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Microfluidic liquid–liquid extraction system based on stopped-flow technique and liquid core waveguide capillary

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Abstract

In this work, a miniaturized liquid–liquid extraction system under stopped-flow manipulation mode with spectrometric detection was developed. A Teflon AF liquid-core waveguide (LCW) capillary was used to serve as both extraction channel for organic solvent flow and adsorption detection flow cell. Gravity induced hydrostatic pressure was used to drive the organic and aqueous phases through the extraction channels. During extraction process, a stable organic and aqueous phase interface was formed at the outlet of the capillary, through which the analyte in the flowing aqueous stream was extracted into the stationary organic solvent in capillary. The absorbance of the analyte extracted into the organic solvent was measured in situ by a spectrometric detection system with light emitting diode (LED) as light source and photodiode as absorbance detector. The performance of the system was demonstrated in the determination of sodium dodecyl sulfate (SDS) extracted as an ion pair with methylene blue into chloroform. The precision of the measured absorbance for a 5 mg L^{-1} SDS standard was 6.1% R.S.D. ($n = 5$). A linear response range of 1–10 mg L^{-1} SDS was obtained with 5 min extraction period. The limit of detection (LOD) for SDS based on three times standard deviation of the blank response was 0.25 mg L^{-1}.

Keywords: Microfluidic system; Liquid–liquid extraction; Stopped-flow technique; Liquid-core waveguide capillary

1. Introduction

Liquid–liquid (L–L) extraction is one of the most widely used tools for matrix isolation and analyte enrichment in analytical chemistry. However, the conventional L–L extraction procedure is time-consuming, labor-intensive, requires large amounts of toxic organic solvent, and often leads to emulsion formation and analyte loss. In recent years, various automated and miniaturized L–L extraction techniques, such as liquid-phase microextraction [1–16] and microfluidic chip based microextraction [17–25], have been developed to overcome these drawbacks. In 1996, Liu and Dasgupta [1] reported a L–L extraction system based on a 1.3 μL single droplet of organic solvent with an in situ spectrometric detector. Almost at the same time, Jeannot and Cantwell [2] reported a similar microextraction system with a microdrop (8 μL) of organic solvent suspended at the end of a Teflon rod immersed in a stirred aqueous sample solution. Thereafter, various L–L extraction systems based on single droplet of organic solvent were developed to perform sample pretreatment [3–8]. In most of these systems, L–L extraction under stopped flow manipulation mode was performed by forming a stationary organic solvent droplet immersed in a larger aqueous sample solution. Droplet volumes of the organic solvents were typically in the microlitres range and sample volumes consumed were in the millilitres range. However, the limitation of these systems is that the microdrops suspended on the needle or other holding devices are sometimes dislodged by the flowing aqueous sample during extraction process [9,15].

From 2000, Kitamori's group has reported a series of microfluidic chip systems performing L–L extraction based on formation of multiple phase laminar flows in microchannels [17–22]. More recently we reported a series of L–L extraction chip systems based on stopped flow and trapped droplet techniques [23–25]. The advantages of chip based solvent extraction systems are low consumption of sample and extractant in nL–μLs ranges and high extraction efficiency due to the microscale effect in microchannels. However, usually expensive equipment and complicated operation were required in the

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fabrication of L–L extraction chips, which may limit their further application in routine analysis.

In this work, a simple and robust microfluidic L–L extraction system under a stopped-flow manipulation mode was developed based on a Teflon AF LCW capillary without requirement for special microfabrication techniques. A stable L–L interface between the stationary organic solvent in the capillary and flowing aqueous sample was formed at the outlet of the capillary by adjusting the liquid level in reservoirs in the system. A miniaturized in situ spectrometric detector to monitor the absorbance of analyte extracted into the organic solvent was built also based on this capillary to achieve sensitivity comparable to other microextraction system [1] and conventional systems [26]. Aqueous solutions of SDS and chloroform were used, respectively, as samples and extractant to demonstrate the performance of the system.

