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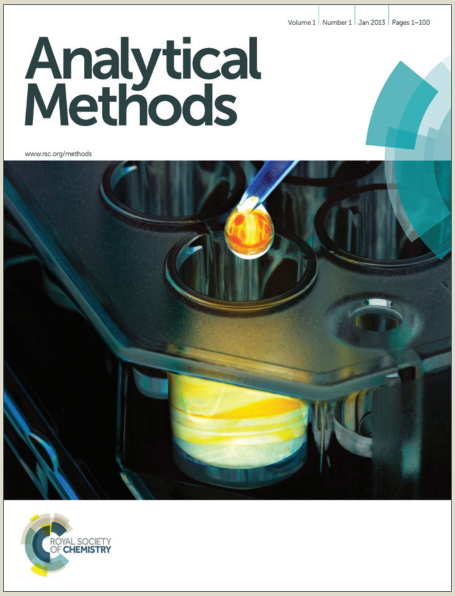

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Multi-commutation flow system with on-line solid phase extraction exploiting the ion imprinted polymer and FAAS detection for chromium speciation analysis in sewage samples

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Abstract

A multi-commutation flow system using on-line solid phase extraction and flame atomic absorption spectrometric detection (MCFA-FAAS) for study of chromium speciation in sewage samples was developed. A new chromium imprinted polymer was prepared and applied as a selective sorbent for separation and preconcentration of Cr(III) ions. The polymer was prepared from the Cr(III)-8-hydroxyquinoline complex as a template, styrene as a functional monomer, divinylbenzene as a cross-linking monomer using 2,2'-azobisisobutyronitrile as an radical initiator by bulk polymerization. The chromium imprinted polymer exhibits good chemical and mechanical stability, sorption capacity and selectivity towards Cr(III) ions. The analyte, Cr(III) ions, was quantitatively retained on the sorbent at pH 9, eluted with 0.3 mL of 0.1 mol L^{-1} HNO_3 and determined by FAAS with a detection limit of 2.1 ng mL^{-1} and 33-fold preconcentration factor obtained for 10 min loading time. The MCFA-FAAS system is fully automated and enables analysis of 1 mL of sample during 4.5 min, what gives the throughput of 12 samples per hour. The accuracy of the proposed method was proved by analysis of reference material of waste water RES 10.2 (ielab). The method was successfully applied to the determination of trace amounts of Cr(III) in municipal sewage samples.

Key words: chromium speciation, 8-hydroxyquinoline, ion imprinted polymer, preconcentration, automation, waste water

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Introduction

Chromium can be introduced into surface water from natural sources as leaching from rocks, deposition of atmospheric aerosols and run-off from the topsoil. However, the significant quantities of this metal are introduced into water system from various anthropogenic sources as metallurgy, electroplating, leather tanning [1,2]. Depending on the character of industrial processes chromium is emitted to effluents in the form of various species in wide range of concentration. The concentration ratio of Cr(III) to Cr(VI) in the environment depends on the sample conditions, such as pH, content of oxygen and organic matter, presence of complexing agents and redox potential.

The information on chromium speciation in water, waste water or drinking water is of significance, because the physiological and toxicological effects of Cr(III) and Cr(VI) on biological systems are totally opposite. Cr(III) is considered as an essential element for proper functioning of living organisms, whereas Cr(VI) shows mutagenic and carcinogenic effects [1,3]. According to the European Council Directive 98/83/EC [4] on the quality of water intended for human consumption, the maximal concentration of total chromium and Cr(VI) in drinking water cannot exceed 0.05 mg L^{-1} and 0.02 mg L^{-1}, respectively. In Poland, the maximal concentration of chromium in sewage, which can be introduced into water or soil, can not exceed 0.05 - 0.5 mg L^{-1} of Cr(VI) and 0.5 - 1 mg L^{-1} of total chromium, depending on a kind of industry [5].

There are several methods available for chromium speciation in environmental samples, such as chromatography [6,7], co-precipitation [8], liquid-liquid extraction [9,10], or solid phase extraction (SPE) [11-14] coupled with different detection techniques. The speciation analysis of chromium using SPE is carried out after retention of one or both chromium forms in a single or dual column systems employing solid sorbents and its/their elution with selective reagents [15-18]. Often the concentration of second form of analyte is determined after its reduction or oxidation in the residual sample [19-22] or as a difference between the concentrations of total chromium and the initially determined chromium form [19-22]. On-line sample pretreatment system coupling efficient separation step with sensitive detection technique allows to minimize the sample/reagents consumption and waste production, reduce the risk of sample contamination, improve the accuracy and precision of measurements as well as increase of sampling frequency. Flame atomic absorption spectrometry (FAAS) is often used for chromium determination in such systems due to continuous character of the detection and fast response to the presence of analyte. This technique is characterized also by good precision, repeatability over extended period of time, low noise, good sensitivity and low costs of analysis. The examples of application the SPE-FAAS systems for chromium speciation analysis in environmental samples are presented in Table 1. As the nature of sorbent strongly influences the successful separation of metal species, the studies on development of new selective and efficient sorbents are still undertaken.

Ion imprinting technology is a technique used for preparing polymers with high recognition abilities, which may be used as selective solid sorbents in SPE [24,25]. The synthesis of ion imprinted polymers (IIP) is performed in the presence of template molecule – a complex of imprinted ion with properly chosen ligand/monomer, which is next polymerized with functional and cross-linking monomers. The high selectivity of IIP arises from active recognition sites, complementary to the shape and size of imprinted ions, and arrangement of functional groups of ligand in the polymeric network, which become available after extraction of imprinted ion with washing solution.

