

Focussing Review

Recent Progress of On-line Combination of Preconcentration
Device with Microchip Electrophoresis

Kenji Sueyoshi*

*Department of Material Chemistry, Graduate School of Engineering, Kyoto University*** To whom correspondence should be addressed.*

Abstract

Microchip electrophoresis (MCE) is one of the versatile separation techniques in microfluidic systems since MCE has remarkable advantages such as high resolution, rapid analysis, small consumptions of samples and reagents, and high integratability to other chemical operations on planer substrate. However, there still remains a significant problem about a poor sensitivity due to a short optical pathlength and extremely small volume of analytes introduced into the separation channel. To overcome this drawback, a lot of on-line sample preconcentration methods have been developed and applied to MCE for improving the sensitivity. In this review, the author highlights recent progresses on combinations of chemical operations integrated onto microfluidic devices for enhancing a detector signal, e.g., solid phase extraction (SPE), affinity capturing, electrokinetic filtrating/trapping, and polymerase chain reaction (PCR) with MCE mainly from 2009. The other integrated techniques and methods which are expected to be combined with MCE are also introduced briefly.

Keywords: microchip electrophoresis, solid phase extraction, affinity capturing, electrokinetic filtering/trapping, polymerase chain reaction

Contents

1. Introduction
2. Solid phase extraction and related techniques
3. Affinity capturing and related techniques
4. Filtering and related techniques
5. Polymerase chain reaction and related techniques
6. Other preconcentration techniques
7. Conclusion

1. Introduction

In micro total analysis system (μ TAS), various chemical/physical operations are integrated into microchannels fabricated on a planar substrate for realizing high-performance analyses using microfluidic devices [1–4]. Among the previously reported operations in microfluidic systems, microchip electrophoresis (MCE) is expected as a versatile separation method since the electroosmotic flow (EOF), which is generated by an electric double layer of an inner surface of the microchannel, is not affected by down-sizing effect of the channel, whereas a back pressure of hydrodynamic

flows dramatically increases upon decreasing the size of a channel [1]. Furthermore, MCE has a lot of useful separation modes, e.g., isotachopheresis, isoelectric focusing, gel electrophoresis, electrokinetic chromatography, electrochromatography, and so on, which allows a wide variety of analytes to be well resolved with a short analysis time and small consumptions of samples/reagents [5–8]. On the other hand, a poor sensitivity of MCE due to its short optical pathlength and/or extremely small amounts of analytes are sometimes problematic. To overcome this drawback, many researchers have been studied on developments of on-line sample preconcentration techniques in electrophoretic analysis as lots of reviews were already reported [9–12]. In this paper, the author focused on basic studies on sample preconcentrators in microfluidics and recent developments of sensitive MCE analyses by on-line integration with preconcentrators related on solid phase extraction (SPE) [13–22], affinity capturing [23–32], filtrating devices [33–47], polymerase chain reaction (PCR) [48–65], and so on [66, 67], onto the microfluidic devices. Additionally, some novel techniques which are expected to be combined with MCE are also listed to

Tel: +81–75–383–2449**Fax:** +81–75–383–2450**E-mail:** sueyoshi@anchem.mc.kyoto-u.ac.jp

contribute to a future progress in MCE [68–75].

2. Solid phase extraction and related techniques

SPE is one of extraction methods using an interaction between analytes and solid phase immobilized onto a packed beads, monolithic structure, inner surface of a column, and so on. The analytes are strongly retained by the solid phase due to hydrophobic, electrostatic, or affinity interaction when they are passing through the solid phase zone. After washing the solid phase, the purified/concentrated analytes are finally released by injecting an eluent. Numerous kinds of extraction cartridges are commercially available in the world, which help us to analyze real samples containing many kinds/large amounts of impurities.

In microfluidic devices for MCE, Kutter *et al.* firstly applied SPE by the modification of the inner surface of the microchannel with octadecyltrimethoxysilane as a solid phase [13]. Then, a lot of SPE and related techniques have been developed in microfluidic devices [14–16], resulting in the selective and sensitive analyses with small consumption of analytes as compared to the conventional off-line procedures.

Regarding the recent studies on combinations of micro-SPE to MCE, Cakal and his coworkers reported a simple combination of MCE and SPE using a polymer monolith [17]. The SPE column was located at the cross injector of the MCE channel made from borofloat glass, and it was formed by a photopolymerization. The fabricated column showed the high stability over 50 times under the electrokinetic fluidic control, and it maintained the analytical performance with an 100-fold improvement of the LOD of

catecholamines as compared to MCE without SPE. Kang *et al.* also fabricated the multi-layer PDMS/glass hybrid microchip with a polymer monolithic column and pressure valves [18]. During SPE, a sample solution was hydrodynamically introduced into a SPE column by controlling the pressure valves. After the elution, the eluted analytes were electrokinetically injected into the separation channel and then detected by the amperometric electrodes fabricated into the channel. In this device, dopamine was 80-fold concentrated by the SPE relative to the conventional microchip zone electrophoresis (MCZE) without SPE. Proczek demonstrated the simple SPE using a polymer monolithic column for electrochromatography [19]. The acrylate-based monolith was synthesized in the glass microdevice by the photopolymerization. Polyaromatic hydrocarbons were directly introduced into the monolithic channel, and then extracted at the head of the column. After introducing the eluting buffer into the channel, the analytes could migrate into the monolithic column, resulting in the good separation based on microchip electrochromatography (MCEC) with a 270-fold sensitivity enhancement within 120 s of preconcentration. Multifunctional protein processing chip with an integrated digestion, SPE, MCE, and electrospray was developed by Wang and Harrison [20]. The trypsin-immobilized beads for the on-line digestion and copolymeric reversed-phase-immobilized beads for SPE were packed into the channel in series by the dam structures as shown in Figure 1–I, providing a rapid on-chip digestion of cytochrome c within 3 min in contrast to the 2 h required for the conventional tryptic digestion in the solution phase. As a result, the observed signal in total ion chromatogram was 20-fold enhanced by SPE in MS detec-

