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Open-channel chip-based solid-phase extraction combined with inductively coupled plasma-mass spectrometry for online determination of trace elements in volume-limited saline samples

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ABSTRACT

In this study, we used an automated online chip-based solid-phase extraction (SPE)–inductively coupled plasma-mass spectrometry (ICP-MS) system for analyzing trace elements in small-volume saline samples (~15 μL). The proposed method involved the adsorption of trace metal ions in the interior of a functionalized poly(methyl methacrylate) (PMMA) channel in order to separate these ions from saline matrices. The adsorption of transition metal ions was presumably dominated by the surface complexation between the carboxylate moieties in the interior of the PMMA channel and the metal ions, which facilitated the formation of metal–carboxylate complexes. The components of the proposed online analytical system used for the simultaneous detection of multiple trace metals in saline samples involved microdialysis (MD) sampling, an established chip-based SPE procedure, and ICP-MS. The SPE–ICP-MS hyphenated system was optimized, and then, the analytical reliability of this system was further confirmed by using it to analyze the certified reference materials—SRM 2670 (human urine) and SRM 1643e (artificial saline water). The satisfactory analytical results indicated that the proposed on-chip SPE device could be readily used as an interface for coupling the MD probe with the ICP-MS system. The dramatically reduced consumption of chemicals and "hands-on" manipulations enabled the realization of a simplified and relatively clean procedure with extremely low detection limits in the range of 5.86–76.91 ng L^{-1} for detecting Mn, Co, Ni, Cu, and Pb in 15-μL samples by ICP-MS. The effectiveness of an online MD–chip-based SPE–ICP-MS technique for continuous monitoring of trace elements in a simulated biological system was also demonstrated. To the best of our knowledge, this is the first paper to report the direct exploitation of a PMMA chip as an SPE adsorbent for online sample pretreatment and trace metal preconcentration prior to ICP-MS measurement.

1. Introduction

In recent times, the importance of trace elements has been emphasized, particularly in view of the emergence of increasing evidence with regard to the vital roles played by these elements in living organisms [1] and environmental systems [2,3]. Clinical research has shown that trace metals play a role in ensuring the normal functioning of the central nervous system [4,5]. However, till date, all commonly used analytical methods cannot determine trace metal ions or their kinetics and spatial distribution with the desired resolution; therefore, direct evidence for clarifying the release and uptake of metal ions in neurological disorders is still lacking [6]. Thus, explaining the physiological roles of trace metal ions in various behaviors is an ongoing challenge in neuroscience [7].

The effectiveness of inductively coupled plasma-mass spectrometry (ICP-MS) for detecting trace elements in biological fluids has been demonstrated; this technique offers the advantages of providing simultaneous multielement/isotopic capability and facilitating ultra-sensitive analysis of samples with large sizes [8]. Despite the advantages offered by ICP-MS in carrying out trace metal analysis, sample pretreatment is usually required to avoid unpredictable suppression or enhancement effects due to the presence of high salt content in the samples [5,9]. Generally, sample pretreatment methods can be broadly categorized in terms of their operating procedure into offline or online processes. Because the use of offline preconcentration systems involves the risk of contamination and requires a relatively large volume of a sample and/or reagent, these systems have been replaced with

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online systems to a great extent. To minimize the amount of labor and errors that arise during the sample pretreatment step, online systems using flow injection (FI) techniques have become increasingly popular because of their potential for automation, minimization of reagent and/or sample consumption, and reduced risk of contamination, all essential prerequisites for trace analysis [10–14].

For trace metal analysis of saline samples, various pretreatment techniques such as solvent extraction, microcolumn solid-phase extraction (SPE) [15–17], in-tube SPE [18], precipitation/coprecipitation [19,20], and knotted reactors [21] have been successfully adapted to FI systems. Komjarova and Blust [22] compared these techniques and concluded that SPE procedures are not only faster, easier, safer, and less expensive but that they also proceed with higher preconcentration factors and lower sample volume requirements when selecting suitable operational parameters. Nevertheless, as described in our previous paper [5], because of matrix-dependent extraction efficiency, leaching of metal impurities contained in the sorbent, and contamination caused by the use of a relatively large amount of reagents during SPE [23], until recently, there have been no reports on the use of FI online solid-phase chelation for separating trace elements from microsamples (e.g., microdialysis samples).

