

High-Throughput Nanoliter Sample Introduction Microfluidic Chip-Based Flow Injection Analysis System with Gravity-Driven Flows

Wen-Bin Du, Qun Fang,* Qiao-Hong He, and Zhao-Lun Fang

Institute of Microanalytical Systems, Zhejiang University, Hangzhou, China

In this work, a simple, robust, and automated microfluidic chip-based FIA system with gravity-driven flows and liquid-core waveguide (LCW) spectrometric detection was developed. The high-throughput sample introduction system was composed of a capillary sampling probe and an array of horizontally positioned microsample vials with a slot fabricated on the bottom of each vial. FI sample loading and injection were performed by linearly moving the array of vials filled alternately with 50- μ L samples and carrier, allowing the probe inlet to enter the solutions in the vials through the slots sequentially and the sample and carrier solution to be introduced into the chip driven by gravity. The performance of the system was demonstrated using the complexation of *o*-phenanthroline with Fe(II) as a model reaction. A 20-mm-long Teflon AF 2400 capillary (50- μ m i.d., 375- μ m o.d.) was connected to the chip to function as a LCW detection flow cell with a cell volume of 40 nL and effective path length of 1.7 cm. Linear absorbance response was obtained in the range of 1.0–100 μ M Fe(II) ($r^2 = 0.9967$), and a good reproducibility of 0.6% RSD ($n = 18$) was achieved. The sensitivity was comparable with that obtained using conventional FIA systems, which typically consume 10 000-fold more sample. The highest sampling throughput of 1000 h⁻¹ was obtained by using injection times of 0.08 and 3.4 s for sample and carrier solution, respectively, with a sample consumption of only 0.6 nL for each cycle.

Flow injection analysis (FIA) is now a well-established discipline for automated solution analysis at 10–10³- μ L levels.¹ Attempts at further miniaturization of FIA systems on the basis of full integration of functional components are well-reflected in the works of Ruzicka and Hansen's group in the mid-1980s, conducted under the concept of integrated microconduits (IMC).² It is not difficult to identify the similarity in concept of IMC with that of miniaturized total analysis systems (μ TAS), introduced by Manz and Widmer³ five years later. However, the two techniques were developed on significantly different technical platforms leading to different degrees of miniaturization. Basically conventional

mechanical fabrication tools were used for producing IMCs, whereas the microelectronic mechanical system (MEMS), developed to an advanced stage at the time, was considered as a basic platform for the realization of μ TAS. The latter technical approach facilitated integration of analytical functional components that allowed a higher degree of miniaturization in μ TAS than IMC. This is well-demonstrated in chip-based microfluidic systems, currently the most far-developed form of μ TAS, where sample handling is typically in the picoliter-to-nanoliter range. However, despite earlier efforts of producing complete MEMS-based μ TAS, including microfabricated pressure-driven pumps,⁴ main developments in the field happened using chips with microfabricated reaction and separation channels employing electrokinetic driving systems. Since the pioneering works of Harrison et al., in chip-based capillary electrophoresis (CE),⁵ this form of μ TAS has achieved tremendous progress.⁶ While the use of electrokinetic means for sample introduction and injection in chip-based CE is now well-established, and stays almost unchallenged, the most suitable drive for other chip-based analytical systems still remains a topic of investigation. Hitherto, MEMS-produced silicon-based pumps and valves for fluid manipulation generally have not lived up to earlier expectations owing to blockage, lifetime, and chemical compatibility problems, and their usage is very limited. Since 1995, Haswell's group^{7–10} has reported a series of works on developing micro-FIA systems fabricated on glass substrates using photolithographic and wet etching techniques, employing electroosmosis flow (EOF) both for the mobilization of reagents and for sample injection in the spectrophotometric determination of orthophosphate,^{7,8} nitrite,⁹ and nitrate.¹⁰ Ramsey's group¹¹ also reported the use of electrokinetic pumping and injection for a chip-based enzymatic assay. The main advantage of electrokinetic pumping is the capability of performing complicated fluid manipulation without requirement for valves, although a programmable multiterminal high-voltage power supply is required instead.

(4) Manz, A.; Fetting, J. C.; Verpoorte, E.; Ludi, H.; Widmer, H. M.; Harrison, D. J. *Trends Anal. Chem.* **1991**, *10*, 144.

(5) Harrison, D. J.; Manz, A.; Fan, Z.; Lüdi, H.; Widmer, H. M. *Anal. Chem.* **1992**, *64*, 1926.

(6) Khandurina, J.; Guttman, A. J. *Chromatogr., A* **2002**, *943*, 159.

(7) Daykin, R. N. C.; Haswell, S. J. *Anal. Chim. Acta* **1995**, *313*, 155.

(8) Doku, G. N.; Haswell, S. J. *Anal. Chim. Acta* **1999**, *382*, 1.

(9) Greenway, G. M.; Haswell, S. J.; Petsul, P. H. *Anal. Chim. Acta* **1999**, *387*, 1.

(10) Petsul, P. H.; Greenway, G. M.; Haswell, S. J. *Anal. Chim. Acta* **2001**, *428*, 155.

(11) Hadd, A. G.; Jacobson, S. C.; Ramsey, J. M. *Anal. Chem.* **1999**, *71*, 5206.

* To whom correspondence should be addressed. Tel.; +86-571-88273496. Fax: +86-571-88273496. E-mail: fangqun@mail.hz.zj.cn.

(1) Ruzicka, J.; Hansen, E. H. *Flow Injection Analysis*; John Wiley & Sons: New York, 1981.

(2) Ruzicka, J.; Hansen, E. H. *Anal. Chim. Acta* **1984**, *161*, 1.

(3) Manz, A.; Graber, N.; Widmer, H. M. *Sens. Actuators, B* **1990**, *1*, 244.

