# Microfluidic chip-based valveless flow injection analysis system with gravity-driven flows<sup>†</sup>

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In this work, a microfluidic chip-based valveless flow injection analysis (FIA) system with gravity-driven flows and liquid-core waveguide (LCW) spectrometric detection was developed. Automated sample injection in the 0.3–6.4 nL range under gated injection mode was achieved by controlling the vertical position of the waste reservoir fixed on a moving platform and the residence time of the reservoir in each position, without the requirement of microvalves or electrokinetic manipulation. An integrated LCW spectrometric detection system was built on the chip by coupling a 20 mm-long Teflon AF 2400 capillary with the microchannel to function as a LCW flow cell, using a green LED as light source and a photodiode as detector. The performance of the system was demonstrated in the determination of  $[NO_2]^{2-}$  based on the Saltzman reaction. Linear absorbance response was obtained in the range of 0.1–20 mg L<sup>-1</sup> ( $R^2 = 0.9910$ ), and a good reproducibility of 0.34% RSD (n = 17) was achieved.

# Introduction

Flow injection analysis (FIA), first introduced by Ruzicka and Hansen in the mid-1970s,<sup>1</sup> is now a well-established discipline for automated solution analysis. Since the first realization of miniaturized total analysis systems (MicroTAS) in the 1990s,<sup>2</sup> rapid progress has been made in the miniaturization of various functional components of analysis systems, including FIA systems. Since 1995, various miniaturized FIA systems based on microfluidic chips have been reported.

Haswell's group has reported a series of works on developing chip-based micro-FIA systems using electroosmosis flow (EOF) pumps to perform fluids driving and valveless sample injection in the picolitre to nanolitre range. These systems were applied in the determination of orthophosphate,<sup>3,4</sup> nitrate<sup>5</sup> and nitrite<sup>6</sup> with electrochemical and spectrophotometric detection. The advantages of EOF driving systems include simple structure and ease of operation. However, the flow rates of EOF driving fluids are easily affected by the variations of pH, ionic strength and composition of the fluids, as well as the surface property of the chip channel.

Leach<sup>7</sup> *et al.* employed poly(dimethylsiloxane) (PDMS) multiple pneumatically driven microvalves and micropumps to control the fluids in the channels to achieve FI operation on a monolithic FIA chip. Recently, Kuwata *et al.* reported a sliding microvalve to perform fluids switching and quantitative sample injection of 10 nL.<sup>8</sup> However, this kind of microvalve usually needs expensive and complicated microfabrication techniques, which may limit its application in routine laboratories. There is another type of miniaturized FIA system, coupling conventional macroscale injection valves and pumps to microfluidic chips to perform sample mixing, reaction and detection in the microchannels.<sup>9,10</sup> Such systems were easy to be built, while the improvements in system miniaturization and sample/reagent consumption (usually in the microlitre range) were limited.

In 2005, we developed a microfluidic chip-based FIA system based on slotted-vial array sample introduction and liquidcore waveguide (LCW) spectrometric detection systems.<sup>11</sup> Highthroughput valveless FI sample injection in the nanolitre range was achieved using the slotted-vial array system. Gravity driving approach was adopted in the system, which has the advantages of simple structure, ease of operation and without the need of additional power supply for driving the system. Guan *et al.* developed a micropump based on capillary and evaporation effects, which was used in a micro-FIA system with slotted-vial array sample introduction and chemiluminescence detection.<sup>12</sup>

In this work, a microfluidic chip-based FIA system under gated injection mode with gravity-driven flows and LCW spectrometric detection was developed. Valveless sample injection in the nanolitre range was achieved by adjusting the liquidlevel difference in the gravity-driven system, without resorting to mechanical valves, EOF manipulation or slotted-vial array systems. The present system was applied in the determination of nitrite<sup>13</sup> to demonstrate the performance.

# Experimental

## Chemicals and reagents

All chemicals were of analytical reagent grade, and demineralized water (Milli Q, Millipore) was used throughout. The stock solution of  $[NO_2]^{2-}$  (100 mg L<sup>-1</sup>) was prepared by dissolving 0.075 g sodium nitrite in 50 mL water. The working standard solutions were prepared daily by suitable dilution of the stock

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solution with 5% acetic acid. The reagent solution was prepared by mixing 10 mL 0.5 g L<sup>-1</sup> *N*-(1-naphthyl) ethylenediamine dihydrochloride (NED, Sinopharm Chemical Reagent Co., China) solution with 2.5 mL acetic acid and 25 mL 10 g L<sup>-1</sup> aminosulfonic acid solution, and made up to 50 mL with water. Before use, all of these solutions were stored at 4 °C.