2. Experimental

2.1. Chemicals

All chemicals were of analytical-reagent grade and deionized water was used throughout. The 100 mg L^{-1} methylene blue (MB) solution was prepared by dissolving 10 mg of MB and 2.83 g of $NaH_2PO_4{\cdot}2H_2O$ in 700 μL cons. H_2SO_4 and 25 mL methanol, and made up to 100 mL with water. The stock solution of sodium dodecyl sulfate (SDS, 100 mg L^{-1}) was prepared by dissolving 10 mg of SDS (Sigma–Aldrich) in 100 mL of water. The series of SDS standard solutions were prepared by sequentially diluting the stock solution with water. The working solution was prepared by mixing one portion of MB solution with three portions of SDS standard solution. All the solutions were prepared at least 24 h prior to use. Chloroform (Zhejiang Deyer Pharmaceutical Co., Hangzhou, China) was used as the extractant without further purification.

2.2. Apparatus

The microfluidic L–L extraction system is shown diagrammatically in Fig. 1. A 10 mm long glass tube (0.8 mm i.d., 1.2 mm o.d.) was used to supply the flowing channel for aqueous sample solution. A 6 mm long fused silica capillary (100 μm i.d., 375 μm o.d.) serving as sampling probe was connected to the inlet of the glass tube via a section of Tygon tubing (380 μm i.d., 900 μm o.d.). The outlet the glass tube was connected with a 70 mm long horizontal plastic tube (4.7 mm i.d., cut from a commercial 1 mL disposable syringe) used as waste reservoir via a 10 cm long Tygon tubing (1 mm i.d., 3 mm o.d.). A 0.4 mm-diameter hole was drilled on the sidewall of the glass tube using a 400 μm-diameter flat-tipped emery drill. A 15 mm long Teflon AF 2400 capillary (250 μm i.d., 500 μm o.d., Random Technologies, San Francisco, USA) was inserted through the hole 200 μm deep into the glass tube channel. The capillary served both as a LCW absorption detection flow-cell as well as an extraction channel for the organic solvent. The other end of the capillary was inserted into a perpendicular plastic tube (15 mm i.d., cut from a commercial 10 mL disposable syringe) that served as a reservoir for the organic solvent, positioned 500 μm from a 1 mm-diameter optical fiber inserted into the reservoir. All connections in the conduit system were sealed with epoxy. The sample vials were produced from the 0.2 mL Microtubes (Porex, Petaluma, USA) with 1.5 mm wide, 2 mm deep slot fabricated on the conical bottom of each tube for pass-through of the sampling probe.

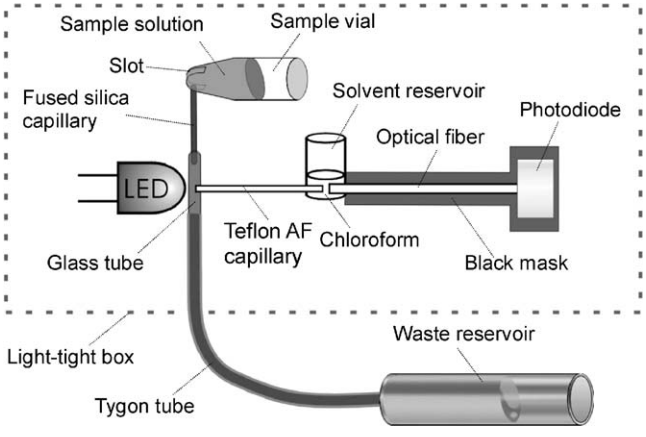

Fig. 1. Schematic diagram of the microfluidic L–L extraction system employing Teflon AF capillary liquid-core waveguide (not to scale).