Over the past decade, microfluidic chip-based systems suitable for use in various biological studies have attracted considerable attention because of their unique advantages: low reagent and power consumption, short reaction time, portability for *in situ* use, low cost, versatile design, and potential for integration with other miniaturized devices [24–27]. Recent advances in the miniaturization of many chemical reactors have facilitated the integration of SPE in microfluidic systems to preconcentrate and clean up samples [28–34]. So far, the channel structure of chip-based SPE devices has been typically classified as open [28], packed [29–31], or monolithic [32–34]. Although both packed and monolithic SPE microchips have higher extraction capacities and provide a wider choice of stationary phases compared to open-channel microchips, the large increase in the hydrodynamic resistance (back pressure of >200 kPa) [35] generated by packing and monolithic materials in the channel can limit the operational flow rate during the SPE process. When downsizing the channel diameter to the micrometer scale, it is important to ensure that the resulting specific interfacial area is large and the molecular diffusion distance is short; this would in turn ensure that a fast phase transfer of analyte species and effective solid-phase [28] and liquid–liquid [36] extraction are achieved. Ramsey [28] and Kitamori [36] reported that although open-channel microchips have limited extraction capacity and contact time for aqueous solutions and organic solvents, they are still effective for extracting trace analytes from microsamples. So far, various microfluidic devices have been successfully developed for metal ion separation based on the classical liquid–liquid extraction theory. However, the open-channel SPE microfluidic devices require a proper inner wall modification for the extraction of metal ions. Although the fabrication of chip-based SPE systems has seen rapid progress, there have been relatively less advances in the use of on-chip SPE for separating trace elements [37].

In this study, we employed the polymeric material poly(methyl methacrylate) (PMMA) to fabricate an open-channel SPE microfluidic device because PMMA facilitates the easy functionalization of the channel surface and has a relatively low cost. The metal–polymer complexation process was optimized for the sorption preconcentration of trace metals in order to determine the selectivity of functionalized PMMA toward metal ions. To solve the "world-to-chip" interface problem [38,39], a valve manifold was designed as an interface that would combine microdialysis (MD) sampling, an SPE microchip, and ICP-MS for achieving a fully automated online chip-based PMMA SPE–ICP-MS system; this system could then be used to determine trace elements in microdialysate samples (~15 μL). The satisfactory analytical results indicated that the proposed SPE–ICP-MS hyphenated system is a promising platform for rapidly determining trace metal ions in microdialysate samples because of its efficient on-chip separation ability and extremely low blank values. Tests performed on this simple, low-contamination, highly sensitive online MD–chip-based SPE–ICP-MS hyphenated system in a simulated biological system confirmed that the proposed hyphenated system certainly facilitated the simultaneous determination of multiple trace metals. To the best of our knowledge, this is the first time that a process for coupling MD sampling to a chip-based SPE device using a valve manifold has been reported; the process is used to establish an automated online system for determining trace elements in volume-limited saline samples.

2. Experimental

2.1. Chemicals and vessels

All chemicals were used as purchased without further purification unless stated otherwise. High-purity water was purified through the Milli-Q apparatus (Millipore, Bedford, MA, USA). Sodium dodecyl sulfate (SDS) (reagent grade), sodium hydroxide (reagent grade), sodium chloride (ultrapure reagent grade), nitric acid (ultrapure reagent grade), and ammonium hydroxide (ultrapure reagent grade) were obtained from J.T. Baker (Phillipsburg, NJ, USA). Maleic acid disodium salt hydrate (reagent grade), and poly(vinyl chloride) (PVC) (reagent grade, MW: 80 000 Da) were purchased from Sigma–Aldrich (St. Louis, MO, USA). PMMA (reagent grade, MW: 35 000 Da) was purchased from Scientific Polymer Products (Ontario, NY, USA). Stock solutions (1000 mg L^{-1}) of analytes were purchased from Merck (Darmstadt, Germany). The perfusion solution (0.9%, w/v, NaCl) used for microdialysis was prepared by dissolving sodium chloride in high-purity water. Fresh working standards for calibration were prepared daily by stepwise dilution of stock solutions using high-purity water. All processes involving reagent preparation were carried out in a class 100 laminar flow hood. To eliminate possible contamination, maleate buffer and perfusion solutions were purified through a column we fabricated that was packed with PVC beads before each experiment. The tubes that were used to connect all of the pieces of the apparatus together were perfused with high-purity water until the contaminants were eliminated.

2.2. Fabrication of the chip-based SPE device

The network of the chip-based SPE device was patterned on PMMA sheets (Kun Quan Engineering Plastics Co. Ltd., Hsinchu, Taiwan) using a commercial CO_2 laser micromachining system (LES-10, Laser Life Co. Ltd., Taipei, Taiwan). The chip was designed by using basic geometric modeling software (AutoCAD, Autodesk Inc., Sausalito, CA). Fig. 1 shows a schematic illustration of layout of the chip-based SPE device (45 mm (L) × 22 mm (W) × 4 mm (H)), which consisted of two separated plates: the cover plate and the bottom plate. The cover plate had an outlet and three channels: sample channel, buffer channel, and eluent channel. Each channel had a corresponding port on the bottom plate, which also contained an extraction channel. The effective extraction channel, which was defined as the distance from the converged point of the flows of the sample and the buffer solution to the confluent outlet, was 2.5 cm. Two separated plates were used instead of one to avoid structure deformation caused by double punches. The channel features were inspected using a high-resolution optical microscope (FS-880ZU, Ching Hsing Computer-Tech Ltd., Taipei, Taiwan). The