The conditions for optimized EOF (pH, ionic strength, buffer composition) often differ substantially from those for the main reaction of the FIA system, and compromising conditions that deviate from optimum may have to be used instead.¹² In electrokinetic pumping, special precautions are also required to avoid decomposition of reagents in the reagent reservoir by electrolytic or thermal effects.⁹ More recently, Leach et al.¹³ reported a microfluidic FIA system incorporated within single two-layer poly-(dimethylsiloxane) monolith multiple pneumatically driven peristaltic pumps, an injection loop, a mixing column, and a transparent window for fluorescence detection, in which the limitations of electrokinetic sample injection were avoided. Besides the miniaturized FIA systems mentioned above, conventional syringe pumps and valves were also used for injecting samples into microfabricated chip-based reaction channels and flow cells.^{14–17} However, reported improvement in performance using such approaches for reducing sample/reagent consumption appears rather limited, owing to the weak compatibility inherent in such combinations.

In this paper, we report a simple and robust microfluidic FIA system based on a new concept for high-throughput chip-based sample changing/loading/injection, characterized by the sweeping of a chip-integrated sampling probe through an off-chip array of bottom-slotted vials holding samples and carrier. To achieve simplicity and reliability, gravity-driven flows were used for realizing reproducible nanoliter sample injection, mixing with reagent, transport, and detection in a liquid-core waveguide (LCW) flow cell under high throughput. Preliminary results obtained using the system were reported in a short communication a few months ago,¹⁸ since then, the system has been fully characterized, further refined, and optimized for achieving sample throughputs of over 1000 h⁻¹ with low carryover.

EXPERIMENTAL SECTION

Chemicals. All chemicals used were of reagent grade unless mentioned otherwise, and demineralized water was used throughout. The stock solution of 2 mM Fe(II) was prepared by dissolving 0.556 g of FeSO₄·7H₂O in 50 mL of 0.1 M HCl and diluting to 1000 mL with water. The series of Fe(II) standard solutions in the range of 1–100 μM were prepared by sequentially diluting the stock solution with water. The *o*-phenanthroline solution was prepared by dissolving 0.5 g of *o*-phenanthroline in 50 mL of 80% ethanol solution, adding 2 g of hydroxylamine hydrochloride, 13.6 g of sodium acetate, and 30 mL of 1 M HCl into the solution, and making up to 1 L. The pH of the *o*-phenanthroline solution was adjusted to 5.00 with 1 M HCl solution. Demineralized water was used as carrier solution for the FI system. A solution of 0.5% brilliant blue food dye was prepared by dissolving 0.5 g of brilliant blue in 100 mL of water and used as an indicator for observing the sample introduction process under a CCD camera.

Fabrication of the Microchip Device. Standard photolithographic and wet chemical etching techniques were used for fabricating channels onto a 1.7-mm-thick 6 × 9 mm glass plate with chromium and photoresist coating (Shaoguang Microelectronics Corp., Changsha, China). The chip with a design shown in Figure 1A was fabricated using a procedure detailed elsewhere.¹⁹ An access hole for the reagent reservoir was drilled into the etched plate with a 1.5-mm-diameter diamond-tipped drill bit at the inlet terminal of the reagent channel. An identical sized blank glass plate was used as cover plate. The reagent reservoir was produced from a 4.7-mm-i.d., 13-mm-section plastic tube, cut from a commercial 1-mL disposable syringe, perpendicularly affixed with epoxy on the etched plate, surrounding the access hole. A 5-mm-long polymer-coated fused-silica capillary (75-μm i.d., 375-μm o.d.; Reafine Chromatography Ltd., Hebei, China), functioning as the sampling probe, was connected to the inlet of the sample introduction channel on the glass chip. A 3-mm section of the polymer coating at the distal end of the capillary was removed by burning and used as is during earlier studies. In later studies, during which most of the data used in this paper were collected, the exposed section was treated with dichlorodimethylsilane for 10 min to produce a hydrophobic surface that reduced sample carryover. A 20-mm-long Teflon AF 2400 capillary (Random Technologies, San Francisco, CA; 50-μm i.d., 400-μm o.d.) was connected to the outlet of the microchannel to function as a LCW absorption detection flow cell. Connection between the capillary and the chip channel was achieved with minimal dead volume mainly following a procedure described by Bing et al.²⁰ Briefly, the terminal of the channel on the edge of the glass chip was first drilled 4 mm deep with a 1.1-mm diamond-tipped drill bit, and the bottom of the hole was drilled 0.7 mm deep with a 400-μm-diameter flat-tipped drill, the inlet terminal of the capillary was inserted into the hole, and the joint was sealed with epoxy.

Micro-FIA System. The high-throughput sample introduction FIA system was composed of a microchip with a capillary sampling probe, reagent reservoir, and an array of microsample vials fixed on a home-built autosampler platform, modified from a chart recorder (LM14-164, Dahua Instruments, Shanghai, China), holding a tray on which fixed positions for 30 vials were reserved. The sample vials were produced from 0.2-mL microtubes (Porex, Petaluma, CA) by fabricating 1.5-mm-wide, 2-mm-deep slots on the conical bottom of the tubes for pass-through of the sampling probe. The slotted sample vials were horizontally fixed on the platform in an array, with the slot of each vial positioned horizontally to allow free passage of the sampling probe through all the vial slots sequentially by linearly moving the platform along the direction of the array. The autosampler platform was fixed to the pen holder of the recorder and driven under computer control along the array direction within a maximum moving distance of 30 cm to programmed positions for defined periods. A step movement between neighboring vials required less than 0.2 s, the timing precision was better than 0.1% for a 2-s stop period, and the spatial precision of the platform was better than 0.25 mm.

The Teflon AF LCW absorption flow cell integrated on the chip was masked from ambient light with a section of black plastic tubing. A green LED (508 nm, Hangke Electronics Co., Hangzhou,

(12) Rainelli, A.; Stratz, R.; Schweizer, K.; Hauser, P. C. *Talanta* **2003**, *61*, 659.

