ANALYTICAL SCIENCES FEBRUARY 2012, VOL. 28

Reviews

Recent Progress of On-line Sample Preconcentration Techniques in Microchip Electrophoresis

Fumihiko KITAGAWA,*† Takayuki KAWAI, Kenji SUEYOSHI,** and Koji OTSUKA****

**Department of Frontier Materials Chemistry, Graduate School of Science and Technology, Hirosaki University, 3 Bunkyo, Hirosaki, Aomori 036–8561, Japan*
***Department of Material Chemistry, Graduate School of Engineering, Kyoto University, Katsura, Nishikyo, Kyoto 615–8510, Japan*

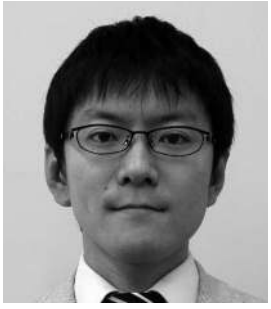
This review highlights recent developments and applications of on-line sample preconcentration techniques to enhance the detection sensitivity in microchip electrophoresis (MCE); references are mainly from 2008 and later. Among various developed techniques, we focus on the sample preconcentration based on the changes in the migration velocity of analytes in two or three discontinuous solutions system, since they can provide the sensitivity enhancement with relatively easy experimental procedures and short analysis times. The characteristic features of the on-line sample preconcentration applied to microchip electrophoresis (MCE) are presented, categorized on the basis of "field strength-" or "chemically" induced changes in the migration velocity. The preconcentration techniques utilizing field strength-induced changes in the velocity include field-amplified sample stacking, isotachophoresis and transient-isotachophoresis, whereas those based on chemically induced changes in the velocity are sweeping, transient-trapping and dynamic pH junction.

(Received November 1, 2011; Accepted November 14, 2011; Published February 10, 2012)

1 Introduction

Microchip electrophoresis (MCE) is one of the most useful separation techniques in micro total analysis systems (μ-TAS). Since several dozen micrometers wide and deep microchannels for MCE exhibit high heat dissipation ability compared to capillary electrophoresis (CE), higher electric field can be applied to the microchannel, resulting in a faster separation of ultra-small amount of analytes within a few minutes. Furthermore, highly flexible configuration of the channels allows paralleled separations on a single microchip. Such

Fumihiko KITAGAWA received a PhD degree from Graduate School of Science, Hokkaido University in 2002 concerning laser trapping-microspectroscopy under the supervision of Prof. N. Kitamura. He started his professional career as an Assistant Professor at Kyoto University in 2003, and was then promoted to a Lecturer in 2009. In 2011, he became an Associate Professor of Graduate School of Science and Technology, Hirosaki University. Currently, his scientific interest is focused on the development of high-performance microscale electrophoretic analysis systems.



Kenji SUEYOSHI is an Assistant Professor of the Graduate School of Engineering, Kyoto University. He received BS (2003), MS (2005), and PhD (2008) degrees from Kyoto University. His recent study involves improving the analytical performance in microscale electrophoresis.

† To whom correspondence should be addressed.
E-mail: kitagawa@cc.hirosaki-u.ac.jp

characteristics in MCE are highly effective for high-throughput analyses of various analytes ranging from small ions to biomacromolecules.

However, poor concentration sensitivity due to a short optical path length and/or a small detection volume in the microchannel is often problematic in MCE, preventing the commercialization of the MCE instruments. To overcome this drawback, two major approaches, including the applications of highly sensitive detection schemes and on-line sample preconcentration techniques, have been devoted to MCE. Among several sensitive detectors, laser-induced fluorescence (LIF),[1-3] mass spectrometry (MS),[4,5] electrochemical (EC) detection[6] and thermal lens microscopy (TLM)[7] are helpful to improve the detectability in MCE. But there are significant limitations in the applications of these detectors, *i.e.*, LIF, EC and TLM require appropriate sample labeling, and the interfacing between the MCE separation device and MS detector has been less developed. Hence, many on-line sample preconcentration techniques developed in CE have been mainly applied to MCE. Besides this, some novel sample preconcentration techniques utilizing specific characteristics of microchips have appeared in MCE.[8]

The on-line sample preconcentration techniques for MCE have four classifications: preconcentration by analyte velocity change in two or three discontinuous solutions system, focusing,[9-20] solid phase extraction,[21-23] and electrokinetic trapping (filtering).[24-27] Among them, novel focusing and electrokinetic trapping techniques have been exhaustively investigated in the recent decade. In the focusing technique, the migration direction of the analytes is reversed in the separation channel, and as a result that they are focused at a point where the migration velocity becomes zero. The focused zones in the microchannel can be simultaneously detected without any mobilization step by employing a whole-channel imaging detector which is suitable for chip-based analysis systems. In addition to classical isoelectric focusing (IEF), electric field gradient focusing (EFGF),[15,16] temperature gradient focusing (TGF)[17,18] and bipolar electrode focusing (BPEF)[19,20] techniques have been developed and applied to MCE. As the development of the fabrication techniques for monolithic and nano-sized structures have progressed, these structure integrated microchannels are employed for the on-line sample preconcentration. Physically and electrokinetically preconcentration techniques using the nanostructures are called solid phase extraction[21-23] and electrokinetic trapping,[24-27] respectively. These approaches can give extremely high preconcentration efficiency, especially for biomacromolecules. However, these focusing, solid phase extraction, and electrokinetic trapping techniques often require highly sophisticated fabrication processes for microdevices and/or complicated experimental procedures. On the other hand, the on-line sample preconcentration based on the changes in the analyte migration velocity at discontinuous solution interfaces is useful in the application to MCE, since such techniques can provide a sensitivity enhancement with a relatively easy experimental procedure and short analysis time.

In the discontinuous solutions system, the analytes dissolved in a sample matrix whose components are different from those in a background solution (BGS) are accumulated around the boundary of the two solutions. To obtain effective velocity changes of the analytes, the differences in the electric field, the retention factor, and pH are formed between the sample and BGS zones. In this article, the on-line sample preconcentration techniques based on the migration velocity change in MCE from the year 2008 are briefly reviewed, with categorization on the basis of field strength- or chemically induced changes in the migration velocity. The sample preconcentration techniques by the field strength-induced velocity change include field-amplified sample stacking[28] and isotachophoresis, whereas those by the chemically induced velocity change include sweeping,[29] transient-trapping[30] and dynamic pH junction[31] techniques.