The literature review shows that only a few IIP have been used as solid sorbents in off-line systems for separation and preconcentration of Cr(III) or Cr(VI) ions prior to detection with different spectrometric techniques [23-27] (Table 1). Polymers selective towards Cr(III) ions were prepared by imprinting of Cr(III)-2-methacryloylhistidine (Cr(III)-MAH) [23] or Cr(III)-(tris[pyrrolidine-1-dithioato-S,S’]) (Cr(III)-PDC) [24] complexes into polymeric network. In another study a complex of Cr(III) with 3-(2-aminoethylamino)propyltrimethoxy-silane (AAPTS) was used for functionalization of silica gel by surface imprinting technique [25]. Polymers selective towards Cr(VI) ions were prepared by imprinting of Cr(VI)-4-vinylpyridine [26] and Cr(VI)-2-methacryloylamidohistidine [27] complexes. In both cases the complexing ligands played a role of functional monomer as well. Hydroxyethyl methacrylate (HEMA) [26,27] or ethylene glycol dimethacrylate (EGDMA) [23,24,26,27] were mostly used as cross-linking monomers. The described above IIP were synthesized by different polymerization methods, such as bulk [24,26], suspension [23], surface imprinting [25] or surfactant-free emulsion polymerization [27]. It must be stressed that only two of these polymers, namely Cr(III)-AAPTS [25] and Cr(III)-PDC [24], were applied for analysis of environmental samples, but none of the cited examples have demonstrated on-line sample processing.

In this work a new fully automated method for selective determination of Cr(III) ions in sewage samples has been developed. The method is based on multi-commutation flow analysis (MCFA) involving on-line solid phase extraction of Cr(III) on a new ion imprinted polymer and FAAS detection. The multi-commutation concept has been recognized as relevant in the field of flow analysis, allowing for higher degree of automation of sample processing [28]. MCFA systems may be characterised by the use of individual commutation devices (typically solenoid valves), which can be arranged as a flow network. The system operation is fully controlled by the software, thus requiring a low involvement of the analyst. Another advantage of such systems is significant reduction of reagent consumption. Chromium(III) imprinted polymer (Cr(III)-IIP) was designed, synthesized by bulk polymerization and used as a minicolumn packing material for selective analyte sorption. A complex of Cr(III) with 8-hydroxyquinoline (8HQ) was used as a template molecule, because 8HQ reacts with Cr(III) more rapidly than other chelating reagents and does not form complexes with Cr(VI) [29,30]. Styrene was used as a functional monomer, divinylbenzene as a cross-linking monomer and 2,2'-azobisisobutyronitrile (AIBN) as an initiator. The sorption properties and selectivity of the Cr(III)-IIP were studied in off-line dynamic system. Finally, the MCFA-FAAS manifold with on-line SPE sample processing was constructed. The introduction of solutions into the analytical path was obtained by aspiration through a single pump channel placed after the SPE column, and by selecting the positions of the respective valves. The MCFA-FAAS method for selective determination of Cr(III) ions was developed and successfully applied to analysis of municipal sewage samples.

Experimental

Instrumentation

A Solaar M6 (Thermo Electron Corporation, UK) atomic absorption spectrometer with flame atomizer and deuterium background correction system was used for chromium determination. The chromium hollow cathode lamp (Photron, Australia) was operated at 6 mA current. The measurements were done in air-acetylene flame (flow rate of acetylene 1.4 L min^{-1}) at λ=357.9 nm with spectral bandpass of 0.5 nm. The absorbance of chromium was measured in a peak height mode. An inoLab pH Level 1 (WTW, Germany) pH meter equipped with an electrode SenTix 21 (WTW, Germany) was used for pH measurements.

A flow system consisted of a peristaltic pump Minipuls 3 (Gilson, France), PTFE tubing of i.d. 0.8 mm and laboratory made glass adsorption columns (i.d. 3.14 mm) packed with the polymer. The two ends of columns were blocked with PTFE membranes. A multi-commutation flow system with on-line SPE column containing 4 solenoid valves (V1-V4) was directly connected to the nebulizer inlet of an atomic absorption spectrometer, as it is shown in Figure 1. The eluate was collected in holding coil (PTFE tubing in diameter 2.6 mm and length 5.6 cm) before injection to atomizer of FAAS. The solenoid valves and peristaltic pump were connected with an electronic interface (KSP, Poland). The computer-controlled measurement device and software KPS (KPS, Poland) was used for MCFA-FAAS measurements with SPE of analyte. The program and interface allow for an independent control solenoid valves and pump during sorption, elution and measurement steps.

The FT-IR absorption spectra (in KBr) of complex and polymers were recorded in the range between 500 and 4000 cm^{-1} with Thermo Nicolet Magna IR 550 Series II (Nicolet, Japan). The particles of polymers were imaged by secondary electron SEM with the use of an Inspect S50 scanning electron microscope from FEI (USA).

Reagents and materials

A stock solution (20 mg mL^{-1}) of Cr(III) as $CrCl_3$ was prepared from Titrisol solution (Merck, Germany). A stock solution (1.001 mg mL^{-1}) of Cr(VI) as $K_2Cr_2O_7$ (Sigma Aldrich, Germany) was used. Working standard solutions of chromium were prepared daily by diluting the stock solutions. Ammonium chloride (p.a.), ammonia solution (25%), acetic acid (p.a.), sodium acetate (p.a.), sodium hydroxide (p.a.) supplied by POCh (Poland) were used for preparation of buffer solutions. Ethanol (96%, p.a.; POCh, Poland) and hydrochloric acid (37%, TraceSelect, Fluka, Germany) were used for sample pretreatment. Nitric(V) acid (69.5%, TraceSelect) obtained from Fluka (Germany) was used as a desorption agent. 8-hydroxyquinoline (8HQ, quinolin-8-ol) supplied by POCh (Poland) was used for preparation of Cr(III)-8HQ complex.

Divinylbenzene (80%, mixture of isomers, Aldrich, Germany), styrene (99%, Fluka, Germany), 2,2'-azobisisobutyronitrile (AIBN, Fluka, Germany) and chloroform (dried and distilled before use) (POCh, Poland) were used for synthesis of the ion imprinted polymer. High-purity argon gas was used to de-aerate the solutions.

For interference studies the solutions of copper(II) nitrate(V) (Sigma Aldrich, Germany), manganese(II) nitrate(V) (Merck, Germany), iron(III), magnesium(II) and calcium(II) nitrates(V) (POCh, Poland) were used. Deionized water of 18.2 MΩ cm^{-1} resistivity obtained from Milli-Q water purification system (Millipore, USA) was used for preparation of all solutions.

