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Interfacing microfluidic chip-based chromatography with flame atomic absorption spectrometry for the determination of chromium(VI)

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ABSTRACT

This work demonstrates the first study on interfacing of microfluidic chip-based chromatography to flame atomic absorption spectrometry (FAAS) for a simple and fast separation or preconcentration and subsequent determination of species. The developed low-cost polydimethylsiloxane microchip includes 12 microcolumns of conventional C18 (5 μm) chromatographic particles, making the parallel chromatographic separation/preconcentration and the direct introduction of the effluent into the spectrometer possible. In order to test this hyphenated microchip–FAAS system, the Cr(VI) was selected to be determined. The adsorbed Cr(VI) was eluted with methanol, and the 30 μL effluents were collected into plastic vessels inserted into the ends of the microcolumns. Then, the effluents were analyzed by FAA spectrometer using micro-injection of the effluents as discrete samples. The separation/preconcentration and FAAS determination of 12 samples needed less than 5 min due to the multiplex feature of the proposed system. The overall capacity of the microcolumn (C18, 5 μm beads, 20 mm × 1 mm × 0.1 mm) was calculated to be 0.45 μg/mm for Cr(VI). Loading only 80 μL samples onto the microchip and nebulizing the methanolic effluent of 30 μL into the FAA spectrometer, 0.0031 μg/mL limit of detection was obtained.

1. Introduction

Microfluidic devices have emerged as novel analytical tools in many areas of biomedical or environmental monitoring. These cheap devices have low power requirements and low sample/reagent consumption and provide the possibilities of fast and possible parallel analysis. For the microfluidic devices, only detectors which have (i) reduced diffusion length between the chip and the detection cell and (ii) the ability to require only a few microliters or submicroliter of sample can be applied. The applicability of many different electrochemical [1], optical and mass spectrometric [2] detection methods has been demonstrated in the last two decades. Since atomic spectrometers typically need at least a few hundreds of microliters for analysis, relatively small number of works can be found in literature about the element-selective detectors hyphenated with microchips. Inductively coupled plasma-mass spectrometry (ICP-MS) has been coupled to chip-capillary electrophoresis for speciation analysis [3,4]. Recently, chip-based magnetic solid-phase extraction was applied before the determination of selenium species using high-performance liquid chromatography (HPLC)-ICP-MS [5]. Besides the ICP-AES or ICP-MS detectors, only one work was found using other type of atomic spectrometric method for interfacing with microchip: Li et al. [6] coupled (flameless) atomic fluorescence spectrometry to microchip, in which electrophoretic separation of mercury species was carried out and the effluents were converted to volatile components. However, no combination of microfluidic chip with the traditional and often used flame atomic absorption spectrometer (FAAS) was found.

Despite the high demand for miniaturized liquid chromatographic (LC) techniques, there are only a few chip-based chromatographic systems compared to chip-based capillary electrophoretic devices [7]. First, Manz et al. [8] described an LC partially integrated onto a silicon chip in which chromatographic C8 particles were retained by frits in the column. Other devices have incorporated a physical barrier [9], tapered capillary geometries [10] or fritless bottleneck [11,12] to trap stationary-phase particles. Hansen et al. [13] reported microfluidic solid-phase chromatography columns formed by multilayer soft lithography. Recently, our group has demonstrated the preparation of simple and permanent multiple chromatographic columns that are suitable for parallel chromatographic separations without frits [14].

Chromium provides a typical example for how the different oxidation forms of the same element may show highly different toxicity: in biological systems, Cr(III) is essential while it is a definitely carcinogenic element in the form of Cr(VI) [15]. Therefore, the accurate individual determination of Cr(VI) is important. For chromium speciation, one of the most effectively used techniques is the on-line combination of HPLC separation with the element-selective detection of atomic spectrometry [16–18]. Following the labour- and time-consuming, yet less accurate extraction, ion-exchange and co-precipitation methods [19,20], in the last two decades, several papers have been published on the combination of

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chromatography and element-selective detection [21,22]. The on-line chromatographic separation/preconcentration of Cr(VI) with FAAS detection has been published by Posta et al. [18,23]. This preconcentration method is based on the ion pairs formed by Cr(VI) with tetrabutylammonium (TBA) salts, as these complexes can be sorbed on C18. After the sorption, the Cr(VI) is eluted with methanol and introduced into the spectrometer.

