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A microchip electrophoresis strategy with online labeling and chemiluminescence detection for simultaneous quantification of thiol drugs

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ABSTRACT

An integrated microfluidic device with online labeling and chemiluminescence (CL) detection was developed for the simultaneous quantification of thiol drugs. In this device, the online labeling, electrophoresis separation and CL detection were compactly integrated onto a glass/poly(dimethylsiloxane) (PDMS) hybrid microfluidic chip. CL detection was based on the oxidation reaction of N-(4-aminobutyl)-N-ethylisoluminol (ABEI) and o-phthalaldehyde (OPA) labeled thiol drugs with NaBrO. Four thiol drugs including 2-mercaptopropionylglycine (2-MPG), captopril (CP), 6-thioguanine (6-TG) and 6-mercaptopurine (6-MP) were employed as model compounds to examine the utility of the system. It was indicated that the separation and detection of four drugs can be completed within 90 s. Detection limits (S/N = 3) for the thiol drugs tested were in the range of 8.9×10^{-9}–13.5×10^{-9} M. The application of the present system was demonstrated by analyzing the thiol drugs in human plasma samples.

1. Introduction

Thiol-containing drugs are widely used for the treatment of many diseases. For example, D-penicillamine and 2-mercaptopropionylglycine (2-MPG) are frequently used as therapeutic drugs in Wilson's disease and rheumatoid arthritis, and as efficient antidotes in heavy-metal poisoning [1,2]. 6-Thioguanine (6-TG) and 6-mercaptopurine (6-MP) are used as anticancer drugs [3]. *N*-acetylcysteine was used as a mucolytic agent for the treatment of chronic bronchitis, and as an effective antidote for acetaminophen poisoning [4,5]. Other thiol-containing drugs such as captopril, mesna and thyreostats are also applied in different clinical fields. However, severe adverse reactions to oral thiol-drugs use have been described in subjects in which abrupt incremental dosing of the drugs were started. This suggests that monitoring of the concentrations of these drugs in biological fluids during disease therapies is warranted. Therefore, there is a need for the development of rapid, simple, and sensitive analytical method to measure these drugs.

Numerous analytical methods have been described for the quantification of thiol drugs, such as spectrophotometry [6,7], spectrofluorimetry [8,9], NMR spectrometry [10], Fourier transform infrared spectrometry [11], colorimetry [12], flow injection analysis (FIA) [13,14], and high-performance liquid chromatography (HPLC) [15,16]. Among these methods, HPLC is the most popular method for the analysis of thiol drugs. However, the assay is sometimes time-consuming, because a pre-treatment of the biological sample (such as solid-phase extraction or pre-column derivatization of analyte) is usually necessary prior to its injection into the instrument. In some cases complex gradient systems are also required, and long analytical times are needed due to prolonged elution times that add up to equilibration time between runs. Capillary electrophoresis (CE) coupled with various detection techniques such as UV, electrochemical and laser induced fluorescence (LIF) have also been developed for the determination of thiol drugs in biological samples [17,18].

Microchip electrophoresis (MCE) has been proven be a powerful separation technique for the analysis of chemical species [19]. Compared with conventional analytical techniques, MCE offers many advantages including extremely small sample size as low as nanoliters to femtoliters, high separation speed and efficiency, ease of integration and automatization. Chemiluminescence (CL) detection was one of the most sensitive detection techniques. MCE couple CL detection has become an attractive and alternative detection scheme for sensitive detection in MCE, and was successfully applied for the determination of metal ions [20], amino acids [21], catecholamines [22] and proteins [23]. Recently, our group developed an MCE–CL assay system for the analysis of biogenic amines in biological sample [24], and high detection sensitivity was achieved by pre-column derivatization.

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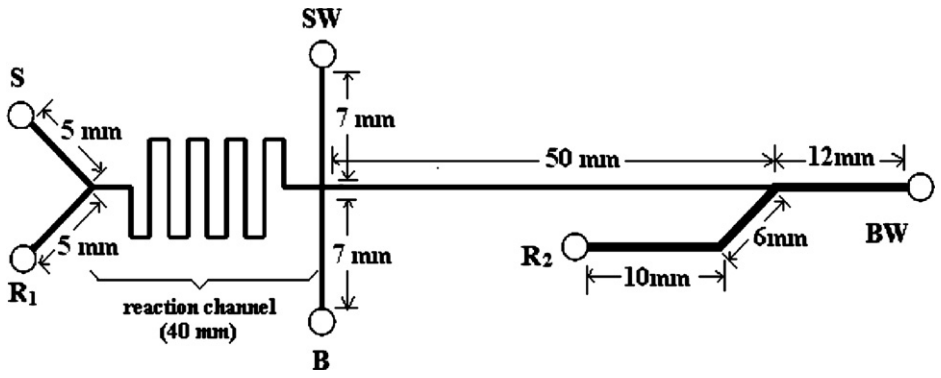

Fig. 1. The layout and dimensions of the glass/PDMS hybrid microchip used in this work. S, sample reservoir; R_1, labeling solution reservoir; B, electrophoretic buffer reservoir; SW, sample waste reservoir; R_2, oxidizer solution reservoir; BW, buffer waste reservoir.