Reference material of waste water from treatment plant with urban origin - RES 10.2 (ielab Calidad, Spain) was used for accuracy control. Sewage samples were delivered from municipal sewage treatment plant (Bialystok, Poland).

Preparation of ion imprinted polymer

The IIP was prepared in two steps: first the binary complex of Cr(III)-8HQ (the template) was formed, next the copolymerization of Cr(III)-8HQ complex with styrene (functional monomer), divinylbenzene (cross-linking monomer) and AIBN (initiator) was performed by bulk method.

To prepare the complex of Cr(III)-8HQ 2 mL of chromium(III) solution (0.077 mmol, 2 mg mL^{-1}) and 0.5588 g (3.85 mmol) of solid 8HQ were added to the acetate buffer solution (20 mL; pH 5.65) and the mixture was stirred for 30 min at 90˚C [31]. Next, the formed complex was extracted twice with 10 mL of chloroform. The excess of ligand present in chloroform layer was destroyed with 0.1 mol L^{-1} NaOH solution (for 5 mL of chloroform 20 mL of NaOH solution was added). The chloroform phase (containing the chromium chelate) was evaporated to dryness. In this way an orange solid (~20 mg) was obtained. The FT-IR (in KBr) spectra of obtained complex was registered and following characteristic bonds were observed: 3434 cm^{-1} - υ(-OH), 1597 and 1575 cm^{-1} - υ(–C=C), 1497 and 1464 cm^{-1} - υ(–C=N), 1281 and 1224 cm^{-1} - υ(C-N), 1200-1000 cm^{-1} - υ(-C-O).

The complex of Cr(III)-8HQ was dissolved in 3 mL of chloroform, next 1.15 mL of styrene (10 mmol) was added and stirred for 30 min at room temp. Then, divinylbenzene (5.6 mL, 40 mmol) and AIBN (50 mg) were added and the polymerization mixture was transferred into glass polymerization ampoules, purged with argon for 10 min, sealed and heated at 80°C for ~20 h. The solid formed was crushed, washed with dichloromethane and ethyl acetate in order to remove excess of reagents, dried at room temperature, ground in mortar and sieved. The FT-IR (in KBr) spectra of obtained polymer was registered and following characteristic bonds were observed: 3100-3000 cm^{-1} - υ(=C-H_{ar}), 1629 and 1600 cm^{-1} - υ(–C=C_{ar}), 1509 and 1486 cm^{-1} - υ(–C=N) from Cr(III)-8HQ, 1280 and 1225 cm^{-1} - υ(C-N), 1200-1000 cm^{-1} - υ(-C-O).

The imprinted Cr(III) ions were leached from portions of the Cr(III)-IIP polymer (1 g) by passing 200 mL of 6 mol L^{-1} HNO_3 solution at a flow rate of 0.8 mL min^{-1}. In the final step the polymer was rinsed with deionized water and dried at room temperature. The particles of 0.2 - 0.3 mm in diameter were selected as column packing.

The control polymer (CP) was synthesized in a similar way, but in the absence of Cr(III) ions. The CP has a similar FT-IR spectrum as the Cr(III)-IIP, indicating the similarity in the backbone structure. Some differences in the fingerprint region have been observed.

Separation procedure

For off-line separation procedure the glass column packed with 0.15 g of polymer was conditioned by passing 3 mL of deionized water at the flow rate of 1 mL min^{-1}. For the analyte retention, the Cr(III) standard, sample solution or reference material of waste water RES 10.2 adjusted to pH 9 with ammonium buffer was passed through the column at the flow rate of 1 mL min^{-1}. The elution of Cr(III) ions was performed with 0.1 mol L^{-1} HNO_3 at the flow rate of 1 mL min^{-1}.

For on-line separation procedure the MCFA-FAAS manifold presented at Figure 1 was used. The glass minicolumn packed with 0.07 g of the Cr(III)-IIP polymer was used in this system. The system was run according to the program given in Table 2. The normal analytical cycle begins turning on V1, V2 and V3 for 60 s for conditioning of the minicolumn. Afterwards the position of V1 was changed and sample solution was loaded on the minicolumn for chosen period of time. The elution of retained analyte was performed by changing the position of V1, V2 and V4, passing the eluent through the minicolumn and collecting the eluate in the holding coil between V3 and V4. Next, the eluate was quickly transferred to the nebulizer of FAAS at flow rate 2.53 mL min^{-1}. Finally, the sorbent was cleaned with HNO_3 solution prior to the next analytical cycle.

All reported retention efficiencies were based on the ratio of the difference between the concentration of Cr(III) in initial solution loaded on the column and the concentration of Cr(III) in effluent, derived from a calibration graph, to the concentration of Cr(III) in initial solution passed through the column. Recovery of Cr(III) was calculated as a ratio of analyte concentration in eluate to the initial concentration of Cr(III) in loaded solution. The concentration of Cr(III) in eluate was calculated from the calibration graph obtained after using developed procedure for separation of Cr(III) on the Cr(III)-IIP column. The concentration of chromium in all solutions was determined by FAAS technique.

Results and discussion

The particles of imprinted and control polymers were characterized using scanning electron microscopy (SEM). The particles display an irregular shape (Figure 2). The surface of the CP particles is very smooth, while the surface of the Cr(III)-IIP particles became patterned after removing of analyte.

Optimization of the Cr(III) separation procedure

The initial studies on assessment of the suitability of the Cr(III)-IIP as SPE material for the selective separation of Cr(III) ions before FAAS determination were carried out in an off-line system.

