Xiao-Feng Fan[1]
Qi Li[1]
Shi-Li Wang[1]
Zhang-Run Xu[1]
Wen-Bin Du[2]
Qun Fang[2]
Zhao-Lun Fang[1]

[1]Research Center for Analytical Sciences, Northeastern University, Shenyang, P. R. China
[2]Chemistry Department, Institute of Microanalytical Systems, Zhejiang University, Hangzhou, P. R. China

Received May 7, 2008
Revised August 4, 2008
Accepted August 22, 2008

Research Article

High-throughput analysis of DNA fragments using a miniaturized CE system combined with a slotted-vial array sample introduction system

An automated nanoliter sample introduction system was combined to a liquid-core waveguide (LCW)-based microfluidic CE system for high-throughput analysis of DNA fragments. The main component of the sample introduction system was a motor-driven plate, on which a circular array of bottom-slotted vials containing sample/buffer solutions was placed. A 7 cm-long LCW capillary served as both the sample probe and separation channel. The inlet terminal of the capillary could pass through the slots of the vials for electrokinetic sample introduction, and the capillary outlet was immersed in the solution of a reservoir, behind which a PMT facing directly to the outlet was positioned. A diode laser was used as excitation source for LCW LIF detection. Performance of the system was demonstrated through the separation of DNA fragments. Baseline separation was achieved for all 11 fragments of ΦX174-*Hae*III digest DNA with a throughput of 33/h. Theoretical plate number for 603 bp fragment was 7.3×10^6/m, corresponding to a plate height 0.14 μm. The detection limitation for 603 bp fragment was 0.4 ng/μL with a precision of 2.2% RSD for the peak height. Automated sample changing and introduction were achieved with only 0.3 nL gross sample consumption for each cycle.

Keywords:
CE / High-throughput analysis / Liquid-core waveguide / Miniaturization / Sample introduction
DOI 10.1002/elps.200800287

1 Introduction

Since 1990s, an increasing number of researchers have directed their attention to the fields of omics sciences and systems biology. For these researches, high-throughput and dynamic data are essential, thus the progress in analytical technologies would greatly facilitate the studies at various omic levels. CE plays a vital role in the omics field owing to its rapid separation, high efficiency, and low consumption [1–4]. Despite all these advantages, enhancing the throughput of CE is still a topic of investigation. Various approaches were attempted for improving the throughput of CE, one of which is capillary array electrophoresis (CAE) [5–7], where requirement for bundles of capillaries brings about those disadvantages as complicated sample introduction and physical operation, as well as high apparatus costs. Therefore, CAE systems are not versatile enough to be routinely used in laboratories.

The throughput can also be improved by developing high-speed microfluidic separation systems. Rapid progresses of microfluidic analysis have been made in CE separation area since the concept of μTAS was introduced [8]. Microfluidic CE systems possess great potential in high-throughput analysis with various advantages [9–11] including: rapid separation, parallel operation, automated analysis, low cost, and ease of coupling to portable instruments for field applications or point-of-care testing. The miniaturized CE systems allow the integration of analytical functional components into a single microchip to achieve high-throughput and automated analysis. Such systems have been frequently used for rapid analysis of nucleic acids [12–14]. In our group, a miniaturized CE device based on a short capillary has been developed for fast separation of DNA fragments without requiring complicated microfabrication techniques [15].

Automated continuous sample introduction is essential to high-throughput analysis. However, in many cases, the sample introduction and changing operations are usually performed manually. For a typical sample change performed in most of the chip-based CE systems, the

Correspondence: Dr. Zhang-Run Xu, Research Center for Analytical Sciences, Northeastern University, Box 332, Wenhua Road 3-11, Heping District, Shenyang 110004, P. R. China
E-mail: xuzr@mail.neu.edu.cn
Fax: +86-24-8367-6698

Abbreviations: CAE, capillary array electrophoresis**; HV,** high-voltage**; LCW,** liquid-core waveguide**; SI,** sequential injection

high-voltage (HV) power supply has to be interrupted before removing the microchip from the detection system, and the sample reservoirs are emptied, washed several times, and refilled with the new sample solution, then the microchip is replaced, inserting electrodes into the reservoirs, adjusting the detection point, and switching on the HV power supply again. These time-consuming operations dramatically decrease the overall throughput of the system. It could be cumbersome that more separation channels in capillary array system entail more required reservoirs and peripheral equipments. Smith *et al.* [16] reported a novel interface to solve this problem. In their study, only a single capillary was employed to inject sample into an array of separation channels on a microchip. The tip of the capillary was sequentially inserted into each channel to introduce sample plug. A computer-driven micromanipulator repeated the injection process by moving the capillary back and forth across the width of the chip. This repeated sample introduction could be carried out continuously without interrupting the separation process and without user intervention. In Zhang's work [17], a 96-well microwell plate with V-shaped bottom was vertically placed in front of the microchip. A sample-loading capillary connected the microwell and the microdevice. Sample solution remained in the wells because of the surface tension when the plate moved under the control of computer for the sample change. