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Fluorescence

DETECTION OF COPPER ION WITH LASER-INDUCED FLUORESCENCE IN A CAPILLARY ELECTROPHORESIS MICROCHIP

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A capillary electrophoresis microchip coupled with a confocal laser-induced fluorescence (LIF) detector was successfully constructed for the analysis of trace amounts of heavy metals in environmental sources. A new fluorescence dye, RBPhOH, synthesized from rhodamine B, was utilized in a glass microchip to selectively determine copper with high sensitivity. A series of factors including running buffer concentration, detection voltage, and sample loading time were optimized for maximum LIF detector response and, hence, method sensitivity.

Keywords: Capillary electrophoresis microchip; Copper ion; Laser-induced fluorescence

INTRODUCTION

Micrototal analysis systems (μTAS), known as lab-on-a-chip, are analytical tools which could be tracked back to the development of silicon chip based gas chromatographs and ink-jet printers in the late 1970s (Kuswandi, Huakens, and Verboom 2007). The μTAS dramatically increases sample analysis efficiency by integrating sample treatment, chemical reaction, component separation, and detection into a single microchip, which could be made as small as a credit card or even as a stamp. This high integration makes μTAS portable, requiring minimal reagent quantities and highly efficient, rendering μTAS "a technology for this century" (Daw and Finkelstein 2006). For decades, μTAS had been widely used in biomedical and pharmaceutical analysis (Koutny et al. 1996; Ludwig, Kohler, and Belder 2003; Cho et al. 2004), clinical diagnostics (Tian et al. 2000; Zhou et al. 2004; Werling et al.

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2008), environmental monitoring (Chena, Lin, and Wang 2006), and forensic investigations (Wallenborg and Bailey 2000; Wang et al. 2002; Karlinsey and Landers 2006). Capillary electrophoresis microchips, the first generation of μTAS, are well developed analytical devices, and they have incurred more and more attention because of their high efficiency and high sensitivity in analyzing a wide range of samples including heavy metals.

Heavy metals are vital components in ecological systems. They could be essential for organisms but toxic when accumulated in vivo to a certain concentration. Food and environment contaminated by heavy metals have arisen more and more concerns due to their long biological half-lives and difficulty to degrade, resulting in inacceptable accumulation in human bodies and causing serious health hazards as diseases, chronic toxicity and liver damage.

Atomic absorption spectroscopy (AAS) is the most popular analytical technique in the field of heavy metal analysis (Cabon 2002; Soriano, Netto, and Cassella 2007). However, it has not the miniaturization advantage of μTAS. The application of microchips to analyze metal ions was limited the lack of sensitive detection methods. Currently, electrochemical detectors have been employed in microchip systems to detect metals including K^+, Na^+ (Pumera et al. 2002; Lichtenberg, de Rooij, and Verpoorte 2002; Qu et al. 2006; Y. Lee et al. 2006), Li^+ (Qu et al. 2006; C. Y. Lee et al. 2006), Mg^{2+}, and Ca^{2+} (C. Y. Lee et al. 2006). Although research on detecting heavy metals using microchips has been reported, the poor consistence of the ion signal sequence (C. Y. Lee et al. 2006) and low sensitivity (Tanyanyiwa and Hauser 2002; Chailapakul et al. 2008; Li et al. 2007) make efficient and sensitive metal analysis. For example, the detection limit of using this method to analyze Cu^{2+} was 7.4 μM (Li et al. 2007). Thus, searching for more sensitive methods to analyze Cu^{2+} is of great importance.

Compared with electrochemical detectors, optical detectors are more sensitive and reproducible. Different optical methods were also employed in microchips to detect metal ions, including absorbance (Malcik et al. 2005; Du et al. 2005), reflectance (Caglar et al. 2006), chemiluminescence (Liu et al. 2003), photodiode array detection (Collins and Lu 2001), and colorimetric methods (Deng and Collins 2003). Currently, the most sensitive optical detection method is based on Laser-induced Fluorescence (LIF) (Xu, Li, and Weber 2007). The optical arrangement of detection devices plays an important role for sensitivity and detection limit (Xu et al. 2007; Fu et al. 2006). Of the two major arrangements, confocal and non-confocal, the confocal LIF system is more sensitive and has lower LOD in separation and detection and has been employed to analyze biomolecules (Sandlin et al. 2005). To the authors' best knowledge, up to date, no studies on detecting metals in microchips with LIF have been reported.