2 Preconcentration by Field Strength-induced Changes in Migration Velocity

2·1 Field-amplified sample stacking (FASS)

Among various on-line sample preconcentration techniques, field-amplified sample stacking (FASS) is the simplest and most fundamental approach.[28] When the FASS technique is applied to MCE, a large volume of a sample solution with a low electrolyte concentration is introduced into the separation channel filled with a BGS containing a large amount of electrolytes. Since the electric field strength in the sample zone is higher than that in the BGS zone due to the difference in the conductivity, the electrophoretic migration of ionic analytes in the sample zone is faster than that in the BGS. The ionic analytes, which move at a faster velocity in the long sample zone, slow down when they pass the sample/BGS boundary. As a result, the analytes are "stacked" into a shorter zone than the original sample plug length. To obtain higher preconcentration efficiency in FASS, one needs a large difference in the conductivity between the two solutions and a large volume injection of the sample solution.

In MCE, FASS is commonly used to improve the sensitivity for various ionic analytes such as amino acids, peptides, proteins, DNA, amines, and metal ions.[8] In these applications, LIF detection has been mainly employed, so that a fluorescence labeling of analytes have been accompanied. In the recent years, on the other hand, universal capacitively coupled contactless conductivity detection (C^4D) schemes and EC detectors have been combined with FASS-MCE to enhance the sensitivity for food dyes,[32] inorganic anions,[33] amines,[34] tetracycline antibiotics,[35] and endocrine disruptors.[36] To achieve further sensitivity enhancement, FASS have been combined

Koji Otsuka received a D. Eng. degree from the Graduate School of Engineering, Kyoto University in 1986 concerning micellar electrokinetic chromatography under the supervision of Prof. T. Ando. After working under a JSPS Fellowship for Japanese Junior Scientists, he joined Osaka Prefectural College of Technology in 1988. In 1995, he moved to Faculty of Science, Himeji Institute of Technology. In 2002, he became a Professor of the Graduate School of Engineering, Kyoto University. His research area includes microscale electrophoresis, micro total analysis systems, and nano-biochips.

Takayuki Kawai is a Doctor course student of the Graduate School of Engineering, Kyoto University. He received a BS degree from the University of Tokyo, and an MS degree from Kyoto University (no photograph).

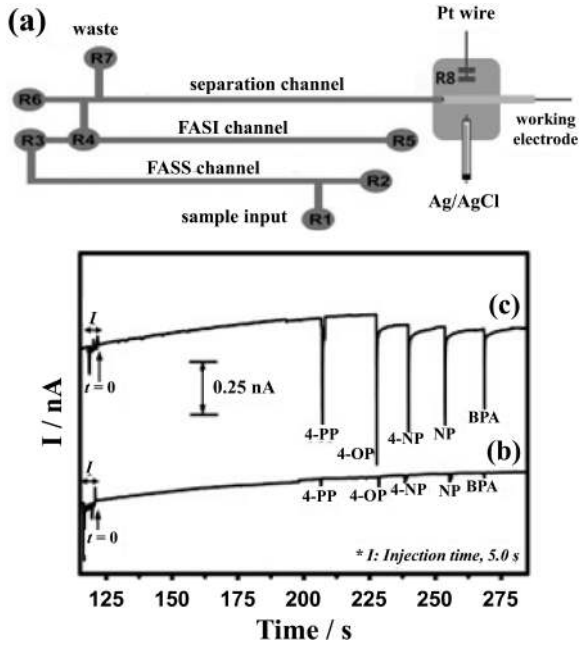


Fig. 1 (a) Schematic diagram of the microfluidic device and electropherograms for five endocrine disruptor phenolic compounds obtained with (b) only FASI step and (c) both FASS and FASI steps. Sample concentration: (b) 15.0, (c) 5.1 nM. Reprinted with permission from Ref. 36 (Copyright 2010 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim).

with a field-amplified sample injection (FASI) technique. In FASI, a low-conductivity sample solution is electrokinetically injected into the separation channel filled with a high-conductivity BGS, resulting in the injection of a larger amount of the analytes as a narrow band. In the application of both FASS and FASI to MCE, a microchip consisting of three parallel channels is employed: the first two channels are for FASS and subsequent FASI steps, while the third one is for the MCE separation (Fig. 1a).[32,35,36] Since concentrated analytes are introduced into the separation channel without the loss of the effective separation length on the three parallel channel chip, both high sensitivity and high resolution can be obtained. As a typical result, a baseline separation of five phenolic endocrine disrupters is successfully attained by FASS-FASI-MCE-EC detection with a limit of detection (LOD) of 7 – 11 fM (Fig. 1b).[36]

2·2 Large volume sample stacking with electroosmotic flow pump (LVSEP)

Large volume sample stacking with electroosmotic flow pump (LVSEP), which is the on-line sample preconcentration technique developed in CE,[37] is based on the stacking of extremely large volumes of an anionic sample solution. In the original LVSEP technique, an electroosmotic flow (EOF) in a bare fused-silica capillary is suppressed by employing acidic BGS, allowing sample stacking and separation to proceed continuously without intermediate polarity switching. A typical experimental procedure of LVSEP is very simple. First, a bare fused silica capillary is exhaustively filled with a low-conductivity sample solution, typically dissolved in deionized water, next a constant voltage is applied between the inlet and outlet reservoirs filled with acidic and high-conductivity BGS. During the stacking of anionic analytes at the sample/BGS boundary, the concentrated analytes move toward the cathode by a faster EOF generated in the sample zone. After the stacking process, the removal of the sample matrix (SM) and the introduction of the acidic BGS into the capillary suppress the EOF, so that the analytes start to migrate toward the anode. Finally, they are separated by zone electrophoresis. In the LVSEP technique, both high enrichment efficiency and high resolution can be obtained. Since the sample solution filled into the whole capillary is concentrated to a narrow zone and the separation starts from the cathodic end, the total capillary length is utilized both for the preconcentration and as the effective separation length. Although this is a simple and effective approach for the on-line sample preconcentration, only a few reports on the application of LVSEP have appeared. This may be because the separation BGS used in LVSEP is limited to acidic buffers.

