



A soft material for chromium speciation in water samples using a chemiluminescence automatic system

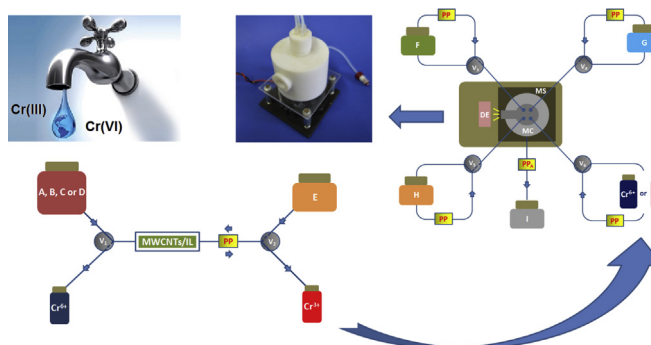
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HIGHLIGHTS

- A sorbent based on MWCNTs and ionic liquid was used for the speciation of chromium.
- The speciation and detection of chromium was performed by using an automatic system.
- Cr(III) and Cr(VI) were detected by chemiluminescence reactions.
- Cr(VI) was determined by the oxidation of luminol with the $\text{Cr}_2\text{O}_7^{2-}$ desorbed.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:

Available online 29 December 2017

Handling Editor: Petra Petra Krystek

Keywords:

Automatic system
Chromium speciation
Ionic liquids
Multiwalled carbon nanotubes
Chemiluminescence detection

ABSTRACT

A soft material formed by multiwall carbon nanotubes and 1-butyl-3-methyl imidazolium chloride was used as sorbent material to perform the chromium speciation in natural waters. This soft material was not yet used for the speciation of metals as chromium. Thus, a multicommutated flow system containing a minicolumn packed with the soft material was designed. The procedure was based on the capacity of the sorbent to retain Cr(VI) as $\text{Cr}_2\text{O}_7^{2-}$ and allow to pass Cr(III) through the column. Then, a fully automated flow-batch analysis system was developed to quantify both species using chemiluminescence detection. Thus, Cr(III) was determined as catalyst of the luminol and hydrogen peroxide reaction and Cr(VI) as oxidant of luminol reaction. This represents a new approach because the oxidation of luminol using $\text{Cr}_2\text{O}_7^{2-}$ has not been reported in literature. The variables of the two systems were optimized. The limits of detection were $1.4 \mu\text{g L}^{-1}$ for Cr(VI) and $4.0 \mu\text{g L}^{-1}$ for Cr(III). The precision of the method was 3.8% and 7.0% for Cr(VI) and Cr(III), respectively. The present method was applied to real water samples with recoveries between 95% and 107%. Besides, these results were in accordance with those obtained using inductive coupled plasma-optical emission spectrometry technique.

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Abbreviations: BmimCl, 1-Butyl-3-methylimidazolium-chloride; C, channel; CNT, carbon nanotubes; EA, electronic actuator; FBA, Flow-batch analysis; IL, Ionic liquids; MDC, mixing detection chamber; MFS, multicommutated flow system; MWCNT, Multiwalled carbon nanotubes; V, solenoid valve; PP, peristaltic pump.

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<https://doi.org/10.1016/j.chemosphere.2017.12.178>
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1. Introduction

Chromium speciation is always important because of the bioavailability and toxicity of some of its chemical forms. Even though there are many oxidation states of chromium, the most commonly found are Cr(III) and Cr(VI), especially as environment

