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A microscale solid-phase extraction poly(dimethylsiloxane) chip for enrichment and fluorescent detection of metal ions

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ABSTRACT

A rapid and simple enrichment system was developed on microfluidic chip which was integrated with on-line complexing and fluorescence detection. Microparticles of ion-exchange resin were trapped into the microchannel by a fabricated weir-structure in the end of the microchannel to construct a micro-solid-phase extraction (μ-SPE) device. Some commonly existing metal ions in environment were served as models to evaluate the performance of the proposed microdevice, in combination with on-line derivatization with 8-hydroxyquinolin-5-sulfonic acid (HQS) and fluorescence detection. The concentration and pH value of HQS solution were optimized for metal–HQS fluorescent derivatization. The parameters, which affected the efficiency of the developed method, including composition and concentration of eluent, pH value and the flow rate of HQS solution and elution, were also investigated. Under the optimal conditions, Ca^{2+}, Mg^{2+}, Zn^{2+} and Pb^{2+} were successfully determined by the μ-SPE device on-chip. The experimental enrichment factors for Ca^{2+}, Mg^{2+}, Zn^{2+} and Pb^{2+} were up to 520, 565, 578 and 487 folds, respectively.

1. Introduction

Microsize-based sample pretreatment techniques have been increasingly popular in chemical and biological assays owing to the compatible advantages of microfluidics integration and microscale assay with low sample/reagent consumption [1]. One of the potential applications is in the field of sample concentration. As is known to all, the exposing of metal ions in environment is greatly significant to human health and ecological system, thus has caught much attention since the past decades [2–4]. For these reasons, many studies have been devoted to the determination of metal ions, such as Ca^{2+}, Mg^{2+}, Zn^{2+} and Pb^{2+}. The common methods for metal ion assay mostly consist of photospectrometric techniques, inductively coupled plasma-mass spectrometry (ICP-MS), ion chromatography, capillary electrophoresis, and so on [5–7]. However, these approaches are often expensive instruments demanded or suffering from problems of complex matrix or trace amount of targets. Therefore, the preconcentration or separation procedure, such as solid phase extraction (SPE), ion exchange, cloud point extraction (CPE), solvent extraction, or co-precipitation, is greatly demanded prior to instrumental determination [8–10]. Besides, there are also some noticeable issues in these methods, such as requirement of large amounts of reagent and time consumption, as well as high cost for sample analysis. Therefore, it is very necessary to develop a simple, sensitive and rapid analytical method for sample pretreatment.

In recent years, micro-total analysis systems (μ-TAS) has attracted increasing interest in environmental analysis and biological researches because of small reagent consumption, low cost, high throughput, miniaturization and automation. The μ-TAS can also integrate several processes into a single microchip including sample injection, chemical reaction, separation and detection [11]. Thus, microfluidic device was recognized as a powerful platform for multistep analysis. For example, a multi-functional microdevice was developed to study cell metabolism that simultaneously integrated the drug injection, bioreactor, micro-SPE, and mass spectrum detector [1]. This micro-platform was also used to monitor the pharmacokinetics of tumor cells. To perform the environmental analysis on microfluidic chip, solid phase extraction (SPE) is of great importance for sample purification and enrichment. And miniaturized SPE system for sample preparation can cater to the μ-TAS, the most popular method is to integrate a μ-SPE system on the chip. Karwa et al. [12] immobilized nano and micro-silica or octadecylsilica into PDMS channel by using sol–gel chemistry to serve as μ-SPE device, and used for extraction/

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purification of DNA. Another strategy for building a SPE unit on PDMS chip was demonstrated by Hu and his co-worker [13], who used a two-level (deep/shallow) PDMS microchannel network to confine the fluorous reversed-phase silica beads within the chamber for a micro-SPE. Besides, monolithic columns were also developed for sample preparation; Yu et al. [14] constructed a monolithic column by using UV-initiated polymerization of porous polymer in a microchannel. Although some great improvements have been obtained in this field, there is still in a high demand in simplifying the process of packing a solid phase extraction microchannel and the preventing of the microparticle leakage.