We have recently reported the application of LVSEP to the MCE analysis of oligosaccharides.[38] To analyze oligosaccharides by LVSEP-MCE, we have modified the original LVSEP technique. In our modified LVSEP, a poly(vinyl alcohol) (PVA)-coated single straight microchannel is used for the preconcentration and the separation. Although it is well-known that the PVA coating is employed for suppressing both EOF and nonspecific adsorption of biomolecules, we have found that the EOF velocity of the PVA-coated channel is enhanced up to 4.4×10^{-4} cm^2/V·s in the low-conductivity sample zone, while in the high-conductivity BGS zone the EOF rate is suppressed to 1.0×10^{-5} cm^2/V·s. We have anticipated that such EOF velocity change by the conductivity is suitable for LVSEP-MCE.

By fluorescence imaging of the LVSEP process in the 40 mm-long PVA-coated channel, one can observe the preconcentration behavior of anionic fluorescein dye, as shown in Fig. 2. In the figure, the moving preconcentration boundary is traced from the anodic end. After the voltage is applied, the analytes are stacked from the anodic side (Fig. 2a). The concentrated analytes move toward the cathode (Fig. 2b) and the vacant SM plug is quickly pumped out of the inlet at the cathodic end by the enhanced EOF due to the low conductivity of the SM. When the analytes reach the channel position of 3 – 4 mm from the cathodic end, the apparent velocity of the analytes is reduced drastically, as shown in Figs. 2c and 2d, and then the moving direction of the concentrated analyte zone is inverted to the anode (Fig. 2e). This indicates that the electrophoretic velocity of the analytes exceeds the EOF velocity because the introduction of the high-conductivity BGS into most of the microchannel suppresses the EOF in the PVA-coated microchannel. After the turn, the analytes migrate with almost the same velocity to the anodic end (Fig. 2f). In this way, the sample stacking and zone electrophoretic separation can be consecutively performed under the constant voltage without the loss of effective separation length. The fluorescence imaging clearly demonstrates that only manual sample injection throughout the single straight channel and the application of the constant voltage between the both ends of the single channel are required in LVSEP-MCE. Due to the combination of the LVSEP technique with MCE, therefore, a complicated electrokinetic sample injection process required in a conventional MCE can be omitted, allowing to simplify experimental procedures and improve the detection sensitivity in MCE.

To evaluate the analytical performance, we conducted the LVSEP-microchip zone electrophoresis (MCZE) and the conventional MCZE analyses of oligosaccharides on the 80 mm-long straight channel microchip and a cross channel microchip with the 40 mm-long separation channel, respectively. In the conventional MCZE analysis of the glucose ladder, G1 – G10 are well separated but oligomers longer than G10 cannot be detected as shown in Fig. 3a. In spite of 500-fold

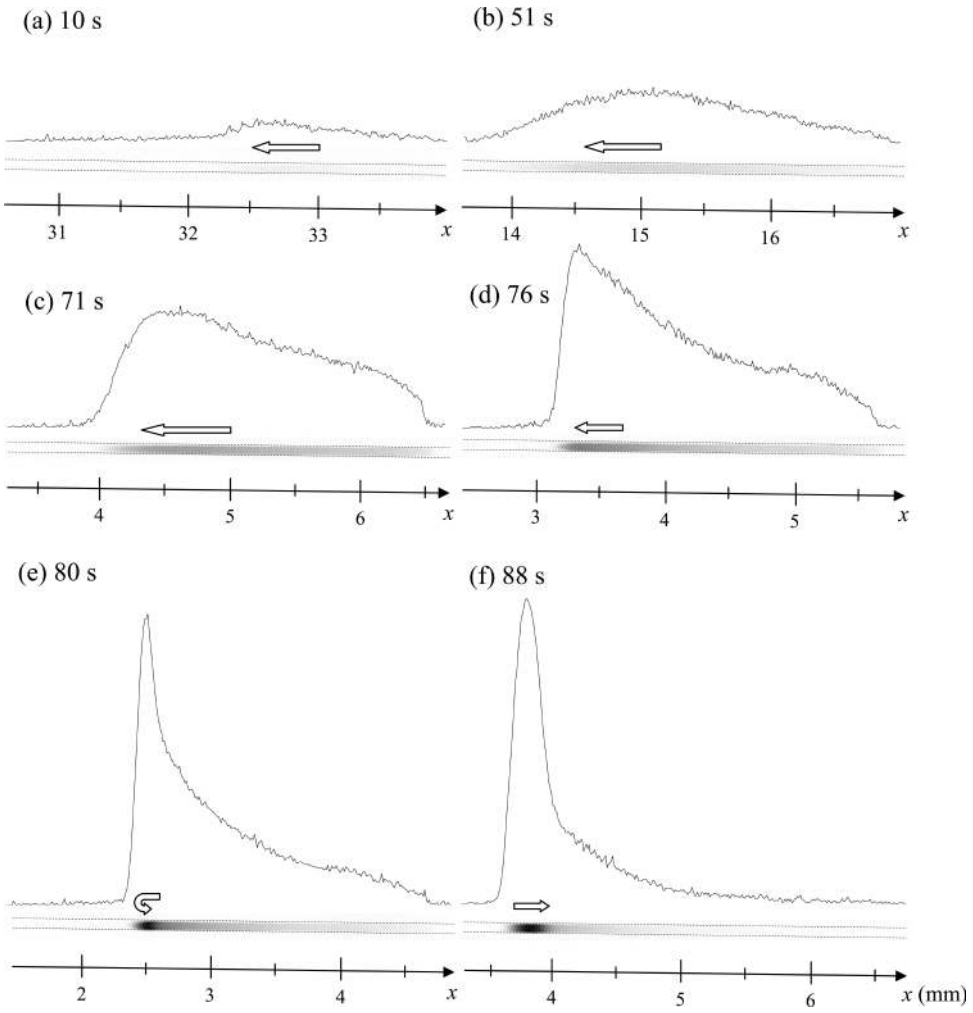


Fig. 2 Fluorescence images and intensity profile of fluorescein concentrated by LVSEP-MCE in a 40 mm-long straight channel. The abscissa axis means the distance from the anodic channel end. The length of the arrow is proportional to the apparent velocity of the analyte zone. Reprinted with permission from Ref. 38 (Copyright 2010 American Chemical Society).

sample dilution, on the other hand, the separated peaks for G1 – G20 appear in the LVSEP-MCZE analysis (Fig. 3b). The sensitivity enhancement factor (SEF), which is calculated by comparing the peak height obtained in the LVSEP condition with that in the conventional PI-MCZE taking into account the dilution factor regardless of the injection volume of the sample solution, is estimated to be 930 – 2900. In the analysis of *N*-glycans released from bovine ribonuclease B, five oligosaccharides are well separated by LVSEP-MCZE, with the SEFs ranging from 1900 to 2200. Since the PVA coating can suppress the nonspecific surface adsorption of biomolecules, the modified LVSEP-MCE technique will provide effective concentration and separation of a wide variety of anionic biomolecules including DNA, peptides, proteins, organic acids, and metabolites.

