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The use of a micropump based on capillary and evaporation effects in a microfluidic flow injection chemiluminescence system

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Abstract

The performance of a micropump operating on evaporation and capillary effects, developed for microfluidic (lab-on-a-chip) systems, was studied employing it as the fluid drive in a microfluidic flow injection (FI) system, with chemiluminescence (CL) detection. The micropump featured simple structure, small dimensions, low fabrication cost and stable and adjustable flow-rates during long working periods. Using a micropump with 6.6 cm^2 evaporation area, with the ambient temperature and relative humidity fluctuating within 2 h in the ranges 20–21 °C and 30–32%, respectively, an average flow-rate of 3.02 μL/min was obtained, with a precision better than 1.2% R.S.D. ($n = 61$). When applied to the microchip FI-CL system using the luminol/hexacyanoferrate/H_2O_2 reaction, a precision of 1.4% R.S.D. ($n = 11$) was obtained for luminol at a sampling frequency of 30 h^{-1}.

Keywords: Microfluidics; Micropump; Evaporation effect; Capillary effect; Flow injection; Chemiluminescence

1. Introduction

Chip-based microfluidic systems are currently generally accepted as the most promising venue for achieving the ultimate goals of micro total analysis systems (μTAS) or lab-on-a-chip systems, which aim at full integration of all analytical functional components. In any microfluidic system, the fluid drive is indispensable, and in fact functions as its "heart" for fluid transportation and distribution between various components of the system. Although the use of electrokinetic means for sample introduction and injection in chip-based capillary electrophoresis is now well established, the high voltage power drives required are yet too bulky to be integrated on a chip system. On the other hand, the most suitable drive for other chip-based analytical systems, to a large extent, still remains to be a topic of investigation. Despite extensive efforts for producing microfluidic systems with integrated microfabricated pressure driven pumps [1], hitherto, the use of microelectromechanical system (MEMS) produced silicon-based pumps as fluid drives generally has not lived up to earlier expectations owing to blockage, lifetime and chemical compatibility problems. An important recent contribution to micropump development was that made by Quake's group using multilayer soft lithographic microfabrication [2]. These pneumatically driven monolithic pumps, produced from polydimethyl siloxane (PDMS), are highly integratable in large scales [3], and used successfully in a number of microfluidic applications [4]. However, despite the sub-millimeter scale of the pumps themselves, their actuation system, which consists of an array of computer controlled solenoid valves and a pressurized gas source, are much larger. More recently, a micro FI system based on gravity driven flows has been developed, demonstrating extremely good reproducibility [5], and requiring no external driving force except that exerted by a 60 cm water column. However, the need for maintaining a sufficiently tall water column still presents an obstacle for further miniaturization of the system.

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A few years ago, a micropump was proposed by Effendhauser et al. [6], in which the fluid flow was induced by controlled evaporation of liquid through a membrane into a gas chamber containing a sorbent. As long as the sorbent was not saturated by the fluid vapor, liquid will be drawn into the space of the pump chamber, inducing a flow. However, the pump was designed to be a disposable device that was limited by the capacity of the sorbent. Juncker et al., developed a microfluidic system with an integrated capillary pump as the fluid drive, using a group of capillaries fabricated on the chip substrate [7]. Considering the low capacity of the capillaries network, apparently the system was also designed to be disposable; and issues on continuous usage of the pump were not addressed. Goedecke and Manz [8,9] reported a micropump, also featuring a network of microfabricated capillaries that generated liquid flow via capillary and evaporation effects. The pump could be employed for continuous operation though forced evaporation using a blower, although sacrificing the compactness of the device.

In an earlier report [10], we briefly described the design and performance of a micropump based on capillary and natural evaporation effects, which was fabricated at low cost without resorting to sophisticated microfabrication techniques, and characterized by liquid sorption using sandwiched filter paper layers. The pump was capable of continuously providing stable flow-rates over hours of operation. Preliminary studies on the performance of the pump showed that the stability of flow-rate was little influenced by variations in temperature and humidity ranges normally encountered in the laboratory environment. In this work, the performance and usefulness of the micropump was further validated, characterized and optimized for its adaptation to a microFIA system with chemiluminescence detection (μFIA-CL system). Peristaltic pumps often used as fluid drives in FIA-CL systems [11–13], are substituted by the micropump developed in this work to produce a more compact system.