Sample pH is one of the most important parameters influencing sorption of analyte on the sorbent. Effect of the pH on retention of Cr(III) and Cr(VI) on the Cr(III)-IIP was studied in the range from 1.0 to 10.0 (Figure 3). It was observed that the retention of Cr(VI) was at the level of 3-10% in the studied pH range. The retention of Cr(III) was increasing along with the increase of sample pH and achieved maximum at pH 8.0-9.5, while in more alkaline solutions (pH > 10) it rapidly decreased. It is known that 8HQ has amphoteric properties due to the presence of donor nitrogen atom and acceptor phenolic hydroxyl group. Low efficiency of retention of the analyte from acidic solutions can be explained by protonation of the nitrogen atom of the imprinted ligand (8HQ), that diminishes interaction between the ligand and a cationic form of the analyte. Under alkaline conditions 8HQ can be present in anionic form and can easily interact with cationic species of Cr(III), e.g. $Cr(OH)(H_2O)_2^{2+}$, formed during replacement of water hydration molecules in $[Cr(H_2O)_6]^{3+}$ by OH^- groups, so the retention of analyte increases. The similar pH effect on Cr(III) sorption on sorbents modified with 8HQ was previously observed [14,32]. The detailed discussion of sample pH influencing analyte sorption was presented by Bruhn et al. [32]. Herein, it must be stressed that the retention of Cr(III) on the ion imprinted polymer is not only the effect of interaction between the ligand and the analyte but also of the size and geometry of analyte species.

The retention of Cr(III) from standards of pH 9 prepared in 0.1 mol L^{-1} ammonium buffer was at the level 92-95%, while the retention of Cr(III) from standards prepared in acetic buffer dropped to 77%. It is probably an effect of the partial formation of Cr(III)-acetate complexes, which are not retained on active sites of the polymer. In further experiments the samples were prepared in ammonium buffer. The solutions of 0.1 mol L^{-1} ammonium buffer, 0.1 mol L^{-1} acetic buffer and deionized water were tested for conditioning of the Cr(III)-IIP. Because the type of conditioning agent had no effect on the efficiency of analyte retention (93-96%, n=3), deionized water was further used for sorbent conditioning.

A loading flow rate of the sample solution is a parameter influencing the efficiency of retention of analyte on the polymer. At sample flow rate in the range from 0.25 to 1.5 mL min^{-1}, the efficiency of retention of Cr(III) ions was at the level of 83-93%. The maximal retention of analyte (93 ± 2%, n=3) was obtained at flow rate of 1.0 mL min^{-1}.

In order to provide the multiple using of sorbent the retained analyte should be eluted quantitatively from the sorbent. For the elution of Cr(III) 2 mL of HCl (0.1 to 3.0 mol L^{-1}) or

HNO_3 (0.01 to 0.5 mol L^{-1}) solutions were tested as stripping agents. The recovery of Cr(III) increased from 56% to 92% along with the increase of HCl concentration and achieved maximum for 3 mol L^{-1} of HCl. The recovery of Cr(III) with HNO_3 was in the range from 77% to 91%, with highest value (91 ± 5%, n=3) obtained for 0.1 mol L^{-1} HNO_3. Such solution was used in further studies, as it is less corrosive for FAAS instrument.

The effect of an eluent flow rate on the recovery of Cr(III) from the sorbent was studied in the range of 0.25-1.5 mL min^{-1}. The recovery of Cr(III) (n=3) was 76 ± 5% at flow rate of 0.25 mL min^{-1}, 81 ± 5% at flow rate of 0.5 mL min^{-1}, 90 ± 7% at flow rate of 1.0 mL min^{-1}, and 84 ± 5% at flow rate of 1.5 mL min^{-1}. Then, 0.1 mol L^{-1} HNO_3 at flow rate of 1 mL min^{-1} was selected.

The minimum volume of eluent required for quantitative elution of Cr(III) from columns filled with 0.07 g and 0.15 g of sorbent was evaluated as follows. The solution of 0.1 mol L^{-1} HNO_3 was passed through the Cr(III)-IIP and portions of eluate (0.05-0.10 mL) were successively collected, appropriately diluted and measured by FAAS. It was found that the volume of 0.3 mL is sufficient for quantitative recovery of analyte from both columns (Figure 4). The minicolumn filled with 0.07 g of Cr(III)-IIP was further used in on-line sample processing system. Such experiment was also performed simultaneously on the CP column. Different profiles of elution of the analyte from both polymers were observed (Figure 4a), what indicates that Cr(III) is more specifically bound to the Cr(III)-IIP than to the CP.

Under optimized experimental conditions mentioned above the efficiency of retention of Cr(III) on the Cr(III)-IIP for 6 successive analytical cycles was 91 ± 1%. The retention of analyte on the CP was 75 ± 3%, what indicate that non-specific interactions between the analyte and the polymer surface also occur. The recovery of analyte from the Cr(III)-IIP was 91 ± 5% and from the CP was 71 ± 3%. The reproducibility of the procedure for 5 µg mL^{-1} of Cr(III), expressed as RSD, was better that 5%.

The sorption capacity of the Cr(III)-IIP and the CP was determined by passing Cr(III) solution (5 µg mL^{-1}) through columns packed with 0.2 g of sorbents and quantifying the content of chromium in each of 4 mL fractions of the effluent by FAAS. The experiment was completed when the efficiency of the Cr(III) retention decreased by 20%. On this basis, the sorbent capacity for Cr(III) was calculated as 8.5 mg g^{-1} and 2.8 mg g^{-1} for the Cr(III)-IIP and the CP, respectively.

Selectivity studies

The selectivity of the Cr(III)-IIP towards Cr(III) ions was tested in the presence of coexisting ions. The Ca(II) and Mg(II) ions, which are commonly present in natural water, and Cu(II) ions, which have an ionic radius and a reactivity towards 8HQ similar to Cr(III) ions, were selected for these studies. The distribution ratios (*D*, mL g^{-1}), selectivity coefficients (α) and relative selectivity coefficients (α_r) of Cr(III), with respect to Cu(II), Mg(II) and Ca(II) ions were calculated from the equations described in [33]. A comparison of *D* values for Cr(III) ions on the Cr(III)-IIP and the CP shows that higher distribution ratios have been achieved on the imprinted polymer (Table 3). The obtained values of selectivity coefficients of the Cr(III)-IIP significantly exceed such values of the CP. The relative selectivity coefficients in the range 4.0 - 10.7 prove that the Cr(III)-IIP is more selective towards Cr(III) ions than the CP. The obtained values of the relative selectivity coefficients for Cr(III) are similar to those reported for polymers with imprinted Cr(III) [23] and Cr(VI) ions [26,27] in the presence of Cr(III)/Cr(VI), Co(II) and Ni(II) ions.