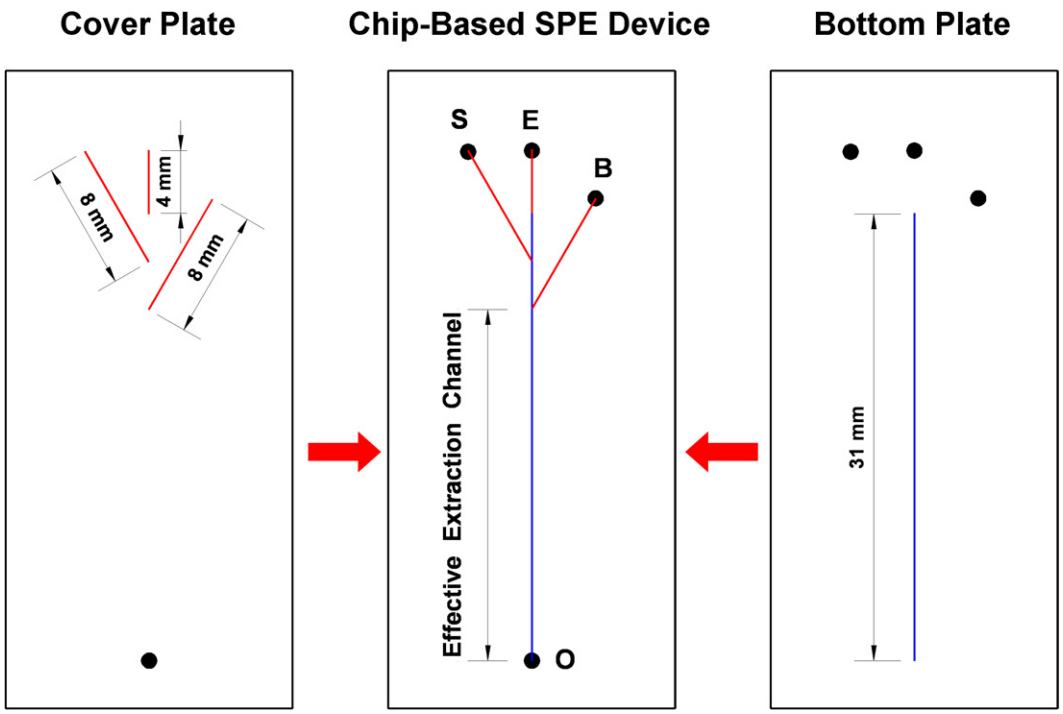

Fig. 1. Schematic illustration of layout of the chip-based SPE device: S, E, and B represent the introduction ports of sample, eluent, and buffer solutions, respectively; O represents the outlet.

dimensions of the effective extraction channel were 2.5 cm (L) × 422.2 μm (W) × 367.4 μm (D).

Prior to bonding, access holes were drilled in the PMMA substrates to create sample- and reagent-introduction ports and a confluent outlet. Both the cover plate and the bottom plate were subjected to a three-step cleaning process with the aid of ultrasonic agitation (0.1% SDS, D.I. water and D.I. water); each step for 10 min and then the plates were dried with nitrogen. The cleaned plates were aligned and thermally bonded under compression at 105 °C for 30 min. Finally, poly(aryletherketone) (PEEK) tubings (Upchurch Scientific, Oak Harbor, WA) were directly inserted into the inlet and outlet holes and secured using an adhesive (Letbond 4403 Instant Adhesive, Sunny Rise Co. Ltd., USA).

2.3. Channel activation of the chip-based SPE device

PMMA, which is mainly composed of esters, has no hydrophilic or active groups on its surface. Channel surfaces were hydrolyzed to carboxylic groups by flushing with saturated NaOH for 12 h, as described by Jeon et al. [40]. Thus, the activated PMMA channel surfaces were expected to exhibit sufficient carboxylation to enable their use as sorbents for metal ions at the appropriate pH.

2.4. Apparatus and instrumentation

A diagram of the chip-based SPE–ICP-MS hyphenated system is shown in Fig. 2. This system consisted of three main parts: the