pollutants. While Cr(III) is considered an essential trace element for preservation of glucose, lipids and protein metabolism (Shah et al., 2012), Cr(VI) is irritant and toxic to human body tissue due to its water solubility and its oxidizing potential. It has been demonstrated that inorganic species of Cr(VI) are carcinogenic (Saçmacı et al., 2012). For these reasons, the determination of the different species of chromium presents greater interest than the determination of total chromium. There are several methods to carry out the speciation of chromium in environmental samples applying different techniques. In most of them, the direct determination of Cr(VI) is proposed and Cr(III) is determined after its oxidation to Cr(VI) (Tuzen and Soylak, 2007). Ren et al. present the determination of Cr(III) by electrothermal atomic spectrometry with the reduction of Cr(VI) to Cr(III) (Ren et al., 2007). On the other hand, separative techniques as ion chromatography, capillary electrophoresis and LC-ICP-MS also have been used (Séby et al., 2003; Lin et al., 2016) but all these methods need a pretreatment step to remove the analytes. Usually, environmental pollutants such as heavy metal and organic compounds have been extracted using solid phase extraction with different sorbent materials. These materials, like activated carbon (Feist and Mikula, 2014), resins (Tokalioglu et al., 2017) carbon nanofibers (Mubarak et al., 2017), and carbon nanotubes (CNTs) (Tarigh and Shemirani, 2013; Thines et al., 2017; Mubarak et al., 2015) have an outstanding role in the enrichment efficiency of the analytes. It is well known the excellent properties of carbon nanotubes as sorbent material but one of the major disadvantages is their tendency to form aggregates. For this reason, different alternatives were proposed to disperse the nanotubes using different solvents such as surfactants, organic solvents and ionic liquids (ILs) (Lu et al., 2012). In recent years, ILs have been combined with sorbent materials to improve the efficiency and selectivity of the extraction procedure (Tokalioglu et al., 2016). The role of ILs is to enhance the dispersion of CNTs, creating in this way a new kind of sorbent material called soft material. This material was used to extract and preconcentrate organic analytes (Polo-Luque et al., 2013). Until now, to the best of our knowledge, there are not reports in which this soft material was applied to evaluate chromium speciation. Only a few works report this speciation in a previous step to the determination of analytes. One of these uses nanostructured α -alumina (Monasterio et al., 2009) and another uses MWCNTs for the adsorption of Cr(VI)-ammonium pyrrolidine dithiocarbamate chelate (Tuzen and Soylak, 2007). On the other hand, up to now, the use of chemiluminescence technique for determining both species of chromium has not been reported. Seitz et al. described the determination of Cr(III) by chemiluminescence analysis using the analyte as catalyst of the luminol reaction (Seitz et al., 1972). Some of the advantages of using chemiluminescence technique are high selectivity and specificity. Moreover, it presents a high compatibility and easy coupling to automatic systems. The automatic systems based on multi-commutation and flow-batch analysis are interesting alternatives to make feasible a method or to transform batch processes into automated processes. Both MFS and FBA systems may perform different steps in the analytical procedure, such as separation and/or preconcentration of analytes and determination that may include the coupling to conventional analytical instruments. Conformation and advantages of FBA systems have been described in literature (Diniz et al., 2012). Particularly, the complete programmability of the process (including, control of sampling, flow rates, filling of mixing chamber, detection and evacuation) and the possibility to perform the measurements of the analytical signal directly inside the mixing chamber can be highlighted. Both are important facts for the automation of methods where chemiluminescence detection is used (Grünhut et al., 2011).

In this work, a new analytical method to determine Cr(VI) and Cr(III) in water samples was developed. For this purpose, a MFS to perform the speciation of chromium, which included a minicolumn packed with a soft material, was designed. The soft material, composed of MWCNTs and IL, improved the absorption of Cr(VI) as $\text{Cr}_2\text{O}_7^{2-}$ in comparison to MCNTs, due to the synergic effect between them. Additionally, this soft material allowed the separation of both chromium species. Then, a FBA system with chemiluminescence detection was developed to carry out the quantitation of Cr(VI), as oxidant of luminol, and Cr(III) as catalyst in the reaction of the luminol and hydrogen peroxide, both in a basic medium. According to our knowledge, this is the first time that the chemiluminescence technique is used to determine both analytes, particularly using the same automatic system.

2. Materials and methods

2.1. Reagents and solutions

All solutions were prepared by using analytical-grade reagents and ultra-pure water ($18\text{M}\Omega\text{ cm}^{-1}$) from a Milli-Q System (Millipore, USA). A 0.2 mol L^{-1} potassium chloride (Merck) solution was mixed with a 0.2 mol L^{-1} sodium hydroxide (Merck) solution in order to obtain a buffer solution of pH 12.00. A 0.01 mol L^{-1} luminol stock solution was prepared by dissolving 0.1770 g of luminol (Sigma-Aldrich) with 100 mL of the buffer solution. This solution was stored in a dark bottle at 4°C and it was stable for one week. The luminol working solution ($5 \times 10^{-3}\text{ mol L}^{-1}$) was daily prepared by appropriate dilution of stock solution in buffer solution. Hydrogen peroxide solution was prepared by diluting of 100 v/v H_2O_2 solution to obtain a 0.01 mol L^{-1} work solution. A $5 \times 10^{-3}\text{ mol L}^{-1}$ EDTA solution was prepared by diluting of 0.1 mol L^{-1} stock solution (Merck Titrisol). Stock solutions of Cr(III) and Cr(VI), both of 100 mg L^{-1} , were prepared by dissolving $\text{Cr}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ (Mallinckrodt) and $\text{K}_2\text{Cr}_2\text{O}_7$ (Sigma-Aldrich) in water, respectively. These solutions were acidified with nitric acid. Standard solutions of Cr(III) and Cr(VI) were freshly prepared by dilution of the stock solutions. MWCNTs with external diameters of 40–60 nm and purity >95% (Bayer) and a 0.5 mol L^{-1} BmimCl (Merck) solution were used to perform the speciation of chromium.