2. Experimental

2.1. Reagents and materials

All reagents were of analytical reagent grade except stated otherwise, and de-mineralized water was used throughout.

Luminol was purchased from Shanxi Normal University and a stock solution of 10 mM luminol was prepared in 0.1 M sodium carbonate. The series of luminol standards in the range of 0.2–1.4 mM was prepared by sequentially diluting the 10 mM solution with 0.1 M sodium carbonate. Potassium hexacyanoferrate (Shenyang First Reagent Works, Shenyang, China) was used to prepare a 50 mM aqueous solution. Thirty percent hydrogen peroxide (Yuanda Co. Ltd., Shanghai, China) was diluted to 1.5% aqueous solution in water. A mixed chemiluminescence reagent containing 48 mM hexacyanoferrate and 2 mM peroxide was used in the studies, except mentioned otherwise.

A 1.5 mm thick PMMA plates (Yongkui Organic Materials Co., Ltd., Shenyang, China) and qualitative filter paper (Fuyang Paper Works, Hangzhou, China) were used for a micropump fabrication. Glass plates precoated with chromium and photoresist (Shaoguang Microelectronics Corp., Changsha, China) were used for microfluidic chip fabrication.

2.2. Fabrication of the micropump

The schematic structure and a photograph of the micropump are shown in Fig. 1a–c, respectively. The fabrication of the pump was detailed in an earlier report [10]. A slightly modified design was used in this study. Briefly, the micropump was composed of four layers, the upper and lower ones being 35 mm × 25 mm × 1.5 mm PMMA plates. On the lower plate was fabricated a 6 mm × 3 mm × 0.5 mm reservoir, with a 1 mm i.d. fluid inlet and outlet vent, and 15–20 2.5 mm diameter holes, which functioned as evaporation apertures. The same number of holes was drilled on the upper plate. Two layers of filter paper were sandwiched between the two plates, with a 1 mm thick PDMS gasket surrounding the reservoir. The four layers were then fixed by four stainless-steel bolts. A 35–45 cm long 0.5 mm i.d. PTFE tube was connected to the pump inlet, functioning both as a connection conduit and as a waste-storage tube.

2.3. Fabrication of the microchip

The microfluidic chip was fabricated using standard photolithography, wet etching and a thermal bonding procedure described elsewhere [14]. Channels with a configuration shown in Fig. 2 were etched onto a 1.7 mm thick 20 mm × 30 mm glass plate with chromium and photoresist coating. Access holes for the reagent and sample inlets and for waste outflow were drilled into the etched plate with a 1 mm diamond drill bit at the inlet and waste terminals of the reactor. An identical sized blank glass plate was used as the cover plate, and thermally bonded to the etched plate. Fifteen millimeter sections of 75 μm i.d., 375 μm o.d. silica capillary (Reafine Chromatography Ltd., Hebei, China), were epoxied to the sample and reagent inlets after inserting into appropriately bored PDMS tubing fixed in the access holes. A 5 mm section of a 2 mm i.d., glass tube was fixed around the chip outlet using epoxy and used for connection to the micropump.