2·3 Isotachophoresis (ITP)

ITP is also recognized as one of the most effective on-line preconcentration techniques in CE and MCE, especially for ionic analytes dissolved in a high salt concentration matrix. In ITP, an ionic sample solution is injected between leading (L) and terminating (T) ion zones. L and T ions exhibit higher and lower electrophoretic mobilities, respectively, than those of analyte ions. When a voltage is applied, a higher and lower electric field is produced in T and L ions, respectively. Due to the difference in the field strength between the two zones, the sample zone is separated into divided zones. If the concentrations of the analytes are lower than that of LE, the separated analytes are concentrated according to the Kohlrausch regulating function.[39] To obtain both effective preconcentration and separation, ITP is often coupled with another separation mode, *e.g.*, capillary zone electrophoresis (CZE) or capillary gel electrophoresis (CGE). Such combination is called transient ITP (tITP).[40]

In ITP, three solutions including sample, L and T electrolytes are successively injected for the preconcentration. To perform the successive injection in MCE, a complicated voltage control is required on a microchip with multiple injectors, *e.g.*, multi-T channel chip. Such an injection process often reduces the analytical reproducibility and requires bothersome optimization for the voltage program. To overcome this issue, we simply injected a sample solution containing L or T ions into the separation channel in tITP. Figure 4 shows the schematic of tITP-CZE using the sample matrix containing L ions and the BGS with T ions. In the first step, the sample zone is divided into discrete L and analyte zones, resulting in the ITP

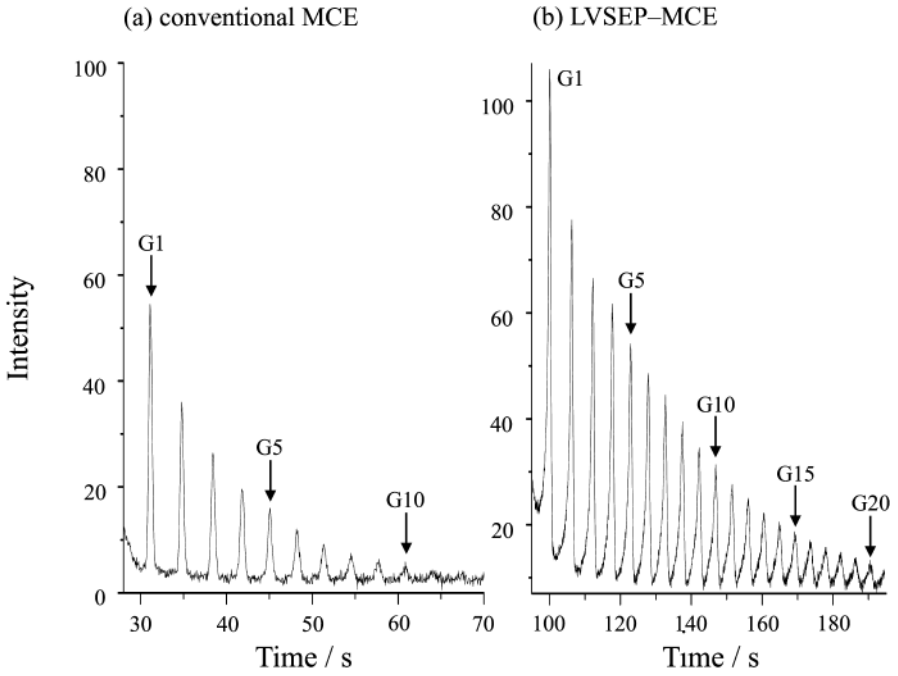


Fig. 3 Electropherograms of glucose ladder obtained with (a) conventional MCE and (b) LVSEP-MCE. Concentration of glucose ladder: (a) 160 ppb, (b) 320 ppt. Reprinted with permission from Ref. 38 (Copyright 2010 American Chemical Society).

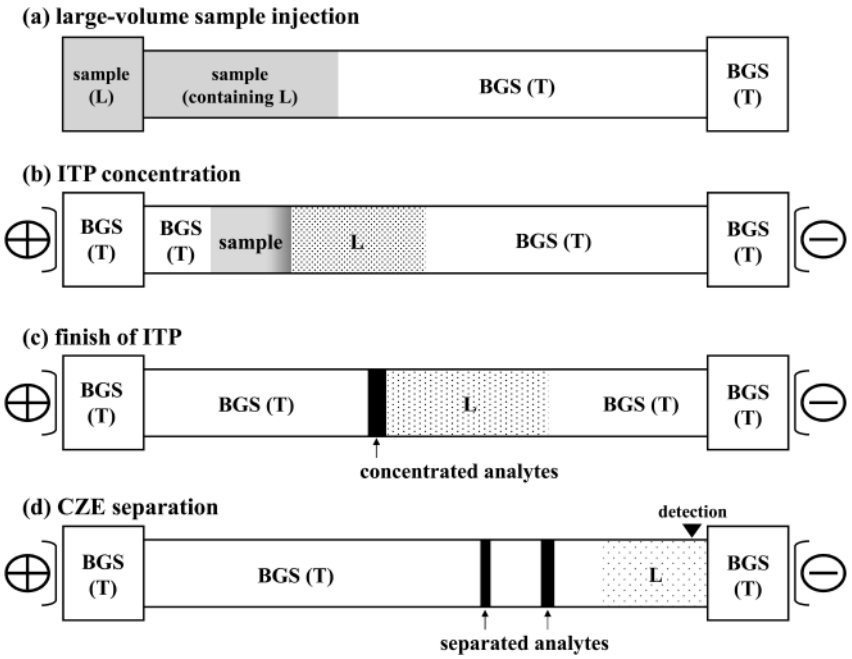


Fig. 4 Schematics of t-ITP-CZE in employing the sample solution containing L ions and the BGS with T ions.