2.2. Instrumentation and software

An Aminco Bowman® Series 2 luminescence spectrometer with the photomultiplier operated at 900 V was used. The emission of photons was registered at 425 nm. A Gilson® Minipuls 3 and an Ismatec® peristaltic pumps and Tygon® tubes were used to pump the fluids. The rest of the tubing was made of Teflon® (0.5 mm i.d.). NResearch® three-way solenoid valves were used to handle all solutions into the systems. The MDC was constructed with Teflon® and was designed in our laboratory in order to prepare solutions and perform the detection in the same place. A lab-made stirrer system was designed to improve the mixing inside the MDC. An EA connected to a Pentium® 4 microcomputer was used to control all the components of the systems. The software used for controlling both, MFS and FBA systems, was developed in Labview® 5.1 visual programming language. A model 710 A Orion® pHmeter with an Orion-Ross® model 81-02 electrode was used to perform the pH measurements.

The composition of the soft material was examined by X-Ray Energy Dispersive Microanalysis (EDS) (X-Max50, Oxford). The physical structure and morphology of the soft material, before and after the sorption of Cr(VI), were analyzed by the Scanning Electron Microscope (SEM) (Leo, 40 XVP, England). In both cases, the sample preparation was carried out metallizing it with gold in a Sputter

Coater (Pelco 91000).

2.3. Chromium speciation procedure

A soft material based on the combination of BmimCl and MWCNTs was prepared to perform the speciation of chromium. A MFS that included a minicolumn packed with this material was developed (Fig. 1). The soft material was prepared weighing 0.1 g of MWCNTs and adding 0.5 mL of BmimCl. This mixture was homogenized in a mortar and it was kept at 4 °C for 24 h. Then, a Tygon® tube (length: 30 mm; inner diameter: 4 mm) was used to pack this material. The MFS consisted of two three-way solenoid valves (V_1 and V_2) and only one channel (C_1) where the minicolumn was inserted. As it can be seen in Fig. 1, different steps were performed in the MFS. When V_1 and V_2 valves were OFF, the soft material was conditioned passing 1 min of water and 2.5 min of 0.05 mol L⁻¹ of BmimCl solution through it. Then, 5.0 mL of standard/sample solution was loaded and a vial 1 was placed at the end of the channel. At this time, the Cr(III) contained in the standard/sample passed through the column and it was collected in the vial 1, while the Cr(VI) was retained on the soft material. After this, the vial 1 was replaced by a waste vial and the column was washed through it to remove the water. All these steps were performed at 0.85 mL min⁻¹. Finally, the elution step was performed at 1 mL min⁻¹ by turning ON V_1 and V_2 valves and in reversal flow mode. In this way, 300 μ L of BmimCl alkaline solution with NaCl passed through the column. When the volume of eluent was all inside the column, the flow was stopped for 10 min. Then, the flow was restored to collect the amount of Cr(VI) in vial 2, at the end of the channel.

2.4. FBA system with chemiluminescence detection

A schematic diagram of the proposed FBA system is showed in Fig. 2a. The system consisted of five channels, through which luminol (C_2), sample (C_3), hydrogen peroxide (C_4), water (C_5) and waste (C_6) flowed. The direction of the flow in the C_2 to C_5 channels was controlled by three-way solenoid valves. Then, when the valves (V_3 to V_6) were OFF, the solutions were recycled to the respective flasks. When the valves were ON, the respective solutions were pumped to the MDC. As it can be seen in Fig. 2b, the lab-made MDC has been designed with four inlets for incoming solutions and one output for emptying. It was equipped with a quartz window, which was located at the bottom part of the chamber in order to minimize the final volume of the reaction mixture (1.50 mL). In this way, the MDC was used to perform both the chemical reaction and the chemiluminescence detection. In addition, a little magnetic stirring

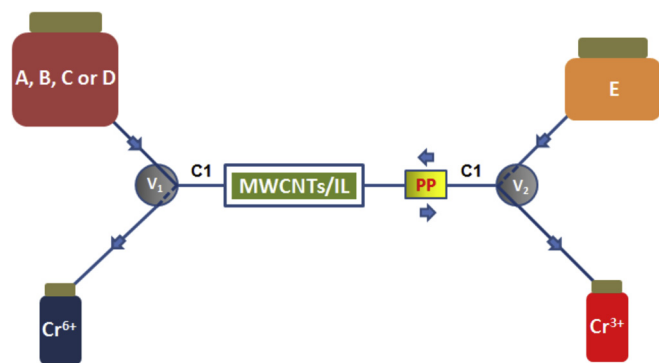


Fig. 1. MFS for chromium speciation. A: conditioner; B: sample; C: water; C1: channel 1; D: air; E: eluent; MWCNTs/IL: Multiwalled carbon nanotubes/ionic liquid; V: solenoid valve; PP: peristaltic pump.