Our work aims at developing a low-cost, microchip-based multiple chromatographic technique that can be coupled with a flame atomic absorption spectrometer. In order to test this system, the toxic Cr(VI) was chosen to be separated/preconcentrated on the microchip prior to the FAAS determination.

2. Experimental

2.1. Apparatus and reagents

Determination of chromium was carried out with a 240FS atomic absorption spectrometer (Agilent Technologies, Santa Clara, CA, USA) equipped with flame atomization (a mixture of air (13.5 L/min) and acetylene (2.9 L/min)). The chromium hollow cathode lamp (Cathodeon) operated at a current of 10 mA. Chromium was determined on the wavelength of 357.9 nm with a spectral bandwidth of 0.2 nm. Deuterium correction of background was used, and the signal was measured in an integration mode for 30 s. Signal peaks were recorded by the software of the spectrometer (SpectrAA 5.2 Pro, Agilent), and figures showing the signals demonstrated in this work were cut from the report automatically generated by the software.

Solution of Cr(VI) was prepared by the dilution of a 1000 mg/L potassium dichromate stock solution produced by Fluka. A detailed description of the chromatographic separation/preconcentration method of Cr(VI) was provided in an earlier publication [18]. The ion pair complex of Cr(VI) with TBA salts (Sigma) was retained quantitatively on a microchip-based C18 microcolumn (Varian 100-C18 5 μm) in an acetic acid medium at pH 3 in the presence of $5 \cdot 10^{-2}$ mol/L TBA. After loading the sample onto the microcolumn, the adsorbed Cr(VI) was eluted with methanol (VWR) and transferred to the FAA spectrometer.

2.2. Preparation of multiple chromatographic packings in a microchip

Microfluidic chips made from polydimethylsiloxane (PDMS) were prepared by using a mold created by soft photolithography mainly according to the procedure described by Whitesides [24]. The channel pattern was printed as a high-resolution (4000 dpi) photomask. In order to get a thickness of around 100 μm (that is, much thicker than usual in microfluidics), the negative type photoresist (SU-8 2025, Microchem, Newton, MA) was spin-coated onto a 3″ silicon wafer only with 500 rpm for 30 s. The PDMS chip was fabricated by a cast molding of a 10:1 mixture of PDMS oligomer and cross-linking agent (Sylgard 184, Dow Corning, Midland, MI). The PDMS chip was sealed onto a glass slide of 1.2 mm thickness after oxygen plasma treatment (PDC-32G, Harrick, Ithaca, NY).

The microchip includes 12 parallel channels (width: 1 mm, height: 100 μm), which merge into a single port E (elution) of a 0.3 mm diameter. At the other end of each channel, similar ports S (sample) are created by punching the PDMS, and a compressed small cotton wool (<1 μg) serving as a frit for retaining the chromatographic particles is pressed down to the bottom of the port (Fig. 1a) using a metal rod.

For the preparation of chromatographic packing, similar process was applied to the one used in our earlier works [14]. The 12 channels of the microchip were packed simultaneously from port E, pumping a single plug of C18 suspension splitted toward the other ends of the parallel channels. Briefly, a suspension (~100 μL) of freshly ultrasonicated, methanolic C18 particles of 5 μm was manipulated through a small-bore tubing (0.25 mm ID) using a peristaltic pump. The tubing was connected to the port E (Fig. 1a,b), and the particles were washed with methanol (~10 μL/min) toward the frit at the end of the channels (ports S). The obtained packing (microcolumn) was rinsed with methanol with intermittent (4–5 s) application of pumping pressure (~2 bars) to improve the compactness of the packing. The packings were conditioned with 10 min washing with methanol and 5 min with the mobile phase before the chromatographic separation/preconcentration. A schematic depiction of the packing preparation procedure and the picture of the obtained microchip are given in Fig. 1a,b.

Samples to be analyzed should be injected to the microcolumns through the S ports using peristaltic pump(s). It is possible to inject even 12 different samples parallelly to separate chromatographic packings (6- or 8-channel peristaltic pumps are quite common in the market). Then, the adsorbed (preconcentrated) Cr(VI) can be eluted from the microcolumn by pumping the eluent from the common port E (elution). In this case, all adsorbed Cr(VI) will be eluted from all microcolumns at the same time, but the elution from a given channel can be prevented by blocking the port S of any microcolumns. A schematic depiction of the sample loading and elution is given in Fig. 1c.