Rhodamine is a xanthene-related compound. It has been extensively used as fluorescence dye in biological analysis (X. F. Yang, Guo, and Zhao 2002; X. F. Yang, Guo, and Li 2003; Kenmoku et al. 2007) because of its spectroscopic properties including a large molar extinction coefficient, a high fluorescence quantum yield, and longer absorption and emission wavelengths (500–600 nm) (Kenmoku et al. 2007; D. Y. Wu et al. 2007; Zheng et al. 2006). Using rhodamine derivatives to detect heavy metals such as Fe^{3+} (Xiang and Tong 2006; M. Zhang et al. 2007; Bae and Tae 2007), Cu^{2+} (Dujols, Ford, and Czarnik 1997; Xiang et al. 2006; Mei et al. 2007;

X. Z. Zhang, Shiraishi, and Hirai 2007; M. H. Lee et al. 2008; Xiang et al. 2008), Hg^{2+} (Y. K. Yang, Yook, and Tae 2005; Ko et al. 2006; Chen et al. 2007; J. S. Wu et al. 2007; M. H. Lee et al. 2007; H. Yang et al. 2007), Pb^{2+} (Kwon et al. 2005), and Cr(VI) (Xiang et al. 2007) has also been reported.

The RBPhOH synthesized from rhodamine B by Xiang et al. (2006) could form 1:1 stable complex with Cu^{2+} rapidly, so it was used to analyze the Cu^{2+} by fluorescence. In this report, a microchip system coupled with an LIF detector was constructed and used to analyze Cu^{2+}. Due to the use of the molecular fluorescence, RBPhOH, Cu^{2+} could be detected with high efficiency as well as high sensitivity and selectivity and could be used for analyzing Cu^{2+} from a variety of samples including wastewater, sewage and seawater.

EXPERIMENTAL

Reagents and Chemicals

The RBPhOH ($C_{35}N_4O_3H_{36}$, 560 g mol^{-1}) was provided by Professor Aijun Tong of Tsinghua University. Copper(II) nitrate, ferrous sulfate, ferric nitrate, chromic(III) sulfate, cadmium chloride, lead nitrate, mercuric chloride, and acetonitrile were purchased from Beijing Chemical Reagents Company. All chemicals were analytical grade and used without any further purification. The running buffer solution for detection was prepared with Tris, pH 6.0. High-purity deionized water (18.2 MΩ) was obtained by passing distilled water through a Milli-Q Plus water purification system. Metal stock solutions (Cu^{2+}, Fe^{2+}, Fe^{3+}, Cr^{3+}, Cd^{2+}, Pb^{2+}, and Hg^{2+}) were prepared by dissolving the corresponding salts in the run buffer. Aqueous solutions containing metal ions were mixed with acetonitrile solutions of RBPhOH and diluted with running solution to prepare sample solutions containing 10% acetonitrile. Then the complex was injected into sample cell. All solutions for electrophoresis experiments were filtered through a 0.2 μm membrane filter before use.

Apparatus

A schematic diagram of the confocal LIF detection system is shown in Fig. 1.

A 532-nm semiconductor laser (output power 50 mW, Beijing Lambdapro Technologies Ltd., Beijing, China) was used as light source. The excitation laser power was adjusted by an attenuation filter to prevent overly saturated photon emission from fluorophores. The laser beam passed through a 532-nm band-pass filter (Shenyang HB Optical Technology Co., China), reflected by a mirror and focused by a 40 × microscope objective lenses (0.65 NA, 2.95 mm working distance, Chongqing MIC Optical & Electrical Instrument Co., Chongqing, China). Alignment of the μTAS position was achieved by an X-Y-Z translation stage (Beijing Optical Instrument Factory, Beijing, China). The excited fluorescence emissions from the sample were collected and focused by the same objective lenses, then successively transmitted through a 560-nm dichroic mirror (Beijing Film Machinery Research Institute, Beijing, China), a spatial filter (dia. = 500 μm) and three pieces of 560-nm long-pass filters (Beijing Film Machinery Research Institute, Beijing, China), into a photomultiplier tube (PMT-CR114, Hamamatsu, Japan), which transferred the