Fluorescein sodium (Sangon Biotechnology Co., Shanghai, China) was used as indicator for observing the sample injection process under a CCD camera.

#### Fabrication of microchip based FIA system

The micro-FIA system was composed of a microchip and a computer controlled sample injecting platform. The glass chips were fabricated using a procedure detailed elsewhere.<sup>14</sup> The configuration design of the microchip is shown in Fig. 1A. The channels were etched to a depth of 25  $\mu$ m and a width of 100  $\mu$ m. Access holes A, B and C with a diameter of 1.5 mm were fabricated at the terminals of the channels. Two 30 mm-long glass tubes with an inner diameter of 4.7 mm were affixed with epoxy on the chip surrounding hole A and B, serving as reagent and sample reservoir, respectively. A 1 mm-deep, 400  $\mu$ m-diameter hole (D) was drilled at the end of the reaction channel on the edge of the glass chip with a 350  $\mu$ m-diameter drill.<sup>15</sup> A 20 mm-long Teflon AF 2400 capillary (50  $\mu$ m i.d., 400  $\mu$ m o.d., Random Technology, San Francisco, USA) was inserted into hole D and sealed with epoxy, to function as a LCW absorption



**Fig. 1** Schematic diagram (A) of the structure for the microchannels in the FIA chip and (B) of the whole chip-based FIA system (not to scale).

detection flow-cell. The epoxy-freezing approach was empolyed in the sealing operation in order to minimize the dead volume at the interface of the capillary and the chip channel.<sup>16</sup> The outlet end of the LCW capillary was inserted into the T-shaped channel (0.5 mm i.d.) of a Plexiglass connector, sealed with silicone sealer, facing the window of the photodiode detector (Model OPT301, Texas Instruments, Yucson, USA), which was installed beside the glass wall of the connector block. A 30 mmlong, 4.7 mm-i.d. glass tube serving as waste reservoir 1 was connected to access hole C through a 15 cm-long tygon tube. Another 15 cm-long tygon tube was used to connect the Tshaped channel connector and waste reservoir 2, which was made from a 4.7 mm i.d., 30 mm-long glass tube. A green LED (538 nm, Hangke Electronics Co., Hangzhou, China) was used as light source, positioned close to the polished right side of the chip, 10 mm from the LCW capillary inlet without further focusing. All connections in the chip conduits were sealed with epoxy unless mentioned otherwise. The optical detection system of the micro-FIA system was shielded from ambient light using black vacuum cement.

The translation platform for sample injection was modified from a chart recorder (LM14–164, Dahua Instruments, Shanghai, China) by perpendicularly positioning the recorder to allow the pen holder of the recorder to move in the vertical direction. Waste reservoir 1 was horizontally fixed on the pen holder, and its movement in the vertical direction was under control of a computer program written in Labview (National Instruments, Austin, USA).<sup>11</sup> The detection signals were processed using LabVIEW software. Flow rate measurements were made by measuring the increase of liquid volume in waste reservoir 1 and 2 within a defined period.

#### Procedures

Before use, the chip conduits, connecting tubes and waste reservoirs were filled with water. The chip was oriented vertically and the reservoirs were all oriented horizontally. 300 µL NED solution was added into the reagent reservoir, and 300 µL sample solution into the sample reservoir. In the initial state, waste reservoir 1 was set at lower position L (Fig. 2(A1)) with a 6 cm liquid-level difference between the sample/reagent reservoir and waste reservoir 1. The sample solution flowed through the sample loading channel (B-O-C) (Fig. 1A) into waste reservoir 1, the reagent solution used as carrier mainly flowed into the reaction channel (A–O–D) with a split stream to waste reservoir 1. Sample introduction under gated injection mode was performed by raising the waste reservoir 1 to higher position H (Fig. 2(B1)), remaining at this position for 1 s to achieve sample injection to the reaction channel, and then recovering the reservoir to the initial position (Fig. 2(C1)). The introduced sample plug was mixed with the reagent solution in the reaction channel, and flowed through the LCW flow-cell into waste reservoir 2.