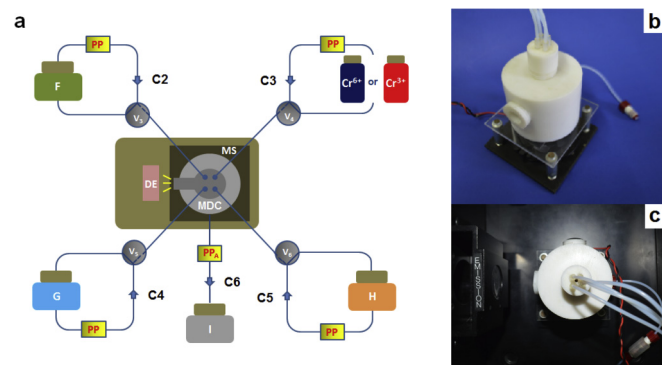


Fig. 2. a) FBA system for Cr(VI) and Cr(III) determination. C2 to C6: channel 2 to 6; F: luminol; G: hydrogen peroxide; H: water; I: waste; MDC: mixing detection chamber; MS: magnetic stirrer; V: solenoid valve; PP: peristaltic pump; PP_A: auxiliary peristaltic pump. b) MDC coupled to the stirring system. c) MDC and the stirring system coupled to the detector.

system was constructed using a cooler fan motor obtained from an Intel® microprocessor and placed below the MDC (Fig. 2b). The MDC coupled to the stirring system was placed in the spectrofluorimeter by means of a lab-made cell holder (Fig. 2c). On the other hand, the fifth channel (C_6) was used for emptying the MDC by means of an auxiliary peristaltic pump (PP_A). Thereby, when the chemiluminescence reaction finished, the solution in the MDC was pumped to the waste.

2.5. FBA procedure

As an initial step, the flow rate for each channel was studied in order to establish the time of activation of each solenoid valve. These times corresponded to the precise volumes of solution that were introduced into the MDC. For this purpose, each channel was filled with bidistilled water and the water was collected and weighed in an analytical balance by activating the peristaltic pump for 30 s. The corresponding volume was calculated by means of the density data taking into account the experimental conditions (25 °C and 1 atm). Finally, the flow rates obtained for each channel were 2.30, 2.60, 2.65, 2.30 and 2.28 mL min⁻¹ for luminol (C_2), sample (C_3), hydrogen peroxide (C_4), water (C_5) and waste (C_6), respectively. Before starting the procedure, the valves were set in a way that all solutions in their respective channels were recycled toward their flasks (OFF position). Then, V_3 , V_4 , V_5 and V_6 valves were switched ON for 10 s and the solutions were pumped toward the MDC in order to fill the channels between the valves and the MDC. Immediately, the PP_A was switched ON and the excess of the solutions contained in the MDC was aspirated to the waste for 20 s. This operation consumed a total time of 30 s. Then, the system was ready to perform the preparation of the calibration for Cr(III) and Cr(VI) and the sample analysis. In the case of Cr(III) determination, the analysis of standard solutions and the samples was performed by sequentially switching ON valves V_3 , V_4 and V_5 during previously defined intervals of time (26, 7 and 7 s, respectively). Thus, 1.00 mL of luminol, 0.30 mL of Cr (III) and 0.20 mL of hydrogen peroxide were pumped toward the MDC. For Cr (VI) determination, the analysis of standard solutions and the samples was performed by sequentially switching ON valves V_3 and V_4 during previously defined intervals of time (31 and 7 s, respectively). In this way, 1.20 mL of luminol and 0.30 mL of Cr (VI) were pumped toward the MDC. In both cases, the corresponding chemiluminescence signal at 425 nm was immediately measured as peak height. When the register of the signal was finished, the solution was aspirated and thrown into the waste by switching ON the PP_A for 45 s. The

magnetic stirring was ON during all the analysis. This procedure was repeated for each Cr (III) and Cr (VI) standard solution and each sample. On the other hand, the system was always cleaned between measurements. The MDC cleaning procedure was carried out by switching ON V_6 valve and the magnetic stirring for 40 s. The total emptying of the MDC was assured by switching ON the PP_A for 45 s.