preconcentration stage (Figs. 4b – 4c). After the ITP concentration, the concentration of the L ions decreases with time, and then the analytes begin to migrate in a CZE manner. Finally, the enriched analytes are separated by CZE, as shown in Fig. 4d. DNA, RNA, proteins, and oligosaccharides are concentrated and separated on conventional cross- or T-channel chips by combining tITP with CZE or CGE.[41-45] On the other hand, a multi T-channel chip is used to inject L, sample and T solutions successively in the ITP and tITP analysis. In the recent years, α-fetoproteins,[46] DNA ladder,[47,48] and PCR products[49] are well concentrated and resolved by employing the multi T-channel chips. As a very unique approach, Bottenus *et al*. have reported the application of a microchip with two reductions in the cross-sectional area along the axial direction of

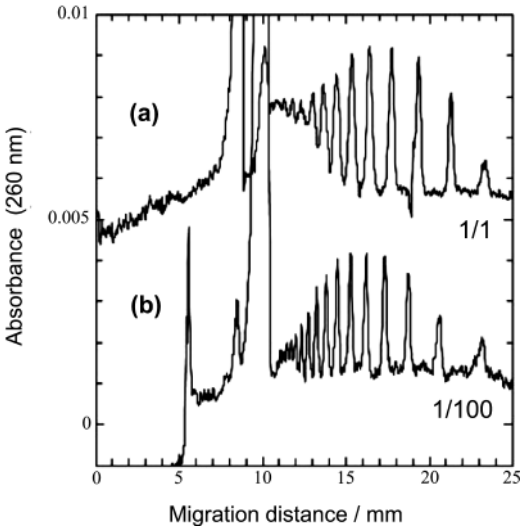


Fig. 5 Electropherograms of DNA fragments obtained with (a) conventional MCE (original concentration) and (b) the FEKS (1:100 diluted). Reprinted with permission from Ref. 51 (Copyright 2008 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim).

the channel to the ITP preconcentration of the biomarker cardiac troponin I and a fluorescent protein R-phycoerythrin.[49] Since the concentration is inversely proportional to the cross-sectional area, improved concentration efficiency can be obtained on the microchip with reduced cross-sectional areas. As a typical result, the concentration factors greater than 10000 are attained.

To improve the preconcentration efficiency, ITP and tITP have been coupled with a large volume sample injection. Davis *et al*. have reported the combination of gradient elution ITP (GEITP) with CZE in a single microcolumn.[50] In GEITP, continuous electrokinetic sample injection with variable hydrodynamic flow is utilized to form a discontinuous ionic interface within the sample reservoir. When L and T ions are present in the BGS and the sample matrix, the ITP preconcentration of the analytes occurs at the interface. The interface and enriched analytes are pulled into a microchannel, and then the sample solution is replaced with the BGS for CZE. By employing GEITP-CZE to a single straight channel chip, effective enrichment and separation of four amino acids is achieved in effective lengths of one centimeter. Hirokawa *et al*. have developed a novel sample preconcentration technique named floating electrokinetic supercharging (FEKS).[51,52] In FEKS, electrokinetic injection (EKI) and ITP sample preconcentration are performed in a separation channel on a cross-geometry microchip. In the preconcentration stage, a voltage is applied between sample and waste ports, whereas two BGS ports filled with an L solution are electrically floated. After the ITP-stacked zones pass the cross-part, they are migrated by introducing L ions from the BGS port to change from the ITP concentration to the CZE separation stage. Without any degradation of resolution, the LODs of DNA fragments are improved around ten times compared to those of conventional MCE (Fig. 5). Since these ITP and tITP techniques are suitable for concentrating sample solutions containing high-concentration salt, they should be effective for highly sensitive analysis of ionic analytes in various biomatrices.

3 Preconcentration by Chemically Induced Changes in Migration Velocity

3·1 Sweeping and transient-trapping (tr-trapping)

In the on-line sample preconcentration by sweeping, a sample solution containing no pseudostationary phase is introduced into a separation channel filled with a BGS containing pseudostationary phase. When the analytes are incorporated by pseudostationary phase at the sample/BGS boundary, the migration velocity of the analytes is decreased. As a result, the analytes are concentrated to a narrow zone. In the original sweeping technique, sodium dodecyl sulfate (SDS) micelle is employed as the pseudostationary phase to preconcentrate neutral and/or hydrophobic analytes.[29] In the recent four years, no application of the sweeping with SDS to MCE has been reported. Pan *et al*. have reported a combination of FASI with the sweeping with bovine serum albumin (BSA) for the ultrasensitive detection of green fluorescent protein (GFP).[53] A low-conductivity GFP solution void of BSA is introduced by FASI into a cross sectional region on a cross-channel chip, and then GFP is concentrated by the sweeping with BSA around the entrance of the separation channel. BSA is very effective stationary phase for the sweeping of GFP, resulting in the SEF of 3570 and the LOD of 8.4 pM. This highly effective preconcentration method is successfully applied to the detection of the GFP content in single *E. coli* cells. This is the first demonstration of the possibility of protein concentration by using BSA as a novel stationary phase in the sweeping technique. Applications of the BSA-sweeping to a wide variety of proteins are expected for highly-sensitive analysis of proteins in MCE.

