



Short communication

# Nonaqueous based microchip separation of toxic metal ions using 2-(5-bromo-2-pyridylazo)-5-(*N*-propyl-*N*-sulfopropylamino)phenol

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## Abstract

The colorimetric metal chelating agent, 2-(5-bromo-2-pyridylazo)-5-(*N*-propyl-*N*-sulfopropylamino)phenol (5-Br-PAPS), was demonstrated on a capillary electrophoresis microchip in the separation and detection of six metal ions of environmental concern, Cd<sup>2+</sup>, Pb<sup>2+</sup>, Cu<sup>2+</sup>, Co<sup>2+</sup>, Ni<sup>2+</sup>, and Hg<sup>2+</sup>. The inclusion of methanol in the buffer was found to improve both the separation efficiency and sensitivity, in addition to making the technique directly amenable to the application of solid-phase extraction. The combination of metal chelation with solid-phase extraction on a C<sub>18</sub> silica gel microcolumn gave several hundred fold improvements in detection limits for the CE microchip measurements of toxic metal ions in water and extracted from a solid Plexiglas surface.

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## 1. Introduction

Due to the deleterious effect heavy metal and transition metal cations have on the environment, the development of new technologies for their real-time characterization and monitoring is becoming a topic of growing interest to the US Environmental Protection Agency (EPA) and to those institutions, e.g. private industry and government, falling under EPA's mandate. The US Department of Energy (DOE), for example, currently has the enormous task of deactivating and decommissioning toxic metal contami-

nated equipment and structures that remain from the United States' involvement in nuclear weapons development over the last 50 years [1]. In a separate issue, the US Navy is currently investigating the development of an environmentally sound ship, for example, which would be capable of remediating hazardous waste materials originating from normal ship operation [2]. Both agencies are in need of fieldable sensors capable of rapidly detecting trace metal cations in waste water, e.g. bilge water, or contaminating building material or laboratory infrastructure, e.g. steel, concrete, Plexiglas, glass.

Recently, miniaturized high-performance analytical systems, so-called laboratory-on-a-chip or capillary electrophoresis microchip systems, have attracted significant interest, finding applications in

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environmental sensing that include the detection of organic peroxides [3], organic acids [4], dissolved organic carbon [5], chlorophenols [6], explosives [7,8], acetylcholinesterase inhibitors [9], alkylmethylphosphonic acids [10], inorganic anions [11,12], and metals [13–18]. These miniaturized devices provide the potential for greatly enhancing the speed of an analytical separation, while dramatically reducing the system size or weight, and the consumption of samples and reagents. All of these features are highly desirable for the development of truly portable devices capable of sensitively and rapidly monitoring hazardous waste metal cations in the field.

Several groups have previously reported on CE-based microchip systems for the separation and detection of metal cations [13–18]. By applying colorimetric metal complexation dyes bearing significantly red-shifted absorbance wavelengths ( $>500$  nm), it becomes possible to incorporate compact and inexpensive light sources, such as light emitting diodes (LEDs) and laser diodes, and detectors, such as photodiodes, into the sensor design. This approach has been successfully applied to the CE microchip for the direct detection of  $\mu\text{g/l}$  levels of uranium and lanthanide metal ions using Arsenazo III [6,7], and low mg/l levels of various transition and heavy metal ions using 4-(2-pyridylazo)resorcinol (PAR) [8]. While PAR was very effective at rapidly separating seven transition metal ions in under a minute, several toxic metal ions were conspicuously missing from this separation, e.g. mercury and lead, due to their inadequate colorimetric complexation by PAR.

In an effort to expand the number of metal ions monitored simultaneously on the microchip, the metal chelating agent, 2-(5-bromo-2-pyridylazo)-5-(*N*-propyl-*N*-sulfopropylamino)phenol (5-Br-PAPS), was examined for its suitability on the microchip platform. Recently, Motomizu et al. reported the simultaneous analysis of various transition and heavy metal ions by conventional CE with 5-Br-PAPS, obtaining detection limits in the  $\mu\text{g/l}$  regime [19]. The initial goal of our research was to demonstrate the applicability of 5-Br-PAPS to the microchip for the separation and determination of a metal cation mixture containing  $\text{Cd}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Ni}^{2+}$ , and  $\text{Hg}^{2+}$ . The inclusion of the nonaqueous solvent, methanol, in the buffer was found to improve both

the separation efficiency and sensitivity, in addition to making the technique directly amenable to the application of solid-phase extraction (SPE). By combining metal chelation with SPE on a  $\text{C}_{18}$  silica gel microcolumn, it is possible to gain several hundred fold improvements in detection limits for the CE microchip measurement following water and solid surface (Plexiglas) sampling.

