Determination of Heavy Metal Ions by Capillary Electrophoresis with Contactless Conductivity Detection after Field-amplified Sample Injection

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A method has been developed for determining of heavy metal ions by field-amplified sample injection capillary electrophoresis with contactless conductivity detection. The effects of the 2-*N*-morpholinoethanesulfonic acid/histidine (MES/His) concentration in the sample matrix, the injection time and organic additives on the enrichment factor were studied. The results showed that MES/His with a low concentration in the sample matrix, an increase of the injection time and the addition of acetonitrile improved the enrichment factor. Four heavy metal ions (Zn^{2+} , Co^{2+} , Cu^{2+} and Ni^{2+}) were dissolved in deionized water, separated in a 10 mM MES/His running buffer at pH 4.9 and detected by contactless conductivity detection. The detection sensitivity was enhanced by about three orders of magnitude with respect to the non-stacking injection mode. The limits of detection were in the range from 5 nM (Zn^{2+}) to 30 nM (Cu^{2+}). The method has been used to determine heavy metal ions in tap water.

(Received February 10, 2005; Accepted March 8, 2005)

Introduction

Capillary electrophoresis (CE) for the analysis of ionic compounds is even-more concerned due to advantages of high resolution, rapid speed and small sample consumption,¹⁻³ where conductivity detection is a very good selection method for moderate sensitivity and easy selection of a background electrolyte compared to the indirect UV-absorption detection usually used. Contact conductivity detection in CE has the disadvantages of contamination of the detection electrodes when samples containing complicated matrixes are analyzed, and interference of the separation high voltage with the detection signal, since the electrodes are in direct physical contact with electrolyte solutions. Capacitively coupled contactless conductivity detection (C_4D)^{4,5} used a very simple detection cell and overcame the disadvantages mentioned above. Hauser⁶ demonstrated a high-voltage C_4D , which achieved limits of detection (LODs) on the order of 50 nM for alkali, alkaline earth metal and ammonium ions, and 1–5 μM for heavy metal ions (Zn^{2+} , Co^{2+} , Cu^{2+} and Ni) by indirect detection.

The analysis of trace heavy metal ions has been gaining much attention in environmental samples. Although spectral analysis, such as atomic absorption spectrometry (AAS),⁷⁻⁹ inductively coupled plasma-atomic emission spectrometry (ICP-AES)¹⁰⁻¹³ and inductively coupled plasma mass spectrometry (ICP-MS),^{14,15} are standard methods for analysis of metal elements, CE as a good alternative is becoming even-more recognized. In order to obtain efficient detection sensitivity, many preconcentration techniques are usually adapted in trace analysis. Burgi and Chien^{16,17} demonstrated the field-amplified

sample injection (FASI) technique for on-line preconcentration of analytes in CE. In FASI, samples are prepared in a low-conductivity buffer and injected electrokinetically into a capillary containing a high-conductivity running buffer. Because of the low conductivity of the sample, a high electric-field strength is established at the capillary inlet. A large amount of sample ions migrate into the capillary and stack at the boundary between the sample zone and the running buffer. An enhancement of detection sensitivity about several hundreds or even thousands times is generally achieved by FASI,¹⁸⁻²¹ which provides a convenient on-line preconcentration method for the analysis of trace or ultra-trace analytes in environmental samples. Cheng²² reported a method of on-line chemiluminescence detection with field-amplified injection in CE, where the sensitivity was improved by approximately six orders of magnitude, and sub-fM level cobalt detection was achieved. Isoo²³ achieved an LOD of 3.8 ppt for Co^{2+} with UV-detection by sweeping in conjunction with FASI, where a 140000-fold improvement for Co^{2+} ions was realized. In this paper, we describe the analysis of heavy metal ions by using FASI-CE with C_4D detection. The detection sensitivity is enhanced about three orders of magnitude with respect to the non-stacking injection mode and LODs of nM are achieved.

Experimental

Instrumentation

A homemade CE system was used for all experiments. It consisted of two high-voltage power supply (0–20 kV and 0–30 kV, Dongwen, China) units, one (0–30 kV) for CE separation while the other for injection, and a fused-silica capillary (65 $\times$ 50 μm i.d. $\times$ 375 μm o.d., L_{detector} = 51 cm, Yongnian, China). A new capillary was treated by rinsing with

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Fig. 1 Effect of the concentration of MES/His in sample solutions on the limit of detection of Zn^{2+} (nM). Separation electrolyte, 10 mM MES/His at pH 4.9 adjusted by 5% acetic acid; separation voltage, 20 kV; sample injection, 5 kV for 30 s; C₄D, 200 kHz, 220 V_{p-p}.

1 M hydrochloric acid and 1 M sodium hydroxide solutions for 3 min in turn, followed by deionized water for 5 min, and finally by running buffer for 3 min. The capillary was rinsed with the buffer for 1 min before each injection. Kefen chromstation (Scien-Tech Instrument Inc., Dalian, China) was used for data acquisition.