The effect of coexisting ions on the separation of Cr(III) ions on the Cr(III)-IIP was also studied. Standard solutions of Cr(III) (25 µg) containing individually added Fe(III) and Mn(II) ions and a mixture of Fe(III), Mn(II), Cu(II), Mg(II) and Ca(II) ions in different concentrations (with total amount of coexisting metal ions up to 75 µg) were passed through the column. It was found that the presence of studied ions did not affect neither retention (98 - 100%) nor recovery (87 – 94%) of Cr(III) from the sorbent.

The analytical performance of MCFA-FAAS method with on-line SPE for chromium speciation analysis

For on-line sample processing the multi-commutation flow manifold presented at Figure 1 was used. The conditions applied for separation of Cr(III) on the Cr(III)-IIP in off-line mode were re-optimized for the MCFA-FAAS system with on-line SPE on the minicolumn packed with 0.07 g of sorbent. The efficiency of the retention of Cr(III) on the Cr(III)-IIP was similar to that obtained in off-line system and was equal to 91 ± 2% (n=6), while the recovery of Cr(III) was 93 ± 2% (n=6). The reproducibility of the procedure for 2 µg mL^{-1} of Cr(III), expressed as RSD, was below 3.0%.

The using of MCFA-FAAS system with on-line SPE affected in shortening the time of conditioning, elution steps, and column cleaning with 0.1 mol L^{-1} HNO_3 to 60 s, 30 s, and 60 s, respectively. The time of sample loading was in the range 15-600 s. The overall time for preconcentration and elution of 1 mL of sample was 4 min 30 s, what gives the sample throughput at the level 12 samples per hour while the overall analysis time for batch procedures using ion imprinted polymers often exceeds 2.5 h per sample [23,26,27].

The calibration graph, prepared by loading standard solutions of Cr(III) of different concentrations for 60 s on minicolumn and its elution with 0.3 mL of 0.1 mol L^{-1} HNO_3, was linear up to 20 μg mL^{-1} giving following regression equation: $y = (0.0472 \pm 0.0030)\ x - (0.0136 \pm 0.0340)$ (r^2=0.9986; n=4, α=0.05). The calculated enhancement factor, based on the ratio of slopes of calibration graphs obtained with and without preconcentration, equals 3.8. The mass of analyte, which can be loaded on minicolumn and on-line determined by FAAS in one cycle without broadening the linear range of calibration graph of analyte, was evaluated by passing the standard solution of Cr(III) (2 μg mL^{-1}) through the Cr(III)-IIP minicolumn for increasing period of time (from 15 s to 15 min at flow rate 1 mL min^{-1}). It was found that the absorbance of Cr was proportional to the loaded mass of analyte up to 20 μg (10 min of loading time). The preconcentration factor of Cr(III) obtained after preconcentration of sample for 10 min was 33. The limit of detection (LOD) of the method was calculated as the concentration of analyte equal to 3-fold standard deviation of the absorbance of blank (ammonium buffer loaded on the column and eluted with the eluent solution) divided by the slope of the calibration graph ($LOD = 3SD_{blank}/a$), while the limit of quantification (LOQ) was calculated as $10SD_{blank}/a$ [34]. The LOD for 10 successive analytical cycles was 0.070 μg mL^{-1}, while LOQ was 0.23 μg mL^{-1}. Taking into account 10 min sample preconcentration time the LOD and LOQ were 2.1 ng mL^{-1} and 7.0 ng mL^{-1}, respectively. The obtained values of LOD and preconcentration factor are comparable to those found in literature (Table 1).

The ability of the Cr(III)-IIP to preconcentrate low concentrations of Cr(III) (0.5 – 5 μg mL^{-1}) from larger volumes of sample (5-20 mL) was also studied. It was found that for 10-20 mL of sample at concentration of analyte below 1 μg mL^{-1} the recoveries were in the range 82 – 85% (n=3), while for higher analyte concentrations recoveries were in the range 90 – 92% (n=3). The results confirmed that the procedure may be applied to the separation of Cr(III) from volumes up to 20 mL.

The procedure was tested for the separation of Cr(III) and Cr(VI) ions. Mixture solutions containing different concentrations of Cr(III) and Cr(VI) ions (listed in Table 4) were loaded to the column, and Cr(III) was eluted with 0.1 mol L^{-1} HNO_3 and determined. Total chromium concentration was determined after reduction of Cr(VI) to Cr(III) based on the procedure described in [35]. For that propose 5 mL of ethanol and 0.1 mL of concentrated HCl was added to 0.5 mL of Cr(VI) (25 μg mL^{-1}) and Cr(III) (25 μg mL^{-1}) mixture solution. The mixture was heated at 50°C until the solution turned colourless and next the ethanol was evaporated on hotplate. The remaining solution was diluted with ammonium buffer up to 2.5 mL and the separation procedure was applied. The obtained results (Table 4) indicate that the method can be used for the separation of Cr(III) from Cr(VI) species, as well as for the determination of total concentration of chromium.

As the certified reference materials for chromium speciation analysis in sewage are not commercially available, the method was applied to analysis of reference material of municipal waste water RES 10.2 with certified value of total concentration of chromium (4.55 μg mL^{-1}). The concentration of Cr(III) in RES 10.2 determined after separation procedure was in good agreement with the certified value for total chromium (Table 5), thus indicating that only Cr(III) is present in RES 10.2. To verify this hypothesis the spectrophotometric method with diphenylcarbazide [36] was used to quantify Cr(VI) in RES 10.2. It was found that the absorbance signal for Cr(VI) in reference material was below the blank value, therefore it was proved that the total concentration of chromium corresponds to the concentration of Cr(III). In this way the accuracy of the developed procedure was confirmed.

Tested polymer shows high stability during working conditions, as its continuous use for more than 170 cycles does not lead to a loss of sorption properties. In that respect studied material is superior to other Cr-imprinted polymers, which saved their adsorption/desorption properties only for 5-6 analytical cycles [25-27].