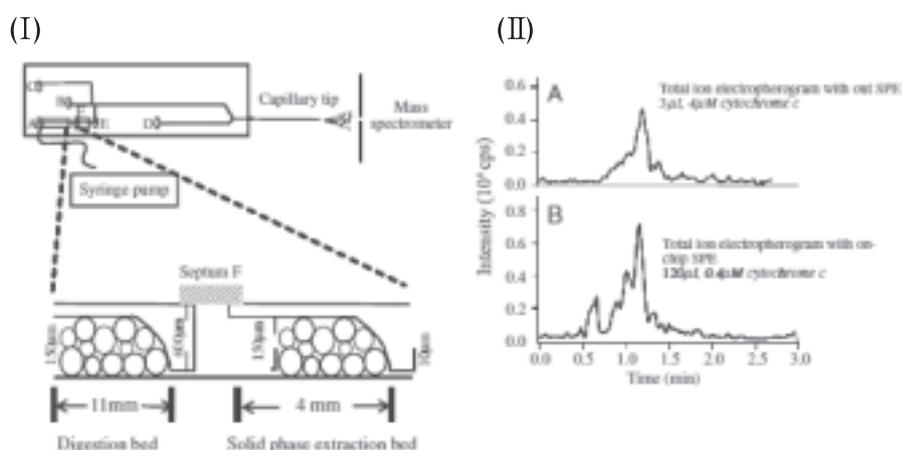


Figure 1. (I) Schematic of the protein preparation chip, with integrated tryptic digestion and SPE beds, a double-T injector for CE and an electrospray tip. The lower drawings show the detail of the 150 μm deep, 800 μm wide “fat channels” in which 40 to 60- μm diameter beads are trapped. (II) Total ion electropherograms (TIE) for cytochrome c digested off-chip with and without integrated SPE. (A) TIE of 4 μM cytochrome c without SPE step; (B) TIE of 0.4 μM cytochrome c with SPE, using an integrated 4 mm long SPE bed and a 20 times volume ratio (120 μL in/6 μL out). Reproduced from ref. [20] with permission.

tion relative to that without SPE (Figure 1–II), increasing the capability of protein processing. The other type of the SPE device using microbeads was reported by Tennico and Remcho [21]. Functionalized magnetic particles of which core/clad were iron oxide/silica, respectively, were synthesized and the surfaces were then modified with octadecylsilane. The magnetic beads can be easily immobilized in a capillary/microchannel by applying a magnetic field relative to the packed beads column which needs a frit structure. The preconcentration efficiency was not described but the highly reproducible SPE–CE/MCE analysis was demonstrated with the simple experimental procedure. The similar techniques using magnetic microparticles modified with an antigen were reported by Ambrosi et al. [22]. In the paper, the EOF was used to move the modified magnetic particles and sample solution, while an electrophoretic separation was not conducted. However, this technique will be applied to the MCE analysis of biogenic compounds in the near future.

In brief summary, recent progresses in a fabrication of microfluidics allow SPE and related techniques to be easily applicable to MCE, providing a highly sensitive analysis of the various compounds on a chip. Further improvements of fabrications of stationary phases in microfluidics will demonstrate more highly effective and sensitive analyses in MCE.

3. Affinity capturing and related techniques

Affinity capturing is also an important procedure for the specific preconcentration and separation based on the interaction between analytes and affinity ligands. Briefly, affinity ligands are encapsulated or immobilized onto a column fabricated in a microchannel. When a sample solution is introduced into the channel, only the analytes having a specific interaction with ligands are captured onto the column, whereas those having no interaction penetrate. The captured analytes are then released by introducing an elution buffer after washing the column, providing the highly specific preconcentration. This technique also has been applied to CE and MCE formats, e.g., an application to two-dimensional MCE [23], immunoaffinity gel electrophoresis [24, 25], chemical immobilization onto a monolithic column for affinity electrophoretic analysis [26], encapsulation of the biomolecules based on the sol-gel technologies [27], and so on [28], realizing the specific and rapid analyses of the biogenic compounds with small consumptions of samples/reagents. Recently, many researchers focus on the encapsulation of ligands into the hydrophilic gels since the affinity of the encapsulated ligands is well kept as compared to the other chemical immobilization techniques.

Many applications of affinity capturing using gel columns formed in a microchannel to MCE have been reported by Mathies and his coworkers [29, 30]. Yeung developed a streptavidin-immobilized short gel column in a microchannel nearby a cross-

type injector [29]. The DNA containing specific configuration of a base was amplified by PCR using a biotin-modified primer. When the solution of the PCR product was then introduced into the gel column, the only the specific double-stranded DNA was captured at the column due to the strong interaction between streptavidin and biotin-modified DNA, resulting in the purification of the analytes. The captured double-stranded DNA was then melted by increasing temperature and released from the column (Figure 2–I). The unreacted primers and dyes were well removed by the affinity column as shown in Figure 2–II, so that the detected signals were 10–50-fold enhanced as compared to the traditional methods. Liu developed a twelve-lane capillary array microchip and scan system for further high-throughput DNA analysis, allowing 12 short tandem repeat analysis of DNA to be completed in 30 min without any manual process intervention [30]. At the same time, the sensitivity was improved at least 10-fold relative to the conventional cross injection scheme. Hokkanen and his colleagues developed the silicon-glass hybrid microchips with a SPE cavity, electrophoresis channel, and conductivity detection electrodes [31]. The cavity was filled with a recombinant anti-testosterone Fab-fragments-immobilized Sepharose gel. As a result of the affinity capturing–MCZE operations, the selective preconcentration of fluorescently labeled testosterone and subsequent MCZE analysis were demonstrated in the fabricated device. An integrated microfluidic device containing affinity column was also reported by Yang and Woolley [32]. A part of an inner surface of a poly (methyl methacrylate) (PMMA) microchannel was modified with an antigen as affinity column by using photopolymerization of acrylate derivatives. The proposed device could quantify fluorescently labeled α -fetoprotein at *ca.* 1 ng/mL levels in *ca.* 10 μ L of human serum in a few tens of minutes.