Recently, we have reported a novel on-line sample preconcentration technique, which is called “transient-trapping (tr-trapping)”, based on the combination of a partial filling with the sweeping techniques using SDS micelle in MCE.[30] To perform the injection of the micellar and sample solutions into the separation channel, we employed a 5 way-cross channel chip (Fig. 6). In the tr-trapping technique, a short plug of the SDS micelle is partially injected into the separation channel filled with a BGS containing no SDS (Figs. 6a – 6b). Immediately after injecting the micellar solution, a sample solution void of SDS is injected as a long plug (Fig. 6c). In the case of using SDS as a pseudo stationary phase, neutral and hydrophobic analytes are suitable for the preconcentration by tr-trapping. When a separation voltage is applied to the separation channel, the analytes in the sample zone migrate toward the micellar zone by a fast EOF. In the tr-trapping condition, the decrease in the conductivity of the micellar plug is induced by the difference in the ionic transfer between the sample/micellar solutions. This decrease in the conductivity causes a strong electric field localized on the micellar plug, resulting in the formation of a highly-concentrated and sharp SDS micelle zone around the boundary. The analytes which reach the sample/micellar solution boundary are strongly retained and concentrated by the concentrated SDS micelle zone. Since the analytes cannot penetrate into the micellar zone, they are focused on the boundary as an extremely narrow band (Fig. 6d). We have termed the phenomenon as the “trap” mechanism. At the same time, the concentration of the SDS micelle is gradually decreased by the diffusion. As the retention of the analytes by the SDS micelle is decreased, the analytes trapped around the boundary are released into the micellar zone in the order of the hydrophobicity (Fig. 6e). Consequently, the analytes are separated by the difference in the release time. We have called the release time difference-based separation as the

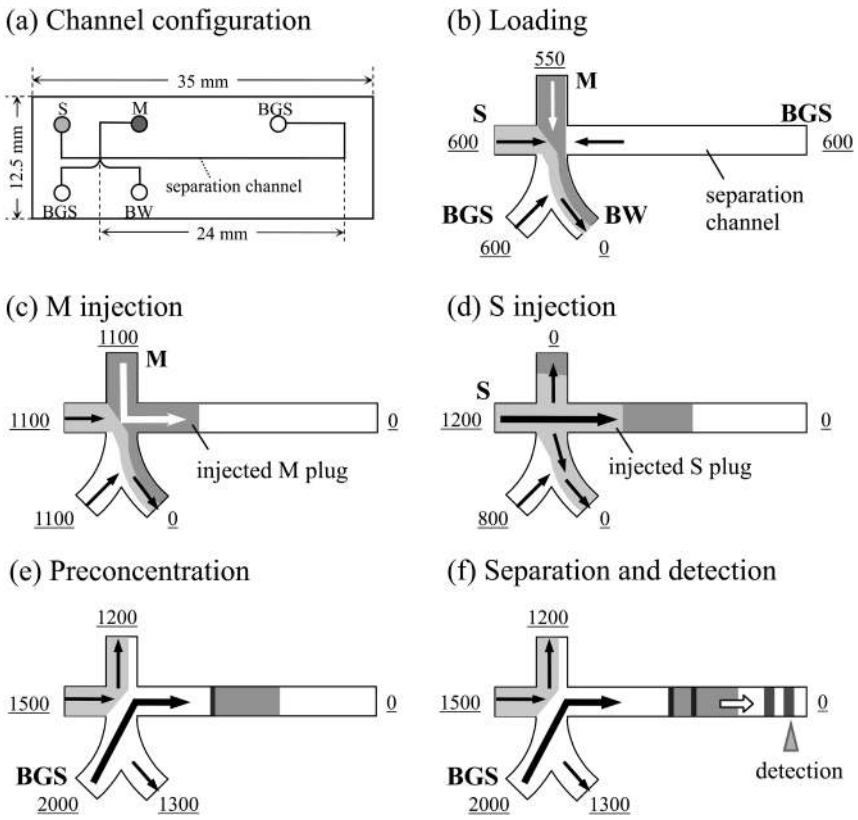


Fig. 6 Schematics of tr-trapping on a 5 way-cross microchip. Underlined values indicate the applied voltages (V). Reprinted with permission from Ref. 30 (Copyright 2008 American Chemical Society).

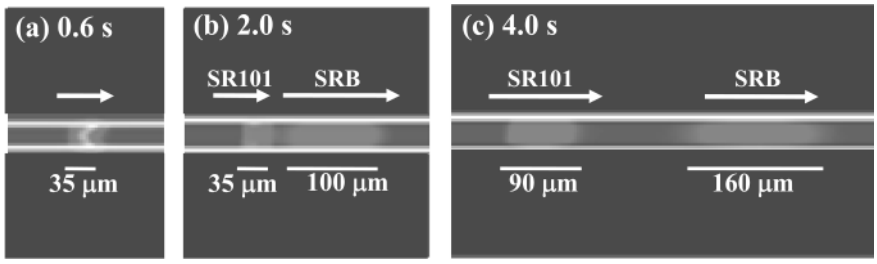


Fig. 7 Fluorescence imaging of the tr-trapping process. Reprinted with permission from Ref. 30 (Copyright 2008 American Chemical Society).

"release" mechanism. After the analytes are released, they migrate toward the detection point (Fig. 6f).

Fluorescence imaging of the tr-trapping process in MCE has clearly revealed the trap-and-release mechanism (Fig. 7). In this experiment, anionic fluorescent dyes, sulforhodamine 101 (SR101) and sulforhodamine B (SRB), are employed as the model analytes. After the sample plug injection with its length of *ca*. 1.2 mm, the analytes are completely concentrated at the separation time of 0.6 s as shown in Fig. 7a. At 2.0 s, more hydrophilic SRB is released from the boundary, resulting in the release-time difference separation of SRB and SR101 (Fig. 7b). The released SRB band is broadened to 100 μm during the migration in the micellar zone, whereas SR101 remains as the narrow band. At 4.0 s, SR101 is released and broadened to 90 μm during the migration in the micellar plug (Fig. 7c). These results show that extremely concentrated analytes are rapidly separated within 2.0 s by utilizing the separation length of *ca*. 1.2 mm. In the tr-trapping condition, the SEF of SR101 in is estimated to be 400 relative to the conventional MCE analysis. Interestingly, the resolution of SRB and SR101 is also improved. Therefore, highly effective preconcentration and ultra-fast separation is realized by the tr-trapping technique in MCE.