The adsorbed amounts of Cr(VI) were eluted into small hydrophobic plastic vessels (5 mm part length of the bottom of a 0.1-mL polypropylene micropipette tip, ~40 μL) positioned just at the ends of the chromatographic microcolumns (Fig. 1d). The plastic vessels could be easily and tightly inserted into the flexible ports S; no leakage outside around the vessel could be observed. Since the inner diameter of the funnel-shape vessel gradually reduced from 3 mm to 0.4 mm, the end of the sampling capillary of the pneumatic nebulizer of the FAA spectrometer could be simply introduced into the lower part of the vessel. The liquid content of the plastic sampling vessel was instantly sucked out by the PTFE capillary of the nebulizer when it was immersed into the vessel. The length of the capillary was reduced to 10 cm to minimize the dispersion of the liquid sample on the surface of the capillary between the microchip and the nebulizer (Fig. 1e).

3. Results and discussion

3.1. Harmonizing the sample volume needed for FAAS to the sample volume eluted from the chip-based chromatographic packings

With FAA pneumatic nebulizers, samples are taken up continuously consuming a few milliliters of sample. After a few seconds of aspiration (several hundreds of μL of the sample), a continuous signal can be obtained. However, small (<200 μL) sample volumes can also be injected. The small volumes of the samples were injected from a funnel or hemisphere-shape cup made from a hydrophobic PTFE plate. Various researches [25,26] have studied this micro-injection technique of discrete samples, proving that the nebulization of a 100 μL sample gives the same sensitivity as the one obtained by conventional nebulization. Others [27] reported that using even a 60 μL volume of a sample the maximum sensitivity can be achieved with a better precision than 1.5 RSD%. The peak height of the analytical signal depends on the injected volume, the wetting of the surface of the sampling vessel and the nebulizer capillary by the liquid sample as well as the solution flow rate of the nebulizer.

The small volumes of the samples were injected to small hydrophobic plastic vessels (cup) by a micropipette (Biohit). The end of the PTFE capillary of the nebulizer was immersed into the bottom of the microsampling cup, then the whole volume of the liquid was completely and immediately sucked into the nebulizer providing a transient signal. The signals rose sharply, and then fast declining with some tailing could be observed due to the dilution of the aerosol at the signal end. In Fig. 2, the effect of the injected sample volume (10–100 μL) on the absorption signal for 5 μg/mL Cr(VI) using micro-injection of the discrete samples is shown. The spike-like signals largely increase with the injected volume up to 30–35 μL and then the increase gets smaller, while the signal intensities for the sample volumes above 100 μL remain

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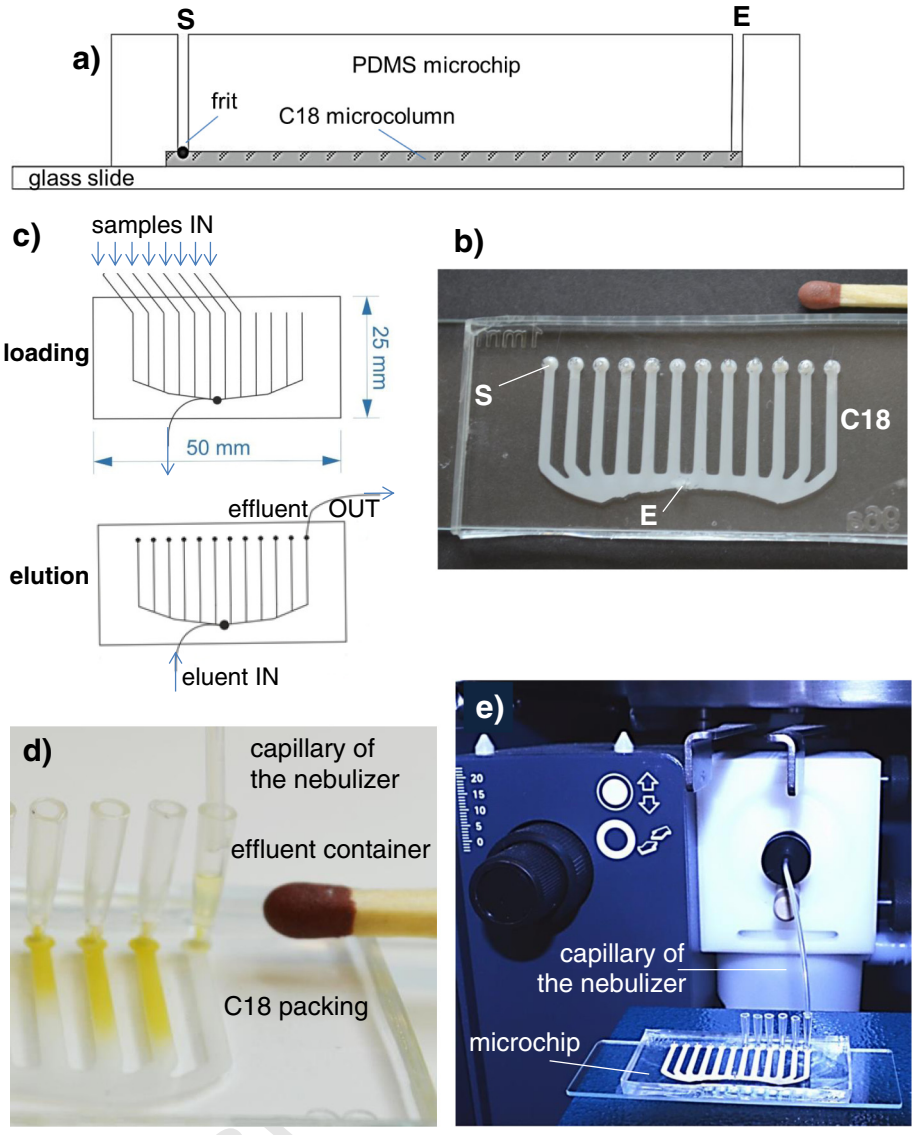