2.4. Setup of the μFIA-CL system

The experimental setup of the μFIA system is shown in Fig. 3. A photomultiplier tube (PMT) (Model H5784-02, Hamamatsu Photonics, Japan) was butted directly against the meandering channels of the chip for chemiluminescence detection, and the entire setup was housed in a light–tight box, with three small holes accommodating the sampling probe, reagent reservoir probe and waste conduit. The latter was connected to the micropump via the storage tube. A 2 mm i.d.,

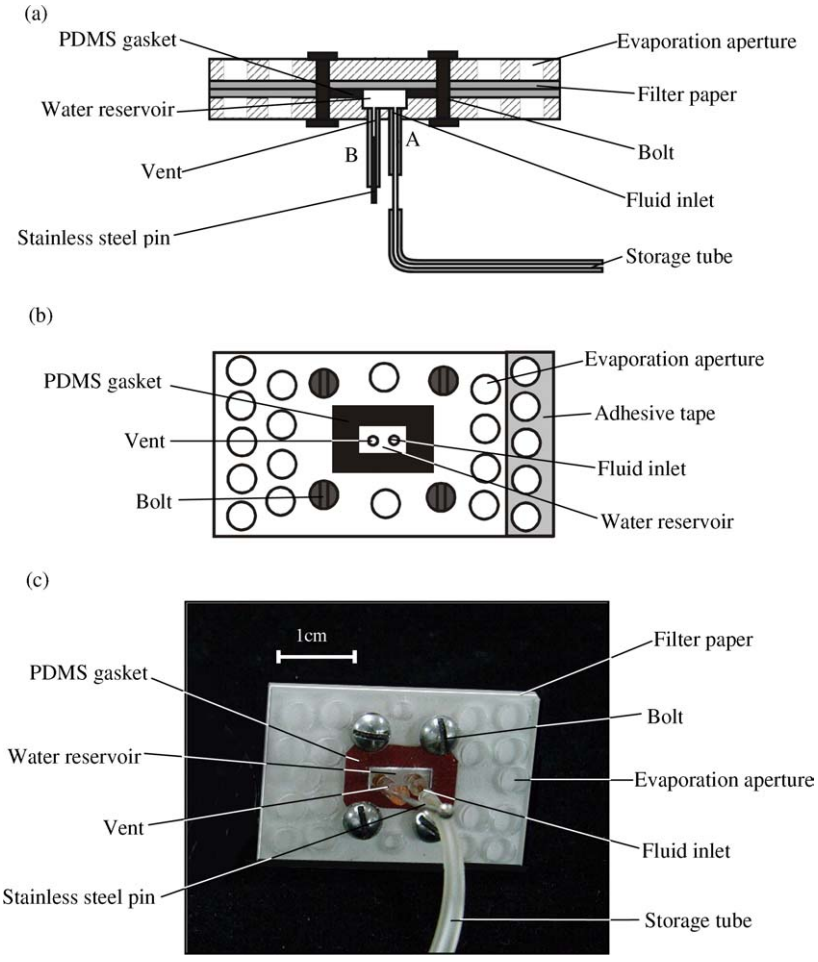

Fig. 1. Schematic diagram of the pump structure (a) side view, (b) bottom view and (c) photograph of the micropump based on capillary and evaporation effects. The adhesive tape shown in (b) was used to regulate the evaporation area (and flow-rate), by blocking some of the holes, and could be removed for achieving maximum flow-rate.

12 mm section glass tube was epoxyed around the extending reagent probe capillary, acting as the reagent reservoir.

2.5. Operations

Water was injected into the micropump from the storage tube before connecting to the microfluidic chip. With the outlet vent of the water reservoir positioned at an upper level, the reservoir in the pump was filled with water using a syringe, the filter paper layers were wetted and air segments trapped in the reservoir were released from the vent. The vent was then blocked using a stainless steel pin. Also using a syringe, water was then injected into the microchip from the waste outlet to fill the channels, with the chip slightly elevated at the outflow end to facilitate release of trapped air from the capillary probes. The water in the reagent tube was removed using a syringe and filled with the mixed hexacyanoferrate/peroxide reagent. The microchip was then connected to the micropump via the storage tube, taking care that no air bubbles were introduced during the connection and pumping was initiated. Luminol sample and blank solutions (functioning as carrier) were contained in plastic microcentrifuge vials with 1 mm wide slots cut 4 mm deep into the conical bottom of the vials, and fixed in an array on a holding platform in the sequence: sample A-blank-sample B-blank, etc. The sampling probe of the microchip was allowed to 'scan' through the slots of the vials of the array by linearly moving the platform relative to the position of the sampling probe, stopping