3. Results and discussion

3.1. Characterization of the soft material before and after adsorption of Cr(VI)

The results obtained by EDS (Fig. 3) showed that the soft material was free from impurities. The five areas marked in Fig. 3a showed the same results, as it was expected. In the analysis the only elements found were the corresponding ones to the composition of the soft material: carbon, oxygen, nitrogen and chloride as it can be seen in Fig. 3b. (corresponding to the analysis of area 3 in Fig. 3a). On the other hand, Fig. 4 shows the physical structure and morphology of the soft material before and after adsorption of Cr(VI) obtained by SEM. Smooth MWCNTs were observed forming an intertwined pattern, probably due to its combination with the ionic liquid (Fig. 4a). Besides, the morphology of the soft material shows that the MWCNTs have uniform diameters between 35 and 45 nm, which demonstrate that the soft material is homogeneous. In general, the tubes became wider, especially at the ends, after the adsorption process (Fig. 4b), showing slightly bigger diameters between 47 and 52 nm. Also, the surface of the tubes was not as smooth (Mubarak et al., 2016) and looked more compact, probably due to the incorporation of chromium into the material.

3.2. Optimization of speciation variables

In this work, a soft material was prepared to perform the speciation of chromium. A combination of BmimCl and MWCNTs was used instead of MWCNTs, because the soft material presented more efficiency to adsorb Cr(VI) as $Cr_2O_7^{2-}$, while Cr(III) passed through the column. This fact is based on the synergic effect between the IL and the CNTs. It has been demonstrated that in a soft material, CNTs are surrounded by ILs, taking into account that the atoms of the alkyl groups are nearer to CNTs surface than the nitrogen and carbon atoms of imidazolium rings (Wang et al., 2008). A physisorption phenomenon is produced, where electrostatic interactions and Van der Waals forces take place on the MWCNTs surface and charged ion clusters are surrounding this surface (Martinis et al., 2017). In this way, the anion of IL (Cl^-) is interchanged with $Cr_2O_7^{2-}$ and the analyte is retained on the soft material, while Cr(III) passes through the column. This mechanism is showed in Fig. 5. In order to validate this selectivity of the soft material, the

typical spectrophotometric reaction for Cr(VI) with 1,5-diphenylcarbazide was used (Bose, 1954). Cr(VI) was determined after its elution and Cr(III) was determined after oxidation with hydrogen peroxide (Andersen, 1998). The obtained results demonstrated that Cr(III) was not retained on the soft material.

As mentioned before, the separation process was carried out in the minicolumn inserted in a channel of the MFS, packed with the soft material. So, it was necessary to optimize all variables of the MFS. Table 1 shows the range of all these variables and their optimum values. On the other hand, the direction of elution and type of eluent were also optimized. For these purposes, three solutions were tested: IL, alkaline IL and alkaline IL with NaCl. The pH of alkaline IL solution was varied between 8.00 and 12.00 and the best recoveries were obtained at pH 11.00. At this pH, the predominant specie of Cr(VI) is CrO_4^{2-} . So it was necessary to add a drop of concentrated nitric acid to the eluate, in order to obtain $Cr_2O_7^{2-}$ and then get it into the FBA system. Then, to enhance the recovery values, 0.01 g mL⁻¹ of NaCl was added to test the salting out effect and effectively, the recoveries were better. Taking into account the contact time of the eluent with the analyte, it was supposed that the best results would be obtained with low flow rates for the elution. However, the best chemiluminescence signals were obtained when higher flow rates were used and a stop flow step was included. The results were improved when the elution was performed in a reverse mode regarding the sample load direction. This elution mode avoids the tight packing of the sorbent and decreases the dispersion of the concentrate during the elution. Thus, the selected optimum values are showed in Table 1.

Moreover, the soft material needed a conditioning step. For this purpose, a 0.05 mol L⁻¹ BmimCl solution, with and without NaOH, was passed through the column during different times (1 min–3 min). The IL solution without NaOH (pH = 5.00) flowing for 2.5 min gave the best results in terms of recovery values for Cr(VI). Besides, two different lengths of the column were tested (15 and 30 mm) and the best results, based on the best chemiluminescence signals, were obtained with the longer column.

3.3. Optimization of the chemiluminescence reactions and the FBA system

The luminol in presence of an oxidant and in alkaline medium produces a chemiluminescence signal at 425 nm. So, using $Cr_2O_7^{2-}$ as oxidant it was possible to determine Cr(VI). According to the literature, this is the first time that Cr(VI) is determined by chemiluminescence, using the analyte as oxidant for the luminol reaction. Usually, Cr(VI) is reduced to Cr(III) and is determined as catalyst of the luminol reaction. On the other hand, taking into account that the chemiluminescence reaction of luminol can be catalyzed by different metals, the determination of Cr(III) was

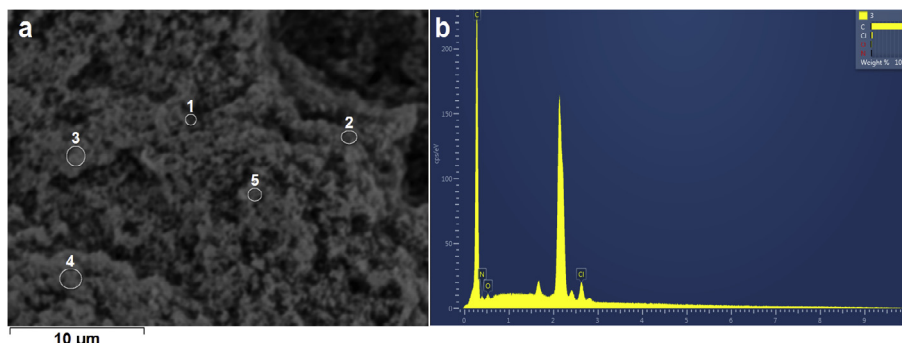


Fig. 3. X-Ray Energy Dispersive Microanalysis: (a) five different analyzed areas, (b) analysis of area 3.

