PMMA microreactor for chemiluminescence detection of Cu (II) based on 1,10-Phenanthroline-hydrogen peroxide reaction

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Revised: 7 September 2015 / Accepted: 14 September 2015 / Published online: 22 September 2015
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Abstract A microreactor for the chemiluminescence detection of copper (II) in water samples, based on the measurement of light emitted from the copper (II) catalysed oxidation of 1,10-phenanthroline by hydrogen peroxide in basic aqueous solution, is presented. Polymethyl methacrylate (PMMA) was chosen as material for fabricating the microreactor with mill and hot bonding method. Optimized reagents conditions were found to be 6.3×10^{-5} mol/L 1,10-phenanthroline, 1.5×10^{-3} mol/L hydrogen peroxide, 7.0×10^{-2} mol/L sodium hydroxide and 2.4×10^{-5} mol/L Hexadecyl trimethyl ammonium Bromide (CTMAB). In the continuous flow injection mode the system can perform fully automated detection with a reagent consumption of only 3.5 μ L each time. The linear range of the Cu (II) ions concentration was 1.5×10^{-8} mol/L to 1.0×10^{-4} mol/L, and the detection limit was 9.4×10^{-9} mol/L with the S/N ratio of 4. The relative standard deviation was 3.0 % for 2.0×10^{-6} mol/L Cu (II) ions ($n=10$). The most obvious features of the detection method are simplicity, rapidity and easy fabrication of the microreactor.

Keywords Microreactor · Chemiluminescence detection · Copper (II) ions · 1,10-phenanthroline

Introduction

Research on microreactors has become well established, resulting in increased number of publications in various areas

including high throughput synthesis, multiphasic chemical reactions, catalytic reactions, and so on. However, high detection sensitivity for reactants and products with the extremely small size is a challenge.

Chemiluminescence (CL) is an interesting and promising detection technique in analytical chemistry. CL of heavy metal ions has many advantages including high detection sensitivity with simple instruments configuration, wide linear range of signal response, rapid measurement, and convenient setup.

Owing to above characteristics, a good combination can be made between CL detection and the microreactor. Several studies have shown that CL detection on microreactor is a promising alternative detection method for the determination of dissolved trace metals. A streptavidin functionalized capillary immune microreactor was designed for highly efficient flow-through CL immunoassay (Yang et al. 2011). A three-layered microchip that consists of a double spiral channel design for CL detection and a passive micromixer to facilitate the mixing of reagents were introduced (Lok et al. 2012). A PMMA microchip with an integrated passive micromixer based on chaotic advection was reported. The micromixer with staggered herringbone structures was used for luminol-peroxide CL detection (Lok et al. 2011). A microchip CE method with chemiluminescence (CL) detection using the reaction of 1,10-phenanthroline and hydrogen peroxide for separation and determination of metal ions is developed (Nogami et al. 2009). A novel enzyme-immobilized flow-through interface was designed for sensitive end-column CL detection in open-tubular capillary electrochromatography (Xie et al. 2013). For solid-state electrochemiluminescence of $\text{Ru}(\text{bpy})_3^{2+}$ /TPA system, three-dimensionally ordered macroporous gold structure has been presented (Gao et al. 2007). A determination method for Co(II), Fe(II) and Cr(III) ions by luminol- H_2O_2 system using chelating reagents is presented (Kim et al. 2012). The effect of detection chip

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geometry on CL signal intensity of tris(1,10-phenanthroline)-ruthenium(II) peroxydisulphate system for analysis of chlorpheniramine maleate in pharmaceutical formulations was investigated (Lawati et al. 2010). CL has been used in the determination of chromium(III) and total chromium using dual channels on glass chip (Waraporn et al. 2007).

In this study, we presented a simple PMMA microreactor for determining the concentration of Cu (II) in water based on CL. The microreactor has many advantages including simple structure, easy fabrication, fast analysis and so on. In addition, the microreactor can be used repeatedly, and permits a drastic decrease in reagent consumption. It can finish detection in less than 2 min. The detection system can be assembled with fairly simple devices.

Materials and methods

Chemicals

All chemicals used were commercially available and of analytical grade, and deionized water was used throughout. 30 % (v/v) hydrogen peroxide (H_2O_2) was obtained from Shanghai Caoxing Zhongli Chemical Reagent Co. (Shanghai). Copper sulfate pentahydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) and sodium hydroxide (NaOH) was obtained from Xi'an Chemical Reagent Co. (Xi'an). 1,10-Phenanthroline and CTMAB were obtained from Sangon Biotech Co., Ltd. (Shanghai).