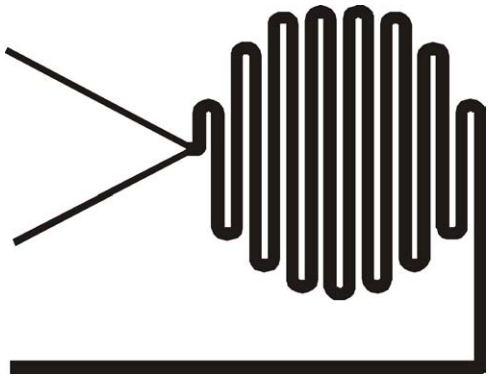

Fig. 2. Channel design of the microfluidic chip.

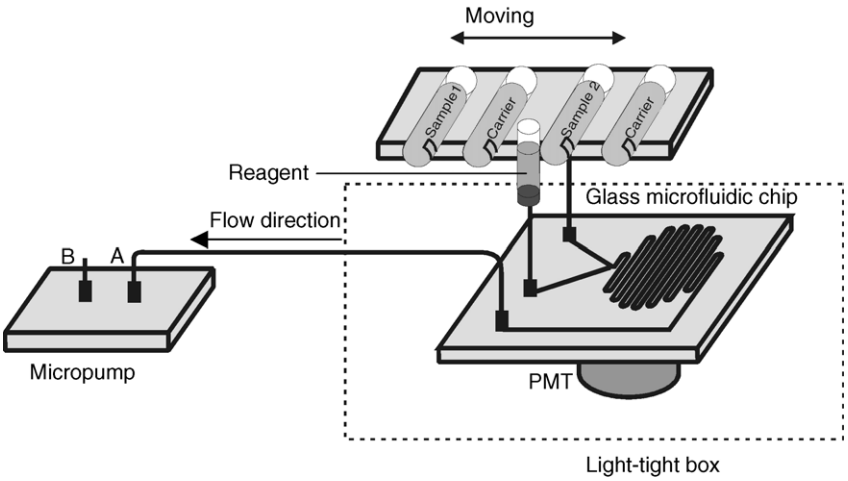

Fig. 3. Schematic diagram of the microfluidic flow injection CL system.

the movement for defined periods with the probe staying in each vial. The injected samples merged with the reagent flow and were mixed to generate chemiluminescence in the meandering reaction channel. The output signal from the PMT was processed by a computer software written in LabView, and peak signals were recorded.

3. Results and discussion

3.1. Design and performance of the micropump

The micropump used in this study was designed to continuously provide stable flow-rates of a few microliters per minute for fluid delivery in microfluidic chips within a few hours of operation. The micropump fulfilled the requirements of relatively small volume of 25 mm × 15 mm × 3 mm, low cost, easy fabrication, and reliable operation in the microliters per minute range for relatively long periods. The flow-rate is also easily adjustable by varying the evaporation area controlled by the number and diameter of evaporation apertures. The main differences in design of the present micropump from other capillary-effect based pumps are summarized as follows. First, by fabricating a shallow water reservoir on the lower chip, with a water layer directly contacting a filter paper layer, the fluid–capillary interfacing area was significantly enhanced, compared with direct extension of the flow inlet to branches of capillaries. This allowed a higher flow capacity without using microfabrication techniques. Second, the exposed filter paper layer also facilitated continuous operation of the pump through simple evaporation without using forced air flows, and flow-rate was easily and reproducibly controlled by simply adjusting the exposed area of the pump (e.g., the flow-rate could be easily reduced by covering some of the evaporation apertures using adhesive tape, as shown in Fig. 1b). Third, a storage tube served both as fluid inlet and a reservoir for holding the waste flows from the chip, to prevent them entering the water reservoir within the pump, and contaminating the filter paper. If that happened, the flow-rate could be affected through variations in vapor pressure. The present design is also modified on the basis of the previous design by increasing the evaporation area to enhance the flow-rate range without increasing the dimensions of the micropump. This was achieved by substituting most part of the PDMS gasket, that used to cover the entire area of the lower PMMA plate, by a second layer of filter paper, leaving only a circular gasket around the reservoir. This was sufficient for avoiding leakage, while the evaporation area was doubled by additionally exposing the lower paper layer to evaporation apertures machined on the lower PMMA plate.