## **Results and discussion**

#### System design

Gated injection is a time-dependent quantitative sample injection technique for microchips, which was first proposed by



Fig. 2 Schematic diagrams and CCD images of sample injection process under gated injection mode.  $10^{-3}$  mol L<sup>-1</sup> fluorescein solution was used as indicator in the CCD images. (A) Initial state; (B) sample injection state; (C) recovered to the initial state.

Ramsey and co-workers<sup>17</sup> in 1994, and had been widely used in chip-based capillary electrophoresis (CE) systems. In 1999, the same group applied this injection approach in a chip-based FIA system for an acetylcholinesterase (AChE) inhibitor assay.<sup>18</sup> Usually, the gated injection on a microchip was conducted using a high-voltage supply capable of achieving high voltage switching between multiple electrodes. Lacharme et al.<sup>19,20</sup> used a high voltage power supply coupled with a computer controlled pressure pulse generation device to perform sample injection under gated injection mode in a CE chip, by mechanically deflecting a PDMS membrane placed on a dedicated chip reservoir. Schlund et al.<sup>9</sup> reported a chip-based pressure-driven liquid/solid chromatographic system employing gated injection mode. In the system, a gas source with a high-precision pressure regulator and gauge was required to control fluid pressures in the head-space over the analyte and mobile phase solutions, and four solenoid isolation valves were required to manipulate the fluid flows. Recently, Moehlenbrock et al.<sup>10</sup> described a chipbased FIA system using a conventional syringe pump and 6-port injection valve to achieve gated injection in the chip channels. Baldock et al.21 reported a gravity fed fluid handling system consisting of a component board fitted with four commercial solenoid valves and three solution reservoirs (formed from 20 mL syringe barrels) to perform sample introduction under gated injection mode for an isotachophoresis chip.

In this work, a gravity driven system was adopted in the FIA chip without the need of an EOF driven system or mechanical pumps. In most of the above-mentioned systems, usually a multiple-electrode, high-voltage switching device or commercial valves coupled with the driving systems were required to perform gated sample injection on microchips. However, in the present system, valveless gated sample injection was readily achieved by changing the vertical position of the reservoir.

In the preliminary experiments,  $10^{-3}$  mol L<sup>-1</sup> fluorescein solution was used as a model sample, and a microscope and CCD camera were used to observe the sample injection process. The flowing of the fluids in the microchannel network was controlled by the hydrostatic pressure created by the liquid level differences between the reservoirs. In the initial state, two separated streams of sample and reagent (as shown in Fig. 2(A2)) were formed in the sample loading and reaction channels, respectively, with a liquid-level difference of 6 cm between the sample/reagent reservoir and waste reservoir 1, and 10 cm between the sample/reagent reservoir and waste reservoir 2. A phase interface between the two laminar streams was formed at cross section O of the channels, with a minor portion (usually less than 1/4) of the stream of reagent solution flowed into the sample loading channel in order to avoid the leakage of sample solution into the reaction channel and detection flow-cell. Sample injection could be achieved by changing the vertical position of either waste reservoir 1 or reservoir 2. In this work, for convenience, waste reservoir 1 was chosen as the moving component to perform the sample injecting operation. When raising waste reservoir 1 to position H, the liquidlevel difference between the sample/reagent reservoir and waste reservoir 1 decreased, and a split sample stream flowed into the reaction channel to achieve sample introduction (Fig. 2(B2)). The injected sample volume was determined by the height difference between position H and L (i.e. the moving distance for waste reservoir 1) and the residence time of the reservoir at position H. With a moving distance of 4 cm for waste reservoir 1, a flux ratio of ca. 6 : 1 between the sample and reagent streams in the reaction channel was obtained, which was high enough to perform sample injection. Shorter moving distance would result in lower flux ratio, such as 1 : 6 corresponding to moving distance of 1 cm, which led to the sample dilution by the reagent solution during the sample injection process. Therefore, the height difference of 4 cm between position H and L was chosen in the following experiments, and the sample injection volume was adjusted by changing the sample injection time (see section "Effects of sample injection volume").