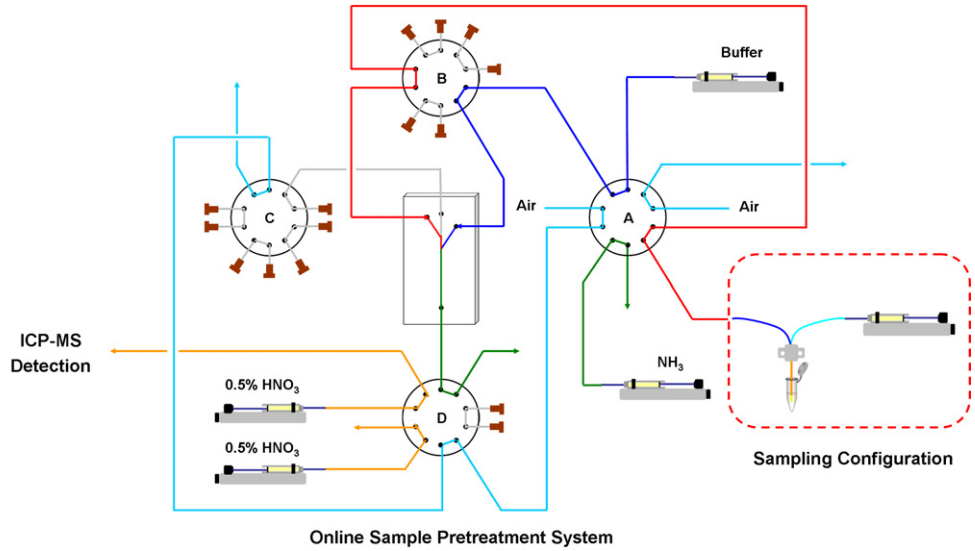

Fig. 2. Chip-based SPE–ICP-MS hyphenated system.

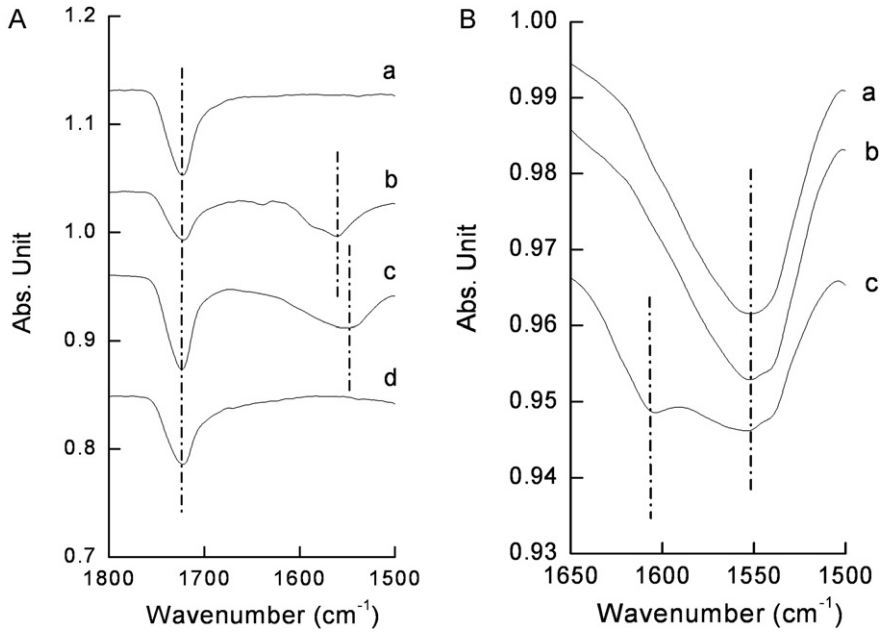

Fig. 3. (A) FTIR spectra of pure PMMA (a), functionalized PMMA (b), functionalized PMMA treated with Ni^{2+} ions (c), and functionalized PMMA treated with HNO_3 (d). (B) FTIR spectra of functionalized PMMA treated with 2500 (a), 5000 (b), and 10 000 (c) mg L^{-1} Ni^{2+} ions.

sampling configuration system, the online sample pretreatment system, and the ICP-MS detection system.

The sampling configuration system could be divided into two subsystems: (1) the direct syringe-injection system and (2) the MD sampling system. The MD sampling system consisted of a syringe infusion pump (KDS-100, Scientific Instrument Service, Ringoes, NJ, USA) and a 24-mm-long MD probe (CMA 20, CMA, Solna, Sweden); the probe had a 4-mm-long and 0.5-mm-diameter metal-free poly(arylethersulfone) (PAES) membrane that had a molecular weight cut-off (MWCO) of 20 kDa. A fluorinated ethylene polypropylene (FEP) tubing (internal volume of 1.2 μL per 100-mm length) purchased from CMA (Solna, Sweden) was used to connect the syringe infusion pump to the MD probe inlet. The outlet tubing of the MD probe was modified for attachment to the electric actuator valve *via* tubing sleeves (Upchurch Scientific, Oak Harbor, WA). In the direct-syringe injection mode, the MD probe was replaced with the syringe.

The online sample pretreatment system consisted of four 10-port electric actuator valves (C2H-2340E, Valco Instruments, Houston, TX, USA) and a laboratory-built chip. All of the valves were programmed and controlled by a personal computer *via* a serial valve interface (SIV-110 and I-22041, Valco Instruments, Houston, TX, USA). Polytetrafluoroethylene (PTFE) tubes (Alltech Associates Inc., Deerfield, IL, USA) were used as conduits to connect all the pieces of the system together. Table S1 gives the operational sequence of the online system. To retain the desired ions, a conditioning solution (0.2%, v/w, NH_3) was introduced to transform the carboxylic moieties on the channel wall of the chip to the NH^{4+} form. The collected samples and maleate buffer (40 mM $C_2H_2(COONa)_2$) were simultaneously delivered to the chip through the corresponding ports. After the sample-loading step, a 0.5% (v/v) HNO_3 solution was used to detach the adsorbed analyte in the microdevice and deliver it to the ICP-MS detection system.