Consequently, it is indicated that high-throughput, highly sensitive, and selective analyses of biogenic compounds can be demonstrated by the combination of affinity capturing and MCE. In the future, various affinity ligands will be employed to realize μ TASs of biogenic compounds for clinical/pharmaceutical diagnoses.

4. Filtering and related techniques

Filtering techniques are also useful pretreatments in real sample analyses, especially in analyses of large biogenic compounds containing protein, DNA, and so on. These large molecules are accumulated by the filtering membrane of which pore size is smaller than the analyte molecules, providing the effective preconcentration and purification of the bioanalytes. In a microfluidic device, the filter is made up into the microfluidic device during the fabrication of the microchannel or formed in the microchannel after fabricating the microfluidic device [33–36]. Especially, the filters made from polymeric gels or monolithic structures are advantageous be-

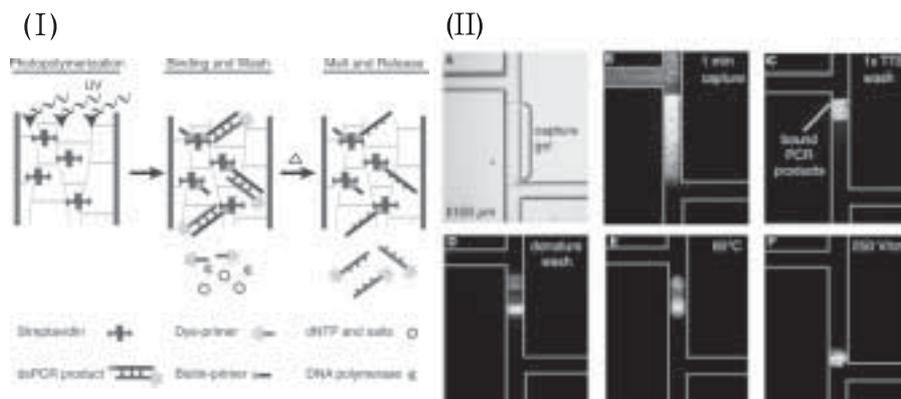


Figure 2. Schematic of the streptavidin-gel capture method for purifying and concentrating forensic PCR products. STR samples are generated with one primer labeled with fluorescent dye and one with biotin. The PCR reaction is electrophoresed through the photopolymerized cross-linked polyacrylamide gel network where the double-stranded DNA products bind strongly to the streptavidin-modified gel. Unbound PCR reactants are washed off, effecting purification, concentration, and desalting. The fluorescently labeled single-stranded DNA is then released for electrophoresis by melting the duplex DNA. (A) Fluorescence images showing the progress of a typical streptavidin affinity capture–MCE operation. (B) The biotin-PCR products bind to streptavidin and concentrate at the entrance of the capture gel within one minute. (C) Unbound PCR products are washed off with 1×TTE. (D) After the denaturant wash, the captured DNA band disperses across the capture gel. (E) The device is then heated up to 65 °C to release the products and (F) electrophoresed at 250 V/cm toward the anode. Reproduced from ref. [29] with permission.

cause they can be formed anywhere and their filtrating characteristics can be controlled by the conditions of preparation. It was difficult to change the pore size after a preparation of the membrane, while the membrane filtration provided the 100–10 000-fold sensitivity enhancement in MCE [24, 36].

In 2003, Dai and Crooks reported a novel filtration technique for ionic compounds using a membrane with nanopores located between the fabricated microchannels on the PDMS substrates [37]. Wang and Han then investigated that the enrichment effect was based on the polarization/depletion of an ion concentration [38]. Briefly, the preconcentration is based on a suppression of a transportation of charged compounds into nano-sized channels by an electrostatic repulsion. When an inner surface of a channel is positively/negatively charged, electric double layer (EDL) is formed nearby the inner surface of the channel and it prohibited an approach of an ion with a same polarity (co-ion). Debye length, which means a thickness of the EDLs, is in order of a few nanometers under a uniform surface charge distributions and electrolyte concentration of a buffer. Thus, the EDL does not affect the migration of charged molecules in the conventional microchannels of which depth and width are 10–100 μm. On the other hand, the EDLs sometimes can overlap in submicron/nano-size channels/pores under a low electrolyte concentration below 0.1 mM. The counter-ions, which have an opposite polarity to the inner surface, can be transported through the nanochannel/nanopores, while the co-ions cannot due to the electrostatic repulsion. As a result, the co-ions are accumulated in front of the charged nanochannel/

nanopores due to the ion depletion. The electrokinetic preconcentration using a nano-channel can demonstrate a million-fold enhancement of the sensitivity [39], so that the many researchers have studied for the highly effective preconcentration of ionic compounds. Related techniques based on the similar concept were often called electrokinetic trapping and applied to the various analyses such as immunoassay [40], enhanced enzyme assay [41], and also MCE [42].