In the original tr-trapping with SDS micelle, the analytes for the preconcentration are limited to hydrophobic compounds, since the trap mechanism requires high retention factors to the SDS micelle. To extend the applicability of the tr-trapping to hydrophilic compounds, we have introduced hydrophobic labeling with a 6-((4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-propionyl)-amino) hexanoic acid (BODIPY) dye.[54] Due to the labeling ability of BODIPY for biomolecules containing amino groups, amino acids are selected as test

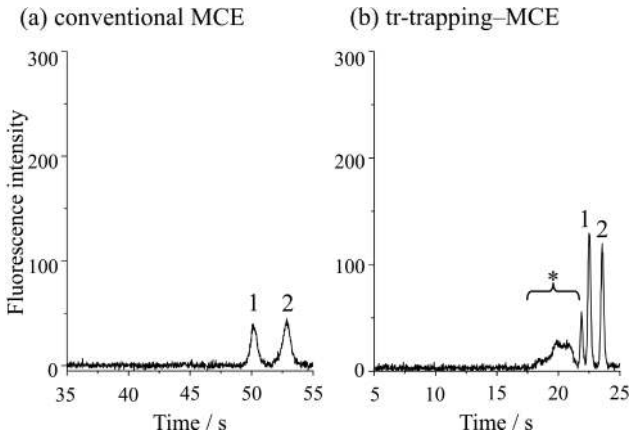


Fig. 8 Electropherograms obtained with (a) conventional MCE and (b) tr-trapping-MCE. Sample concentrations: (a) 500, (b) 25 nM. Observed peaks are identified as 1, Lys; 2, His; ∗, system peak of the free BODIPY dyes. Reprinted with permission from Ref. 54 (Copyright 2011 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim).

analytes. In the tr-trapping-MCE analysis of two BODIPY labeled amino acids, 80 – 160-fold enhancement of the peak intensity and a baseline separation is also achieved within 30 s as shown in Fig. 8. These results demonstrate that hydrophilic analytes containing amino groups can be analyzed by tr-trapping-MCE with the high sensitivity, resolution and short analysis time. Since the tr-trapping based on the partial filling technique is compatible with MS detection, the combination of the tr-trapping and MS detector is expected to obtain structural information about the minor compounds.

3·2 Dynamic pH junction

A dynamic pH junction is the sample preconcentration technique based on the pH difference between the sample solution and the BGS.[31] Since the dynamic pH junction technique is suitable for the concentrations of weak acids, bases, and their zwitter ions, it is employed especially for the analysis of biomolecules. In the dynamic pH junction, a sample solution containing analytes with higher isoelectric point or pK_a than the pH of the SM is injected as a long plug into a separation channel filled with a BGS with high pH. Since the charge states of the analytes differ among the SM and BGS, the migration of the analytes should decelerate at the pH junction, resulting in the concentration of the analytes.

In CE, the dynamic pH junction technique has been employed to the analysis of adrenalins, nucleotides, purines, estrogens, flavines, catecholamines, amino acids, peptides, proteins, and so on.[55] On the other hand, the applications to MCE have not been reported till lately. Recently, Kazarian *et al*. have applied the dynamic pH junction technique to the MCE analysis of monosaccharides and disaccharides.[56] By labeling neutral saccharides with amino ethyl fluorescein which has a carboxyl group, rapid preconcentration and separation is obtained within 120 s. As a result, the 10-fold decrease in the LOD is obtained by the preconcentration effect compared to the conventional MCE. Since efficient concentration of biomolecules such as peptides and proteins is expected by the dynamic pH junction technique, the coupling with the MCE-mass spectrometry system will realize high-performance analysis of trace peptides for proteomic studies.

4 Conclusion

In this review, the recent progress of on-line sample preconcentration techniques field strength- and chemically induced changes in the migration velocity at discontinuous solution interfaces is overviewed in MCE. In the recent years, the on-line sample preconcentration on simple microchip devices, *e.g*., LVSEP, t-ITP and FEKS, has attracted much attention because these techniques provide both sufficient preconcentration efficiencies and good separation performance with simple and easy experimental procedures. Furthermore, the preconcentration techniques compatible with MS detection, *e.g*., tr-trapping and dynamic pH junction, have been applied to MCE. The coupling of MCE-MS with the on-line sample preconcentration should be helpful for proteomic and metabolomic researches. On the other hand, MCE is expected to work as a separation part in portable miniaturized-analyzers. In such analytical devices, simple micro-detectors with relatively low sensitivity are employed, so that these preconcentration techniques are indispensable for improving the detectability. Therefore, further improvements of the preconcentration and separation efficiencies with short analysis time, high reproducibility and robustness are still required in the on-line sample preconcentration in MCE.

5 Acknowledgements

This work was supported in part by the Grant-in-Aid for Scientific Research (No. 22750069) from the Japan Society for the Promotion of Science (JSPS) and SENTAN, JST.

6 References

1. D. J. Harrison, P. G. Glavina, and A. Manz, *Sens. Actuators, B*, **1990**, *10*, 244.
2. S. C. Jacobson, R. Hergenröder, L. B. Koutny, R. J. Warmack, and J. M. Ramsey, *Anal. Chem*., **1994**, *66*, 1107.
3. S. C. Jacobson, L. B. Koutny, R. Hergenröder, A. W. Moore Jr., and J. M. Ramsey, *Anal. Chem*., **1994**, *66*, 3472.
4. I. M. Lazar, R. S. Ramsey, S. Sundberg, and J. M. Ramsey, *Anal. Chem*., **1999**, *71*, 3627.
5. Y. Tachibana, K. Otsuka, S. Terabe, A. Arai, K. Suzuki, and S. Nakamura, *J. Chromatgr., A*, **2003**, *1011*, 181.
6. R. P. Baldwin, *Electrophoresis*, **2000**, *21*, 4017.
7. K. Uchiyama, A. Hibara, K. Sato, H. Hisamoto, M. Tokeshi, and T. Kitamori, *Electrophoresis*, **2003**, *24*, 179.
8. K. Sueyoshi, F. Kitagawa, and K. Otsuka, *J. Sep. Sci*., **2008**, *31*, 2650.
9. J. Ou, T. Glawdel, C. L. Ren, and J. Pawliszyn, *Lab Chip*, **2009**, *9*, 1926.
10. G. Sommer, A. K. Singh, and A. V. Hatch, *Anal. Chem*., **2008**, *80*, 3327.
11. K. Shimura, K. Takahashi, Y. Koyama, K. Sato, and T. Kitamori, *Anal. Chem*., **2008**, *80*, 3818.
12. F. Kitagawa, S. Aizawa, and K. Otsuka, *Anal. Sci*., **2009**, *25*, 979.
13. J. Wen, E. W. Wilker, M. B. Yaffe, and K. F. Jensen, *Anal. Chem*., **2010**, *82*, 1253.
14. R. Ishibashi, T. Kitamori, and K. Shimura, *Lab Chip*, **2010**, *10*, 2628.
15. S. L. Lin, Y. Y. Li, H. D. Tolley, P. H. Humble, and M. H. Lee, *J. Chromatogr., A*, **2006**, *1125*, 254.