In MCE, the microfluidic channels are extremely small, which affects negatively the detection sensitivity. Actually, the sensitivity for the MCE–CL methods reported previously was not impressive, the detection of limits was usually in the range from 10^{-6} to 10^{-7} M. Using CL labeling approach, the sensitivity for CL detection can be remarkably improved because no CL reagent was added to the running buffer, and therefore, the background of the CL detection was extremely low. The aim of this work was to develop a rapid, sensitive and selective MCE–CL system for the simultaneous quantification of thiol drugs in biological samples. Therefore, a new microfluidic device integrated with precolumn and postcolumn reactor was designed. Conditions for online labeling, electrophoresis separation and CL detection of the targeted analytes were studied, and the quantification of the thiol drugs in human plasma was demonstrated.

2. Experimental

2.1. Chemicals and solutions

Captopril (CP), 2-mercaptopropionylglycine (2-MPG), 6-thioguanine (6-TG), 6-mercaptopurine (6-MP) and o-phthalaldehyde (OPA) were provided by Sigma Chemicals (St. Louis, MO, USA). N-(4-aminobutyl)-N-ethylisoluminol (ABEI) was purchased from Fluka (Buchs, Switzerland). Sodium dodecyl sulfate (SDS) was obtained from Taopu Chemicals (Shanghai, China). All other chemicals used in this work were of analytical grade or better. Water was purified by employing a Milli-Q plus 185 equip from Millipore (Bedford, MA, USA), and used throughout the work. The electrophoretic buffer was 20 mM borate buffer (pH 9.6, adjusted with 1 M NaOH solution) containing 18 mM SDS. The oxidizer solution was 40 mM sodium carbonate buffer (pH 12.0, adjusted with 1 M NaOH solution) containing 1 mM NaBrO. The labeling solution was 0.1 mM ABEI and 0.05 mM OPA in electrophoretic buffer. Stock solutions of ABEI and OPA were prepared in methanol and diluted with 20 mM borate solution (pH 9.0). Stock solutions of thiol drugs were prepared in 20 mM borate solution (pH 9.6). All solutions were filtered through 0.22 μm membrane filters before use.

2.2. Apparatus and microfluidic chip

The assay was carried out using a laboratory built MCE–CL system as described previously [25]. The microchip assembly was mounted on the *X–Y* translational stage of an inverted microscope (Olympus CKX41) that also served as a platform of CL detection. CL signal was collected by means of a microscope objective. After passing a dichroic mirror and a lens, CL photons were detected by a photomultiplier (PMT, Hamamatsu R105). The PMT was mounted in an integrated detection module including HV power supply, voltage divider, and amplifier. The output signal of PMT was recorded and processed with a computer using a Chromatography Data System (Zhejiang University Star Information Technology, Hangzhou, China). A multi-terminal high voltage power supply, variable in the range of 0–8000 V (Shandong Normal University, Jinan, China), was used for sample injection and electrophoresis separation. The inverted microscope was placed in a black box.

A schematic layout of the glass/PDMS hybrid microchip is shown in Fig. 1. The width of all microchannels except oxidizer introduction channel (250 μm) is 70 μm; the depth of all microchannels is 25 μm. All reservoirs were 4.0 mm in diameter and 2.0 mm deep. The channel from reservoirs S and R_1 to the cross intersection was used for sampling and labeling, the channel between B and BW was used for the separation. The channel between the cross intersection and SW was used for delivering sample waste, and the channel between R_2 and BW was used for the oxidizer introduction. The join-point of the oxidizer introduction channel with the separation channel was used for the collection of CL.

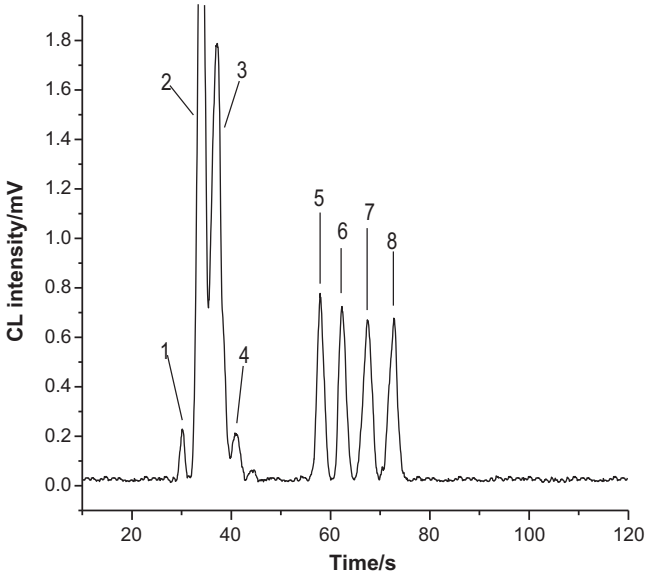

Fig. 2. Electropherogram obtained from the separation of a mixture of standard thiol drugs. Electrophoretic electrolyte was 20 mM borate buffer (pH 9.6) containing 18 mM SDS. The oxidizer solution was 40 mm sodium carbonate buffer (pH 12.0) containing 1 mM NaBrO. Labeling solution was 20 mM borate buffer (pH 9.6) containing 0.1 mM ABEI and 0.05 mM OPA. Potential applications for sample labeling, injection and separation were described in experimental section. All analytes concentrations are 4.0×10^{-7} M. Peaks: (1, 3, 4) decomposition product of tagging reagent; (2) ABEI; (5) 6-MP; (6) 6-TG; (7) 2-MPG; (8) CP.