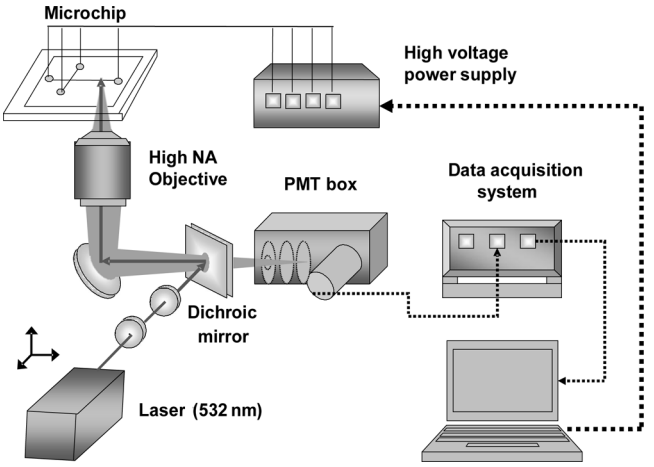

Figure 1. The structure of confocal LIF detection system for μTAS.

fluorescence signal into an electric signal. The electric signal obtained from the PMT was collected by a HP 34970A data acquisition unit (Agilent Technologies, Palo Alto, CA). Data process was performed in HP BenchLink DataLogger. A high voltage power supply with an adjustable voltage range from 0 to +5000 V (The College of Chemistry, Chemical Engineering & Material Science, Shandong Normal University, China) was used for sample injection and Capillary Electrophoresis (CE) separation.

Methods

A cross-pattern glass microchip (60 mm long × 20 mm wide × 2.2 mm deep) was purchased from Dalian Institute of Chemical Physics, CAS. The chip had a 45 mm long separation channel (from the cross to the buffer waste reservoir) and 10 mm long injection channel (between the sample and buffer reservoir). All channels were etched to a depth of 20 μm and a width at the top of the channel of 60 μm. The glass chip was placed on a laboratory-built plastic holder for fixing the chip. Platinum wire electrodes were inserted into the reservoirs. Before measurement, the glass microchip was flushed with 0.1 M NaOH, deionized water, and running buffer solution sequentially for 10 min each.

RESULTS AND DISCUSSION

The Optimize of Conditions

The RBPhOH was dissolved in acetonitrile forming a colorless solution, which turned into claret upon the addition of Cu^{2+} aqueous solution, even with Cu^{2+} concentration as low as 1×10^{-6} mol L^{-1}. An experiment was then set up to analyze Cu^{2+} with a confocal detection system in a glass microchip. Because the RBPhOH powder was difficult to dissolves in water but dissolved easily in acetonitrile, a glass

microchip was used to protect the microchannels from erosion by acetonitrile. If the concentration of RBPhOH is lower, the concentration range would be short. The RBPhOH itself shows weak fluorescence at relatively high concentrations. As a result, the concentration of RBPhOH was fixed about 3×10^{-5} mol L^{-1}. The results suggested that no fluorescence was detected from the sample solution containing 3×10^{-5} mol L^{-1} RBPhOH only, and a strong fluorescent signal was detected with the addition of Cu^{2+}. This result confirmed that Cu^{2+} could be detected by a LIF detector at the presence of RBPhOH in a glass microchip. The fluorescence intensity slightly decreased when pH changed from 5.0 to 8.0 by using Fluorescence Spectrophotometer (VARIAN, CARY Eclipse, U.S), thus, pH 6.0 was employed.

The running buffer (tris buffer) concentration was optimized next. The result revealed that the buffer concentration had obvious effects on signal intensity. Increasing buffer concentration caused electrostacking, which suppressed peak dispersion and formed relatively higher concentration of the sample plug to give an intensive signal (Deng and Collins 2003). However, a further increase of the running buffer concentration also increased ionic strength, which, in turn, increased the current and heat in the microchannel, resulting in sample dispersion. The results suggested that the signal intensity also increased when running buffer concentration was increased from 10 to 20 mM. After that, increased running buffer concentration resulted in decreased peak height. As a result, 20 mM Tris was optimal for Cu^{2+} detection.

The sample loading time affected the amount of sample in the cross-channel. A short injection resulted in a smaller sample conveyed into the cross-channel and lower signal intensity. A prolonged sample loading time caused sample spreading in the channel, which broadened the signal peak. A 40 s sample loading time was optimal for obtaining maximum peak intensity.

The influence of detection voltage was also studied. Higher voltages increased the electric field and decreased sample migration time. However, excessive high voltage caused high currents and overheating, which increased sample dispersion and decreased the fluorescence signal. In addition, air bubbles were always produced at higher voltages, which affected signal detection as well. The detection voltage for Cu^{2+} was then fixed at 900 V for maximum peak intensity.

Linear Range and Detection Limit

Under optimized conditions as described, a series of solutions of Cu^{2+} with a concentration range from 6.03×10^{-7} mol L^{-1} to 1.56×10^{-5} mol L^{-1} were detected at the presence of RBPhOH by determining the peak height. The correlation coefficient of calibration curve was 0.99969. The detection limit for Cu^{2+} was found to be 1.34×10^{-7} mol L^{-1} ($S/N = 3$) and the relative standard deviation of the migration time was 1.53%.