# Effects of liquid level differences between the sample/reagent reservoir and waste reservoir 2

The effects of the liquid level differences between the sample/reagent reservoir and waste reservoir 2 in the 5-16 cm in 1 cm intervals were studied with a sample injection time of 1 s. The results are as shown in Fig. 3. The increase of the liquid level differences led to the increase of reagent flow rate as well as sample flow rate in the reaction channel during the sample injection process, which resulted in the decrease of





**Fig. 3** (A) Typical recordings of absorbance *vs.* time curves with different liquid level differences between sample/reagent reservoir and waste reservoir 2, including 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 and 16 cm, corresponding to a flow rate in the reaction channel of 0.09, 0.12, 0.14, 0.16, 0.19, 0.21, 0.23, 0.26, 0.28, 0.30, 0.32 and 0.35  $\mu$ L min<sup>-1</sup>. (B) Effect of the liquid level differences between sample/reagent reservoir and waste reservoir 1 on response signals (n = 3). Sample injection time, 1 s;  $[NO_2]^{2-}$  concentration, 10 mg L<sup>-1</sup>.

signal peak width and the increase of sample injection volume under fixed sample injection time, respectively (Fig. 3A). In 5– 12 cm liquid level difference, the peak heights increased due to the increase of sample injection volume. The decrease in peak height above 12 cm liquid level difference may be caused by the reduction of reaction time of sample and reagent in the reaction channel under higher flow rate. The liquid level difference of 10 cm between sample/reagent reservoir and waste reservoir 2, corresponding to reagent flow rate of  $0.21 \,\mu L \,min^{-1}$ , was selected to obtain higher detection sensitivity.

#### Effects of sample injection time

The effects of the sample injection time of 0.2, 0.4, 0.8, 1, 1.5, 2, 3, 5 and 8 s, corresponding to injection volume of 0.3, 0.7, 1.3, 1.6, 2.1, 2.5, 3.4, 4.7 and 6.4 nL, were studied with a distance of 4 cm between position H and L for waste reservoir 1. The signal peak height and the analysis time for each cycle increased with the sample injection time and volume (as shown in Fig. 4). These results agreed with those reported in most of FIA systems. Larger injected sample volume significantly increased the analysis time for each cycle. A sample injection time of 1 s

**Fig. 4** (A) Typical recordings of absorbance *vs.* time curves with different sample injection volumes. (B) Absorbance (n = 3) *vs.* sample injection volume curve. Sampling time, 0.2, 0.4, 0.8, 1, 1.5, 2, 3, 5 and 8 s, corresponding to a sample volume of 0.3, 0.7, 1.3, 1.6, 2.1, 2.5, 3.4, 4.7 and 6.4 nL. Flow rate in the reaction channel, 0.21  $\mu$ L min<sup>-1</sup>. Other conditions as Fig. 3.

corresponding to an injection volume of 1.6 nL was selected, mainly to increase analysis throughput.

#### Analytical performance

The performance of the system was tested in the determination of sodium nitrite based on the Saltzman reaction under optimized conditions, including 1 s of sample injection time, and 10 cm of liquid level difference between sample/reagent reservoir and waste reservoir 2. A precision of 0.3% RSD (n =17) was achieved using 10 mg L<sup>-1</sup> [NO<sub>2</sub>]<sup>2-</sup> solution (Fig. 5). The sample consumption for each cycle was 1.57 nL, and the analysis throughput was 113 h<sup>-1</sup>. A linear range of 0.1–20 mg L<sup>-1</sup> for detection of [NO<sub>2</sub>]<sup>2-</sup> was obtained with a limit of detection (3 $\sigma$ ) of 0.19 mg L<sup>-1</sup>.

#### Conclusions

We demonstrated a simple and efficient chip-based FIA system without the need of a mechanical pump and valve. The present system supplied a simple operation approach for sample injection in microchips under gated injection mode by changing the vertical position of the waste reservoir.



**Fig. 5** Typical recording of repetitively injected sample to show the repeatability of the system. Sample injection time, 1 s;  $[NO_2]^{2-}$  concentration, 10 mg L<sup>-1</sup>; flow rate in the reaction channel, 0.21 µL min<sup>-1</sup>.

In this work, for convenience, a recorder with a relatively large size was used as the moving platform to achieve waste reservoir switching between two positions. If necessary, more compact platforms using small motors or modified from a CD driver could be readily adopted to achieve the same operation. Although the LCW absorption detection was employed in the present work, the micro-FIA system could be coupled with other detection approaches, such as fluorescence, chemiluminescence, electrochemistry or mass spectrometry. Since the gated injection mode allows a continuous sample flow in the system during the whole analysis process, the application of the present system could also be extended to on-line process monitoring.

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