   |
| Br⁻             | 900                    |
| NH₄⁺            | 750                    |
| K⁺              | 450                    |
| SO₄²⁻           | 200                    |
| Pb²⁺            | 125                    |

| Interfering ion | Tolerance limit/ mg L⁻¹ |
|-----------------|------------------------|
| NO₃⁻            | 12300                  |
| Na⁺             | 6500                   |
| Cl⁻             | 5000                   |
| CH₃COO⁻         | 1600                   |
| I⁻              | 1150                   |
| Br⁻             | 900                    |
| NH₄⁺            | 750                    |
| K⁺              | 450                    |
| SO₄²⁻           | 200                    |
| Pb²⁺            | 125                    |

### Table 2

Analytical performance of the on-line preconcentration system for Cr(III) determination using AXAD16-XO resin

| Parameter                                | Figures of merit |
|------------------------------------------|------------------|
| Preconcentration time/PT, s              | 60               |
| Sample consumption/mL                    | 5.0              |
| Dynamic range/μg L⁻¹                     | 0.84 - 600       |
| Limit of detection/μg L⁻¹                 | 0.25             |
| Limit of quantification/μg L⁻¹            | 0.84             |
| Sample throughput/h⁻¹                    | 30               |
| Preconcentration factor                  | 33               |
| Correlation coefficient                  | 0.9999           |

Regression equation (6 standards, Cr(III)/μg L⁻¹) (with preconcentration): $A_{60} = 0.00226 [\text{Cr(III)}] - 0.00195$ $A_{120} = 0.00512 [\text{Cr(III)}] - 0.00250$

Regression equation (6 standards, Cr(III)/mg L⁻¹) (without preconcentration): $A = 0.07 [\text{Cr(III)}] + 0.00025$ $A = 0.07 [\text{Cr(III)}] + 0.00012$

Precision (%RSD): $1.57, [\text{Cr(III)}] = 50 \mu g L^{-1}$ $1.08, [\text{Cr(III)}] = 100 \mu g L^{-1}$

### Table 3

Analytical results for application to industrial water samples

| Sample                  | Added/μg L⁻¹ | Found/μg L⁻¹ | Recovery, %b |
|-------------------------|--------------|--------------|--------------|
|                         | Cr(III)      | Cr(VI)       | Cr(III)      | Cr(VI)      | Total Cr | Cr(III) | Cr(VI) |
| Anand Parbat industrial area, Delhi, India | —            | —            | 27.6 ± 1.2   | 12.7 ± 1.1  | 40.3 ± 3.2 | 96.8       | 96.0     |
| —                       | 20           | 20           | 46.1 ± 0.9   | 31.4 ± 3.9  | 77.5 ± 4.8 | 97.2       | 101.5    |
| Seelampur industrial area, Delhi, India | —            | —            | 23.2 ± 2.0   | 6.8 ± 1.9   | 30.0 ± 5.0 | 97.2       | 101.5    |
| —                       | 20           | 20           | 42.0 ± 2.6   | 27.2 ± 0.5  | 69.2 ± 3.1 | 100.0      | 95.2     |
| Wazirpur industrial area, Delhi, India | —            | —            | 28.9 ± 1.6   | 11.6 ± 1.1  | 40.5 ± 2.9 | 100.0      | 95.2     |
| —                       | 20           | 20           | 48.9 ± 2.4   | 30.1 ± 2.6  | 79.0 ± 3.0 | 100.0      | 95.2     |

a. Spiking with Cr(III) and Cr(VI) solutions traceable to NIST. b. Confidence interval 95%.
hence there is no need for re-packing of the chelating resin. The reagents. The mini-column used does not show leaching, and for the speciation of Cr(III) and Cr(VI) has been developed. The total chromium line SPE based systems (Table 4). The method has been applied chelating resin shows good sorption for Cr(III), and a reduction in the analysis time as well as the consumption of reagents. The proposed method offers better selectivity, excellent precision, high preconcentration factor, high sampling frequency and improved detection limits as compared to other reported on-line SPE based systems. Table 4 gives the analytical performance of some reported on-line FAAS methods.

**Conclusions**

A highly sensitive and rapid on-line solid-phase extraction method using Amberlite XAD-16 modified with xylenol orange for the speciation of Cr(III) and Cr(VI) has been developed. The use of a flow-injection system interfaced to FAAS leads to a reduction in the analysis time as well as the consumption of reagents. The mini-column used does not show leaching, and hence there is no need for re-packaging of the chelating resin. The chelating resin shows good sorption capacity for Cr(III), and stands out amongst its contemporaries. The total chromium determination by reduction was found to be a suitable method for the determination of Cr(III) and Cr(VI) in a given unknown sample. The proposed method offers better selectivity, excellent precision, high preconcentration factor, high sampling frequency and improved detection limits as compared to other reported on-line SPE based systems (Table 4). The method has been applied to industrial water samples; spike studies show that good recovery of Cr(III) has been achieved within the 95% confidence interval. For validation of the method, the proposed method was applied to NIST Standard Reference Material 1643e; it was found that the certified and observed results were in close agreement. Thus, the proposed method for on-line speciative determination of chromium has been successfully applied.

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**Table 4**  Comparison of some reported on-line preconcentration FAAS methods with the proposed work

| Support                        | Chelating agent                  | Eluent | PT/s | Sample volume/mL | RSD, % | DL/μg L⁻¹ | PF | Ref. |
|--------------------------------|----------------------------------|--------|------|------------------|--------|-----------|----|-----|
| Amberlite XAD-16              | Xylenol orange                    | HNO₃   | 120  | 10               | 1.08   | 0.11      | 73 | This work |
| Divinyl benzene               | Poly 2-(5-methylisoxazol)-metal  | HNO₃   | 210  | 5.8              | 2.3    | 0.05      | 48 | 39  |
| Polysilicate matrix           | Funaria hygrometrica             | HNO₃   | 180  | 6                | 1.7    | 1.15      | 22 | 41  |
| FeC nanoparticles             | Chitosan                         | HCl    | 4000 | 100              | 2.5    | 0.0524    | 100| 33  |
| Llama Fibres                 | —                                | NaOH   | 750  | 25               | 4.3    | 0.3       | 32 | 40  |
| MWCNT’s                      | —                                | HNO₃   | 180  | 6                | 1.7    | 1.15      | 22 | 41  |
| Silica                        | Al₂O₃/TiO₂                       | HCl    | 120  | 15               | 4.6    | 0.34      | 23 | 44  |
| Silica gel                    | Zirconium oxide                  | HNO₃   | 225  | 15               | 3      | 1.9       | 20.8| 45 |

PT, Preconcentration time; RSD, relative standard deviation; DL, detection limit; PF, preconcentration factor.
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