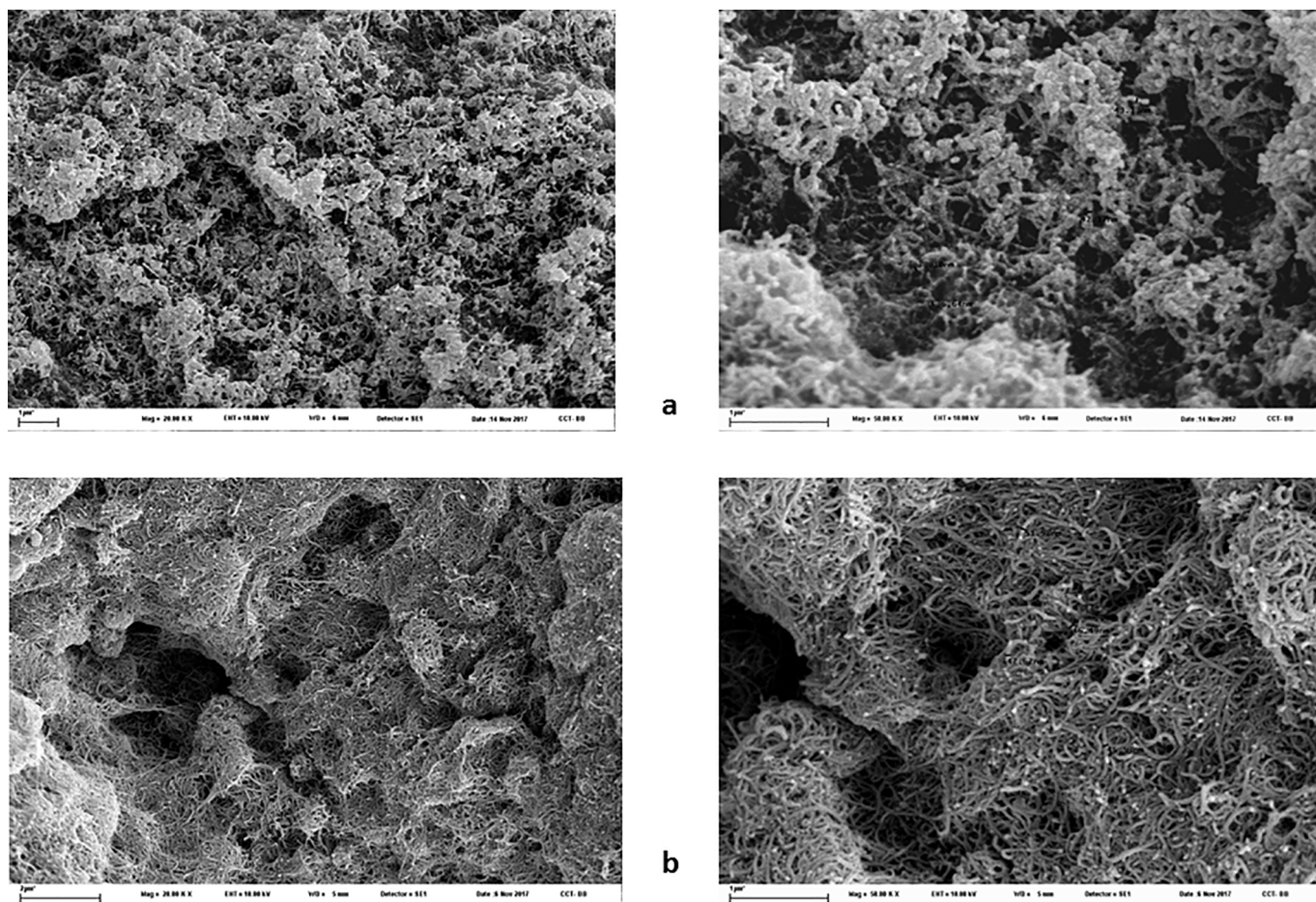


Fig. 4. SEM images of soft material: (a) before chromium adsorption (20000x and 50000x), (b) after analyte adsorption (20000x and 50000x).