An Agilent 7500a system (Agilent, CA) was used as the ICP-MS system. A Micromist nebulizer (AR35-1-EM04EX, Glass Expansion, Victoria, Australia) was fitted to a Scott-type quartz double-pass spray chamber. Because similar results were obtained when analyzing either the peak heights or the peak areas, this study quantified samples only in terms of their peak areas. Table S2 lists the instrument operating conditions selected for achieving optimal sensitivity and low background noise.

3. Results and discussion

3.1. Metal ion interaction with PMMA

The functionalized channel surface of the proposed PMMA chip-based open-channel SPE device was tested for its efficiency in separating trace metal ions from samples with high salt content. To investigate the retention behavior of the metal ions of interest toward the functionalized interior of the PMMA channel, PMMA powder was immersed in maleate buffer solutions with and without Ni^{2+} ions and then evaporated the solvent prior to Fourier transform infrared (FTIR) spectroscopic analysis (see Supplementary Data for PMMA characterization protocol). Because IR spectroscopy is sensitive to varying carboxylate moieties but not to metal-centered vibrational modes [41,42], metal–carboxylate interactions were elucidated by analyzing the vibrational spectra of the carboxylate moiety of PMMA after protonation, deprotonation, and metal complexation.

Fig. 3A shows the characteristic features of the IR spectra corresponding to native, deprotonated, Ni-complexed, and protonated functionalized PMMA. The spectrum for native PMMA showed no peaks that could be assigned to carboxylic carbonyls (C=O; 1500–1650 cm^{-1}), whereas the characteristic absorption peak corresponding to the PMMA carbonyl stretch was observed at 1722 cm^{-1}. With regard to functionalized PMMA, an absorption band associated with asymmetric ($\nu_{as}(COO^-)$) stretching vibrations of the derived carboxylate group (hereafter referred to as ν_{as}) appeared in the range of 1500–1610 cm^{-1}. As indicated in Fig. 3A, the ν_{as} band between 1500 and 1610 cm^{-1} shifted to a lower frequency only when Ni^{2+} ions were mixed with the functionalized PMMA suspension. These results suggested that the interaction between the functionalized PMMA and Ni^{2+} ions was very effective and that the polymer matrix coordinated to the Ni^{2+} ions *via* the carboxylate group of the polymer.

This hypothesis was further tested by examining the manner in which Ni^{2+} ion concentration affected the complexation

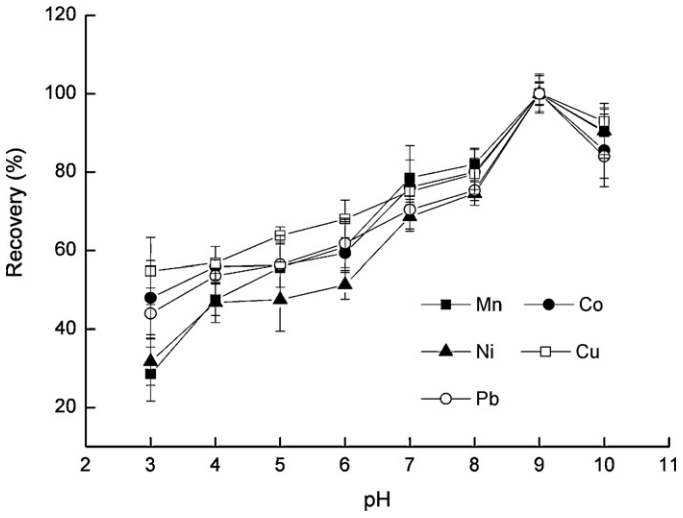

Fig. 4. Variation in the recovery of analytes with respect to pH.

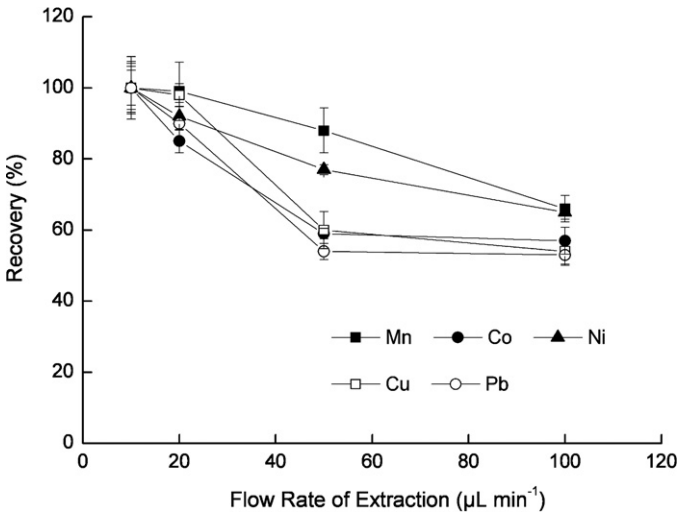

Fig. 5. Variation in the recovery of analytes with respect to extraction flow rate.