Meanwhile, optical detection technique on-chip is much more popular because it can be performed with noncontact, easy construction and fast reading. The widely used methods are absorbance [15], fluorescence [16] and chemiluminescence [17]. While for the microscale analysis, highly sensitive fluorescence detection is the mostly useful and popular in microfluidic system. Consequently, the fluorescence method integrated with μ-SPE column will be an easy performance to validate the fabricated micro-system in the determination of metal ions with the advantages of simplicity and sensitivity.

Herein, we have successfully developed a microfluidic-based preconcentration channel integrated with on-line fluorescent derivatization technique and validated by metal ions (Ca^{2+}, Mg^{2+}, Zn^{2+} and Pb^{2+}) detection. In this work, micro-weirs were specially designed to trap solid phase carrier of ion-exchange resin microparticles in μ-SPE column for target enrichment. 8-Hydroxyquinolin-5-sulfonic acid (HQS) was used as fluorescent reagent for on-line metal ion derivatization. The detection was performed in situ with the noncontact fluorescence method by the invert fluorescence microscope, which was equipped with a Xe lamp and a charge-coupled device (CCD) detector. The developed microdevice could be recognized as a simple and rapid analytical platform, promising in microscale assay and instrumental measurement.

2. Experimental

2.1. Materials and reagents

Poly(dimethylsiloxane) (PDMS) prepolymer (Sylgard 184) was purchased from Dow Corning Co. (Midland, MI, USA). SU-8 negative photoresist was obtained from Microchem (Newton, MA). Trichloro-(1H, 1H, 2H, 2H-perfluorooctyl) silane was purchased from Sigma-Aldrich (Tianjing, China). The glass plates used to fabricate chips were purchased from Changsha Shaoguang Chrome Blank Co., Ltd. (Hunan, China). Silicon wafers were purchased from Xilika Co., Ltd. (Tianjin, China). The microparticles of ion exchange resin were obtained from the SPE cartridge of the bond elute plexa PRS (Varian, America). Metal salts of $CaCl_2$, $MgCl_2$, $ZnCl_2$, $Pb(NO_3)_2$, oxalate acid ($H_2C_2O_4 \cdot 2H_2O$) and ethanediamine (en) were purchased from Beijing Chemical Plant (Beijing, China). HQS was purchased from Alfa Aesar Co. Ltd. (Tianjing, China). All reagents were of analytical grade and used without further purification. Pure deionized water was used throughout the preparation of all aqueous solutions.

2.2. Instrumentation

A layer of SU-8 photoresist was spun on the silicon wafer via a spin coater (Karl Suss GmbH, Germany) and heated at 65 °C for 10 min. Exposure machine (J KG22A, Shanghai Optical Machinery Company, China) was used to transfer the pattern of the photomask to the silicon surface. The oxygen plasma device (PDC-32G, Ithaca, America) was used for surface treating when bonding PDMS replica with the glass plate. The micro-syringe pump (Harvard PHD2000, Holliston, MA) was used to deliver solutions into the chip. The fluorescence detection was performed on an inverted fluorescence microscope (Leica DMI4000 B, Germany), which was equipped with a Xe lamp light source and a CCD detector. The CCD images were recorded and analyzed using Image J software, then the data were imported into another data analyzer software of Origin 8.0 for further analysis.

2.3. Fabrication of the chip and the μ-SPE

The PDMS/glass microchip was fabricated as the reported method [18]. Briefly, pattern of the channels was designed with Adobe Illustrator CS4 software and printed out as a high resolution photomask. A totally cleaned silicon wafer was used as substrate and spin-coated with a layer of ca. 70 μm-thick photoresist (SU-8 2050), then exposed to the UV light to translate the pattern of the designed photomask to generate a mold. The patterned SU-8 structure of mold can be acquired with following sequences: UV–vis exposure (7 min), post exposure baking (65 °C, 6 min), development and cleaning, silanization (30 min) with trichloro-(1H, 1H, 2H, 2H-perfluorooctyl) silane. For the PDMS replica fabrication, PDMS prepolymer and curing agent were mixed with the mass ratio of 10:1 and poured on the SU-8 mold after being degassed (30 min) in vacuum, followed by thermocuring at 65 °C for 2 h. Finally, the PDMS replica was peeled off from the mold, trimmed and punched, then bonded with a cleaned glass slide after oxygen plasma treatment.

The microparticles of cation-exchange resin (~45 μm in diameter) modified with propylsulfonic group were used to pack into the μ-SPE microchannel. Firstly, the microparticles were suspended in deionized water, then the suspension (~0.5 mL) was delivered into the microchannel using 1.0 mL syringe by pump. Finally, deionized water was introduced at 10 μL min^{-1} by pump for compact conformation.

2.4. Procedure of the μ-SPE and analysis on chip

In our experiments, $CaCl_2$, $MgCl_2$, $ZnCl_2$, $Pb(NO_3)_2$ were used to prepare standard stock solutions, series of working solutions of Ca^{2+}, Mg^{2+}, Zn^{2+} and Pb^{2+} were prepared by diluting the store solutions (10 mmol L^{-1}) to the appropriate concentrations. 10 mmol L^{-1} HQS store solution was prepared in 5 mmol L^{-1} HCl–tris buffer solution, and the HQS aqueous solution was adjusted to pH 8.0 by using 1 mol L^{-1} NaOH and HCl solutions. The analysis procedures involved several steps including reagent injection, analyte enrichment and on-line derivatization, as well as fluorescence detecting on-chip. After the μ-SPE column was conditioned by CH_3OH/H_2O (5:95,v/v), 50 μL metal ion solution was injected into the μ-SPE microchannel at the flow rate of 3 μL min^{-1} by a micro-syringe pump. Then the oxalic acid/en eluent (each at 2.5 mmol L^{-1}, mixed with volume ratio of 3:1, pH 4.5, 0.5 mL) was used to elute the metal ions, and HQS chelate reagent was also injected into the other parallel channel for metal ions derivatization. At the same time, CCD was used to monitor the fluorescence intensity of the metal–HQS complexes on-chip.

3. Results and discussion

3.1. Setup and performance of the μ-SPE detection system

The chip was designed with two parallel introducing channels, one microchanel with the size of 24.5 mm × 400 μm (length × width) was used for μ-SPE column packing; the other with the size of 24.5 mm × 300 μm (length × width) was aimed to introduce HQS fluorescent complex reagent (Fig. 1a). A zigzag cross channel (3.0 mm in length and 400 μm in width) with V-cofferdam was

designed for the aim of efficiently mixing the metal ions with HQS reagent. The depth of the microchannel was about 70 μm. The designed detection window near the end of the outlet positioned the detection point for metal–HQS complexes, with the aim to avoid the effects resulted from mixing and reaction time. To fabricate the μ-SPE system, the cation-exchange resin microparticles (~45 μm in diameter) modified with propylsulfonic group were packed into the μ-SPE microchannel (Fig. 1b). The weirs with 30 μm gaps were fabricated at the end of the packing microchannel, contributed to trap the microparticles in the channel. The eluted cations were mixed and derivatized with HQS reagent in the zigzag cross channel, in which V-cofferdam weirs were fabricated (Fig. 1c). The weirs were designed to increase the vortex of the fluids and diffusion speed, thus enhancing the efficiency of mixing and complexing [19].

The metal ion enrichment and detection by the μ-SPE integrated chip were carried out by the constructed system. As shown in Fig. 2, a precise syringe pump was used to deliver reagents. For the μ-SPE investigation, 50 μL metal ion standard solution was injected into the μ-SPE microchannel at the flow rate of 3 μL min^{-1}. Followed by on-line elution by the oxalic acid/en eluent (each at 2.5 mmol L^{-1}, mixed with volume ratio of 3:1, pH 4.5) at the flow rate of 3 μL min^{-1}, the eluate was then mixed and reacted with HQS solution (2.0 mmol L^{-1}). Finally, the fluorescence signal of metal ion–HQS complexes was detected by CCD of the inverted fluorescence microscope.

3.2. Optimization of the fluorescence detection on-chip

To effectively validate the microdevice, reaction conditions were investigated. The fluorescence signal was obtained from the metal–HQS complexes, thus the mixing efficiency between analyte and complex reagent was very important and could influence the detection sensitivity. Some parameters, including

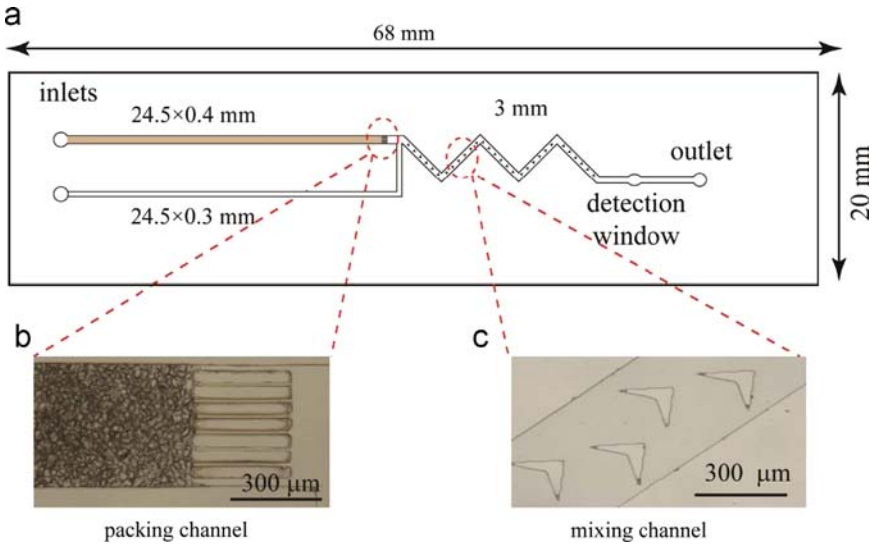

Fig. 1. (a) Schematic diagram of the designed chip combined with μ-SPE integration and fluorescence detection. (b) Microscope photograph of ion-exchange resins packed microchannel with the fabricated weir structure. (c) Microscope photograph of the V-cofferdams structure in the zigzag channel.