During 2009–2011, some papers were reported on the combination of the electrokinetic trapping with MCE [43–45]. Kuo *et al.* developed the electrokinetic trapping of DNA using a closed valve fabricated in the PDMS microchannel as shown in Figure 3–I [43]. The inner surface of the channel and closed valve were covered with ionic polymer bilayer, providing the formation of the negatively charged nano-scale channel at the valve. DNA was successfully concentrated by using the closed valve (Figure 3–II), and *ca.* 3600-fold improvement of the sensitivity within 1.9 min of operation and separation in microchip gel electrophoresis were demonstrated by opening/closing the valve. The other type of preconcentrator based on electrokinetic trapping was developed by Nge and Woolley [44]. An ion-permeable membrane consisting of acrylamide, *N,N'*-methylene-bisacrylamide, and 2-(acrylamide)-2-methylpropane-sulfonate was formed in the microchannel nearby the injection intersection. Anionic proteins were excluded from the porous membrane based on both the size and charge, resulting in the effective preconcentration prior to the separation by MCE. As compared to the protein analysis without an electrokinetic trapping,

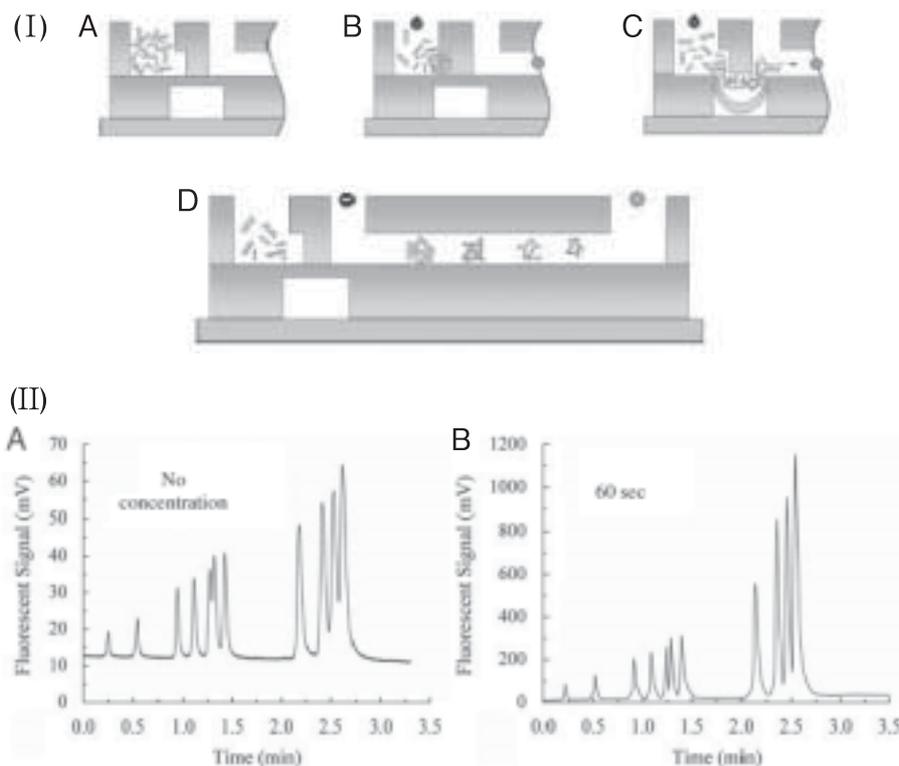


Figure 3. Schematic illustration of the micro-CE chip capable of online DNA pre-concentration and separation analysis. A cross-sectional view of the micro-CE chip shows that the DNA samples are pre-concentrated and separated by controlling the pneumatic suction of the normally closed valve. (I–A) Initial condition, (I–B) electrokinetic trapping of DNA nearby the closed valve, (I–C) Introduction of focused DNA into the separation channel by opening the valve, and (I–D) closing valve and electrophoretic separation of DNA. (II) Electropherograms of the dye-labeled ϕ X 174 DNA/*Hae*III markers obtained in (II–A) the traditional cross-shaped PDMS micro-CE chip and (II–B) in the new micro-CE chip at the pre-concentration times of 60 s. Reproduced from ref. [43] with permission.

10–40-fold enhancements of the observed signals were achieved with 1–4 min pre-concentration. Yamamoto and Suzuki also reported about the electrokinetic trapping using an acrylamide-made gel membrane comprising 2-acrylamidoglycolic acid as a permselective pre-concentrator [45]. In this device, weak acids of which pK_a is approximately 6 can be trapped in front of the gel membrane, whereas a strong acid of which pK_a is below 2 cannot. The authors clarified that the permselectivity of the gel membrane depended on the dissociation of carboxylate of glycolic acid of which pK_a of *ca.* 4. This method displays a sensitivity enhancement factor of up to 10^5 within 3 min.

The other electrokinetic trapping devices using polycarbonate nanoporous membrane [46] or titania nanoporous membrane [47] were also reported without connecting MCE. An application of these techniques to the MCE-based analysis is expected for the realization of a simple and highly sensitive diagnosis with a small sample consumption.

5. Polymerase chain reaction and related techniques

Microchip gel electrophoresis (MCGE) is widely used as a powerful separation tool for genomic researches [48–50]. In DNA analysis using a microfluidic device, however, we often face a significant issue that the amount of the obtained DNA is less than that required for the detection. SPE and filtering can be employed to improve the detectability of DNA analyses in microfluidic devices [51, 52], while it is difficult for these techniques to concentrate specific DNA related on single nucleotide polymorphisms. For molecular biology, genomics, diagnosis, and so on, PCR is often employed to amplify the specific DNA. In PCR, briefly, DNA was mixed with a PCR matrix containing a DNA polymerase and then amplified under a repetition of a thermal cycle consisting high temperature ($\sim 94^\circ\text{C}$) for denaturation, low one for annealing ($50\text{--}60^\circ\text{C}$), and middle one for extension ($\sim 70^\circ\text{C}$) steps. In a microfluidic device of which volume of microchambers/channels is extremely small, temperature of a sample solution can be varied easily and rapidly. Thus, a further high-throughput PCR with a shorter amplification time can be employed as compared to commercialized

batch PCR procedures. The various microfluidic PCR devices have been developed for these years [52–56], and some of them were directly combined with MCE on a chip to improve an efficiency of gene assay [57, 58].