between the carboxylate group of the polymer and the cations. Based on the spectra shown in Fig. 3B, it was inferred that our results were consistent with those reported by Strathmann and Myneni [43]; the ν_{as} band weakened, broadened, and tended to split into two peaks upon increasing the concentration of Ni^{2+} ions. This result confirmed that the polymer matrix coordinated to the divalent cations *via* the carboxylate group of the functionalized polymer. The surface complexation between the carboxylate moieties in the interior of the PMMA channel and metal ions was proposed to be the main mechanism that facilitated the retention of transition metal ions when the pH of the sample solution was set to 9. Moreover, the inhibition of the ν_{as} band (Fig. 3A) also confirmed that elution with an acid can be used as a simple protocol for detaching the retained cations from the functionalized PMMA.

3.2. Optimization of the chip-based SPE device

To optimize the analytical performance of the functionalized PMMA chip as a sorbent medium for collecting analyte ions from saline solutions, we tested the effects of two main parameters on the extraction efficiency: the pH of the sample solution and the sample flow rate.

3.2.1. Effect of pH

Because the reaction between metal ions and the carboxylate group strongly depended on the pH of the sample solutions, the acidity of the sample solution was expected to be the main factor that determined the extraction efficiency of metal ions. To investigate the effect of pH on the adsorption of analyte ions in the interior of the PMMA channel, normal saline solutions containing analyte ions and buffered with maleate buffer were transported through the chip at an extraction flow rate of 20 μL min^{-1}. As shown in Fig. 4, the extraction efficiency of all of the trace elements increased with increasing the pH of the solution and reached the maximum value at pH 9. In the low pH region, the competition between hydronium and metal ions toward the carboxylated channel surface was expected to inhibit the extraction efficiency. When the pH of the sample solutions was as high as 10, the extraction efficiency of all of the tested elements decreased, probably because of the formation or precipitation of anionic hydroxyl complexes. To maximize the extraction efficiency, a pH of 9 was selected as the working pH for subsequent experiments because maximum signal intensity of analyte ions was observed at this pH level.

3.2.2. Effect of extraction flow rate

The flow rate in the extraction process was another important factor that not only affected the extraction efficiencies of the analyte ions but also controlled the analysis time. In this study, because both the sample and buffer solutions were directly loaded into the chip channel, the dynamic mixing and quantitative extraction of all the analyte ions were achieved simultaneously during the sample-loading step. Thus, the flow rate in the extraction process was defined as the total flow rate of the sample and buffer solutions. To ensure that the extraction flow rate was selected such that sufficient time was available for the mixing of the sample and buffer solutions and for the retention of the analyte ions on the channel surface, the effect of the extraction flow rate on the extraction efficiency was examined in the range of 10–100 μL min^{-1}. As shown in Fig. 5, the intensity of the obtained analyte signals decreased with an increase in the flow rate. The maximum adsorption of analyte ions occurred when the mixture of the sample and buffer solutions passed through the channel at a flow rate of 10 μL min^{-1}. Fig. 5 shows that the combined mixing and extraction in the chip channel reduced the residence time because of an increase in the flow rate, which apparently reduced the extraction efficiency. Even so, almost complete adsorption of all tested ions was accomplished instantly in comparison with conventional column methods [44,45] and the sample residence time was also significantly reduced from 120 to 15 s. These results also proved that the unique characteristics of the microspace in the extraction channel, such as the high surface-to-volume ratio and short molecular diffusion distance, certainly assisted the chip-based SPE device in carrying out rapid and effective extraction. To achieve maximum extraction efficiency and a short analysis time, an extraction flow rate of 20 μL min^{-1} was adopted for subsequent experiments.

3.3. Analytical characteristics

After optimizing the PMMA chip-based SPE procedure, the effect of Na^+ ions present in 0.9% (w/v) NaCl solution, which has the same salinity as microdialysate, on the adsorption of analytes toward the functionalized PMMA channel was investigated. The experimental results indicated that except for Mn^{2+} (57%), 80% or more analyte ions could be extracted from a saline solution in which the concentration of Na^+ ions was 90 000 times that of the analyte ions. Although the salt suppression effect could have been eliminated by reducing salinity, undiluted samples were transferred to the chip channel for extraction to avoid any possible contamination during the dilution procedure.

Table 1
Analytical characteristics of the online chip-based SPE–ICP-MS system.