(13) Leach, A. M.; Wheeler, A. R.; Zare, R. N. *Anal. Chem.* **2003**, *75*, 967.

(14) Xu, Y.; Bessoth, F. G.; Eijkel, J. C. T.; Manz, A. *Analyst* **2000**, *125*, 677.

(15) Yakovleva, J.; Davidsson, R.; Bengtsson, M.; Laurell, T.; Emneus, J. *Biosens. Bioelectron.* **2003**, *19*, 21.

(16) Jorgensen, A. M.; Mogensen, K. B.; Kutter, J. P.; Geschke, O. *Sens. Actuators, B* **2003**, *90*, 15.

(17) Greenwood, P. A.; Greenway, G. M. *Trends Anal. Chem.* **2002**, *21*, 726.

(18) Fang, Q.; Du, W.-B.; He, Q.-H.; Fang, Z.-L. *Gaodeng Xuexiao Huaxue Xuebao (Chem. J. Chinese Universities)* **2004**, *25*, 1442.

(19) Jia, Z.-J.; Fang, Q.; Fang, Z.-L. *Anal. Chem.* **2004**, *76*, 5597.

(20) Bings N. H.; Wang, C.; Skinner C. D.; Colyer C. L.; Thibault P.; Harrison D. J. *Anal. Chem.* **1999**, *71*, 3292.

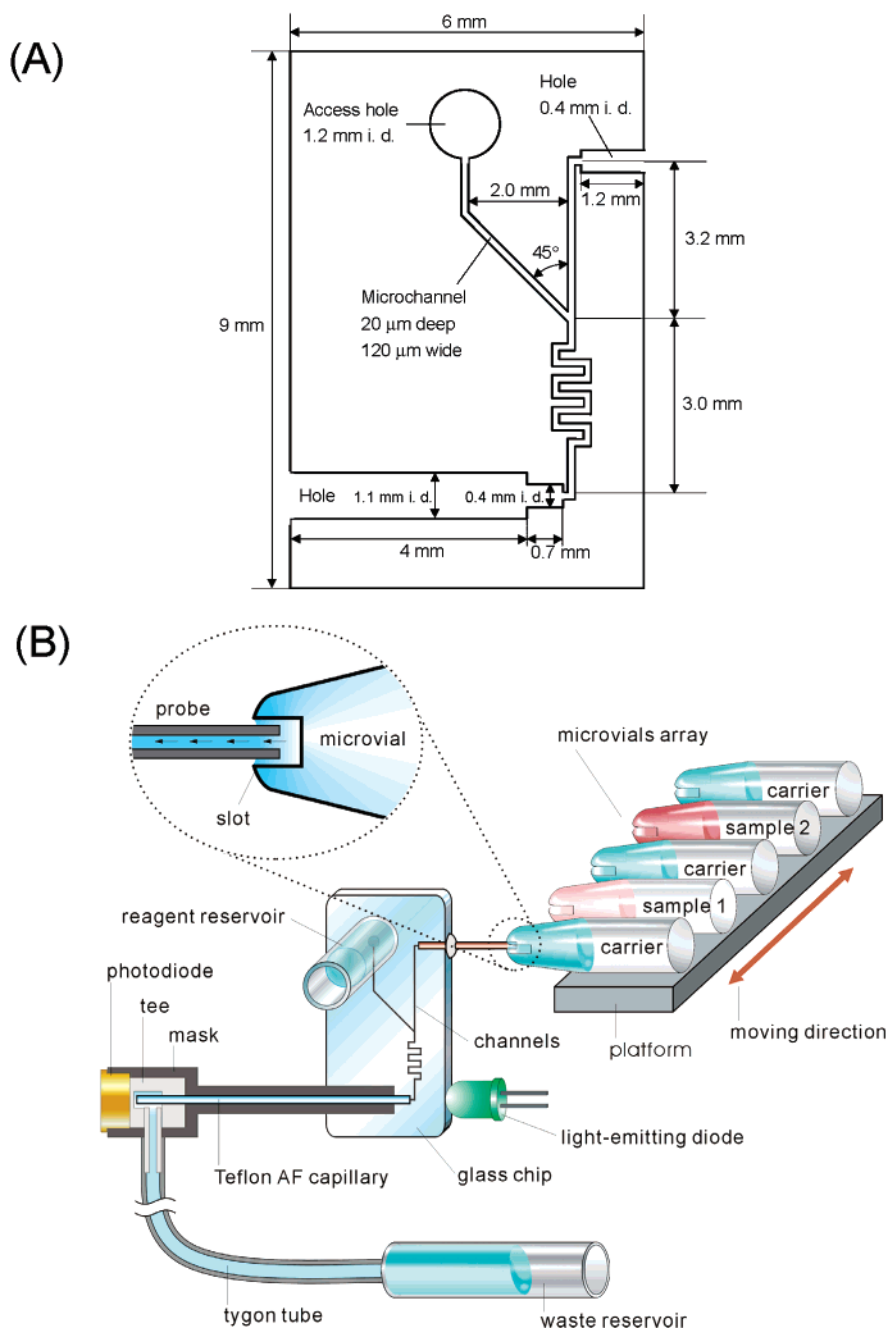


Figure 1. Schematic diagram (A) of the structure for the microchannels in the FIA microchip system and (B) of the microchip-based FIA system (not to scale).

China) was used as light source, positioned 3 mm from the LCW capillary inlet without further focusing. The outlet end of the LCW capillary was inserted into the T-shaped channel (0.5-mm i.d.) of a Plexiglas connector, reaching 0.2 mm to the end of the channel turning point, facing the window of the photodiode detector (model OPT-301, with integrated amplifier, Texas Instruments, Tucson, AZ), which was installed inside the wall of the connector block. LabVIEW software (National Instruments, Austin, TX) was used to process the detection signals. A section of Tygon tubing (110-cm length, 2.0-mm i.d.) was connected to the outlet of the connector via a stainless steel capillary (0.8-mm i.d., 1-mm o.d.), and its distal end was connected to a horizontal plastic tube (4.7-mm i.d., 70-mm length, cut from a commercial 1-mL disposable

syringe) as waste reservoir. All connections in the flow conduit were sealed with epoxy, and the sections of conduit in the detection system were masked from ambient light with black plastic tubing or black paint.