Fig. 1. (a,) Schematic (not scale-true, side-view) and the picture (top-view) (**b,**) of the PDMS microchip including C18 chromatographic packings (20 mm × 1 mm × 0.1 mm, 5-μm beads, port S is for sample IN or elution OUT; port E is for waste or elution pumping). (**c,**) The samples are parallelly injected to the microcolumns through the S ports (loading), and then the adsorbed Cr(VI) can be eluted by pumping the eluent from the common port E (elution). **(d,)** Picture of the elution of Cr(VI) preconcentrated on the chromatographic packing into a plastic vessel for the subsequent direct nebulization into the FAA spectrometer. **(e,)** Arrangement of the microchip coupled to the FAA spectrometer.

constant and equal to that obtained by a larger sample volume or continuous nebulization. The reason for the surprisingly good sensitivity for the only 30 μL sample volume should be found in the construction of the nebulizer of the used FAA spectrometer (240FS, Agilent). Thus, the mass sensitivity obtained for 30 μL was improved by a factor of 11 compared to the (continuous) sucking of 0.5 mL sample volume.

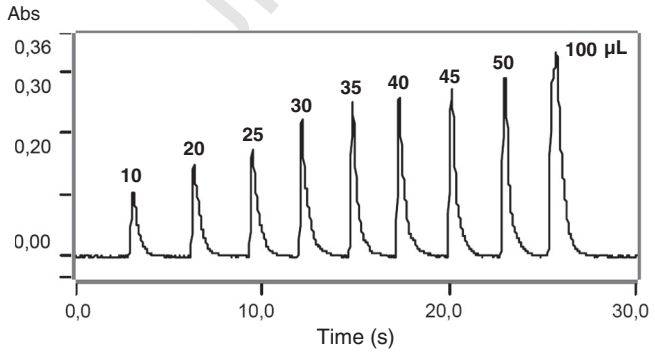

Fig. 2. The effect of injected sample volume (10–100 μL) on the absorption signal for 5 μg/mL Cr(VI) using micro-injection of the discrete samples.

In Fig. 3a, the signals obtained for standard solutions of 0.5–50 μg/mL Cr are shown. The applied sample volume was 30 μL using micro-injection of the discrete samples. From these data, a 0.0089 μg/mL limit of detection (LOD) and a linear detection range of 0.2–50 μg/mL could be concluded. Fig. 3b shows the signals of a number of 30 μL samples containing 4 μg/mL Cr as an example of the reproducibility and the time consumption of the measurements (approximately 20 samples/min). The relative standard deviations ($N = 12$) amounted to 2.9% and 3.8% in signal height and signal area, respectively. Of course, better RSD% values could be obtained for 0.5 mL sample volumes considered as continuous sampling (0.9% and 1.1%). These analytical performance data prove that the sensitivity obtained only from 30 μL sample volume are only 30% worse than using continuous sample introduction, and the precision values are also acceptable. However, by reducing the sample volume below 30 μL, the LOD and precision data become dramatically worse.