A red LED (630 nm, Hangke Electronics Co., Hangzhou, China) was used as light source, positioned 2 mm from the Teflon AF capillary outlet to directly illuminate the capillary outlet through the sidewall of glass tube without further focusing. The light transmitted out from the capillary inlet was conducted by the optical fiber to a photodiode detector (Model OPT-301, with integrated amplifier, Texas Instruments, Tucson, USA). The Teflon AF capillary was masked from ambient light with black plastic tubing. The sections of conduit in the detection system were masked from ambient light using a light-tight box painted black inside. LabVIEW software (National Instruments, Austin, USA) was used to process the detection signals.

The L–L extraction system and detection system were fixed onto a platform, keeping the glass tube in upright position and the Teflon AF capillary in horizontal position, whereas the horizontally positioned waste reservoir was fixed onto another platform which could be perpendicularly moved to vary the position of the waste reservoir in relation to that of L–L interface at the outlet of the capillary.

For direct observing the stability of the L–L interface at the outlet of Teflon AF capillary, a stereo microscope (SZ-45B3, Sunny Instruments Co., NingBo) equipped with a CCD camera (YH-9628, Yonghui Technology Development Co., Shenzhen) was used.

2.3. Procedures

Before use, the conduits in the L–L extraction system were emptied. 100 μL blank solution and aqueous working solutions containing the ion-pair product were pipetted into separate horizontal sample vials. The L–L extraction was performed first by inserting the sampling probe through the slot of the blank solution vial. The aqueous solution driven by gravity flowed through

the glass tube, while the aqueous flow into the Teflon AF capillary was obstructed owing to surface tension effect produced by the hydrophobic properties of Teflon AF. Then the organic solvent reservoir was filled with 500 μL chloroform, which produced a liquid level of 3 mm relative to that of the capillary outlet. A stable aqueous/organic phase interface was formed at the outlet of the capillary based on surface tension by positioning the waste reservoir 4 mm below the capillary outlet. The L–L extraction and analysis for sample solution was performed by inserting the sampling probe into a sample vial instead of the blank vial. The ion-pair product extracted into the organic phase was in situ detected in this LCW capillary flow-cell. After one analytical cycle, the position of the waste reservoir was lowered to 30 mm below the capillary outlet for 5 s to wash out the organic solvent in the capillary, and then the waste reservoir was restored to its original position to obtain a fresh phase interface for the next analytical cycle.

3. Results and discussion

3.1. Design of the microfluidic L–L extraction system

In our previously reported miniaturized L–L extraction system [23–25], a stopped-flow manipulation mode was adopted to perform extraction in microfluidic chip with a "Y" shaped channel, which had the advantages of high enrichment factor and simple operation without requirement for organic/aqueous phase separation. In this work, the L–L extraction system was simplified by using a Teflon AF LCW capillary as a conduit to supply the organic solvent instead of using a chip with microfabricated channels, allowing the system to be built in routine laboratories without requirement for complicated microfabrication devices and operations. Owing to the hydrophobic property of Teflon AF, a stationary phase interface was easily formed at the capillary channel outlet by adjusting the liquid level in the reservoirs (See Section 3.2). In addition to serving as a conduit for organic solvent delivery, the Teflon AF capillary was also used as a flow-cell for the absorption detection making use of its waveguide properties when filled with aqueous solution. Such detection systems had been applied in long-path-length absorption spectrometry for microfluidic chips to improve sensitivity [27,28]. In the present system, for convenience, a 1.5 cm long Teflon AF capillary was used as a detection flow cell.

In the present L–L extraction system, hydrostatic pressure produced from difference in liquid levels between the sample/chloroform containers and the waste reservoir was employed to provide driving force for sample introduction and transfer as well as washout of chloroform from the capillary. Horizontal tubular sample vials and waste reservoirs were used to maintain stable hydrostatic pressure in the extraction channels during prolonged working periods. A slot was fabricated at the bottom of each sample vial to facilitate sample change. Owing to the low consumption of organic solvent (within several hundred nanoliters, see Section 3.3) during extraction process under the stopped-flow extraction mode, an upright reservoir was employed for organic solvent, in which the liquid level showed no observable changes during 3 h extraction periods.