One of the main drawbacks of all micropumps based on capillary and evaporation effects, although rarely addressed in detail in related publications, is the influence of ambient temperature and humidity on flow-rate. However, in our earlier studies using the present micropump [10], we found that under constant humidity, the flow-rate increased by 3% with each degree variation in temperature when the temperature increased from 25 to 30 °C. The influence of humidity was found to be more complicated. Below a relative humidity of 40%, the variation of flow-rate was <1% for unit percentage change in relative humidity, but could approach 10% at high relative humidities of about 90%. However, under reasonable conditions of <70% relative humidity, the variation in flow-rate for each percentage change in humidity is expected to be about 3%. Such a degree of stability should fulfill requirements for most applications. However, in that work the performance of the pump was only tested without workloads, and has not been used with real analytical systems. Since the pump can only be used in the draw-out mode, the force supplied by the pump was studied in this work by measuring the flow-rate at different heights of water column suspended below the pump, using the storage tube as the water column, prolonged when necessary. The relation between flow-rate and pressure is shown in Fig. 4. The results showed that capillary force provided by the filter paper layers of the micropump was able to support a 240 cm water

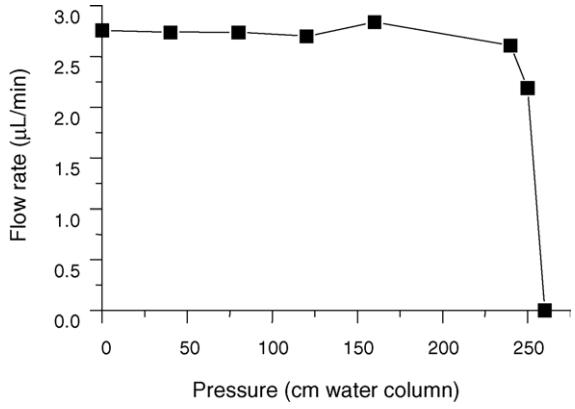

Fig. 4. Micropump flow-rate as a function of pressure in terms of suspended water column heights. Temperature range, 20–21 °C; relative humidity range, 36–38%; pump evaporation area, 6.6 cm^2.

column, corresponding to 23.5 kPa, below which height the flow-rate remained almost constant, but abruptly degraded to zero when larger than that. This compares favorably with the pressure produced by more sophisticated diaphragm type micropumps with piezo, thermo-pneumatic, or electrostatic actuation, which typical supply output pressures of a few tens kilopascal or less [15]. Experiments using different evaporation areas (by blocking some of the apertures) showed that the height of the water column was not affected by the area exposed, while flow-rates were varied. This is quite understandable, since the column is supported by the capillary forces created in the filter paper, and not by evaporation effects.

The microfluidic chip described in Section 2 was employed as a workload for the micropump to study the stability of flow-rate within a few hours of continuous operation. The outlet of the chip was connected to the pump, while one of the two inlets was blocked and the other connected to a transport conduit addressing a water reservoir. The latter was covered by a layer of silicon oil to avoid evaporation, and positioned on the weighing pan of a balance to measure the liquid outflow rate, as detailed elsewhere [10]. Measurements were made automatically every 2 min under computer control, and stored. Using a micropump with an evaporation area of about 6.6 cm^2, and with the ambient temperature and relative humidity fluctuating in the ranges 20–21 °C and 30–32%, respectively, within 2 h, the average flow-rate in the period was 3.02 μL/min, with a precision better than 1.2% R.S.D. ($n = 61$). During the study, no attempts were made to control conditions of air convection in the ambient environment. The performance of the pump was thus considered to be sufficiently reliable and robust for most microfluidic applications.