## 2. Experimental

### 2.1. Reagents

The metal cation chelating reagent, 5-Br-PAPS, was obtained from Dojindo Labs. (Kumamoto, Japan). High-purity deionized water ( $18 \text{ M}\Omega \text{ cm}$ ) was obtained by passing distilled water through a Milli-Q Plus water purification system (Millipore). The metal ion stock solutions ( $1000 \text{ mg/l}$ ) were purchased from Sigma as atomic absorption standard solutions in 5%  $\text{HNO}_3$ .

### 2.2. Microchip instrumentation

The borofloat glass microchips utilized in this study have been described previously, prepared by Micalyne, to contain  $100 \mu\text{m}$  deep microchannels for improved absorbance detection [8]. Deeper microchannels can be tolerated due to the lower conductivity of the nonaqueous buffer systems utilized. Before measurement, the glass microchips were flushed with deionized water,  $1 \text{ M}$   $\text{HNO}_3$ , water,  $0.1 \text{ M}$   $\text{NaOH}$ , water, and running buffer solution. The high-voltage switching apparatus consisted of two  $8 \text{ kV}$  high-voltage power supplies (Bertan High Voltage) and an array of high-voltage reed relays (Crydom), the operation and configuration of which have been detailed elsewhere [8]. Colorimetric detection of the 5-Br-PAPS metal chelates was accomplished using an instrumental setup very similar to one described previously, with the exception that a green LED light source ( $570 \text{ nm}$ , Purdy Electronics) was utilized for all absorbance measurements [8].

### 2.3. Microcolumn preparation

The C<sub>18</sub> silica microcolumn was prepared using TFE PTFE tubing (1.5 mm I.D.×3.0 mm O.D., Supelco), packed with 15 mg of silica gel C<sub>18</sub> (particle size 50–150 μm, pore size 125 Å, Waters). Upchurch SF polyether ether ketone (PEEK) ferrules (pore size 2 μm) were fixed on both ends of the microcolumn.

### 2.4. Solid-phase extraction

Using a flow-rate of 3 ml/min, an 80 ml solution of 5-Br-PAPS ( $3.7 \cdot 10^{-6}$  M) containing Cd<sup>2+</sup> (25 μg/l), Pb<sup>2+</sup> (10 μg/l), Co<sup>2+</sup> (1.0 μg/l), and Ni<sup>2+</sup> (1.0 μg/l) was passed through a C<sub>18</sub> silica microcolumn that had previously been washed and activated sequentially with 0.1 M HCl (20 ml), water (50 ml), and methanol (50 ml). A nitrogen tank coupled to a Supelco capillary rinsing apparatus was used to force the solution through the column. Following this SPE step, 20 ml of water were sent down the microcolumn to remove any precipitated salts. For the elution step, pure methanol was pumped through the column at a flow-rate of 25 μl/min. A dark blue solution (25 to 50 μl) was collected and diluted with an equal volume of pH 4.9 acetate buffer (8 mM) to give the sample solutions analyzed on the microchip.

### 2.5. Plexiglas surface preparation and sampling

A mixture of metal nitrate salts in methanol (600 μl) was spiked onto a 100 cm<sup>2</sup> surface of Plexiglas, providing a metal cation contaminated surface with 18, 4.2, and 6.0 ng/cm<sup>2</sup> of Pb<sup>2+</sup>, Co<sup>2+</sup>, and Ni<sup>2+</sup>, respectively. Following complete evaporation of the methanol solution, the contaminated surface was immersed in 15 ml of 0.1 M HNO<sub>3</sub> solution. The washing process was performed twice and all collected solutions were combined. After adjusting the pH to ~7.0 with 1 M NaOH, 19 mg sodium acetate trihydrate was added and the pH adjusted to 4.9 with acetic acid. The metal chelates were formed by adding 0.1 ml of the 5-Br-PAPS stock solution ( $1.3 \cdot 10^{-3}$  M, pH 4.9 acetate buffer). The resulting sample

solution was applied to the SPE microcolumn, and the eluent analyzed on the CE microchip.