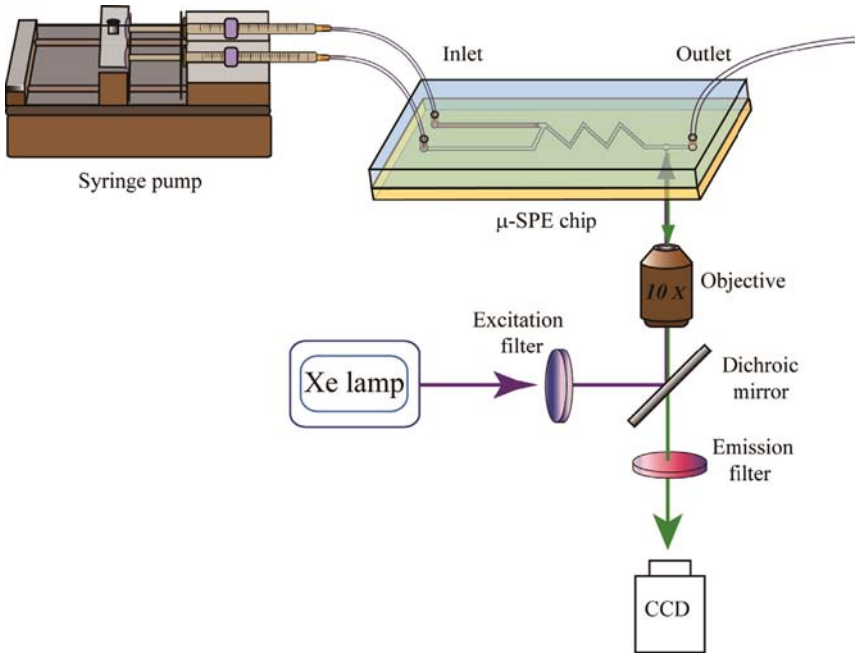

Fig. 2. Schematic illustration of the setup used in the work.

the composition and pH of the buffer, concentration of the complex reagent and the flow rate of solutions, were optimized. Meanwhile, high flow rate will lead to laminar flow and reduce the mixing efficiency, and thus decrease the detection sensitivity [20]. In this work, HQS was used as the fluorecence complex reagent for metal ions to realize fluorescence detection. The maximum wavelengths of excitation and emission were 372 nm and 502 nm, respectively. As HQS has the phenolic hydroxyl group and sulfonic group, its solubility and derivatization is related with solution pH. As shown in Fig. 3, pH value shows a significant effect on the fluorescence intensity of the metal complexes. The derivatization of Ca^{2+}, Mg^{2+} and Pb^{2+} has optimal fluorescence intensity at pH 8.0, and Zn^{2+} at pH 7.0. In the following work, pH 8.0 was chosen for the four metal ion detection. In addition, 5.0 mmol L^{-1} Tris–HCl buffer was used to prepare the HQS solution with the aim of decreasing pH effect [21]. At the same time, the concentration of HQS ranging from 1.0 to 5.0 mmol L^{-1} was also investigated. While the results showed that it was no significant effect on the metal–HQS fluorescence intensity. Thus, 2.0 mmol L^{-1} of HQS was used.

3.3. μ-SPE on-chip

In order to enrich the metal ions, cation-exchange resin microparticles were used to pack μ-SPE microchannel. The V-coff-erdam structured zigzag cross channel was designed to increase fluids diffusion and improve the fluorescent derivatization reaction. The operation included two steps. First, the μ-SPE microchannel was conditioned by using CH_3OH/H_2O (5:95, v/v) for 1.0 min at the flow rate of 3 μL min^{-1}. Then, the standard samples were loaded into microchannel, adsorbed by the μ-SPE column and then rinsed by H_2O, and finally eluted by the oxalic acid/en buffer. To investigate the efficiency of the fabricated μ-SPE system, 50 μL Zn^{2+} (1×10^{-6} mol L^{-1}) standard solution was loaded into the μ-SPE microchannel. Then, the oxalic acid/en buffer (~0.5 mL) was used to elute the metal complex. At the same time, HQS solution at the optimal conditions was introduced into another channel. The eluted metal ions were mixed and reacted with HQS in the zigzag cross channel. Finally, the derived metal–HQS complex was detected.

The experimental procedure was investigated to obtain the optimal conditions for elution and fluorescence detection. The eluent composition is important for exchanging ions from the packed microchannel. To improve the eluent efficiency, the mixture of weak metal complexing agents $H_2C_2O_4$ and en were used to prepare eluent. It is well known that the two reagents are always used in ion chromatography and liquid chromatography for metal ions separation [22,23] due to their chelation with metal ions. The concentrations of $H_2C_2O_4$ and en which affected the elution of metal ions were optimized as shown in Fig. 4a and b. By using the confirmed mixing volume ratio ($H_2C_2O_4$:en=3:1), different concentrations of $H_2C_2O_4$ solution at 1.0, 1.5, 2.0, 2.5 and 3.0 mmol L^{-1} were mixed with 2.0 mmol L^{-1} en, then different concentraions of en solution at 1.0, 1.5, 2.0, 2.5 and 3.0 mmol L^{-1} were mixed with 2.5 mmol L^{-1} $H_2C_2O_4$. It was found that the highest sensitivity was