Analysis of real samples

The method was applied to determination of Cr(III) in tap and municipal sewage. Samples were filtered through a 0.45 μm Supelco membrane filter, adjusted to pH 9 with ammonium buffer and left for equilibration. The concentration of Cr(III) in analyzed samples determined by the developed method was below LOD. Therefore, the procedure was applied to samples spiked with Cr(III) and Cr(VI) as listed in Table 5. The recovery values of Cr(III) were in the range 90-109% (Table 5), what indicates the suitability of the Cr(III)-IIP for the efficient separation of Cr(III) ions from water and sewage samples. The reproducibility of the separation procedure for different samples was in the range from 0.4 to 4.0% (Table 5). The MCFA-FAAS method with on-line SPE is suitable for speciation analysis of chromium in such samples.

Conclusions

In this work the new MCFA-FAAS method with on-line SPE sample processing was developed. In the multi-commutation flow system the concept of time-base insertion of sample and analyte separation/concentration with using minicolumn was implemented. The new ion imprinted polymer, based on imprinted Cr(III)-8HQ complex, was designed and used as solid sorbent for separation of Cr(III) ions. The prepared Cr(III)-IIP has some advantages over other ion imprinted polymers proposed for separation of Cr under dynamic [24,25] or static conditions [23,26,27] as long-term stability and fast kinetic of separation process. Efficient retention of Cr(III) on the Cr(III)-IIP and its total elution allow for application of the Cr(III)-IIP in multi-commutation flow system for on-line separation and determination of Cr(III) ions. The constructed MCFA-FAAS system enables to automate the separation procedure of Cr(III) ions on Cr(III)-IIP, reduce the volume of sample and eluent, shorten the time of analysis and improve repeatability of separation process (RSD<3%). The system operation is fully controlled by the software, thus requiring a low involvement of the analyst. The throughput of sample achieved on Cr(III)-IIP (12 samples/h) is better than on other ion imprinted polymers [24,25]. The main advantage of the MCFA-FAAS method is the possibility of determination of Cr in complex samples such as municipal sewage without influence of matrix ions. The use of the developed method for determination of Cr speciation in other environmental samples is also possible.

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Table 1. Solid-phase extraction methods for determination of Cr in aqueous samples in dynamic and static systems by FAAS

Sorbed form of analyte	Sorbent	Sample pH	Eluent	PF (EF[a])	LOD, ng mL^{-1}	RSD, %	Analyzed sample	Ref.
			Dynamic system - off-line mode					
Cr(VI)	Dowex M 4195	2	4 mol L^{-1} NH_3 H_2O	31	1.9	10	surface water, industrial waste	19
Complex of Cr(VI) with DPC	Amberlite XAD-16	1	0.05 mol L^{-1} H_2SO_4 in methanol	25	45	1	tap water	21
Complex of Cr(III) with dithizone	Chromosorb 108	8	2 mol L^{-1} HNO_3 in acetone	71	0.8	3.6-8.2	surface water, coffe, tobacco	11
Cr(III) Cr(VI)	Amberlite XAD-1	5 1	2 mol L^{-1} HNO_3 2 mol L^{-1} NaOH	100 140	20 40	2.0	industrial waste	18
Complex of Cr(III) with 8HQ	graphene	8	2 mol L^{-1} HNO_3	125	0.5	4.3	tap, river and sea water	14
Cr(III)	IIP: Cr(III)-PDC	4	0.2 mol L^{-1} HNO_3	10	0.018[b]	5.8	tap and river water, municipal sewage	24
Cr(III) Cr(III) and Cr(VI)	IIP: Cr(III)-AAPTS	2 7	1 mol L^{-1} HNO_3	40[a]	0.004[c]	4.4	lake, tap and well water	25
			Dynamic system - on-line mode					
Cr(VI)	Nb_2O_5/ SiO_2	1	2 mol L^{-1} HNO_3	23	0.3	4.6	tap, river and well water	12
Cr(VI)	NH_2-SBA-15	2	0.5 mol L^{-1} NH_3 H_2O	44	0.2	2.1	waste water	15
Complex of Cr(VI) with APDC	PTFE	1	methyl isobutyl ketone	80[a]	0.8	3.2	natural water, sediments	17
Cr(III)	IIP: Cr(III)-IIP	9	0.1 mol L^{-1} HNO_3	33	2.1	3.0	municipal sewage	This work
			Static system – batch mode					

Cr(III)	IIP: Cr(III)-MAH	7	0.1 mol L^{-1} succinic acid	50	1.2	2.6	model solutions	23
Cr(VI)	IIP: Cr(VI)-VP	4	0.1 mol L^{-1} formic acid		^{d}		artificial waste water	26
Cr(VI)	IIP: Cr(VI)-MAH	4	0.1 mol L^{-1} formic acid		^{d}		model solution	27

Notes: PF - preconcentration factor; EF[a] - enrichment factor; IIP – ion imprinted polymer; DPC - diphenylocarbazide; 8HQ - 8-hydroxyquinoline; APDC - ammonium pyrrolidine dithiocarbamate; NH_2-SBA-15 - mesoporous silica material; PTFE - polytetrafluoroethylene; Cr(III)-PDC – Cr(III)-(tris[pyrrolidine-1-dithioato-S,S']) complex; Cr(III)-AAPTS – silica gel with imprinted Cr(III) -3-(2-aminoethylamino)propyltrimethoxy-silane complex; Cr(III)-IIP - ion imprinted polymer with Cr(III)-8-hydroxyquinoline complex; Cr(III)-MAH - Cr(III)-methacryloylhistidine complex; Cr(VI)-VP - Cr(VI)-4-vinylpyridine complex; Cr(VI)-MAH - Cr(VI)-methacryloylhistidine complex;

[b] ETAAS; [c] ICP-MS; [d] UV-VIS

Table 2. Operational sequence of the MCFA-FAAS system with on-line separation of Cr(III) on Cr(III)-IIP (V1, V2, V3, V4 - solenoid valves; position of valve: 0 – channel A is open, channel B is closed; 1 – channel A is closed, channel B is open; see Figure 1).