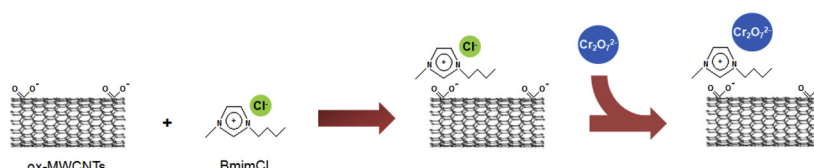


Fig. 5. The mechanism of chromium adsorption on the soft material.

Table 1

Studied ranges and optimum values for chromium speciation variables.

Variables	Studied ranges	Optimum values
Load flow rate (mL min^{-1})	0.80–4.04	1.21
Elution flow rate (mL min^{-1})	3.03–8.08	7.88
Sample volume (mL)	5–15	5
Elution volume (μL)	50–500	300
Stop-flow time (min)	5–15	10

performed using hydrogen peroxide as oxidant and Cr(III) as catalyst. The concentration of all chemical reagents involved in both reactions and the reaction media should be optimized. The luminol concentration was studied between $1 \times 10^{-4} \text{ mol L}^{-1}$ and $1 \times 10^{-2} \text{ mol L}^{-1}$ and the optimum value was $5 \times 10^{-3} \text{ mol L}^{-1}$ for both analytes. The studied pH range was 10.00–13.00. Again, the optimum value (12.00) was the same for the two analytes. These

facts represented an advantage in the determination of both analytes. The other optimized variables were only for the determination of Cr(III) and they were: H_2O_2 concentration (studied range: $1 \times 10^{-4} \text{ mol L}^{-1}$ and $3 \times 10^{-2} \text{ mol L}^{-1}$, optimum value: $1 \times 10^{-2} \text{ mol L}^{-1}$), EDTA concentration (studied range: $1 \times 10^{-3} \text{ mol L}^{-1}$ and $1 \times 10^{-2} \text{ mol L}^{-1}$, optimum value: $5 \times 10^{-3} \text{ mol L}^{-1}$). Also, the variables related to the FBA system were optimized in order to obtain the best signal. FBA variables such as order and volumes of the reagents added into the MDC and stirring time were optimized based on the higher chemiluminescence signal and the repeatability of the measurements obtained for both Cr(III) and Cr(VI) determinations. As regards to the order of the reagents added into the MDC, three different combinations were tested: luminol and oxidant, oxidant and luminol, and luminol-oxidant simultaneously. The optimum order was luminol and oxidant. On the other hand, the total volume of reaction was 1.50 mL, which included 1.00 mL of luminol, 0.20 mL of sample and 0.30 mL of hydrogen peroxide for Cr(III) and 1.20 mL

of luminol and 0.30 mL of sample for Cr(VI). Optimal chemiluminescence signals were obtained when the corresponding oxidant (H_2O_2 for Cr(III) and $\text{Cr}_2\text{O}_7^{2-}$ for Cr(VI)) was added so fast as possible to the MDC (See section 2.5). Finally, the MDC coupled to the stirring system was designed in order to obtain a rapid and efficient mixture of the solutions. As the speed of stirring presented a significant effect in the analytical signal, it was necessary to maintain a high and stable speed of stirring (500 rpm) during the chemiluminescence reaction.

3.4. Analytical performance

The different analytical parameters were calculated using the optimized proposed methods. The calibration curve for Cr(VI) was $y = (59.396 \pm 569) x + (1968 \pm 30)$, $R^2 = 0.9998$, and for Cr(III) was $y = (75.250 \pm 3088) x - (1254 \pm 162)$, $R^2 = 0.9960$. In both cases, y : chemiluminescence signal and x : $[\mu\text{g L}^{-1}]$. The limit of detection (LOD) and limit of quantitation (LOQ) for Cr(VI) were $1.4 \mu\text{g L}^{-1}$ and $38 \mu\text{g L}^{-1}$, respectively. For Cr(III) the LOD was $4 \mu\text{g L}^{-1}$ and the LOQ was $12 \mu\text{g L}^{-1}$ (Miller and Miller, 2010). The precision of the method, represented by repetitively (% RSD) was 7.0 and 3.8 for Cr(III) and Cr(VI) respectively, calculated from eight independent measurements of a standard solution containing $50 \mu\text{g L}^{-1}$ for both analytes.