The electrodes of C₄D were two identical stainless-steel tubes (400 μm i.d. and 700 μm o.d.) 5 mm in length, placed 1 mm apart from each other. A thin-grounded copper plane with a 0.4 mm diameter central hole, through which the separation capillary was passed, was placed between the electrodes to minimize the coupling capacitance through the air between the electrodes. The electronic circuitry of the C₄D was analogous to that described in Ref. 5 except that a high-voltage excitation source was used, which comprised a home-made transformer and a EE1641B1 function generator (Xinlian Electronic Corp., Nanjing, China).

Reagents and method

All reagents used were of analytical grade, and high-purified water from a Milli Q (Millipore, Bedford, MA, USA) was used throughout. Stock solutions (20 mM) of heavy metal ions were prepared from their nitrate (Ni^{2+} , Co^{2+}), sulfate (Cu^{2+}) and acetate (Zn^{2+}) salts. The sample solutions were prepared fresh before use by dilution of stock solutions with the water. 2-*N*-morpholinoethanesulfonic acid (MES) (Biomo, USA) and histidine (His) (Shanghai Chemical Reagent Corp., China) were purchased and used. Fresh electrolyte solutions were prepared daily by mixing 0.1 M MES and His stock solution and degassed in an ultrasonic bath and subsequently filtered with 0.45 μm syringe filters before use; 5% acetic acid was used to adjust the pH of the electrolyte. To avoid contamination of ions from the glass vessel, plastic vials were used for filling the sample solutions. The vials were washed with hydrochloric acid, followed by high-purified water before use. The sample injection time was 5–60 s at 5 kV. The separation voltage of CE was kept at 20 kV throughout the study. All experiments were carried out at $25 \pm 1^\circ\text{C}$.

Results and Discussion

Effect of the concentration of MES/His in the sample solution

The stacking efficiency in FASI can be described by the following equation:²¹

Fig. 2 Electropherogram of 20 nM Zn^{2+} . The conditions are the same as in Fig. 1.

$$E_s = \frac{E_1 \mu_1}{E_2 \mu_2} \quad (1)$$

where E_s is the stacking efficiency, E_1 and μ_1 are the electric field strength and the effective electrophoretic mobility of the analytical ion in the sample zone; E_2 and μ_2 are the corresponding parameters in the buffer zone. According to Eq. (1), the larger in the difference of the electric field strength between the sample and the buffer zone, the larger in the stacking efficiency, which indicates that a sample solution with low conductivity should be prepared for high stacking efficiency and detection sensitivity. In this study, heavy-metal ions were prepared in a buffer containing 0–10 mM MES/His electrolyte to demonstrate the effect of the conductivity of the sample buffer on the stacking efficiency. Figure 1 shows that the effect of the MES/His concentration on LOD of Zn^{2+} , is similar to that in Ref. 22. It can be seen that the LOD in pure water and a buffer of low concentration is lower than that in a high electrolyte concentration, and that the LOD in pure water is three orders of magnitude (1.3×10^3) lower than that in 10 mM MES/His buffer. Figure 2 is electropherogram of 20 nM Zn^{2+} prepared in water. It can be seen that the peak of the Zn^{2+} is comparatively sharp, which indicates that the analyte can be stacked in a narrow zone. The calculated LOD of Zn^{2+} is 5 nM, based on a signal-to-noise ratio of 3. Similar results were also obtained for Co^{2+} , Cu^{2+} and Ni^{2+} . The enhancement of detection sensitivity was over 10^3 for Co^{2+} and Ni^{2+} , and 800 for Cu^{2+} .

Effect of the injection time

The influence of the injection time on the enrichment factor (detection sensitivity) is shown in Fig. 3. As expected, the peak height is proportional to the injection time in the range from 5 to 60 s, and the separation efficiency (height plate) keeps almost constant below 50 s. A further increase in the injection time causes peak broadening, and thus reduces the separation efficiency. Hence, subsequent studies are carried out below an injection time of 50 s.

Effect of the concentration of the running buffer

According to the basic theory of FASI, an increase in the conductivity (concentration) of the running buffer results in a larger conductivity difference between the buffer and the sample zone, and a higher stacking efficiency can be achieved, and thus higher sensitivity; however, a higher running buffer concentration reduces, the conductivity difference between the Zn^{2+} and Co^{2+} ions and the buffer, and thus lower the peaks.

Fig. 3 Effect of the injection time on the peak height and the plate height of Zn^{2+} . Sample concentration, 100 nM Zn^{2+} ; injection time, from 5 s to 60 s. All other conditions are the same as in Fig. 1.