## 3. Results and discussion

### 3.1. Effect of methanol content on microchip separation

The composition of the electrophoretic running buffer has a profound effect upon the microchip separation of the metal chelates mixture. Because methanol was an essential component to ensure a high recovery efficiency for the SPE of 5-Br-PAPS metal chelates, the effect of varying percentages of methanol in a sodium acetate (pH 4.9) buffer on the microchip separation of six transition and heavy metal contaminants, Cd<sup>2+</sup>, Pb<sup>2+</sup>, Cu<sup>2+</sup>, Co<sup>2+</sup>, Ni<sup>2+</sup>, and Hg<sup>2+</sup>, was examined (Fig. 1). The sodium acetate buffer system was selected as a suitable starting point based upon the work of Motomizu et al. [9]. Fig. 1a depicts the separation electroferogram attained in purely aqueous, 4 mM sodium

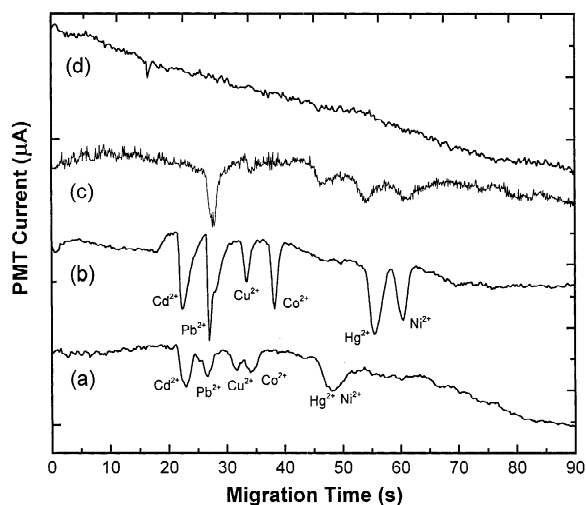


Fig. 1. Effect of methanol percentage on the CE microchip separation of a mixture of six different metal cations, Cd<sup>2+</sup> (10.2 mg/l), Pb<sup>2+</sup> (10.0 mg/l), Cu<sup>2+</sup> (0.5 mg/l), Co<sup>2+</sup> (0.5 mg/l), Hg<sup>2+</sup> (11.6 mg/l), and Ni<sup>2+</sup> (1.0 mg/l), with 5-Br-PAPS ( $3.0 \cdot 10^{-4}$  M): (a) sodium acetate (4 mM, pH 4.90); (b) 50% methanol in sodium acetate (8 mM, pH 4.90); (c) 75% methanol in sodium acetate (8 mM, pH 4.90); (d) 100% methanol with LiClO<sub>4</sub> (5 mM) as supporting electrolyte.

acetate at pH 4.9. In this case, the six 5-Br-PAPS metal chelates were unsuccessfully separated under this field strength (980 V/cm) and separation length (8 cm) due to general peak overlapping and band broadening. The effect of the applied field strength on the separation efficiency and microchip performance was examined in tandem. As expected, increasing the separation field strength from 470 to 980 V/cm linearly decreases the migration times of these metal chelates. However, the high conductivity of this aqueous phase buffer system in the 100  $\mu\text{m}$  deep  $\times$  200  $\mu\text{m}$  wide microchannels being investigated here, caused the microchannel current to exceed 60  $\mu\text{A}$  at the higher field strengths. As a result of the microchip's inability to effectively dissipate this Joule heating, the resolution was insufficient at all field strengths measured to give a complete separation of these six metal ions.

Peak shapes and resolution improved significantly when the methanol percentage in the separation buffer was increased to 50%, as shown in Fig. 1b. In this case, all six different metal chelates were well separated in approximately 1 min. Note that methanol has a lower conductivity than water. The addition of methanol lowers the microchannel separation current to less than 30  $\mu\text{A}$  at an applied field strength of 980 V/cm, giving improvements in the resolution and sensitivity when compared to the purely aqueous buffer system. The theoretical detection limits can be calculated, based on a signal-to-noise ratio of 3:1, to be 1.88, 1.33, 0.17, 0.10, 2.3, and 0.27 mg/l for  $\text{Cd}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Hg}^{2+}$ , and  $\text{Ni}^{2+}$ , respectively. Comparing the migration times from Fig. 1b with those obtained by Motomizu et al. on a commercial CE instrument, the CE microchip enables the detection of individual metal cations six times faster, e.g.  $\text{Cd}^{2+}$  is detected after only 20 s in comparison with the retention time of 2.1 min obtained by Motomizu et al.