Step	Process	Flow rate, mL min^{-1}	Time, s	Position of valves
1	Conditioning of sorbent with H_2O	1	60	V1=0; V2, V3=1; V4=0
2	Loading of sample solution (pH 9)	1	15-600	V1, V2, V3=1; V4=0
3	Elution with 0.3 mL of 0.1 mol L^{-1} HNO_3:			V1, V2=0
	- eluate reaches V3,	1	68	V3=1, V4=0
	- eluate fills the holding coil between V3 and V4,	1	20	V3=0, V4=0
	- eluate is transferred to the nebulizer of FAAS	2.53	10	V3=0, V4=0
4	Cleaning of sorbent with 0.1 mol L^{-1} HNO_3	1	60	V1, V2, V3=0; V4=1

Table 3. Selectivity parameters of Cr(III)-IIP and CP for Cr(III) ions against coexisting ions (sample: 5 mL of 5 µg mL^{-1} Cr(III) +5 µg mL^{-1} other metal ion, pH 9, flow rate: 1 mL min^{-1})

Metal ion, Me	Distribution ratio, *D,* mL/g				Selectivity coefficient, *α*		Relative selectivity coefficient, α_r
	Cr(III)		Me				
	Cr(III)-IIP	CP	Cr(III)-IIP	CP	Cr(III)-IIP	CP	
Cu(II)	1619	74.3	36.1	17.8	44.8	4.2	10.7
Mg(II)	1011	96.2	0.7	0.6	1444	160	9.0
Ca(II)	383	66.3	2.6	1.8	147	36.8	4.0

Table 4. Recovery of Cr(III) on the Cr(III)-IIP from mixture of Cr(III) and Cr(VI) ions obtained by MCFA-FAAS method (sample: 1 mL at pH 9, flow rate: 1 mL min^{-1}; eluent: 0.3 mL of 0.1 mol L^{-1} HNO_3, flow rate 1 mL min^{-1}; mean value ± SD, n=3).

Analyzed solution	Found mass of Cr(III) ±SD, µg	Recovery of Cr(III) ± SD, %
5.4 µg of Cr(III) + 4.9 µg of Cr(VI)	5.5 ± 0.1	102 ± 2
2.5 µg of Cr(III) + 12.2 µg of Cr(VI)	2.7 ± 0.1	108 ± 4
5.3 µg of Cr(III) + 20.4 µg of Cr(VI)	5.4 ± 0.2	102 ± 4
5.0 µg of Cr(III) + 4.9 µg of Cr(VI)	5.1 ± 0.2	102 ± 4
5.0 µg of Cr(III) + 4.9 µg of Cr(VI)	9.3 ± 0.4 (a)	94 ± 4 (a)

(a) after reduction of Cr(VI) to Cr(III)

Table 5. Recovery of Cr(III) ions on the Cr(III)-IIP from water and sewage samples obtained by MCFA-FAAS method (sample: 1 mL at pH 9, flow rate 1 mL min^{-1}; eluent: 0.3 mL of 0.1 mol L^{-1} HNO_3, flow rate: 1 mL min^{-1}; mean value ± SD, n=3).

Sample	Concentration Cr(III), µg mL^{-1}			Recovery of Cr(III) ± SD, %
	in sample	in sample spiked with Cr(III)	in eluate ± SD	
Municipal sewage	< LOD	3.0	3.2 ± 0.1	107 ± 3
Treated municipal sewage	< LOD	3.1	2.8 ± 0.1	90 ± 3
Municipal sewage	< LOD	5.3 µg Cr(III) + 5.0 µg Cr(VI)	5.8 ± 0.1 9.6 ± 0.1$^{(a)}$	109 ± 2 93 ± 1$^{(a)}$
Waste water RES 10.2	4.55 ± 0.055$^{(b)}$		4.70 ± 0.02	103± 1

(a) after reduction of Cr(VI) to Cr(III)

(b) property value of Cr in waste water RES 10.2

Figure captions:

Figure 1. Schematic diagram of the MCFA-FAAS manifold with on-line SPE for determination of Cr(III) (minicolumn: 0.07 g of Cr(III)-IIP; V1, V2, V3, V4 - solenoid valves; A, B - channels of solenoid valves).

Figure 2. SEM images of particles surface of: a) CP and b) Cr(III)-IIP (5000x magnification).

Figure 3. Effect of the sample pH on Cr(III) and Cr(VI) retention on Cr(III)-IIP (sample: 5 mL 5 µg mL $^{-1}$ of Cr, flow rate: 1 mL min^{-1}).

Figure 4. a) Elution profiles of Cr(III) from column filled with 0.07 g and 0.15 g of Cr(III)-IIP and 0.15 g of CP with 0.1 mL portions of 0.1 mol L^{-1} HNO_3 (flow rate 1.0 mL min^{-1}); b) Total recovery of Cr(III) from Cr(III)-IIP and CP with 0.1 mol L^{-1} HNO_3 (flow rate 1.0 mL min^{-1}).

Figure 1.

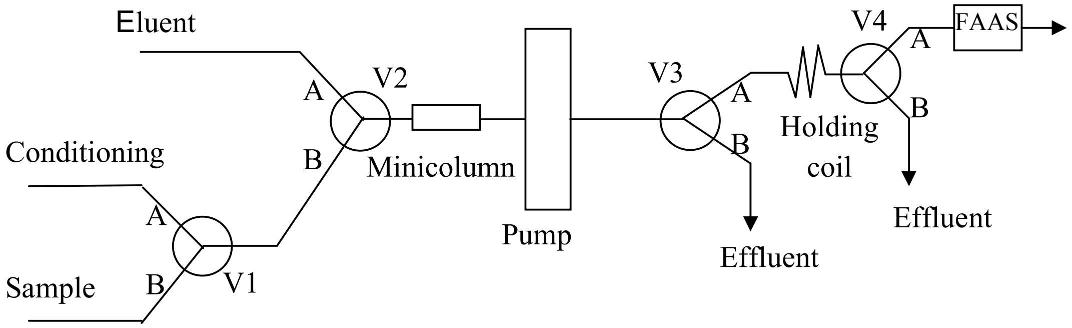
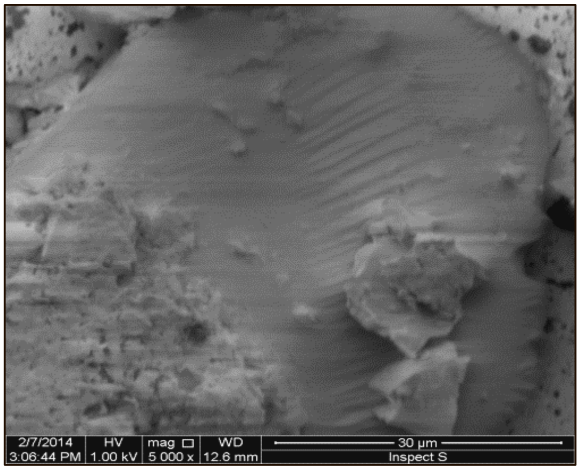