The chip and detection system were fixed onto a 5×5 cm Plexiglas plate in upright position, with the reagent reservoir on top. The horizontally positioned waste reservoir was fixed at a level lower than the outlet of the LCW flow cell. The flow rates of the reagent and sample/carrier flows were adjusted by varying the level of the waste reservoir in relation to that of the reagent reservoir/sampling probe.

For direct viewing and recording of the sample introduction process, a stereo microscope (SZ-45B3, Sunny Instruments Co.,

NingBo, China) equipped with a CCD camera (YH-9628, Yonghui Technology Development Co., Shenzhen, China) was used.

Flow rate measurements were made with a gravimetric approach, by estimating the loss in water weight from an upstream vial within a defined period (for sample loading flow rate) or by estimating the weight of water collected in a downstream vessel at the waste outlet (for total flow rate).

Procedures. The conduits in the chip and the connecting tubes were filled with carrier, and 300 μL of *o*-phenanthroline solution was pipetted into the horizontal reagent reservoir. The fluids in the channels were driven by hydrostatic pressure produced from differences in liquid levels of the reservoirs. The vials of the sample vial array were filled alternately with 10–50- μL samples and blank solution (carrier). Sample injection was performed with the chip position unchanged, while linearly moving the array of vials, allowing the sample probe inlet to sweep into the slots, and residing in the sample and carrier solution vials for predetermined periods, programmed by the autosampler. The sample/carrier solutions were pulled by gravity into the probe capillary and chip channel. Thus, sample injection was performed by linearly moving the array of vials, in order of carrier, sample 1, carrier, sample 2, carrier, etc. The injected samples merged at the Y-juncture with the reagent flow, mixed with the reagent downstream in the mixing channel, and the reaction product was detected in the LCW flow cell.

Safety Considerations. *Wet etching of the glass chips should be performed in a well-ventilated hood, while wearing protective gloves and goggles.*

RESULTS AND DISCUSSION

General Objectives and Considerations in System Design.

The main objective of this work was to develop a simple and robust sample injection system for chip-based FIA, suitable for high-throughput absorbance photometric determinations using nanoliter-volume samples. A subordinate aim was to achieve detection power comparable to conventional FIA on that basis. The challenges associated with this aim were multiple: first, the design and development of a reproducible nanoliter volume injector, suitable for noninterrupted injection of a series of different samples at high throughput and low carryover; second, development of a robust driving system suitable for performing such injections and transporting the injected sample and reagent to a flow-through detector; and third, the design and development of a compact chip-integrated long path length flow cell suitable for absorbance measurements with nanoliter-volume samples. Designing principles followed in this work for facing the above challenges are summarized as follows: (a) glass chip containing microfabricated fluidic channels for sample introduction, reagent merging, and mixing, with on-chip integration of a capillary sampling probe and an LCW flow cell; (b) liquid transport by a gravitational approach with downstream fluid control,²¹ created by differential liquid levels, using horizontally positioned reagent/waste reservoirs; (c) off-chip sample/carrier presentation in microvials with a slot fabricated in the bottom,²² as an aperture for sample/carrier aspiration; (d) sample aspiration (time-based loading) achieved through a capillary probe connected to the microfluidic chip by

sweeping the probe into the slots of horizontally positioned vials and stopping for defined periods; (e) sample injection achieved by programmed movement of an array of sample and carrier vials across the stationary sampling probe; (f) sensitivity enhancement by using a long path length LCW flow cell produced from Teflon AF capillary.

The above principles may be readily identified in the design of the micro-FIA system presented in Figure 1B.

Sample Presentation and Injection. Hitherto, in most microfluidic chip-based analytical systems, on-chip fabricated reservoirs were used to contain sample and reagent solutions. Although on-chip reservoirs improved the degree of integration, each sample change required that the sample reservoir be manually emptied, rinsed, and refilled with the new sample solution. Multiple sample reservoirs may be fabricated on a single chip¹³ for achieving high-throughput serial assays. However, each additional reservoir requires a separate driving control terminal, and in addition to complications in chip-design, sophistication of peripheral equipment also increases proportionally with the number of reservoirs, rendering the approach impractical beyond a limit of, at most, 10–20 samples. Obviously, such operations are not acceptable for chip-based FIA systems, from which, to be practical, at least sampling frequencies of 60–100 h^{-1} are expected. It becomes questionable, therefore, whether on-chip reservoirs (unless with flow-through function) should be used as sample vessels for high-throughput microfluidic systems such as chip-based FIA.

In this work, a sample introduction system comprising an on-chip capillary sampling probe that substituted the sample reservoir and an off-chip sample presentation system with slotted sample vial array was used to achieve sample changing and injection in the FIA system. Automated sample change was achieved by linearly moving a sample vial array in one direction, with the sampling probe sequentially sweeping into the sample/carrier vials for determined periods. The slots fabricated in the sample vials significantly facilitated sequential passing of a capillary sampling probe through each vessel of the vial array and achieved sample change without resorting to two-dimensional movement. High-throughput sample change up to several thousand samples per hour with low carryover may be obtained with the present system by using a sweep–stop sampling mode (see Analytical Performance).

In the present system, the slots in sample vials were fabricated with a width of 1.5 mm to ensure easy sweep-through of the sampling probe (0.38-mm o.d.). Within a maximum slot width of 2.5 mm, the bulk of aqueous sample and carrier solutions in the vials were retained in the vials through surface tension between the slot and vial walls, without leakage through the slots.