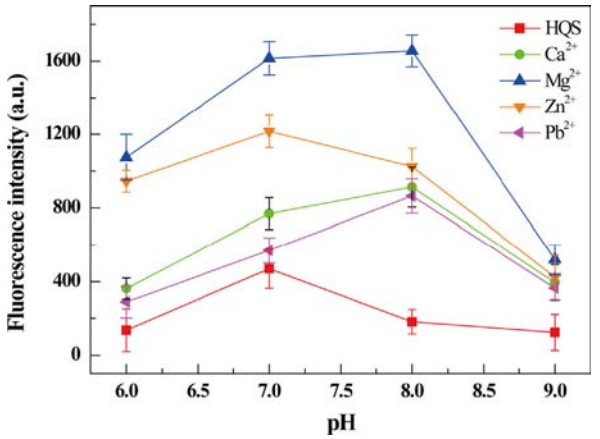

Fig. 3. pH dependence of HQS solution on fluorescence intensity. The detected concentrations of metal ions were 1×10^{-5} mol L^{-1} and HQS 2 mmol L^{-1}.

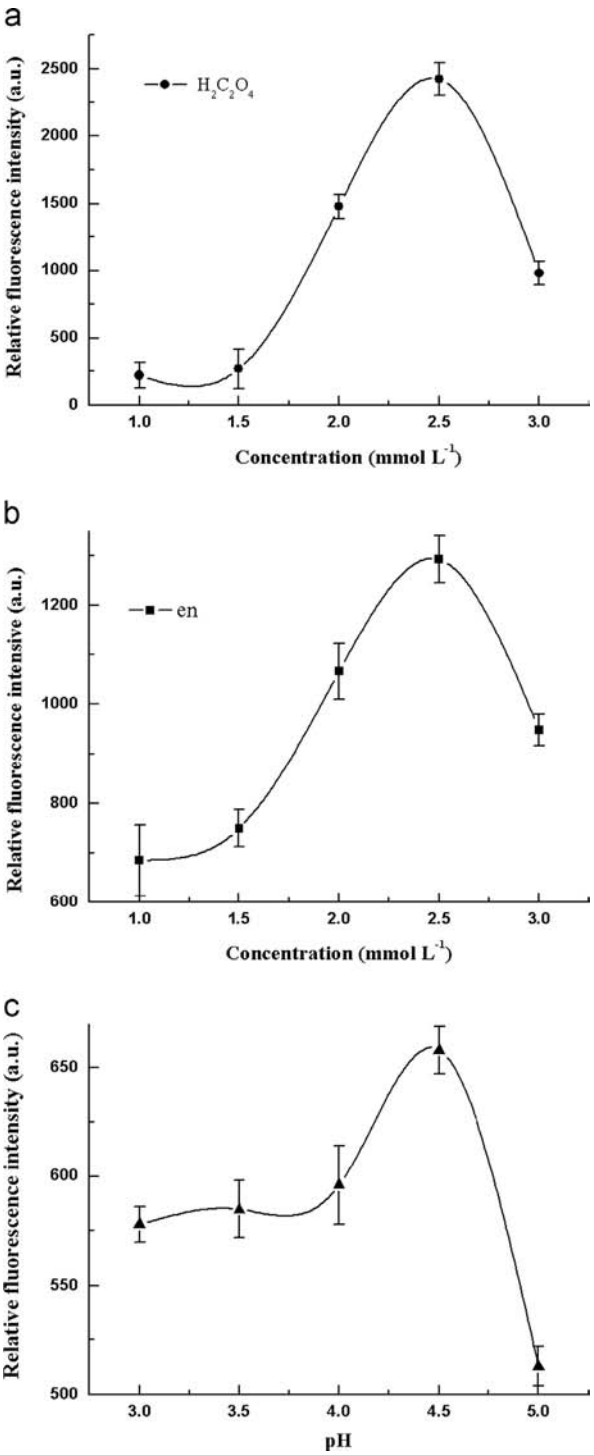

Fig. 4. Effects of the eluate condition on the efficiency of elution. (a) Effect the concentration of $H_2C_2O_4$ (1.0–3.0 mmol L^{-1}), (b) effects of en concentrations (1.0–3.0 mmol L^{-1}), and (c) effects of eluate pH. The other experimental parameters are as follows: $H_2C_2O_4$: en, pH 4.5; loaded volume of Zn^{2+} was (1×10^{-6} mol L^{-1}) 50 μL; HQS solution was 2 mmol L^{-1} (pH 8.0), flow rate was 3 μL min^{-1}.

Table 1
Reproducibility and enrichment efficiency for four kinds of metal ions by μ-SPE performed on-chip.

Metal ions	Ca^{2+}	Mg^{2+}	Zn^{2+}	Pb^{2+}
Enrichment factor	520	562	578	487
RSD (%) ($n=7$)	2.0	1.7	2.5	3.8

obtained for Zn^{2+} when 2.5 mmol L^{-1} $H_2C_2O_4$ was mixed with 2.5 mmol L^{-1} en at volume ratio of 3:1. The eluent pH value was another significant effect parameter on elution. Thus, the pH value of the $H_2C_2O_4$/en eluent was studied in the range of 3.0–5.0. According to the results shown in Fig. 4c, there was a significant influence on the elution efficiency with pH changing. The low elution efficiency happened at low pH, which was attributed to the competition exchange between the hydrogen ions and metal ions on the chelation sites. Thus, pH 4.5 was selected as the optimum for the following work. The effect in flow rate of eluent and HQS at the range of 1–10 μL min^{-1} was also investigated. Our results indicated that the flow rate had an obvious influence in the fluoresence signal. Lower flow rate was a benefit for the ion-exchange of the analytes from the resins, as well as the mixing and complexing with HQS solution, whereas prolonged the detection time. While the higher flow rate caused a decrease in the fluorescence response and made the packed channel damaged because of high pressure in it. Finally, 3 μL min^{-1} was used in the following work.