16. P. Myers and K. D. Bartle, *J. Chromatogr., A*, **2004**, *1044*, 253.
17. D. Ross and L. E. Locascio, *Anal. Chem*., **2002**, *74*, 2556.
18. M. Becker, A. Mansouri, C. Beilein, and D. Janasek, *Electrophoresis*, **2009**, *30*, 4206.
19. R. Dhopeshwarkar, D. Hlushkou, M. Nguyen, U. Tallarek, and R. M. Crooks, *J. Am. Chem. Soc*., **2008**, *130*, 10480.
20. D. R. Laws, D. Hlushkou, R. K. Perdue, U. Tallarek, and R. M. Crooks, *Anal. Chem*., **2009**, *81*, 8923.
21. W. Yang, X. Sun, T. Pan, and A. T. Woolley, *Electrophoresis*, **2008**, *29*, 3429.
22. N. Thaitrong, N. M. Toriello, N. Del Bueno, and R. A. Mathies, *Anal. Chem*., **2009**, *81*, 1371.
23. N. Beyor, L. N. Yi, T. S. Seo, and R. A. Mathies, *Anal. Chem*., **2009**, *81*, 3523.
24. J. Dai, T. Ito, L. Sun, and R. M. Crooks, *J. Am. Chem. Soc*., **2003**, *125*, 13026.
25. S. Yamamoto, S. Hirakawa, and S. Suzuki, *Anal. Chem*., **2008**, *80*, 8224.
26. T. Hahn, C. K. O'Sullivan, and K. S. Drese, *Anal. Chem*., **2009**, *81*, 2904.
27. L. F. Cheow, S. H. Ko, S. J. Kim, K. H. Kang, and J. Han, *Anal. Chem*., **2010**, *82*, 3383.
28. D. S. Burgi and R. L. Chien, *Anal. Chem*., **1991**, *63*, 2042.
29. J. P. Quirino and S. Terabe, *Science*, **1998**, *282*, 465.
30. K. Sueyoshi, F. Kitagawa, and K. Otsuka, *Anal. Chem*., **2008**, *80*, 1255.
31. P. Britz-McKibbin and D. D. Y. Chen, *Anal. Chem*., **2000**, *72*, 1242.
32. K. S. Lee, M. J. A. Shiddiky, S. H. Park, D. S. Park, and Y. B. Shim, *Electrophoresis*, **2008**, *29*, 1910.
33. S. D. Noblitt, F. M. Schwandner, S. V. Hering, J. L. Collett, and C. S. Henry, *J. Chromatogr., A*, **2009**, *1216*, 1503.
34. Q. Guan and C. S. Henry, *Electrophoresis*, **2009**, *30*, 3339.
35. K. S. Lee, S. H. Park, S. Y. Won, and Y. B. Shim, *Electrophoresis*, **2009**, *30*, 3219.
36. H. B. Noh, K. S. Lee, B. S. Lim, S. J. Kim, and Y. B. Shim, *Electrophoresis*, **2010**, *31*, 3053.
37. Y. He and H. K. Lee, *Anal. Chem*., **1999**, *71*, 995.
38. T. Kawai, K. Sueyoshi, F. Kitagawa, and K. Otsuka, *Anal. Chem*., **2010**, *82*, 6504.
39. F. Kohlrausch, *Ann. Phys. Chem*., **1897**, *62*, 209.
40. F. Foret, E. Szoko, and B. L. Karger, *J. Chromatogr*., **1992**, *608*, 312.
41. D. Liu, Z. Ou, M. Xu, and L. Wang, *J. Chromatogr., A*, **2008**, *1214*, 165.
42. D. Liu, B. Chen, L. Wang, and X. Zhou, *Electrophoresis*, **2009**, *30*, 4300.
43. R. B. Schoch, M. Ronaghi, and J. G. Santiago, *Lab Chip*, **2009**, *9*, 2145.
44. J. Wang, Y. Zhang, M. R. Mohamadi, N. Kaji, M. Tokeshi, and Y. Baba, *Electrophoresis*, **2009**, *30*, 3250.
45. H. Nagata, T. Itoh, Y. Baba, and M. Ishikawa, *Anal. Sci*., **2010**, *26*, 731.
46. C. C. Park, I. Kazakova, T. Kawabata, M. Spaid, R. L. Chien, H. G. Wada, and S. Satomura, *Anal. Chem*., **2008**, *80*, 808.
47. C. C. Lin, B. K. Hsu, and S. H. Chen, *Electrophoresis*, **2008**, *29*, 1228.
48. L. Y. Qi, X. F. Yin, and J. H. Liu, *J. Chromatogr., A*, **2009**, *1216*, 4510.
49. D. Bottenus, T. Z. Jubery, Y. Ouyang, W. J. Dong, P. Dutta, and C. F. Ivory, *Lab Chip*, **2011**, *11*, 890.
50. N. I. Davis, M. Mamunooru, C. A. Vyas, and J. G. Shackman, *Anal. Chem*., **2009**, *81*, 5452.
51. T. Hirokawa, Y. Takayama, A. Arai, and Z. Q. Xu, *Electrophoresis*, **2008**, *29*, 1829.
52. Z. Q. Xu, K. Murata, A. Arai, and T. Hirokawa, *Biomicrofluid*, **2010**, *4*, 14108.
53. Q. Pan and M. Zhao, *Anal. Chem*., **2009**, *81*, 5333.
54. K. Sueyoshi, K. Hashiba, T. Kawai, F. Kitagawa, and K. Otsuka, *Electrophoresis*, **2011**, *32*, 1233.
55. A. A. Kazarian, E. F. Hilder, and M. C. Breadmore, *J. Sep. Sci*., **2011**, *34*, 2800.
56. A. A. Kazarian, E. F. Hilder, and M. C. Breadmore, *Analyst*, **2010**, *135*, 1970.