Recently, Mathies and his coworkers have vigorously studied on the microchips containing PCR and MCE for high throughput DNA analysis [59–62]. Limiting after 2009, an integrated PCR–MCE microdevice with an in-line affinity capture gel (ACG) was developed by Thaitrong [59]. The in-line capture gel was synthesized in the microchannel in front of the separation channel for MCE, which provided the 3.5-fold increase in signal intensities and improved resolution compared to the device containing the side arm capturing. This advance is exploited to successfully detect *Escherichia coli* O157 in a 500-fold higher background of *E. coli* K 12. Beyor demonstrated a microfluidic device for pathogen detection integrating cell capturing by immunoaffinity magnetic microbeads, PCR, and MCE [60]. The device realized a sensitive detection of *E. coli* O 157 in the presence of *E. coli* K 12 at a ratio of 1:1000. A further improved microdevice integrating sequence-specific DNA template purification using immunoaffinity magnetic microbeads, PCR employed by a fabricated micro-heater, ACG, and MCE was reported by Liu as shown in Figure 4 [61]. The analyses of 9-plex DNA in human oral swabs were well conducted in the single microdevice for *ca.* 3 hours, which included the 15 min of cell lysis, 45 min of DNA digestion and hybridization, 40 min of DNA template capture and washing, 40 min of DNA amplification, and 30 min of post-PCR clean-up, in-line capturing, and MCE as compared to a conventional forensic STR typing for 7–8 hours. Scherer et al. developed the instrument for the automated multi-channel PCR–MCE device to realize a high-throughput analysis of DNA [62]. In the paper, a compact, self-contained instrument for a control and detection of the high-throughput integrated microfluidic device was developed and named multichannel capillary array electrophoresis portable scanner (McCAEPs), which showed good resolutions and similar signal to noise ratio as compared to those obtained by previous reports [58]. These integrated microsystems will be a significant advance in the development of high-throughput and highly sensitive μ TAS for on-site DNA analysis.

The other PCR–integrated device was demonstrated by Zhong and Lin [63]. They also reported the PCR–MCE device integrating microfabricated heater and resistance temperature detector, allowing 30 complete PCR cycles in about 33 min. As a result, a rapid assay of hepatitis B virus was performed in less than 45 min.

In brief conclusion, the integration of PCR and related techniques with MCE can provide the versatile DNA analysis, especially for the clinical, forensic, and genomic diagnoses. In the near future, it is expected that further connection of microfluidic PCR

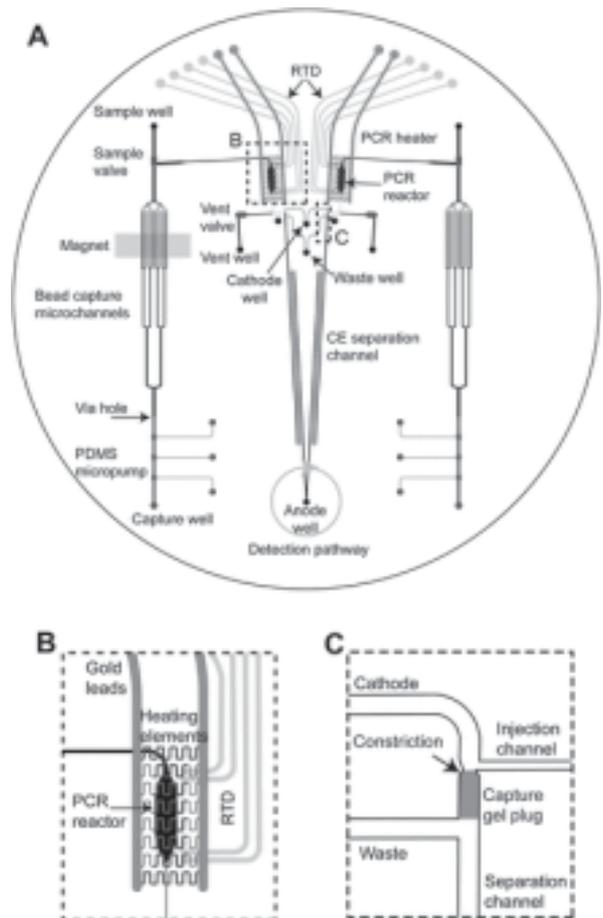


Figure 4. Schematic of fully integrated microdevice for forensic STR analysis. (A) Microchip mask design for performing DNA template purification, PCR, post-PCR cleanup, in-line injection and CE separation. (B) Expanded view of the PCR chamber and the microfabricated heater and RTD. (C) Expanded view of the affinity gel-based capture inline injector. Shaded area indicates the location of streptavidin capture gel. Reproduced from ref. [61] with permission.

devices such as rolling circle amplification [64, 65] with MCE will be developed to realize a high-throughput DNA analysis.

6. Other preconcentration techniques

In the former sections, the integrations of SPE, filtering, electrokinetic trapping, and PCR with MCE were described. In this section, the other type of preconcentration techniques combined with MCE or those expected to be connected with MCE are introduced [66–75].