Based on the present sample introduction system, instead of adopting a volume-based sample injection approach that has been employed in most other chip-based FIA, using both electrokinetic^{7–11} and pressure driving means,¹³ a time-based injection²³ involving sequential aspiration of sample and carrier from separate vessels lined in an array was devised. FIA operation was achieved efficiently simply by introducing carrier into the sampling probe before and after a sample was introduced, i.e., by sweeping the probe through the vial array in the sequence carrier–sample–

(21) Huang, Y.-Z.; Fang, Q.; Li, D.-N. *Gaodeng Xuexiao Huaxue Xuebao (Chem. J. Chinese Universities)* **2004**, 25, 1628.

(22) Fang, Q.; Du, W.-B.; He Q.-H. Chinese Patent, Appl. No. 03114734.8, 2003.

(23) Riley, C.; Rocks, B. F.; Sherwood, R. A. *Talanta* **1984**, 31, 879.

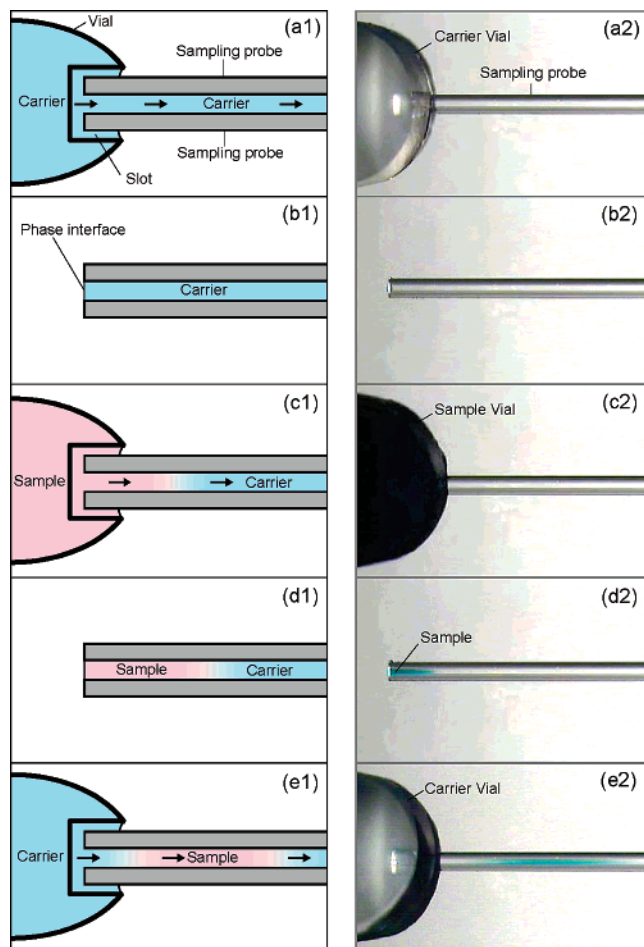


Figure 2. Sample loading and injection with the probe–slot–vial combination (a1), (b1), (c1), (d1), and (e1) working principle and (a2), (b2), (c2), (d2), and (e2) CCD images of the sampling probe during an sample injection cycle. 0.5% (w/v) brilliant blue solution used as sample.

carrier. The carrier and sample plugs flowed into the mixing and reaction channel of the chip under hydrostatic pressure (discussed in detail later). The amount of sample loaded could be changed in the nanoliter range (0.1–100 nL) by adjusting the solution flow rate and residence time of the probe within the vial. Figure 2 shows the working principle and typical CCD images of the sampling probe during the various stages of a FI operation process using 0.5% (w/v) brilliant blue solution as sample and water as carrier, observed with a light background under fluorescent lamp illumination. A video clip of the FI process is available as Supporting Information. Dispersion of the sample zone in the carrier can be observed in Figure 2, with the parabolic shaped front of the injected sample zone (Figure 2 c1 and c2), characteristic of Poiseuille flows, gradually developing following injection into the carrier, and the dispersion increasing (Figure 2 d1, d2, e1, and e2).

Control of Gravity-Driven Flow. In the present FIA system, hydrostatic pressure produced from difference in liquid levels between the sample/reagent containers and the waste reservoir was employed to provide driving power for sample introduction (loading), injection, transfer, and mixing of sample and reagent. Hitherto, the gravity-driven approach has been applied mainly in chip-based continuous-flow analysis systems;^{21,24–26} however, to

the best of our knowledge, it has not been applied for achieving on-chip introduction of a series of samples, presumably owing to challenges in achieving efficient sample change with low carry-over. In this work, the difficulty has been overcome by the combination of an on-chip capillary sampling probe and the slotted-vial array described in the previous sections, combined with gravitational flow in the pump-out mode.

A relatively long (110 cm) and large inner diameter (2 mm) tubing was used downstream of the detection flow cell to increase the overall hydrostatic pressure and flow rate to $\sim 1 \mu\text{L}/\text{min}$ without increasing the length of the channel upstream of the detection zone. The flow rates for the fluids in the chip channels could be adjusted conveniently by changing the vertical position of the waste reservoir relative to that of the sample vials/reagent reservoir. Horizontal tubular reservoirs^{21,25} for reagent and waste, with an inner diameter of 4.7 mm, were used to maintain stable hydrostatic pressure in the chip channels during prolonged working periods. By varying the liquid level within a range of 10–80 cm difference, the sampling flow rate showed a linear relationship with liquid level difference.