As the percentage of methanol is increased to 75% (Fig. 1c) and 100% (Fig. 1d), the separation efficiencies and sensitivities are impaired dramatically. Under these conditions, the measured microchannel currents became very unstable and difficulties were encountered in manipulating the transport of 5-Br-PAPS and its metal ion chelates from one reservoir to another. The addition of different concentrations of  $\text{LiClO}_4$  or  $\text{Et}_4\text{NClO}_4$  as supporting electrolyte in

methanol also proved to be insufficient in stabilizing the microchannel current and enabling control of the reagent transport. High percentages of methanol in the electrophoretic buffer were deemed unsuitable for the 5-Br-PAPS metal chelate separation on a microchip, and, therefore, all subsequent optimization steps were performed in 50% methanol content.

### 3.2. Solid-phase extraction

In the absence of SPE, the CE microchip gives detection limits for metal ions such as  $\text{Cu}^{2+}$  and  $\text{Co}^{2+}$  as low as 165 and 103  $\mu\text{g/l}$ , while heavy metal ions such as  $\text{Cd}^{2+}$  and  $\text{Pb}^{2+}$  are in the low mg/l range. The EPA's National Primary Drinking Water Regulations for these metal ions fall into the 2 to 50  $\mu\text{g/l}$  range, therefore highlighting the importance of preconcentration for this particular approach. Akatsuka et al. have shown that  $\text{C}_{18}$  silica gel can be effectively used as a SPE material for 5-Br-PAPS metal chelates [20]. With a five-fold molar excess of 5-Br-PAPS to the metals, they reported adsorption efficiencies for most metal ions ( $\text{Cu}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Cd}^{2+}$ ) contained within a seawater matrix to be greater than 95%; the recovery efficiencies were in the range of 75 to 95%. For our purposes, a micro-SPE column was packed with 15 mg  $\text{C}_{18}$  silica gel and applied to the SPE of 5-Br-PAPS metal chelates. The adsorption and elution processes were monitored by UV-Vis measurements. The adsorption efficiency was determined to be more than 98% for an 80 ml solution containing  $\text{Cd}^{2+}$  (25  $\mu\text{g/l}$ ),  $\text{Pb}^{2+}$  (10  $\mu\text{g/l}$ ),  $\text{Co}^{2+}$  (1.0  $\mu\text{g/l}$ ) and  $\text{Ni}^{2+}$  (1.0  $\mu\text{g/l}$ ) and 5-Br-PAPS ( $3.7 \cdot 10^{-6}$  M) in 4 mM acetate buffer at pH 4.9. The adsorbed 5-Br-PAPS metal chelates were subsequently eluted in methanol. Elution curves obtained using UV-Vis measurements demonstrated that over 95% of the metal chelates are successfully eluted in the first 50  $\mu\text{l}$  of collected methanol. By miniaturizing the SPE microcolumn, the volume required for attaining maximal recovery efficiency was lowered from approximately 800 to 50  $\mu\text{l}$ , a volume which is more appropriate to the dimensions of the CE microchip.

The preconcentrated metal chelates in methanol solution were subsequently mixed with an equal volume of 8 mM acetate buffer at pH 4.9 and