Noblitt et al. were integrated a water-based condensation growth tube collector with an MCE device, providing the real-time and on-line monitoring of aerosol composition as shown in Figure 5 [66]. As compared to the conventional particle-into-liquid sampler–ion chromatography (PILS–IC), a comparable sensitivity was observed

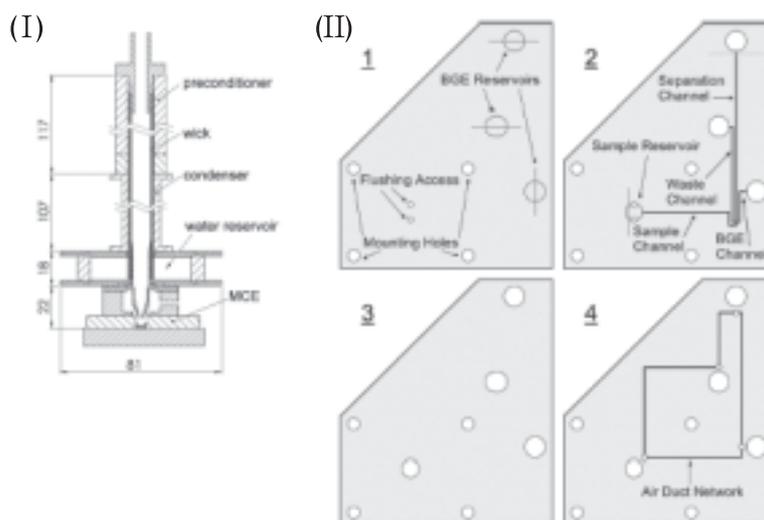


Figure 5. (I) Schematic of the aerosol chip electrophoresis (ACE) system, showing a growth tube collector, water reservoir for passive wetting of the wick, and the microchip into which the aerosols are deposited. All dimensions are shown in millimeters. (II) Exploded view of microchip design. The chip is assembled bottom-to-top, layers 1–4. Reproduced from ref. [66] with permission.

by the developed device with one-third and one-fifth the time resolution in on-site real time monitoring due to the shorter analysis time in MCE than that in IC.

Droplet-membrane-droplet liquid phase microextraction (LPME) was combined with MCE by Sikanen and his coworkers [67]. In the demonstrated device, the sample inlet reservoir was simply covered with a supported liquid membrane (SLM) sustained in the pores of a small piece of a flat poly(propylene) membrane. The model analytes were extracted from sample droplets, through 1-octanol as SLM, and into acceptor droplets. Unfortunately, the recoveries of the model analytes were not so high (13–66%). However, the author believes that a further optimization will allow an improved device based on this simple concept to achieve a further effective extraction.

MCEC analyses are basically connectable to on-line SPE, so that the application of on-line SPE to MCEC analyses by using novel stationary phases such as vertically aligned carbon nanotubes [68], various beads [69], and the other electrochromatographic techniques [70], will provide a highly sensitive and effective analysis in the future.

Liu reported a development of a microfluidic device for fluorescence *in situ* hybridization (μ FlowFISH) [71]. In the device, cells are introduced into the FISH chamber formed into the microchannel by cross-linked poly (acrylamide). The accumulated cells were then treated with fluorescent probes. This device provided the quantitative detection of microbial cells from a complex sample by connecting flow cytometry. The author understands that a connection of μ FlowFISH device to on-line lysis and MCE will con-

tribute to a selective and sensitive analysis of the specific cell lysate.

Additionally, connections of other preconcentration and pretreatment techniques, such as dielectrophoresis and related techniques [72–74], and microdroplet extraction [75], to MCE also will contribute to the sensitive microfluidic analysis in the future.

7. Conclusion

Recent developments and improvements of the on-line coupling of the sample preconcentrator to MCE allow the microfluidic devices to demonstrate the highly sensitive analyses based on the concept of μ TAS. In addition, a lot of studies on on-line sample preconcentrations in CE/MCE have been actively reported as mentioned above, helping us to improve the sensitivity with an easy experimental procedure [9–12]. On the other hand, various sensitive detection schemes have been developed and applied in microfluidics, providing a sensitive detection of the non-fluorescent analytes or selective detection based on an affinity [76]. Furthermore, the studies on a development of small/portable instruments for an on-site or point-of-care analysis have been also in progress [77]. In the near future, the author believes that these different approaches will be combined to realize the highly sensitive, effective, high-throughput analysis with a low cost and easy procedure.

Acknowledgment

The author would like to express his gratitude to Professor Koji Otsuka (Kyoto University) for his supervision and valuable discussions. The author is also very grateful to Associate Professor Fumi-

hiko Kitagawa (Hirosaki University) for his kind guide, comment and encouragement. The author greatly acknowledges to Professor Richard N. Zare (Stanford University) for his generous help in Stanford. The author acknowledges all the collaborators and co-workers in his study.

This work has been supported by in part by a grant from the Grant-in-Aid for Young Scientists (B) and the Grant-in-Aid for the Global COE Program from the Ministry of Education, Culture, Sports, Science and Technology, Japan. This study is also supported by the Japan Society for the Promotion of Science and the Shimadzu Science Foundation.

Finally, the author is very grateful to the Society for Chromatographic Sciences for selecting him as a recipient of the Encouragement Award in 2011 and gave him the opportunity to publish this focusing review.