Although the minimal uptake demand for sensitive FAAS detection is around 30 μL, this sample volume is still enormously large for typical

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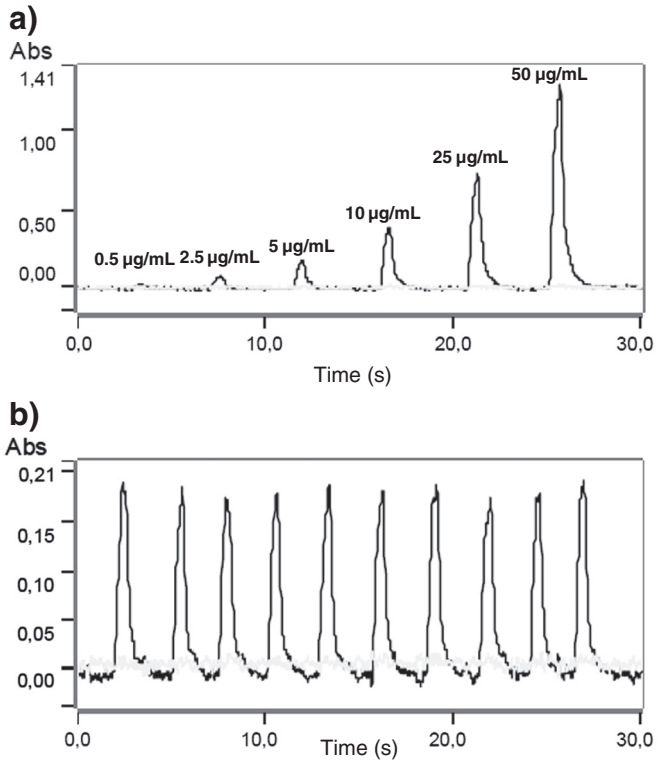

Fig. 3. Signals obtained for standard solutions of 0.5–50 µg/mL Cr **(a,)** and the signals of repeated determinations of samples containing 4 µg/mL Cr **(b,)** using micro-injection of the discrete samples (in each case the sample volumes were 30 µL).

microfluidic applications. In our earlier [11,12] and others' [13] chip-based chromatographic works, even less than 0.1 µL volume of sample was eluted from the microcolumns. In order to increase the effluent volume, the dimensions of microcolumns were maximized. The soft lithographic procedure [24] used for the microchip preparation enables a 0.1 mm maximal height of the microchannel (the SU-8 2025 photoresist was spincoated with a low speed for short time). The width of a microcolumn was increased to 1 mm (in other chip-based chromatographic work, the widths are 0.1 mm [11–13]). The length of the microcolumns was limited by the width of the glass slide. After these considerations, the dimension of the microcolumns was 20 mm × 1 mm × 0.1 mm (Fig. 1b), which was useful to produce effluent (after separation or preconcentration of components) in a range of 10–30 µL. These chip-based chromatographic columns already can serve enough volume of sample for the micro-injection FAAS.

3.2. Separation/preconcentration and determination of Cr(VI)

The on-line HPLC separation and FAAS detection of Cr(III) and Cr(VI) have been proposed by Posta et al. [18] using a reverse phase C18 column (5 µm particle size, 125 mm length, 4.6 mm i.d.) and using TBA salt as ion-pair forming agent. For the separation, 100 µL sample was needed, and more than 50× enrichment factor was achieved using up a sample volume of 5 mL. The applicability of the separation and preconcentration of Cr(VI) in complex sample matrix (seawater) was shown as well [28]. In our work, we used their optimized conditions. The carrier solution was an aqueous solution containing 0.1 mM NH_4 acetate at pH 3. After loading the sample to the microcolumn, the Cr(III) and other ionic components of the sample were passing through the microcolumn, while the Cr(VI) was retained. With the miniaturization of the separation unit, much less sample volume, 10–30 µL and 100–300 µL, was necessary for the separation and preconcentration, respectively.