3.2. Formation of stable L–L interface

In the present microfluidic L–L extraction system, there are four forces exerted on the L–L interface at the outlet of the capillary (as shown in Fig. 2), including the hydrostatic pressure f_o and f_a with different directions produced by the organic solvent reservoir and sample vial, and negative pressure f_w produced from the waste reservoir, as well as the surface tension f_s of the organic solvent at the capillary outlet. The combination of these forces f_c in the horizontal direction could be expressed as

$$f_c = f_o + f_w + f_a + f_{sp} \quad (1)$$

where f_{sp} is the vector force of surface tension f_s in the horizontal direction. When f_c is equal to zero, a stable stationary phase interface can be produced at the outlet of the capillary (as shown in Fig. 3b). If $f_c > 0$, the aqueous solution flows into the capillary to push the organic solvent back (as shown in Fig. 3a). If $f_c < 0$, the organic solvent overflows from the capillary outlet (as shown in Fig. 3c). Therefore, in the present system, the stability of the L–L interface is mainly governed by the liquid levels in the sample vial, waste and solvent reservoir. The former two liquid levels were decided by the vertical positions of the sample vial and waste reservoir, respectively, while the liquid level in the organic solvent reservoir was decided by the volume of organic solvent filled in the reservoir. The effects of the positions of the sample vial and waste reservoir on the stability of L–L interface were studied with the liquid level of solvent reservoir fixed at 3 mm above the L–L interface. The results (Fig. 4) show that corresponding to a defined sample vial position, a position range of approximately 4 mm of the waste reservoir usually existed (the shadowed section shown in Fig. 4), within which a stable phase interface could be obtained (i.e. $f_c = 0$). This is the result of self-adjustment of the organic solvent surface tension at the interface by changing its shape to counteract the position variation of waste reservoir within this range. With the raising of the sample vial position (i.e. to increase f_a), the position region of

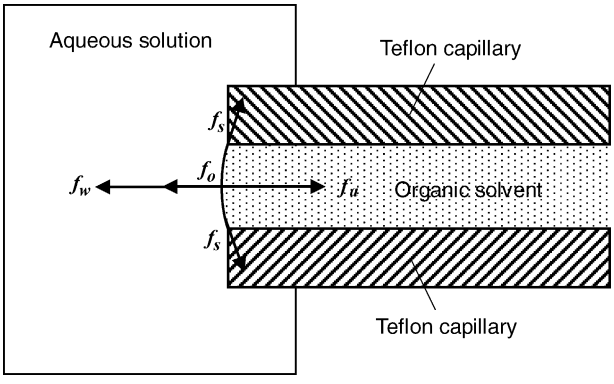

Fig. 2. Schematic diagram of different forces exerted on the liquid–liquid interface at the outlet of the capillary.