A limitation of gravity-driven flows is that its stop-and-go intervals cannot be conveniently controlled. To stop a flow, either the liquid level of at least one of the reservoirs has to be adjusted or a valve system has to be adopted, both leading to complications in the drive system and hampering its simplicity. In the present system, when sample vials are changed either switching from sample to carrier or vice versa, the sampling probe inevitably experiences a period outside the vials. Under normal continuous-flow conditions, air segments would be drawn into the exposed sampling probe and then into the microchannel of the chip, resulting in deleterious effects often experienced in FIA. An important finding in this work, not reported previously, is that, within a well-defined and reasonably wide range for liquid level differences between sampling vial/reagent reservoir and waste reservoir, a stable gas/liquid interface was formed at the inlet of the probe capillary with sufficiently small inner diameter, when the sampling probe was exposed in air while traveling between vials, and no air entered the probe inlet, although the flow from the reagent reservoir continued (see Figure 2 b1, b2, d1, and d2). This phenomenon allowed the achievement of a spontaneously stopped flow in the sampling probe without the requirement for adjustment of liquid level or valve systems and can be explained by the existence of surface tension effects at the air/liquid interface formed at the terminal of the probe capillary. The surface tension produced a capillary rise (denoted by H) on the fluid in the probe channel counteracting the gravity force, as expressed by the equation²⁷

$$H = (2\sigma/\gamma R) \cos \theta \quad (1)$$

where σ is the surface tension of the fluid, θ the contacting angle

(24) Weigl, B. H.; Bardell, R.; Schulte, T.; Williams, C. In *Micro Total Analysis Systems 2000*; Van den Berg, A.; Olthuis, W.; Bergveld, P., Eds.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 2000; p 299.

(25) <http://www.micronics.net>.

(26) Chen, H.; Fang, Q.; Yin, X.-F.; Fang, Z.-L. In *Micro Total Analysis Systems 2002*; Baba, Y.; Shoji, S.; Van den Berg, A., Eds.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 2002; p 371.

(27) Munson, B. R.; Young, D. F.; Okiishi, T. H. *Fundamentals of Fluid Mechanics*, 4th ed.; John Wiley & Sons: New York, 2002; pp 26–28.

between liquid and capillary, γ the specific weight of the fluid, and R the inner radius of the capillary. In the present system, the calculated H value for the 75- μm -i.d. sampling probe was 39 cm of H_2O . This value was confirmed experimentally in this work using a 10-cm-long, 75- μm -i.d. capillary, with which an identical H value was obtained. This implies that when differences in liquid level between sample vial/reagent reservoir and waste reservoir are lower than 39 cm, one can expect that the fluid in the sampling probe will remain stationary during the exposed interval, even though the flow in the chip was not stopped. In fact, in the present system, a measured H value of up to 100 cm was obtained due to the presence of a continuous reagent flow in the reagent channel in the chip. The continuously flowing reagent stream produced a hydrodynamic effect partially counteracting the gravity-driving effect on the fluid in the connecting sampling channel during this stage.

In summary, for H values of 10–100 cm studied in this work, sample flow was stopped on exposure of the sampling probe to air, owing to buildup of surface tension at the probe tip, and immediately resumed when reinserted into a vial containing solution, owing to the now predominant hydrostatic pressure induced by liquid level difference between the sample vial and the waste reservoir.

One possible criticism of gravity-driven flows might be associated with their sensitivity to variations in temperature and viscosity. This is not an exclusive limitation for gravity-driven flows, but also exists for many other driving means, such as driving by electrokinetic, centrifugal, pneumatic, and capillary effects. Effects from temperature and viscosity are usually overcome by appropriate calibration, which was easily and automatically conducted in the present system by periodically inserting standards into the vial array. However, frequent calibration was not found to be necessary in our studies, since ambient temperatures and sample viscosity rarely changed to such an extent as to significantly affect flow rate.

Mixing of Sample and Reagent. In conventional FIA systems, mixing of sample and reagent may be achieved either through dispersion of the injected sample zone into a reagent carrier or by merging of the sample/carrier stream with a continuous flow of reagent. With the channel dimensions in microfluidic systems, however, laminar flows predominate during flow merging. Although several effective mixing strategies in microfluidic systems have been proposed,^{28,29} they all involve complications in chip fabrication. The merging flow approach was adopted in this work to evaluate whether molecular diffusion alone could induce sufficient mixing under the laminar flow conditions in the present chip design. The steady-state absorbance obtained with the $\text{Fe(II)}-o\text{-phenanthroline}$ reaction by injecting a sufficiently large sample at 0.38 $\mu\text{L}/\text{min}$ flow rate was compared with an off-chip fully mixed and reacted sample, with the same sample/reagent proportions, filled into the chip channels. Results obtained in this study show 78% complete mixing within the chip, implying that, at least for relatively small molecules and ions, the channel and flow cell dimensions employed in this work are sufficient for achieving mixing of sample and reagent exclusively via diffusion.

Absorption Measurements in the LCW Flow-Through Detection System. Despite recent attempts for increasing the optical path length of microfluidic systems,^{30–32} for most applications, the path lengths are still restricted to micrometer levels characteristic of microfabricated chip channels and structures, resulting in absorbance sensitivities typically 2–3 orders of magnitude lower than conventional spectrophotometry. In our previously reported work, a long optical path length absorbance detection system on a microchip was developed using an integrated LCW capillary flow cell.³³ In that work, an effective absorption path length only about one-third of the LCW capillary length was achieved as a consequence of light transmission through the thick silica walls of the capillary, with Teflon AF 1600 coated on its outer surface. In this work, the chip-based LCW detection system was modified by using a complete Teflon AF capillary as the detection flow cell. An effective absorption path length of 17.4 mm, which was nearly equal to its length (20 mm), was obtained due to the holophotal property of the Teflon AF surface.³⁴ With the same length of capillary flow cell, the effective absorption path length of the present system was a factor of ~ 3 higher than that in the previous LCW chip system, and the volume of the flow cell was reduced to 40 nL, which was one-sixth of the latter.