3.4.1. Selectivity

The effect of common cations and anions present in water samples in the determination of 0.08 mg L^{-1} of Cr(VI) and 0.07 mg L^{-1} of Cr(III) was examined applying the whole method. Different cations present in water samples could catalyze the luminol reaction instead of Cr(III), so a study of these interferences was performed. The first step in this study was to compare the concentration of a standard solution of Cr(III) (0.07 mg L^{-1}) with the concentration corresponding to the same standard solution containing the interference. 6.00 mg L^{-1} of Cu(II), 0.06 mg L^{-1} of Fe(III), 6.00 mg L^{-1} of Zn(II), 14.00 mg L^{-1} of Ba(II), 10.00 mg L^{-1} of Ca(II) and 10.00 mg L^{-1} of Mg(II) were tested. Only Fe(III) presented interference. This fact was solved adding a $5 \times 10^{-3} \text{ mol L}^{-1}$ EDTA solution to the samples. In this case, it was important to check the formation of Cr(III)-EDTA complex, because under this chemical form, there was not chemiluminescence signal. The Cr(III)-EDTA complex is kinetically slow to form, and the other cations form EDTA complex rapidly. Hamm (1953) studied the Cr(III)-EDTA system and it was showed that the rate of complex formation was independent of EDTA concentration and at a higher concentration of Cr(III) the rate was proportional to this concentration. Besides, the rate of the formation of the complex increased with increasing pH. Thus, a study taking into account these considerations was performed. First of all, it is important to notice that in this case, the Cr(III) concentration levels were low, and the samples were acidified with a drop of $1 \text{ mol L}^{-1} \text{ HNO}_3$. Hence, the EDTA concentration was tested between 1×10^{-2} and $1 \times 10^{-3} \text{ mol L}^{-1}$, working with

the Cr(III) standard solution. The best chemiluminescence signal was obtained with $5 \times 10^{-3} \text{ mol L}^{-1}$. Then, the same standard solution containing different concentrations of Fe(III) was prepared and the respective signals were measured. In this way, 6 mg L^{-1} of Fe(III) did not interfere. It is important to highlight that EDTA should be added just prior to analysis, to avoid the possible formation of Cr(III)-EDTA complex and to obtain a good reproducibility. Conversely, the anions present in the water samples could be adsorbed on the soft material together with Cr(VI), so it was necessary to test these possible interferences. Therefore, different concentrations of anions were added to a standard solution of Cr(VI) (0.08 mg L^{-1}). Thus, it was possible to demonstrate that the proposed method supported 4 mg L^{-1} of Cl^- and NO_3^- , 3 mg L^{-1} of PO_4^{3-} and 2.5 mg L^{-1} of SO_4^{2-} . Due to the analyzed samples usually contain higher concentration levels of SO_4^{2-} than 2.5 mg L^{-1} , this interference was solved by adding BaCl_2 to the samples and filtering them with a $0.45 \mu\text{m}$ nylon filter.

3.5. Analysis of real samples

The proposed method was applied to three different water samples. These real samples showed that both chromium species were not present in them, so they were spiked with the respective standard solutions. A recovery study was carried out and the same spiked samples were analyzed by inductively coupled plasma optical emission spectrometry, in order to validate the method. Table 2 shows the obtained results and the respective recoveries which agreed with those recommended for water samples. On the other hand, the obtained results applying inductively coupled plasma optical emission spectrometry were in accordance with those obtained with the proposed method.

4. Concluding remarks

A new automatic FBA method with chemiluminescence detection was developed to determine Cr(VI) and Cr(III). The speciation of both analytes was performed by using a MFS which contained a minicolumn packed with a soft material. One of the novelties of this work is the use of a soft material composed by MWCNTs and IL that allows the separation of both chromium species. Cr(VI) was retained on the soft material and Cr(III) passed through the minicolumn. Besides, it is the first time that the chemiluminescence technique was used to determine both species, Cr(VI) as oxidant of luminol reaction and Cr(III) as catalyst of the luminol and hydrogen peroxide reaction using the same concentration of luminol and pH value. This was an additional advantage when FBA systems were used.

The new method was applied to real water samples and the obtained results presented good recoveries and were in agreement with those obtained with inductively coupled plasma optical emission spectrometry method.

Table 2
Analysis of real samples.

Sample	Cr(III) [$\mu\text{g L}^{-1}$]			Cr(VI) [$\mu\text{g L}^{-1}$]		
	Added	Found ^a	Recovery (%)	Added	Found ^a	Recovery (%)
Drinking water	40	42 ± 1	105	10	10 ± 1	100
	60	58 ± 3	97	50	49 ± 2	98
Tap water	40	38 ± 2	95	10	10.2 ± 3	102
	60	62 ± 2	103	50	52 ± 5	104
Filtered tap water	40	43 ± 3	107	10	9.5 ± 2	95
	60	58 ± 1	97	50	60 ± 4	102

^a The results are average of five replicates.

Conflicts of interest

The authors declare that they have no conflict of interest.

Acknowledgment

The authors acknowledge financial support of Universidad Nacional de Colombia del Sur. C. Fernández, C. Domini and M. Grünhut also acknowledge to CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas).

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