Eluent
Conditioning
Sample
A
B
V1
A
B
V2
Minicolumn
Pump
V3
A
B
Effluent
Holding
coil
V4
A
B
FAAS
Effluent

Figure 2.

a)


2/7/2014
3:06:44 PM
HV
1.00 kV
mag ☐
5 000 x
WD
12.6 mm
30 µm
Inspect S

b)

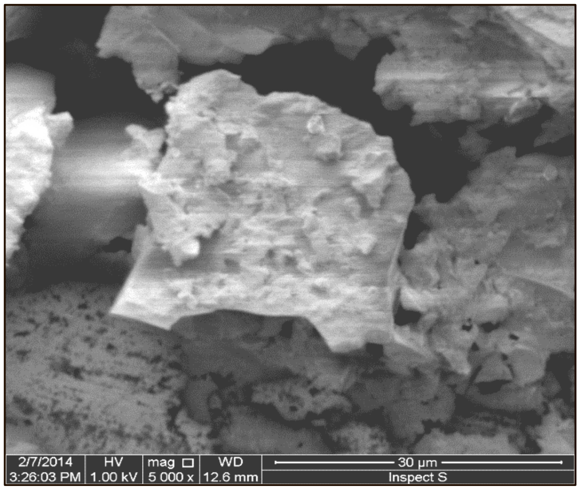

2/7/2014
3:26:03 PM
HV
1.00 kV
mag ☐
5 000 x
WD
12.6 mm
30 µm
Inspect S

Figure 3.

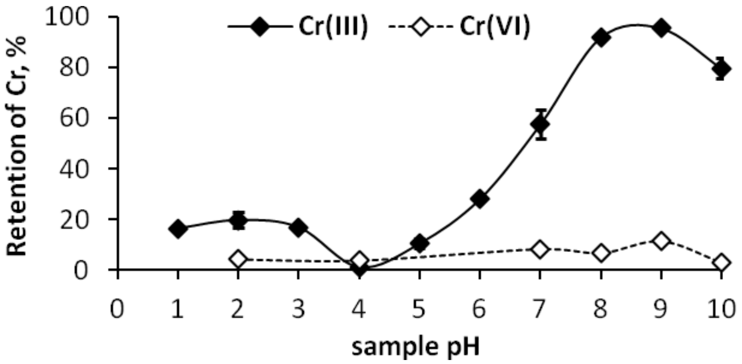
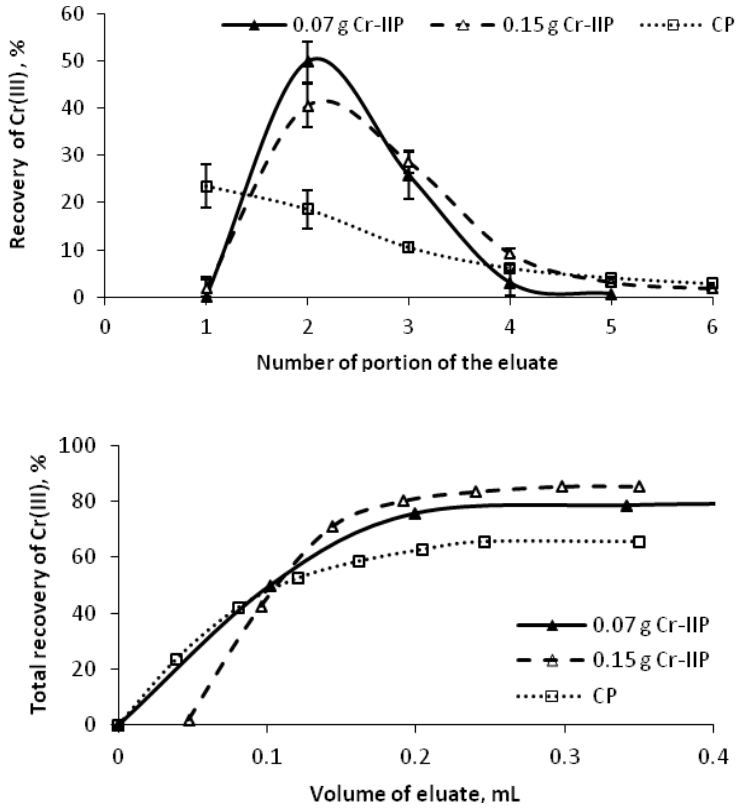

Cr(III)
Cr(VI)
Retention of Cr, %
100
80
60
40
20
0
0
1
2
3
4
5
6
7
8
9
10
sample pH

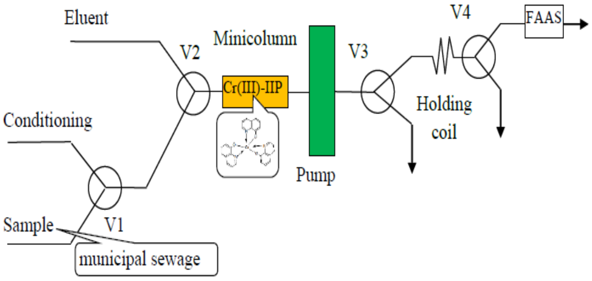
Figure 4



a)

b)

0.07 g Cr-IIP
0.15 g Cr-IIP
CP
Recovery of Cr(III) , %
60
50
40
30
20
10
0
0
1
2
3
4
5
6
Number of portion of the eluate
Total recovery of Cr(III), %
100
80
60
40
20
0
0
0.1
0.2
0.3
0.4
Volume of eluate, mL


Eluent
V2
Minicolumn
Cr(III)-IIP
V3
V4
FAAS
Conditioning
Holding
coil
Pump
Sample
V1
municipal sewage