Reference

- [1] Manz, A.; Graber, N.; Widmer, H. M., *Sens. Actuators B* **1990**, 244–248.
- [2] Vilknær, T.; Janásek, D.; Manz, A. *Anal. Chem.* **2004**, 76, 3373–3386.
- [3] Dittrich, P. S.; Tachikawa, K.; Manz, A. *Anal. Chem.* **2006**, 78, 3887–3907.
- [4] West, J.; Becker, M.; Tombrink, S.; Manz, A. *Anal. Chem.* **2008**, 80, 4403–4419.
- [5] Manz, A.; Harrison, D. J.; Verpoorte, E.; Fettingner, J. C.; Paulas, A.; Lüdi, H.; Widmer, H. M., *J. Chromatogr.* **1992**, 593, 253–258.
- [6] Bruin, G. J. M. *Electrophoresis* **2000**, 21, 3931–3951.
- [7] Wu, D.; Qin, J.; Lin, B. *J. Chromatogr. A* **2008**, 1184, 542–549.
- [8] Kenyon, S. M.; Meighan, M. M.; Hayes, M. A. *Electrophoresis* **2011**, 32, 482–493.
- [9] Breadmore, M. C. *Electrophoresis* **2007**, 28, 254–281.
- [10] Sueyoshi, K.; Kitagawa, F.; Otsuka, K. *J. Sep. Sci.* **2008**, 31, 2650–2666.
- [11] Breadmore, M. C.; Thabano, J. R. E.; Dawod, M.; Kazarian, A. A.; Quirino, J. P.; Guijt, R. M. *Electrophoresis* **2009**, 30, 230–248.
- [12] Breadmore, M. C.; Dawod, M.; Quirino, J. P. *Electrophoresis* **2011**, 32, 127–148.
- [13] Kutter, J. P.; Jacobson, S. C.; Ramsey, J. M., *J. Microcol. Sep.* **1999**, 12, 93–97.
- [14] Wu, Q.; Bienvenue, J. M.; Hassan, B. J.; Kwok, Y. C.; Giordano, B. C.; Norris, P. M.; Landers, J. P.; Ferrance, J. P. *Anal. Chem.* **2006**, 78, 5704–5710.
- [15] Bienvenue, J. M.; Legendre, L. A.; Ferrance, J. P.; Landers, J. P. *Forensic Sci. Int.: Genet.* **2010**, 4, 178–186.
- [16] Reedy, C. R.; Hagan, K. A.; Marchiarullo, D. J.; Dewald, A. H.; Barron, A.; Bienvenue, J. M.; Landers, J. P. *Anal. Chim. Act.* **2011**, 687, 150–158.
- [17] Cakal, C.; Ferrance, J. P.; Landers, J. P.; Caglar, P. *Anal. Bioanal. Chem.* **2010**, 398, 1909–1917.
- [18] Kang, Q.-S.; Li, Y.; Xu, J.-Q.; Li, Y.-T.; Huang, W.-H. *Electrophoresis* **2010**, 31, 3028–3034.
- [19] Proczek, G.; Augstin, V.; Descroix, S.; Hennion, M.-C. *Electrophoresis* **2009**, 30, 515–524.
- [20] Wang, C.; Jemere, A. B.; Harrison, D. J. *Electrophoresis* **2010**, 31, 3703–3710.
- [21] Tennico, Y. H.; Remcho, V. T. *Electrophoresis* **2010**, 31, 2548–2557.
- [22] Ambrosi, A.; Guix, M.; Merkoçi, A. *Electrophoresis* **2011**, 32, 861–869.
- [23] Tia, S. Q.; He, M.; Kim, D.; Herr, A. E. *Anal. Chem.* **2011**, 83, 3581–3588.
- [24] Apori, A. A.; Herr, A. E. *Anal. Chem.* **2011**, 83, 2691–2698.
- [25] Chen, X.; Kapil, M. A.; Hughes, A. J.; Herr, A. E. *Anal. Chem.* **2011**, 83, 6573–6579.
- [26] Tetala, K. K. R.; van Beek, T. A. J. *Sep. Sci.* **2010**, 33, 422–438.
- [27] Sakai-Kato K.; Ishikura, K. *Anal. Sci.* **2009**, 25, 969–978.
- [28] Sung, W.-C.; Chen, H.-H.; Makamba, H.; Chen, S.-H. *Anal. Chem.* **2009**, 81, 7967–7973.
- [29] Yeung, S. H. I.; Liu, P.; Bueno, N. D.; Greenspoon, S. A.; Mathies, R. A. *Anal. Chem.* **2009**, 81, 210–217.
- [30] Liu, P.; Scherer, J. R.; Greenspoon, S. A.; Chiesl, T. N.; Mathies, R. A. *Forensic Sci. Int.: Genet.* **2011**, 5, 484–492.
- [31] Hokkanen, A.; Sirén, H.; Amundsen, L. K.; Kolari, K.; Franssila, S.; Tuomikoski, S.; Stuns, I.; Rovio, S.; Nevanen, T. K.; Takkinen, K.; Söderlund, H. *Mycrosyst. Technol.* **2009**, 15, 611–619.
- [32] Yang, W.; Sun, X.; Wang, H.-Y.; Woolley, A. T. *Anal. Chem.* **2009**, 81, 8230–8235.
- [33] de Jong, J.; Lammertink, R. G. H.; Wessling, M. *Lab Chip* **2006**, 6, 1125–1139.
- [34] Khandurina, J.; Jacobson, S. C.; Waters, L. C.; Foote, R. S.; Ramsey, J. M. *Anal. Chem.* **1999**, 71, 1815–1819.
- [35] Foote, R. S.; Khandurina, J.; Jacobson, S. C.; Ramsey, J. M. *Anal. Chem.* **2005**, 77, 57–63.
- [36] Hatch, A. V.; Herr, A. E.; Throckmorton, D. J.; Brennan, J. S.; Singh, A. K. *Anal. Chem.* **2006**, 78, 4976–4984.
- [37] Dai, J.; Ito, T.; Sun, L.; Crooks, R. M. *J. Am. Chem. Soc.* **2003**, 125, 13026–13027.
- [38] Kim, S. J.; Song, Y.-A.; Han, J. *Chem. Soc. Rev.* **2010**, 39, 912–922.
- [39] Wang, Y.-C.; Stevens, A. L.; Han, J. *Anal. Chem.* **2005**, 77,