In order to visualize the separation/preconcentration process of the Cr(VI) on the C18 microcolumns embedded in the transparent PDMS chip, a mixture of high concentration of Cr(VI) and Cr(III) standard solutions of 10 µL volumes is pumped simultaneously to the parallel columns to retain the Cr(VI) (Fig. 4a). Because the chromate has a relatively intensive yellow color, by taking a picture of the microchip, a densitogram of each microcolumn can be created by means of a densitometric software (e.g. CP Atlas 2.0, freeware thin layer chromatogram evaluation software) [28]. The densitograms, that is the plots of intensity (RGB values) of colors per pixels of the picture of the microcolumn, are shown in Fig. 4c. The yellow area of the Cr(VI) can be simply

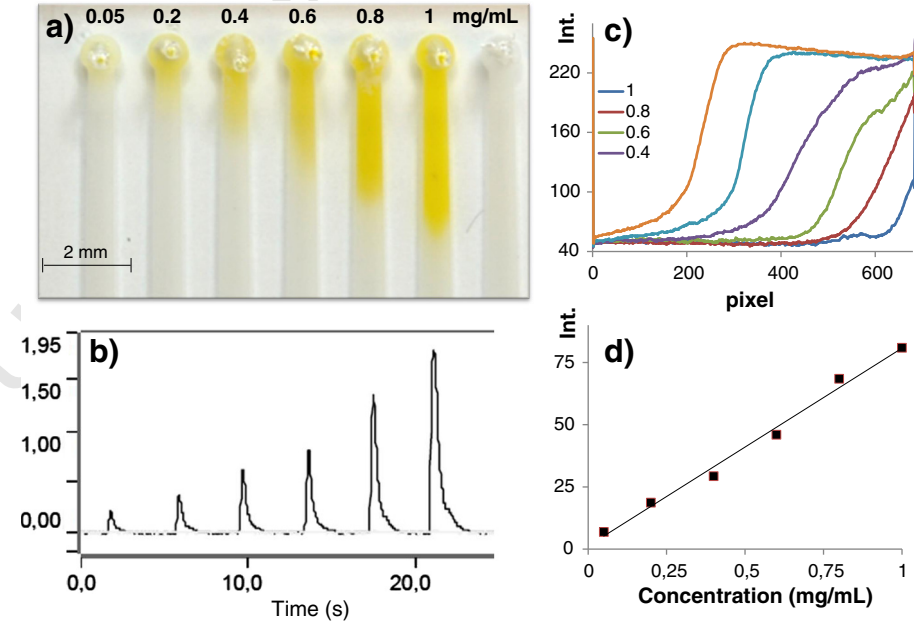

Fig. 4. (a,) Picture of the chip-based microcolumns, on which the mixture of 0.05–1 mg/mL Cr(VI) and Cr(III) standard solutions of 10 µL volumes is loaded for 4 min to retain (separate) the Cr(VI). **(b,)** Signals of the effluents obtained with FAA spectrometer using micro-injection of the effluents as discrete samples of 30 µL. Effluents were collected into a plastic vessel inserted into the ends of the microcolumns. **(c,)** Densitogram of the adsorbed Cr(VI) (measuring the intensity (RGB) of colors on the microcolumns of (a,)). **(d,)** The calibration plot of the Cr(VI) standard solutions adsorbed on the microchip and eluted into the FAA spectrometer.

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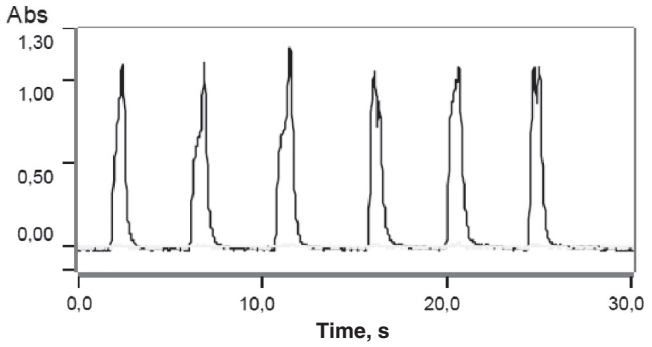

Fig. 5. Reproducibility and time needed for measurements of 30 μL samples of Cr(VI) obtained with FAA spectrometer using micro-injection of the effluents as discrete samples. The effluents were obtained after 0.1 mg/mL solutions of 30 μL volumes are simultaneously loaded to the parallel microcolumns to retain the Cr(VI) and then simultaneously eluted the microcolumns with 30 μL methanol.

integrated by the same software, and this integrated area is proportional to the sample concentration loaded onto the microcolumns (Fig. 4d.). The calibration plot of the Cr(VI) standard solutions adsorbed on the microchip showed good linearity ($R^2 = 0.9902$). Actually, the determination of high concentration (>20 μg/mL) Cr(VI) is possible by this colorimetric procedure (taking photo and making densitogram) without using FAAS. For the detection of samples containing less than 20 μg/mL concentration (10 μL) Cr(VI), more sensitive atomic spectrometric detection is necessary.