The two effects on the detection sensitivity conflict. In our experiments, we found that the peak heights of Zn^{2+} and Co^{2+} were reduced when the concentration of the running buffer was increased from 5 mM to 15 mM, which showed that the effect of the conductivity on the sensitivity was dominant. Because the Ni^{2+} and Cu^{2+} ions produce negative response peaks, which indicates that the two effects are accordant, we found an increase in the peaks when the concentration of the buffer was increased. It is a compromise to select a 10 mM MES/His running buffer for the simultaneous analysis of the four heavy metal ions.

Effect of the addition of acetonitrile

The addition of an organic solvent into the sample matrix can decrease the conductivity and viscosity of the sample matrix, and thus improve the stacking efficiency due to an increase of the electric field strength and the electrophoretic motilities of analytes in the sample zone. By adding 15% acetonitrile into the sample matrix (water), a 1.5-fold sensitivity enhancement was achieved. A higher concentration of acetonitrile was not used due to an increase of the migration time of the ions.

Peaks of light ions

There are always several large peaks before the peak of Zn^{2+} in Fig. 2, which are response peaks of K^+ , Na^+ , Ca^{2+} and Mg^{2+} from the used water. The C_4D combined with FASI can achieve the LODs of nM or sub-nM to these light ions, which are lower than the background concentration of them in the used purified water. It is possible for the C_4D with FASI to be used to analyze trace or ultra-trace alkali and alkaline metal ions in special samples if highly purified background electrolyte is used.

Simultaneous analysis of four heavy metal ions

Figure 4 is an electropherogram of four metal ions (Zn^{2+} , Co^{2+} , Cu^{2+} and Ni^{2+}) that were prepared in purified water and separated in CE by FASI in 10 mM MES/His running buffer at pH 4.9. The four ions were completely separated in less than 7 min. Figure 4 shows that the Zn^{2+} and Co^{2+} produced a positive response, but the Cu^{2+} and Ni^{2+} yielded negative peaks, which indicates that the latter have a smaller conductivity than the buffer. It is obvious that the peak of Ni^{2+} is much wider than those of the other ions; this phenomenon can also be seen in Ref. 6 (in Fig. 4). We can therefore draw that the widening of the Ni^{2+} peak is not attributed to the FASI process, but to a stronger interaction of Ni^{2+} with hydroxyls on the inner wall of the separation capillary.

Fig. 4 Electropherogram of four heavy metal ions. Conditions: 500 nM metal ions, except for Zn^{2+} (100 nM); electrolyte, 10 mM MES/His–15% acetonitrile at pH 4.9. All other conditions are the same as in Fig. 1.

Fig. 5 Electropherogram of metal ions in tap water upon being diluted 10-fold. The conditions are the same as in Fig. 4.

Analytical characteristics

Under the optimized conditions, replicate analyses of the sample ions were carried out. The LODs and relative standard deviations (RSDs) of the migration time (t_s), peaks height (h) and peaks area (S) are given in Table 1. The method shows a good linearity relationship between the peak area and the sample concentration, as can be seen from Table 1.

Analysis of real sample by the presented method

The developed method was applied to the determination of trace metal ions in a tap water sample. Figure 5 is the electropherogram of diluted 10-fold tap water by FASI. The calibration results showed that the water sample contained Zn^{2+} (7.7 μM); no Co^{2+} , Cu^{2+} or Ni^{2+} was found. The high peak before Zn^{2+} covered the peaks of Na^+ , Ca^{2+} , Mg^{2+} .

Conclusions

This study demonstrates that combining C_4D with FASI greatly improves the detection sensitivity (over 1000-fold) and without any loss of the separation efficiency (within an injection time of 50 s). The stacking efficiency in FASI strongly depends on the concentration of MES/His in the sample matrix. The increase in the injection time and the addition of acetonitrile can improve the detection sensitivity. The separation of Zn^{2+} , Co^{2+} , Cu^{2+} and Ni^{2+} was completed in 10 mM MES/His running buffer at pH 4.9 and detected by C_4D ; LODs at the nM level were achieved. The method has been used for the analysis of heavy metal ions in tap water.

Table 1 Limits of detection (LODs) and linearity range of sample ions, and relative standard deviations (RSDs, concentration: 500 nM except for 100 nM Zn²⁺) of the migration time (t_s), peak height (h) and peak area (S) ($n = 6$)

Sample	RSD, %			LOD/nM	Linearity range/nM	Correlation coefficient
	t_s/min	h/mV	S			
Zn ²⁺	0.80 (4.50)	2.88 (7.13)	2.14	5	5 – 1000	0.999
Co ²⁺	0.91 (4.72)	3.30 (7.29)	2.08	10	10 – 1000	0.998
Cu ²⁺	1.37 (6.44)	3.16 (3.63)	1.38	30	30 – 1000	0.998
Ni ²⁺	1.46 (6.68)	4.13 (4.52)	5.58	15	15 – 1000	0.997

Acknowledgements

This work was supported by the National Natural Science Foundation of China (Contract No. 29925514 and No. 20227501), and Youth Science Foundation of Dalian Institute of Chemical Physics (Contract No. DICP S200304).

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