Effect of Sampling/Carrier/Reagent Flow Rates and Injection Volume. In this study, flow rates were adjusted by changing the differences in liquid levels between the sample vial/reagent reservoir and the waste reservoir. Therefore, variation in any one flow rate will always result in proportional variations in all flow rates. The effects of sample loading flow rate in the range of 0.08–0.5 $\mu\text{L}/\text{min}$ (0.21–1.37 $\mu\text{L}/\text{min}$ total flow rate) were studied with 80 μM Fe(II) sample solution. The results in Figure 3A show that the peak height increased and the analysis time decreased with the increase of flow rate. The increase in sensitivity was due to the proportional increase in sample volume with the increase of sample loading flow rate during a fixed sampling period. The reduction in analysis time was due to the proportional increase in total flow rate including those of the sample, reagent, and carrier. Despite the changes in sensitivity, the main relationship observed from the study in Figure 3A is the effect of flow rate on analysis time or sampling frequency. Flow rate in the range of 1.05–1.37 $\mu\text{L}/\text{min}$ (total flow rate), corresponding to the difference in liquid level of 60–80 cm, was chosen mainly for achieving high sampling frequencies.

The effect of sample volume in the range 3.2–64 nL, corresponding to sampling times of 0.5–10 s at 0.38 $\mu\text{L}/\text{min}$ sample loading flow rate (1.05 $\mu\text{L}/\text{min}$ total flow rate) on peak response was studied using a 80 μM Fe(II) sample solution. The results in Figure 3B show a behavior similar to that in conventional FIA, except that the sample volumes were at least 3 orders of magnitude lower than those typically employed in the former with fast photometric reactions. The peak height increased with volume

(28) Bessoth, F. G.; deMello, A. J.; Manz, A. *Anal. Commun.* **1999**, 36, 213.

(29) Dertinger, S. K. W.; Chiu, D. T.; Jeon, N. L.; Whitesides, G. M. *Anal. Chem.* **2001**, 73, 1240.

(30) Liang, Z. H.; Chiem, N.; Ocvirk, G.; Tong, T.; Fluri, K.; Harrison, D. J. *Anal. Chem.* **1996**, 68, 1040.

(31) Verpoorte, E.; Manz, A.; Ludi, H.; Bruno, A. E.; Maystre, F.; Krattiger, B.; Widmer, H. M. *Sens. Actuators, B* **1992**, 6, 66.

(32) Tiggelaar, R. M.; Veenstra, T. T.; Sanders, R. G. P.; Gardeniers, J. G. E.; Elwenspoek, M. C.; Van den Berg, A. *Talanta* **2002**, 56, 331.

(33) Du, W.-B.; Fang, Q.; Fang, Z.-L. *Gaodeng Xuexiao Huaxue Xuebao (Chem. J. Chinese Universities)* **2004**, 25, 610.

(34) Dallas, T.; Dasgupta, P. K. *Trends Anal. Chem.* **2004**, 23, 385.

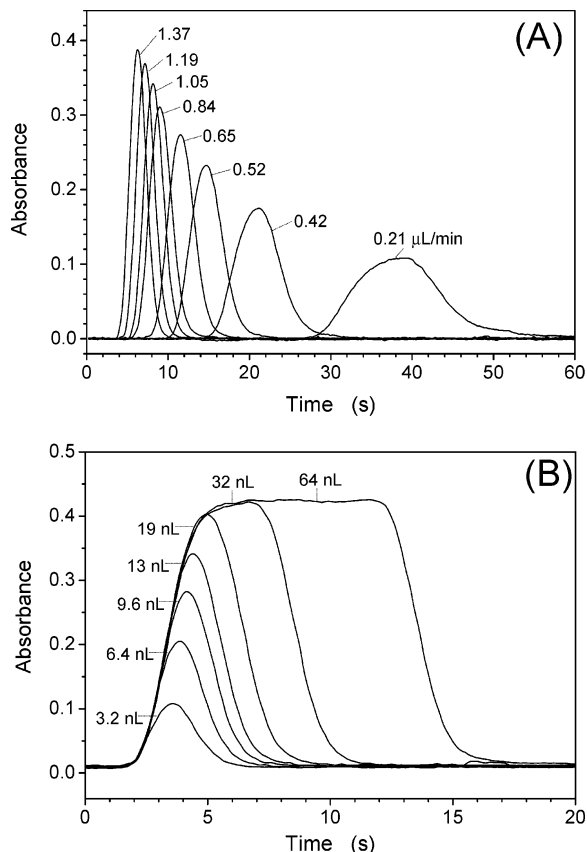


Figure 3. Typical recordings of injected $80\ \mu\text{M}$ Fe(II) sample (A) with 0.21, 0.42, 0.52, 0.65, 0.84, 1.05, 1.19, and $1.37\ \mu\text{L}/\text{min}$ total fluids flow rate, corresponding to 10, 20, 30, 40, 50, 60, 70, and 80 cm of difference in liquid levels between sample vial/reagent reservoir and waste reservoir, to show the effect of sampling flow rate. Injection time for carrier, 60 s; (B) with 0.5, 1, 1.5, 2, 3, 5, and 10 s sampling time, corresponding to 3.2, 6.4, 9.6, 13, 19, 32, and 64 nL sample injection volume at $0.38\ \mu\text{L}/\text{min}$ sampling flow rate, to show the effect of sample injection volume. Injecting time for carrier, 20 s; Other conditions as in Figure 4.

rather steeply at lower volumes, with an $S_{1/2}$ value (dispersion coefficient, 2) of 8 nL. With the increase of sample volume, both the sample loading time and injection time were increased, resulting in the decrease of sampling frequency and throughput. A sample loading time of 2 s (13-nL sample volume) was considered to be a good compromise between sensitivity and throughput.