Alkaline buffer solution was obtained by dissolving NaOH powder in deionised water. A stock solution of 1,10-Phenanthroline with concentration 6.0×10^{-3} mol/L was obtained by dissolving 0.10812 g 1,10-Phenanthroline in NaOH buffer solution and was used after storing at room temperature for a week. 1×10^{-3} mol/L Cu(II) sulfate in deionised water was prepared as stock solution. All working solutions were prepared freshly from the stock solution by dilution with deionized water before the experiments.

PMMA microreactor

Mill and hot bonding method were applied in fabricating the microreactor with two transparent PMMA substrates. Firstly, a PMMA, the base plate, was etched with a mill and formed micro-channels. Secondly, the base plate was washed clearly by alcohol and water sequentially. Lastly, the base plate was bonded to the top plate that was made several holes of diameter of 1 mm at the pressure of 1.2–1.5 MPa at 80° Celsius for 20 min, and then cooled to the room temperature whilst maintaining a pressure of 1.2–1.5 MPa.

The main channel of the microreactor is a cross-channel with four same square waveform channels for improving mixing. There are four reservoirs on the microreactor, named R1, R2, R3, and R4. The channel length is 40.0 mm from R1

to R4, and 20.0 mm from R3 to R4. The height and width of the square waveform channel are 8 mm and 1 mm. The depth and the width of the channel are 80 μm and 250 μm , respectively. The photo of the microreactor is shown in Fig. 1.

Experimental setup

The microreactor was positioned directly facing the window of a photomultiplier tube (PMT, H5784-02, Hamamatsu, Japan) with a distance of 3 mm between the microreactor and PMT. The PMT was interfaced directly to a personal computer via a serial connection in order to acquire data. The entire setup was carefully shielded in a light-tight black box to eliminate background readings during entire experiments. The Cu (II) solution, 1,10-phenanthroline solution, mixed solution of CTMAB and Hydrogen peroxide were simultaneously delivered from three 1 ml disposable syringe through the syringe pump via 0.8 mm I.D. PTFE tubing into R1, R2, R3 in the microreactor. A complete analysis could be finished within 2 min with a reagent consumption of 3.5 μL . The concentration of Cu (II) was quantified by the relative CL intensity. The schematic diagram of experimental setup is shown in Fig. 2.

Results and discussion

Effect of flow rate on CL intensity

The flow rate is an important factor influencing the response of the system, including the relative CL intensity, sampling frequency and lifetime of the microreactor. The flow rate of the syringe pump on the CL response was investigated in the range 10–100 $\mu\text{L}/\text{min}$. Fig. 3 shows that CL intensity increased rapidly with increasing of flow rate up to 62 $\mu\text{L}/\text{min}$, followed by a gradual decline. If the flow rate was faster, the solvents could not mix sufficiently. If the flow rate was

Fig. 1 The square-channel microreactor

Fig. 2 Schematic diagram of working principle of the microreactor

slower, diffusion could lead to a wide peak. Hence, a flow rate of 62 $\mu\text{L}/\text{min}$ was selected as optimal value.

Effect of 1,10-phenanthroline concentration on CL intensity

The 1,10-phenanthroline concentration had an important effect on the CL intensity for the determination of Cu (II) ions. The concentration of 1,10-phenanthroline was varied from 1.0×10^{-6} to 1.0×10^{-4} mol/L with the concentration of hydrogen peroxide keeping at 1.5×10^{-3} mol/L, the concentration of CTMAB keeping at 2.4×10^{-5} mol/L and the concentration of Cu (II) ions keeping at 1.0×10^{-6} mol/L. Fig. 4 shows that the CL intensity increased with increasing 1,10-phenanthroline concentration. When the 1,10-phenanthroline concentration was higher than 6.3×10^{-5} mol/L, the CL intensity decreased. So, 6.3×10^{-5} mol/L 1,10-phenanthroline was chosen as the optimal.