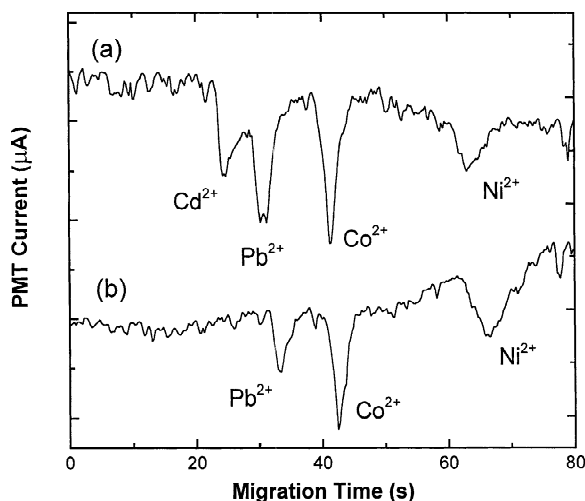


Fig. 2. CE microchip separation of a metal cation mixture on a microchip (a) after SPE of 80 ml of dilute metal chelates solution containing  $\text{Cd}^{2+}$  (25  $\mu\text{g}/\text{l}$ ),  $\text{Pb}^{2+}$  (10  $\mu\text{g}/\text{l}$ ),  $\text{Co}^{2+}$  (1  $\mu\text{g}/\text{l}$ ), and  $\text{Ni}^{2+}$  (1  $\mu\text{g}/\text{l}$ ) at pH 4.9 in 4 mM sodium acetate buffer, and (b) after extraction from the surface of a Plexiglas substrate contaminated with 18, 4.2, and 6  $\text{ng}/\text{cm}^2$  of  $\text{Pb}^{2+}$ ,  $\text{Co}^{2+}$ , and  $\text{Ni}^{2+}$ , respectively.

introduced into the microchip sample reservoir. Fig. 2a depicts the electropherogram obtained for a sample mixture containing four different metal cations. The SPE pre-step results in a loss of resolution from the electropherogram shown in Fig. 1b, due to the increased ionic strength resulting from the 5-Br-PAPS reagent and metal chelates concentrated elution from the microcolumn. To maintain complete resolution in the electropherogram, two metal ions were purposely omitted from the test sample ( $\text{Cu}^{2+}$  and  $\text{Hg}^{2+}$ ). The calculated detection limits based upon a signal-to-noise ratio of 3:1 for  $\text{Cd}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Co}^{2+}$ , and  $\text{Ni}^{2+}$  are 6.0, 1.8, 0.15, and 0.48  $\mu\text{g}/\text{l}$ , respectively. The extent of preconcentration by SPE is quite significant. An improvement of 311-, 730-, 677-, and 556-fold of the previously measured detection limit was achieved for  $\text{Cd}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Co}^{2+}$ , and  $\text{Ni}^{2+}$ , respectively.

### 3.3. Surface sampling and detection

In order to evaluate the feasibility of applying this

SPE to solid surface sampling and ultimately the CE microchip, a Plexiglas surface was chosen as a best case scenario for assessing the details of this extraction. A sample mixture of  $\text{Pb}^{2+}$ ,  $\text{Co}^{2+}$ , and  $\text{Ni}^{2+}$  in methanol solution was spiked and allowed to dry on a 100  $\text{cm}^2$  surface of Plexiglas, resulting in a metal cation contaminated surface containing 18.0, 4.2, and 6.0  $\text{ng}/\text{cm}^2$  for  $\text{Pb}^{2+}$ ,  $\text{Co}^{2+}$ , and  $\text{Ni}^{2+}$ , respectively (the three remaining metal ions were omitted to ensure complete resolution). The metal contaminants were carefully washed off the surface and collected in 30 ml of 0.1 M  $\text{HNO}_3$ . After adjusting the pH to 4.9 with NaOH and sodium acetate, the chelating reagent, 5-Br-PAPS, was added and the solution was passed through the SPE microcolumn. The sample eluent was mixed with aqueous buffer, applied to the microchip, and separated on the microchip to give three well-defined, baseline-resolved peaks, shown in Fig. 2b. The favorable signal-to-noise characteristics of these real-sample data indicate low detection limits of 11, 1.3, and 3.4  $\text{ng}/\text{cm}^2$  based on  $S/N = 3$  for  $\text{Pb}^{2+}$ ,  $\text{Co}^{2+}$ , and  $\text{Ni}^{2+}$ , respectively. The precision and reproducibility were examined from a series of three repetitive analyses of sample mixtures exposed to a Plexiglas surface, yielding relative standard deviations of 5.6, 4.2, and 4.7% for  $\text{Pb}^{2+}$ ,  $\text{Co}^{2+}$ , and  $\text{Ni}^{2+}$ , respectively.

## 4. Conclusions

We have demonstrated the coupling of surface sampling and SPE to CE microchip metal ion analysis. The presence of a nonaqueous solvent in the buffer, a factor imperative to the success of SPE, is not detrimental to the microchip separation, but, in fact, improves the resolution and sensitivity of the 5-Br-PAPS separation. The instrumental simplicity and inexpensive nature of the optical absorbance detection system can be adequately utilized, providing that an SPE sampling protocol is used in conjunction with the microchip separation platform. The CE microchip provides a simple and reliable means for quick separation and determination of metal cation mixtures containing various toxic heavy metal and transition metal ions of environmental concern.

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