Element	Linear range ($\mu g\,L^{-1}$)	R^2	MDL[a] ($ng\,L^{-1}$)	NIST 2670 (urine)		NIST 1643e (artificial saline water)	
				Certified value ($\mu g\,L^{-1}$)	Measured value ($\mu g\,L^{-1}$)	Certified value ($\mu g\,L^{-1}$)	Measured value ($\mu g\,L^{-1}$)
Mn	0.1–100	0.995	64.74	(330)[b]	319 ± 5	38.97 ± 0.45	42.00 ± 0.36
Co	0.1–100	0.999	23.64	–	–	27.06 ± 0.32	27.00 ± 0.10
Ni	0.1–100	0.997	76.91	(300)[b]	278 ± 12	62.41 ± 0.69	60.00 ± 2.32
Cu	0.1–100	0.996	51.34	370 ± 30	344 ± 10	22.76 ± 0.31	22.00 ± 0.57
Pb	0.1–100	0.998	5.86	109 ± 4	100 ± 1	19.63 ± 0.21	22.00 ± 0.17

[a] Sample volume = 15 μL.
[b] Reference value.

The loading capacity of the microdevice was evaluated under the optimized experimental conditions by passing 15-μL aqueous solutions containing varying concentrations of Mn^{2+} ions and then measuring the eluted fractions, which revealed a capacity of 6.17 $ng\,cm^{-2}$ (112.2 $pmol\,cm^{-2}$). In other words, the observed loading capacity was high enough to allow the chip-based SPE device, which consisted of a 2.5-cm extraction channel, to extract about 130 $\mu g\,L^{-1}$ of Mn^{2+} from 15 μL of saline samples. Analysis of the enhancement factors indicated that the ratio of the peak heights of the signals before preconcentration to that after preconcentration was ca. 5 when the injected sample volumes were only 15 μL. This indicated that the proposed system can simultaneously remove the salt matrix and enhance the analytical signal, even for very small sample volumes.

The utility of the online chip-based SPE–ICP-MS hyphenated system for determining trace metal ions was further evaluated in a test of its long-term stability by performing SPE for a continuous 8-h period. There was no significant difference in the recovery of the analyte ions, and the repeatability for all continuous 8-h measurements for all of the analyte ions was less than 9% CV, which suggest that this method is highly practical.

The linear dynamic ranges were determined by passing 15 μL of standard solutions containing 0.1–400 $\mu g\,L^{-1}$ of analyte ions through the chip-based SPE device under optimum conditions. The linearities of all analyte ions were satisfactory up to at least 100 $\mu g\,L^{-1}$ with correlation coefficients higher than 0.995, and the detection limits ranged from 5.86 to 76.91 $ng\,L^{-1}$. The detection limits were reached based on three times the standard deviation of the baseline noise ($n = 7$).

Generally, when chelating resin is used to separate metal ions from saline samples, washing and conditioning steps are essential for improving the extraction efficiency and for removing the residual matrix from the preconcentration column. However, according to Paulson [46] and Straßburg et al. [47], large quantities of insufficiently pure reagents can cause a high blank value or irreproducible results in assays utilizing chelating resin. Because only 34 μL of 0.2% NH_4OH and an air stream were used in the washing and conditioning steps, respectively, for the PMMA chip-based SPE procedure, the results indicated that correction for the elemental concentration in the resultant solution is unnecessary and that the detection limits achieved (Table 1) are comparable to those of the conditioning-free SPE technique that we proposed previously [18].

The accuracy of the present method was demonstrated by analyzing two saline reference materials: NIST 2670 (human urine) and NIST 1643e (artificial saline water). Although the microdevice had a sufficiently high loading capacity for directly extracting all analyte ions from the saline matrices, these two samples were determined with an appropriate dilution to draw the concentration of the target elements within the linear range. Table 1 shows that the analytical results were in reasonably good agreement with the certified values even in the presence of complicated matrices. These satisfactory analytical results indicated that the proposed method accurately determines the concentrations of trace metal ions of interest in microsamples containing complicated matrices.

3.4. In vitro response

Although the effectiveness of ICP-MS for trace analysis in chemically complex microsamples is well established, its detection capability is largely limited by instrument sensitivity, interference effects, and contamination. To overcome these limitations, online sample pretreatment systems developed in recent years have combined MD with ICP-MS for continuous and *in vivo* determination of trace metal ions in living animals [18,48,49]. Given the novel applications of the proposed method for analyzing volume-limited samples and the need to monitor the transfer kinetics of trace elements in living animals in the field, the proposed hyphenated system was combined with an MD probe to test its effectiveness for *in situ* and online monitoring of dynamic changes in trace metal ions concentrations found in microdialysates.