Interference Experiment

To investigate the application of selective and sensitive detection of Cu^{2+} at the presence of RBPhOH in glass microchips, a mixture solution of 5×10^{-6} mol L^{-1} Cu^{2+}, Fe^{2+}, Fe^{3+}, Cr^{3+}, Cd^{2+}, Pb^{2+}, and Hg^{2+} was prepared. Under optimized conditions, only the Cu^{2+} peak was detected by the LIF system, and the peak height

was found to be in accordance with the calibration curve, suggesting no disturbance by other heavy metal ions (data not shown here). The fact that the present microchip system selectively detected Cu^{2+} at the presence of a series of heavy metal ions suggests that Cu^{2+} and RBPhOH forms a more stable complex with a much higher association constant (Ka), compared with the other ions, which resulted in high fluorescent intensity. In conclusion, this method is very selective and sensitive for detecting Cu^{2+} with RBPhOH in microchips with an LIF detection system.

Separation and Detection of Hg^{2+} and Cu^{2+}

Interestingly, when RBPhOH was added into the running buffer as a component of the running buffer, two fluorescence signals were detected but the noise became bigger. Further experiments confirmed that the two peaks are Cu and Hg, suggesting that Hg^{2+} and Cu^{2+} could be separated and detected simultaneously when RBPhOH was added into the running buffer.

As shown in Fig. 2, Hg^{2+} and Cu^{2+} were successively separated and detected by RBPhOH in a microchip coupled with a LIF detector. The concentration of Cu^{2+}, calculated from the calibration curve, was 4.9×10^{-6} mol L^{-1}. The RSD of the peak height was 2.4%. In addition, Fe^{2+}, Fe^{3+}, Cr^{3+}, Cd^{2+}, and Pb^{2+} were also studied but could not be detected by this method.

The result suggested that Hg^{2+} forms more stable complexes with RBPhOH than most other metals except Cu. With no RBPhOH in the running solution, the Hg^{2+} complex can be easily dissociated in the separation channel due to the relatively low association constant of Hg^{2+} with RBPhOH. When RBPhOH was added into the running buffer, it drove the chelation reaction going forward so that

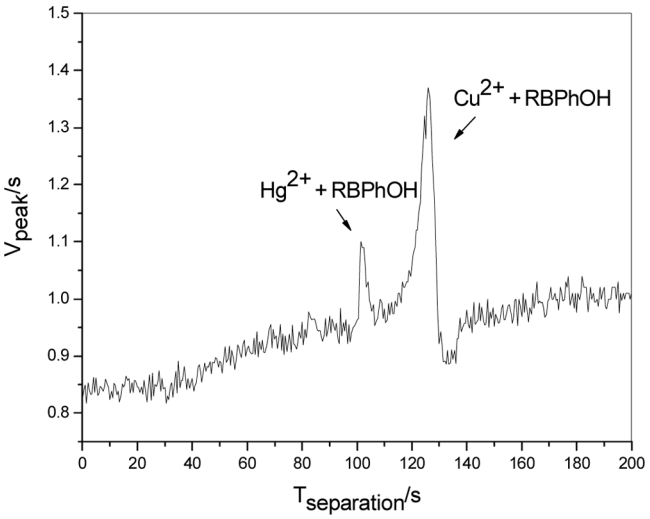

Figure 2. Electropherogram of a mixture of 5×10^{-6} mol L^{-1} Hg^{2+}, 5×10^{-6} mol L^{-1} Cu^{2+} in the running buffer. Running buffer: 20 mM pH 6.0 Tris, 3×10^{-5} mol L^{-1} RBPhOH, 10% CH_3CN. Injection voltage: 400 V; Sample loading time: 40 s; Separation voltage: 900 V.

a certain amount of Hg^{2+} and RBPhOH complex emitted weak fluorescence and Hg could be detected.

CONCLUSION

We have constructed a μTAS coupled with a confocal LIF detector for rapid and facile detection of Cu^{2+} in glass microchips. The detection limit for Cu^{2+} to be 1.34×10^{-7} mol L^{-1} is at least 10 times or even 70 times (Li et al. 2007) more sensitive than conductivity detection, which is widely used to detect metal ions at present. The selectivity and sensitivity of this method depend on the association constant of metal-RBPhOH complex as suggested by the detection of Cu^{2+} and Hg^{2+} when adding RBPhOH to the running buffer.

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