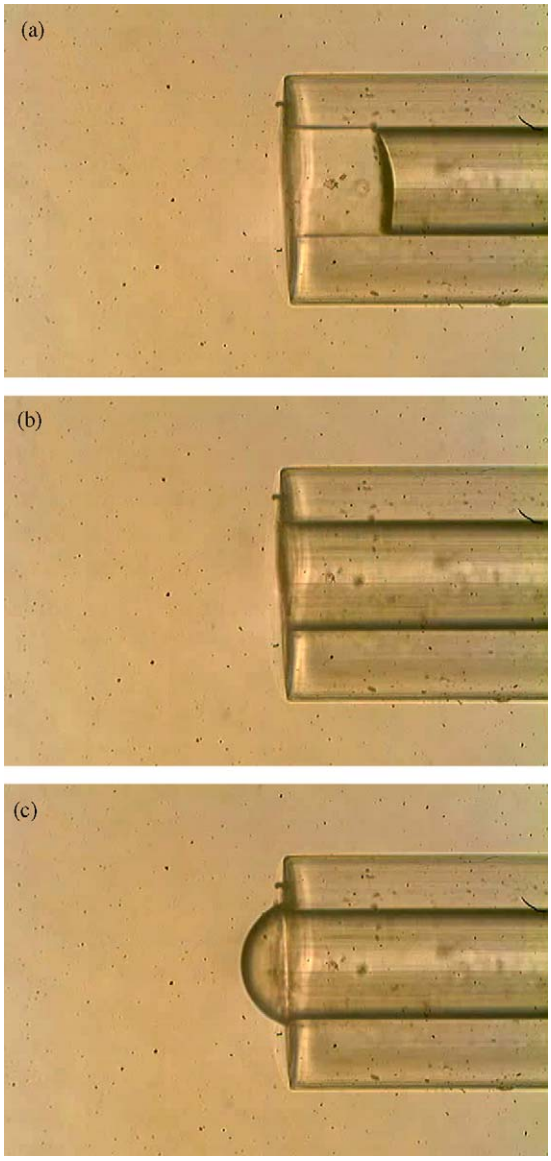

Fig. 3. CCD images of three typical states of the L–L interface at the outlet of capillary under different hydrostatic pressures.

waste reservoir required for stable interface shifted down (i.e. to increase f_w) to maintain the force equilibrium at the L–L interface.

In addition to the above experiments, the effects of the liquid level in the solvent reservoir and the position of the waste reservoir on the stability of L–L interface were studied. The results are shown in Fig. 5. Stable L–L interfaces were obtained within a position range of ca. 6 mm (the shadowed section shown in Fig. 5) of the waste reservoir, corresponding to a fixed liquid level in the chloroform reservoir. With the raising of liquid level in the chloroform reservoir (i.e. to increase f_o), the waste reservoir position required for stable interface shifted up (i.e. to decrease f_w) to maintain the force equilibrium at the phase interface.

Considering the consumption of sample and chloroform, a relatively low position for the sample vial and the liquid level of chloroform at 10 and 3 mm above the phase interface was

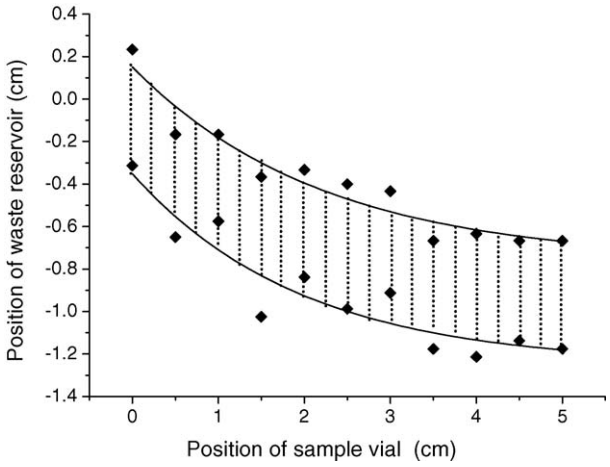

Fig. 4. Effects of positions of aqueous sample vial and waste reservoir on the stability of L–L interface. The liquid level in the chloroform reservoir was fixed at 3 mm above the interface. The positions of the sample vial and waste reservoir were evaluated in relation to that of L–L interface at the capillary outlet.

chosen in the present L–L extraction system, respectively, corresponding to a sample flow rate of 2.0 μL min^{-1} (70 μm s^{-1}). Under these conditions, an optimized position of 4 mm below the phase interface (expressed as −4 mm in Figs. 4 and 5) for the waste reservoir was chosen during the extraction process. This position is at the center of the region of waste reservoir positions that produced stable L–L interfaces.