The adsorbed Cr(VI) was eluted with methanol, and the 30 μL effluents were collected into plastic vessels inserted into the ends of the microcolumns (Fig. 1d). The effluents were analyzed by FAA spectrometer using micro-injection of the effluents as discrete samples. The obtained atomic absorption signals (Fig. 4b) are in a good agreement with the photos and densitograms of the microcolumns.

Making a reproducibility study, 12 effluents were obtained when 0.1 mg/mL sample solutions of 30 μL volumes were parallelly loaded to the microcolumns and then parallelly eluted the Cr(VI) into the effluent containers. The 30 μL aliquots of effluents as discrete samples are micro-injected into the FAA spectrometer. From Fig. 5, it can be concluded that 6 measurements could be carried out within 30 s. The RSD% values of the measurements of the 12 effluents were 3.7 and 5.8 for peak heights and peak areas, respectively. These obtained RSD% values were the summed contributions of the precision of the injections of the sample volumes into the microchip, the reproducibility of the adsorption process on the parallel microcolumns and the elution of the adsorbed components, and the precision of the FAAS detection of the discrete samples.

In order to obtain a maximum enrichment, we should know the volume by which the adsorbed components can be completely eluted. Increasing the volume of the methanol to elute the previously adsorbed Cr(VI), it was found that 10 μL eluent already washed more than 95% part of the adsorbed components into the effluent container. The efficiency of the elution of the adsorbed Cr(VI) was plotted against the volume of the methanol used for the elution (Fig. 6). Although the 15 μL would be a proper elution volume in our chromatographic system to obtain the maximum enrichment, the 30 μL volume of the effluent is required for the more sensitive and precise FAAS detection.

3.3. Analytical performance

One of the main advantages of microfluidics is that a large number of analytical systems (in our work chromatographic separation units) can be arranged on a single microchip; thus, several chromatographic separations, preconcentrations and elutions can be carried out at the same time. Thus, although the chromatographic separation/enrichment procedures require quite a long time, the separation/preconcentration and FAAS determination of 12 samples needed altogether less than 5 min due to the multiplex feature of the proposed system.

The overall capacity of the microcolumn (C18, 5 μm beads, 20 mm × 1 mm × 0.1 mm) was calculated to be 0.45 μg/mm packing for the Cr(VI). Although the demonstrated microchip included only 20 mm length of packings, the lengths can be largely (e.g. 10×) increased if serpentine and curved channels are used for the packing; thus, the capacity of the microcolumn will be increased as well. Theoretically, the capacity of the column could be increased by thicker packings; however, the thickness of the channel created in the PDMS microchip using soft lithography [24] was not allowed to exceed a maximum of around 0.1 mm.

The adsorption of ion-pair formed Cr(VI) on the C18 column is not strong enough to achieve a high preconcentration factor. A maximum 80 μL volume of Cr(VI) could be adsorbed on the 20 mm length of the column, but pumping larger volumes of aqueous solutions will result in the slow immobilization of the adsorbed Cr(VI). The highest preconcentration (8×) was obtained when 80 μL sample volume was loaded onto the microcolumn, and the adsorbed Cr(VI) was eluted into a 10 μL volume. Loading 80 μL samples onto the microchip and