- 4293–4299.
- [40] Wang, Y.-C.; Han, J. *Lab Chip* **2008**, *8*, 392–394.
- [41] Lee, J. H.; Song, Y.-A.; Tannenbaum S. R.; Han, J. *Anal. Chem.* **2008**, *80*, 3198–3204.
- [42] Kim, S. M.; Burns, M. A.; Hasselbrink, E. F. *Anal. Chem.* **2006**, *78*, 4779–4785.
- [43] Kuo, C.-H.; Wang, J.-H.; Lee, G.-B. *Electrophoresis* **2009**, *30*, 3228–3235.
- [44] Nge, P. N.; Yang, W.; Pagaduan, J. V.; Woolley, A. T. *Electrophoresis* **2011**, *32*, 1133–1140.
- [45] Yamamoto, S.; Watanabe, Y.; Nishida, N.; Suzuki, S. *J. Sep. Sci.* **2011**, *34*, 2879–2884.
- [46] Wu, D.; Steckl, A. J. *Lab Chip* **2009**, *9*, 1890–1896.
- [47] Hoeman, K. W.; Lange, J. J.; Roman, G. T.; Higgins, D. A.; Culbertson, C. T. *Electrophoresis* **2009**, *30*, 3160–3167.
- [48] Foster, R. E.; Hert, D. G.; Chiesl, T. N.; Fredlake, C. P.; Barron, A. E. *Electrophoresis* **2009**, *30*, 2014–2024.
- [49] Sun, M.; Lin, J. S.; Barron, A. E. *Electrophoresis* **2011**, *32*, 3233–3240.
- [50] Hou, C.; Herr, A. E. *Anal. Chem.* **2010**, *82*, 3343–3351.
- [51] Price, C. W.; Leslie, D. C.; Landers, J. P. *Lab Chip* **2009**, *9*, 2484–2494.
- [52] Khandurina, J.; McKnight, T. E.; Jacobson, S. C.; Waters, L. C.; Foote, R. S.; Ramsey, J. M. *Anal. Chem.* **2000**, *72*, 2995–3000.
- [53] Kim, J.; Byun, D.; Mauk, M. G.; Bau, H. H. *Lab Chip* **2009**, *9*, 606–612.
- [54] Lekwichai, A.; Porntheeraphat, S.; Bunjongpru, W.; Sripumkhai, W.; Supadech, J.; Rahong, S.; Hruanun, C.; Poyai, A.; Nukeaw, J. *Ad. Mat. Res.* **2010**, *93–94*, 105–108.
- [55] Zhang, Y.; Park, S.; Yang, S.; Wang, T.-H. *Biomed. Microdevices* **2010**, *12*, 1043–1049.
- [56] Jha, S. K.; Joo, G.-S.; Ra, G.-S.; Lee, H. H.; Kim, Y.-S. *IEEE Sens. J.* **2011**, *vol. 11, No. 9*, 2065–2070.
- [57] Liu, C. N.; Toriello, N. M.; Mathies R. A. *Anal. Chem.* **2006**, *78*, 5474–5479.
- [58] Liu, P.; Seo T. S.; Beyor, N.; Shin, K.-J.; Scherer, J. R.; Mathies R. A. *Anal. Chem.* **2007**, *79*, 1881–1889.
- [59] Thaitrong, N.; Toriello, N. M.; Bueno, N. D.; Mathies, R. A. *Anal. Chem.* **2009**, *81*, 1371–1377.
- [60] Beyor, N.; Yi, L.; Seo, T. S.; Mathies, R. A. *Anal. Chem.* **2009**, *81*, 3523–3528.
- [61] Liu, P.; Li, X.; Greenspoon, S. A.; Scherer, J. R.; Mathies, R. A. *Lab Chip* **2011**, *11*, 1041–1048.
- [62] Scherer, J. R.; Liu, P.; Mathies, R. A. *Rev. Sci. Instrum.* **2010**, *81*, 113105-1–6.
- [63] Zhong, R.; Pan, X.; Jiang, L.; Dai, Z.; Qin, J.; Lin B. *Electrophoresis* **2009**, *30*, 1297–1305.
- [64] Li, N.; Jablonowski, C.; Jin, H.; Zhong, W. *Anal. Chem.* **2009**, *81*, 4906–4913.
- [65] Tanaka, Y.; Xi, H.; Sato, K.; Mawatari, K.; Renberg, B.; Nilsson, M.; Kitamori, T. *Anal. Chem.* **2011**, *83*, 3352–3357.
- [66] Noblitt, S. D.; Lewis, G. S.; Liu, Y.; Hering, S. V.; Collett, Jr., J. L.; Henry, C. S. *Anal. Chem.* **2009**, *81*, 10029–10037.
- [67] Sikanen, T.; Pedersen-Bjergaard, S.; Jensen, H.; Kostianinen, R.; Rasmussen, K. E.; Kotiaho, T. *Anal. Chim. Act.* **2010**, *658*, 133–140.
- [68] Wu, R.-G.; Yang, C.-S.; Wang, P.-C.; Tseng, F.-G. *Electrophoresis* **2009**, *30*, 2025–2031.
- [69] Svec F. *Electrophoresis* **2009**, *30*, S 68–S 82.
- [70] Jemere, A. B.; Martinez, D.; Finot, M.; Harrison, D. J. *Electrophoresis* **2009**, *30*, 4237–4244.
- [71] Liu, P.; Meagher, R. J.; Light, Y. K.; Yilmaz, S.; Chakraborty, R.; Arkin, A. P.; Hazen, T. C.; Singh, A. K. *Lab Chip* **2011**, *11*, 2673–2679.
- [72] Çetin, B.; Li, D. *Electrophoresis* **2011**, *32*, 2410–2427.
- [73] Nakano, A.; Chao, T.-C.; Camacho-Alanis, F.; Ros, A. *Electrophoresis* **2011**, *32*, 2314–2322.
- [74] Sasaki, N.; Hosokawa, K.; Maeda, M. *Lab Chip* **2009**, *9*, 1168–1170.
- [75] Jain, A.; Verma, K. K. *Anal. Chim. Act.* **2011**, *706*, 37–65.
- [76] Liu, C.; Qu, Y.; Luo, Y.; Fang, N. *Electrophoresis* **2011**, *32*, 3308–3318.
- [77] Felhofer, J. L.; Blanes, L.; Garcia, C. D. *Electrophoresis* **2010**, *31*, 2469–2486.