Analytical Performance. The performance of the micro-FIA system was demonstrated in the determination of Fe(II) with α -phenanthroline as reagent under optimized conditions. An outstanding reproducibility of 0.6% RSD ($n = 18$) was achieved in 18 consecutive determinations of a $80\ \mu\text{M}$ Fe(II) standard (Figure 4). A linear response range of $1.0\text{--}100\ \mu\text{M}$ Fe(II) was obtained with a regression equation of, $A = 4.10 \times 10^{-3} C + 1.01 \times 10^{-2}$ ($r^2 = 0.9967$) (as shown in Figure 5). The limit of detection for Fe(II) based on 3 times the standard deviation of the blank values was $0.1\ \mu\text{M}$ (3σ). The sensitivity was 1.7-fold of that obtained using conventional FIA systems with a 1-cm path length flow cell, which typically consume 10 000-fold more sample. High sample throughput of $300\ \text{h}^{-1}$ (Figure 5) was obtained using injection times of 2 and 10 s for sample and carrier solutions, respectively, with a carryover of $<1\%$, and sample consumption

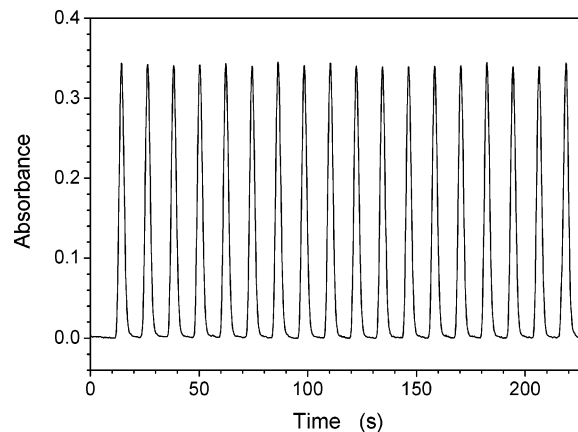


Figure 4. Typical recordings of repetitively injected $80\ \mu\text{M}$ Fe(II) sample to show the repeatability of the system. Carrier, water; sampling time, 2 s; injection time for carrier, 10 s; sampling flow rate, $0.38\ \mu\text{L}/\text{min}$.

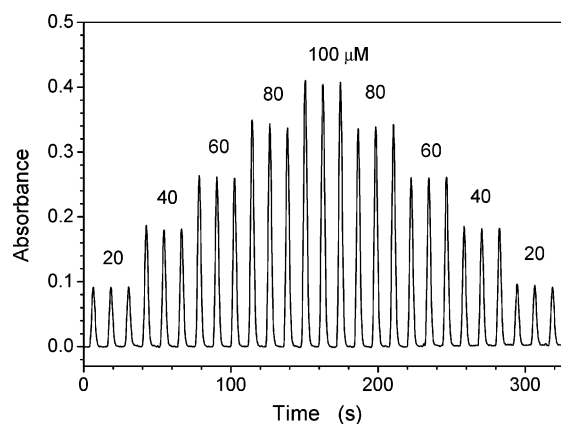


Figure 5. Typical recordings of sequentially injected 20, 40, 60, 80, and $100\ \mu\text{M}$ Fe(II) standards to show the linear relationship between adsorption response and Fe(II) concentration. Other conditions as in Figure 4.

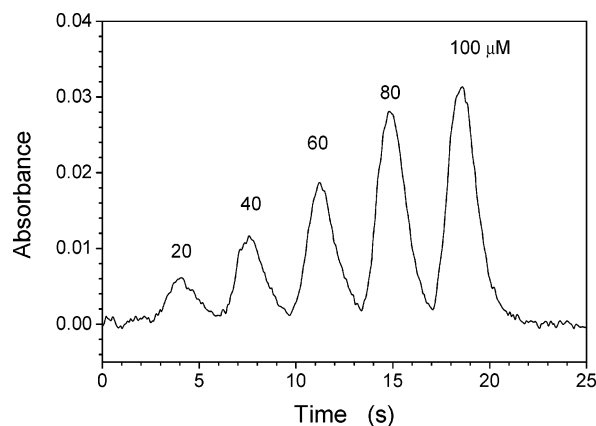


Figure 6. Typical recording of sequentially injected 20, 40, 60, 80, and $100\ \mu\text{M}$ Fe(II) standards to show the highest sampling throughput of $1000\ \text{h}^{-1}$. Sampling time, 0.08 s; injecting time for carrier, 3.4 s; sampling flow rate, $0.5\ \mu\text{L}/\text{min}$.

of 13 nL for each cycle. The highest sample throughput of $1000\ \text{h}^{-1}$ was obtained (see Figure 6) by increasing the sample/carrier/reagent flow rate to $0.5/0.5/0.87\ \mu\text{L}/\text{min}$ (80-cm difference in liquid level between the sample vial and waste reservoir) and decreasing the sample loading and carrier injection times to 0.08 and 3.4 s, respectively, in which case the sample consumption

was further reduced to 0.6 nL (with a carryover of <3%, and reduction of sensitivity by a factor of 12 compared with that using a 13-nL sample).

CONCLUSIONS

The present system proved to be an efficient and robust means for achieving 1000 h⁻¹ high-throughput chip-based FI analysis, with submicroliter sample/reagent consumption, and absorbance detection sensitivity exceeding that of conventional FIA, without resorting to valves and pumps. The study revealed potentials for achieving sample throughputs of over 10 000/h by further decreasing the sample loading time, increasing flow rate, and reducing channel dimensions. To achieve that goal, a specially designed, more compact version of the autosampler is under development, on which interchangeable cartridges of vial arrays can be removed and reloaded in a matter of seconds. The system could be further simplified into a single-channel FIA system by removing the reagent reservoir and substituting the carrier vials with reagent. The present system can also be readily modified into a sequential injection system using an array of different reagent vials together with the sample and carrier. The system also could be conveniently adapted as a nanoinjector for automated

sample introduction with other microfluidic systems, such as chip-based CE or continuous-flow analysis. The spontaneous stop flow phenomenon observed and exploited in this work could also be adopted as a robust and simple approach for achieving stopped-flow operations, such as in kinetic measurements using gravity drive.

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SUPPORTING INFORMATION AVAILABLE

A video clip of the FI process. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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