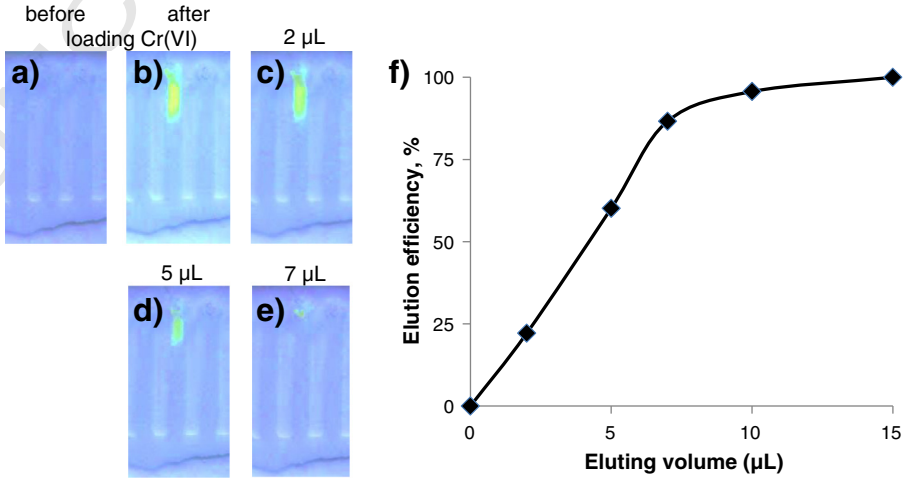

Fig. 6. Efficiency study of eluting Cr(VI) previously adsorbed on C18 microcolumn in microchip. Picture about the microcolumn before **(a,)** and after **(b,)** Cr(VI) is loaded onto the packing and after the column is eluted with **(c,)** 2 μL, **(d,)** 5 μL and **(e,)** 7 μL methanol. The efficiency of elution of adsorbed Cr(VI) was plotted against the volume of the methanol used for elution **(f,)**.

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nebulizing the methanolic effluent of 30 μL into the FAA spectrometer, 0.0031 μg/mL LOD was obtained (2.1× better than obtained with conventional, continuous sample introduction). Based on a few pre-experiments with dyes (e.g. brilliant blue FCF, safranin-O), which adsorb much stronger onto the C18 column, more than 100× preconcentration factors were obtained by loading only 1–2 mL of samples. These pre-experiments show that a chromatographic system with higher enrichment power should be found in future applications.

4. Conclusions and outlook

In this work, we demonstrated for the first time the interface microfluidic chip-based chromatography with flame atomic spectrometry. The developed low-cost microchip includes 12 microcolumns of conventional C18 (5 μm) chromatographic particles, making the parallel chromatographic separation/preconcentration and the direct introduction of the effluent into the spectrometer possible. In order to test the hyphenated microchip–FAAS system, the Cr(VI) was selected to be separated/preconcentrated. Although the LOD of the chromium determination in this system was only slightly better than in the conventional FAAS detection, choosing a chromatographic system in which the analyte is more strongly retained, much higher improvement in LOD could be achieved.

In general, the on-line hyphenations of the chromatography and the atomic spectrometric detection are more preferred than the off-line combinations; however, the proposed simultaneous preconcentrations of several samples on the microchip and the micro-injection of the effluents as discrete samples are optimal and can be considered "more" than off-line. The preconcentration of sample solutions requires a relatively long time (1–100 min); however, in the microchip, a large number (e.g. 12) of samples can be preconcentrated at the same time. For the preconcentrations on the microchip, only a peristaltic pump (or a syringe in the field) is needed, and the simultaneous elutions to get effluents for the direct micro-injection should be accomplished right before the FAAS analysis. The proposed method of preconcentration on the microchip and the introduction of the effluent from the chip directly to the nebulizer capillary of the atomic spectrometer combine the advantageous features of the off-line and the on-line hyphenations.

In this work, the microchip was intended to hyphenate with the most often used atomic spectrometer (FAAS), but the use of graphite furnace AAS seems to be a more advantageous choice, since it only needs a sample of around 10–15 μL, just the volume of the effluent collected at the ends of the microcolumns. On the other hand, there are several FAAS works where only a few-microliter-volume sample was needed for sensitive determination [30,31]. Since C18 reversed-phase silica particles are widely used as the stationary phase in HPLC or solid-phase extraction (SPE), the described chip-based chromatographic system has great potential in many applications (e.g. preconcentration, purification, separation).

Whitesides et al. [32,33] introduced microfluidic paper-based analytical devices, a new class of point-of-care diagnostic devices that are cheap, easy to use and specifically designed for use in developing countries. Carrying out a colorimetric assay on these microfluidic devices, photographing the results with a cellular phone equipped with a camera, and transmitting the image to an expert (clinical, environmental laboratory), obvious advantages of telemedicine can be utilized [32]. We believe that the hereby proposed chromatography-based microchip with colorimetric assay for the detection of toxic Cr(VI) provides the principle of a similar useful tool.

5. Uncited reference

[29]

Acknowledgments

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