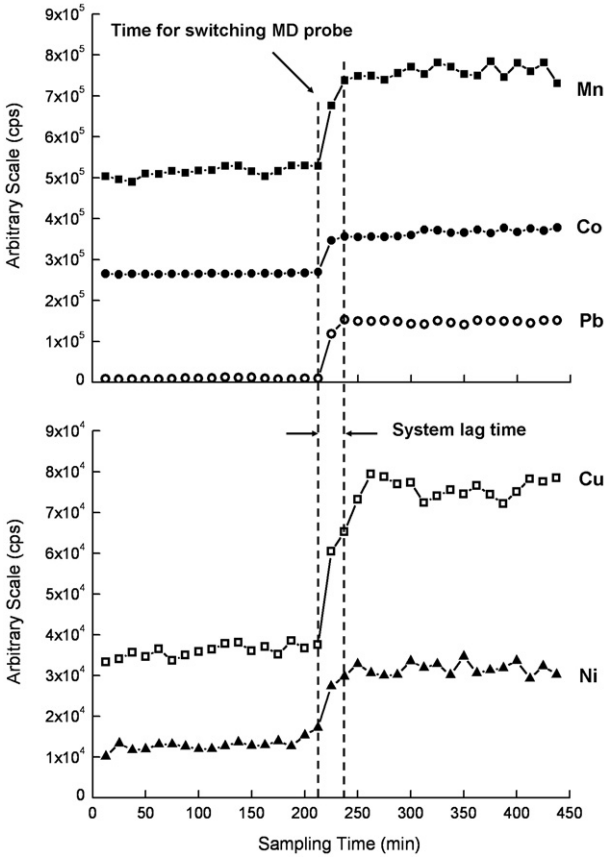

Fig. 6. Variations in analyte signals obtained by switching MD probe from 0.9% NaCl solutions to 0.9% NaCl solution containing 5 $\mu g\,L^{-1}$ of analytes. Each data point was obtained every 12.5 min. The left dash line represents the time ($t = 212.5$ min) at which the concentration of analyte ions was changed from 0 to 5 $\mu g\,L^{-1}$. The time interval (from $t = 212.5$ to 237.5 min) between the two dash lines which defined as system lag time for this experiment was 25 min.

Fig. 6 illustrates the variations in the analyte signals observed in the microdialysates collected from 0.9% (w/v) NaCl solutions with and without analyte ions additions. For the time that the analyte ions spent in the dialysates, with each data point obtained every 12.5 min, the signal profiles for all tested elements exhibited similar features. After continuous measurement for 212.5 min, the probe was switched between the vials containing different concentrations of analyte ions; changes in the signals appeared and reached a steady state 25 min later. The system lag time, defined as the duration needed for the signal intensity to reach a steady state after switching the probe, was 25 min. Because the corresponding volume associated with the length of the connection tubing was ca. 18 μL, transferring the analyte from the probe to the microdevice at a perfusion rate of 3.0 $\mu L\,min^{-1}$ required almost half the entire collection interval. Therefore, the lag time of the system was primarily attributed to the connection accessories. Moreover, to meet the sensitivity requirements of the ICP-MS instrument for identifying elements of interest present in the tested samples in concentrations lower than nanograms per milliliter, a temporal resolution of 12.5 min was required to collect sufficient microdialysate. However, the graph still clearly illustrates that the proposed online analytical system enables the characterization of the concentration–time curve with a temporal resolution of 12.5 min. In future experiments, improved sampling techniques with higher sampling efficiency and instruments with better sensitivity are expected to achieve the ultimate goal of near-real-time monitoring with excellent temporal resolution.

4. Conclusions

In this study, a method for the rapid fabrication of open-channel PMMA SPE chips was developed in order to reduce the cost (lower than 1 USD per device) and reduce the complexity of methods used for fabricating glass-based SPE chips; the method developed in this study involved simple CO_2 laser engraving and channel surface functionalization. Notably, PMMA was not only used for fabricating an on-chip SPE device, but also as the SPE adsorbent after surface functionalization. Because the proposed open-channel SPE chip had a higher surface-to-volume ratio and significantly lower diffusion distance and selective extraction capability in comparison with conventional SPE methods [44,45], almost complete adsorption of all tested ions was accomplished instantly and the sample residence time was also significantly reduced from 120 to 15 s. Regarding the aim of this study which was focused on analyzing volume-limited samples, the chip-based SPE method can minimize the contamination problems so that the procedural blank of metal ions of interest can be easily maintained at a very low level that is very critical to the success of trace metals analysis in tiny samples. The developed online chip-based SPE–ICP-MS hyphenated system had selective extraction capability and could handle small volumes of samples inside the channel; an MD probe could be coupled to the system for continuous monitoring of trace metal ions in small-volume microdialysate samples. Owing to the innate superiority of selective separation and the simplicity of this preconcentration procedure, the proposed chip-based SPE–ICP-MS system had several advantages, namely, high extraction efficiency, low reagent consumption, low blank value, short procedural time, and high cost-effectiveness. Analytical performance tests using simulated samples indicated that this strategy is an important advancement in the *in situ* detection of dynamic changes in trace metal ion concentrations in the extracellular fluid of targeted organs.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2011.02.037.

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