Fig. 3 The interrelation between flow rate and the CL intensity

Fig. 4 The interrelation between 1,10-phenanthroline concentration and CL intensity

Effect of hydrogen peroxide concentration on CL intensity

The concentration of the oxidant also influences the CL intensity. The concentration of hydrogen peroxide was varied from 1.0×10^{-5} to 1.0×10^{-2} mol/L with the concentration of 1,10-phenanthroline keeping at 6.3×10^{-5} mol/L, the concentration of CTMAB keeping at 2.4×10^{-5} mol/L and the concentration of Cu (II) ions keeping at 1.0×10^{-6} mol/L. Fig. 5 shows that the CL intensity increased with increasing concentration of hydrogen peroxide. When the concentration of hydrogen peroxide was higher than 1.5×10^{-3} mol/L, the CL intensity increased slowly, and the higher concentration of hydrogen peroxide easily produced bubbles. So, 1.5×10^{-3} mol/L hydrogen peroxide was chosen as the optimal.

Fig. 5 The interrelation between hydrogen peroxide concentration and CL intensity

Effect of NaOH concentration on CL intensity

The CL reaction of hydrogen peroxide with 1,10-phenanthroline must be in alkaline medium. Preliminary assays showed that the pH had an important effect on the CL intensity for the determination of Cu (II) ions. NaOH was chosen as buffer solution. The results in Fig. 6 shows that the CL intensity increased with increasing NaOH concentration in the range 1.0×10^{-2} – 7.0×10^{-2} mol/L. When NaOH concentration increased from 7.0×10^{-2} mol/L to 1.5×10^{-2} mol/L, the CL intensity slowly decreased. So the NaOH concentration of 7.0×10^{-2} mol/L was chosen as optimum.

Effect of CTMAB concentration on CL intensity

CL signal can be enhanced with the presence of a cationic surfactant in the reaction of 1,10-phenanthroline and hydrogen peroxide for the detection of Cu(II). CTMAB was chosen as cationic surfactant in the experiment. Fig. 7 shows that the CL intensity increased with increasing CTMAB concentration in the range 1.0×10^{-6} – 1.0×10^{-4} mol/L. When CTMAB concentration was higher than 2.4×10^{-5} mol/L, the CL intensity decreased. So the CTMAB concentration of 2.4×10^{-5} mol/L was chosen as optimum.

Effect of Cu (II) ions concentration on CL intensity

In order to analysis the influence of Cu (II) ions on CL, the concentration of Cu (II) ions ranges was changed from 1.0×10^{-8} to 1.0×10^{-4} mol/L. The Cu (II) ions, 6.3×10^{-5} mol/L 1, 10-phenanthroline, 1.5×10^{-3} mol/L H_2O_2 and 2.4×10^{-5} mol/L CTMAB in alkaline buffer solution were introduced into the microreactor. The flow rate was set to $63 \mu\text{L min}^{-1}$. The minimum analytical time for each sample was within 2 min. The

Fig. 6 The interrelation between NaOH concentration and CL intensity

Fig. 7 The interrelation between CTMAB concentration and CL intensity

CL intensity increased with increasing concentration of Cu (II) ions.

Linearity, repeatability and limit of detection

The linearity, repeatability and limit of detection were measured with the optimized conditions. The response to Cu (II) concentration was linear over the range 1.5×10^{-8} – 1.0×10^{-4} mol/L. The regression equation was $I = 830C + 8$, where I and C represented the CL intensity in counts and the concentration of Cu (II) ions in mmol/L. Fig. 8 shows that the CL intensity versus concentration of Cu (II) ions. The detection limit for Cu (II) was found to be 9.4×10^{-9} mol/L with the S/N ratio of 4. The relative standard deviation of the system was examined by making 10 consecutive runs with Cu (II) concentration of 2.0×10^{-6} mol/L and was found to be 3.0 %.

Fig. 8 The effect of concentration of Cu(II) ions on CL intensity (error bars represent the standard derivations)

Conclusions

A simple CL microreactor fabricated in PMMA has been presented, and Cu (II) ions were detected with the microreactor. The results have shown that the linear range of the Cu (II) ions concentration was 1.5×10^{-8} to 1.0×10^{-4} mol/L and the detection limit was 9.4×10^{-9} mol/L (S/N=4). The most significant advantage of the detection method is simplicity and sensitivity. The microreactor can be easily fabricated with mill and hot bonding method, and can be used repeatedly with little reagent consumption. The detection method is not limited for only Cu (II) determination, and offers possibility for the determination of other similar metal ions in water.

Acknowledgments This work was supported by National Natural Science Foundation of China (51405214), Liaoning Province Doctor Startup Fund (20141131), Fund of Liaoning Province Education Administration (L2014241), and the Fund in Liaoning University of Technology (X201301).

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