3.3. Analytical performance

The analytical performance of the present microfluidic L–L extraction system was demonstrated in the determination of SDS with MB as reagent. Fig. 6 shows typical recordings of absorbance of 5 mg L^{-1} SDS during five repetitive extraction cycles. The absorbance response increased almost linearly with extraction time in the range of 0–5 min, beyond that the increase of response gradually levelled off, which implies that the L–L

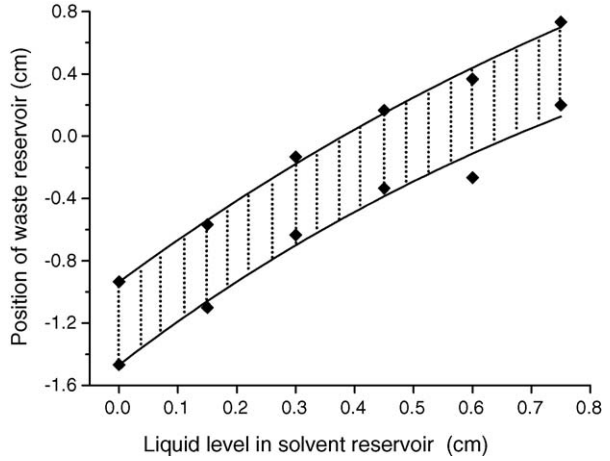

Fig. 5. Effects of liquid level of chloroform and position of waste reservoir on the stability of L–L interface. The position of the sample vial was fixed at 10 mm above the interface. The liquid level of chloroform and position of waste reservoir were evaluated in relation to that of L–L interface at the capillary outlet.

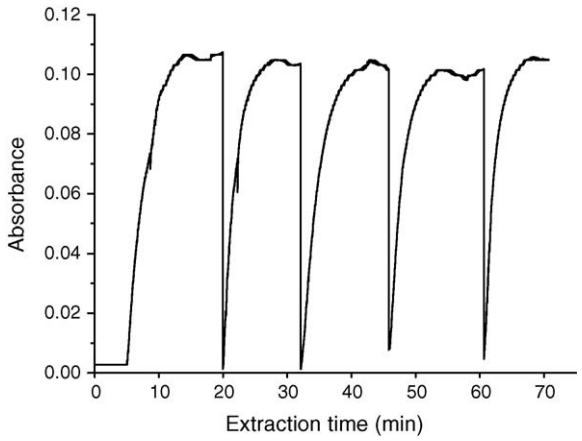

Fig. 6. Typical recordings of absorbance of 5 mg L^{-1} SDS during five repetitive extraction cycles to show the repeatability of the system.

extraction nearly reached equilibrium state. An extraction time of 5 min seemed to be a good compromise between detection sensitivity and analytical throughput. The precisions of a 5 mg L^{-1} SDS standard with extraction times of 5, 10 and 15 min were 6.1%, 3.9% and 3.8% R.S.D. ($n = 5$), respectively (Fig. 6). The analytical signal was found to be linearly related to the SDS concentration in the range of 0–10 mg L^{-1} within a 5 min extraction period: $A = 0.01936C - 0.00456$ ($r^2 = 0.9946$), where A is the absorbance response and C is the concentration of SDS solution in mg L^{-1}. The limit of detection (LOD) for SDS based on three times the standard deviation of the blank values was 0.25 mg L^{-1}. The shortest total analysis time for one cycle was 5.5 min, including 5 min extraction time and 0.5 min conduit washing and sample changing time. The consumptions of sample and organic solvent in one analytical cycle were 10 μL and 740 nL, respectively.

4. Conclusions

The present system proved to be an efficient, robust and organic solvent-economic means for achieving L–L extraction under stopped flow mode. The use of Teflon AF LCW capillary significantly facilitated the construction of the L–L extraction system and spectrometric detector in routine chemical laboratories. Such systems should be widely applicable for various analytical purposes involving L–L extraction with spectrometric detection. Although not pursued further in this work, the sample consumption of the system could be further reduced by decreasing the inner diameter of the aqueous phase channel, and sample consumption less than 1 μL is foreseeable.

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