3.4. Enrichment efficiency and reproducibility

Under the optimized conditions, the microchip integrated with μ-SPE column was used to enrich and determine Ca^{2+}, Mg^{2+}, Zn^{2+} and Pb^{2+}. As shown in the Table 1, the enrichment factors (EF) for the four metal ions determined in this work were in the range of 487–578, which were calculated by the ratio of the relative fluorescence intensity with and without enrichment. The reproducibility and stability of the μ-SPE device were also examined. In the experiments, the extractions of Ca^{2+}, Mg^{2+}, Zn^{2+} and Pb^{2+} were carried out independently. The relative standard deviations (RSD) for seven measurements at 1.0 μmol L^{-1} with 50 μL loading volume were 2.0%, 1.7%, 2.5% and 3.8%. These results indicate that the integrated μ-SPE device can realize the online μ-SPE and sensitive fluorescence detection.

4. Conclusion

In conclusion, an integrated μ-SPE device was successfully developed for metal ion analysis, which combined with on-line μ-SPE and HQS fluorescence derivatization. Under the optimized conditions, the ion-exchange resins packed μ-SPE microchannel effectively enriched the metal ions of Ca^{2+}, Mg^{2+}, Zn^{2+} and Pb^{2+} by 520, 565, 578 and 487 folds, respectively. The sensitive fluorescence detection was realized by on-line derivatization of eluted metal ions in the V-cofferdam structured zigzag cross channel. The results demonstrated that the integrated microdevice offers a good alternative for rapid, low-cost and sensitive analysis of trace metal ions in aqueous samples. The developed microdevice is expected for different analyte preconcentration by using different solid phase microparticles packing, as well as applied to the sample pretreatment methods in instrumental analysis.

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