3.2. Design of the microfluidic chip

The chip design reported by Du et al. [5], which was used for flow injection (FI) photometric detection employing a gravitational drive, was adapted to study the performance of the micropump in a FI chemiluminescence (CL) detection system. The channel design of the microfluidic chip is shown in Fig. 2, with a Y-configuration to allow merging of reagents with the sample/carrier stream. The meandering channel downstream of the merging point served both as a mixing reactor and as the flow-cell for detection, with the channel area coinciding with that of the PMT window, which was positioned directly below the chip. This allowed efficient collection of CL emission with a simple setup. Silica capillary probes fixed to the two inlets were used to address the slotted sample/carrier vials and the mixed CL reagent, respectively. When the outlet channel was connected to the micropump via the storage tube, the sampling probe was used for performing valveless nanoliter sample injection by linearly moving the vial array in the sequence: carrier-sample A-carrier-sample B-carrier, etc.

3.3. Performance of the micro FI-CL system driven by the micropump

The performance of the micro FI-CL system driven by the micropump based on capillary and evaporation effects was studied using a model system involving the well-known luminol/hexacyanoferrate/H_2O_2 CL reaction, employing the procedure described in Section 2, using luminol as a sample. Samples were injected for 10 s at 110 s intervals, during which carrier solution was aspirated through the sampling probe. No efforts were made in this work to optimize the CL reaction conditions, and the work here was conducted only to show the feasibility of using the micropump as a fluid drive for the micro FI system. The recordings for the analysis of a series of samples are shown in Fig. 5. The results showed a linear response in the range 0.2–1.4 mM with a regression equation

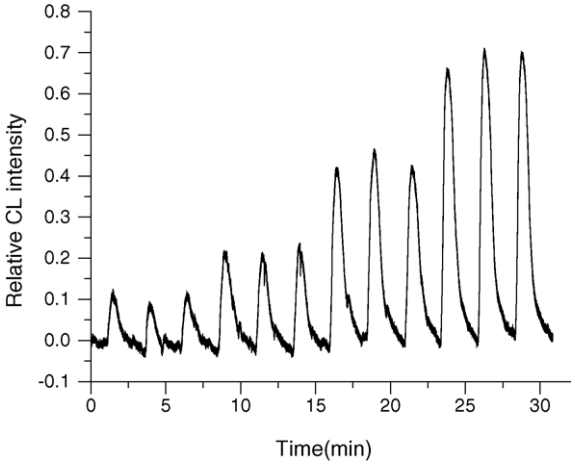

Fig. 5. Typical recordings of sequentially injected 0.2, 0.6, 1.0 and 1.8 mM luminol standards. Samples were injected for 10 s at 110 s intervals, during which water carrier was aspirated through the sampling probe. CL reagent, 48 mM hexacyanoferrate + 2 mM H_2O_2; temperature range, 20–21 °C; relative humidity range, 30–32%; pump evaporation area, 6.6 cm^2; total flow-rate, 3.02 μL/min.

of: $y = 2 \times 10^{-3} + 0.46C$ ($r^2 = 0.9971$), where y is the CL signal intensity and C the luminol concentration. A precision of 1.4% R.S.D. ($n = 11$) was achieved using a 10 mM luminol sample.

4. Conclusion

The feasibility of using a simple micropump operated via capillary and evaporation effects as a liquid drive for microfluidic analytical systems is demonstrated in this work. Obviously, its usefulness is not restricted to the CL reaction system used in this study, but could be extended to a broad range of applications involving fluid sampling and reactions. The pump has proved to be sufficiently robust and reliable for continuous operation in the microliters per minute range for at least a few hours. Although not pursued in this work, the micropump offers full potentials for on-chip integration on a platform not exceeding the size of a credit card, without using any peripheral equipment, such as valves and/or pressure sources. Further work is being conducted toward these directions.

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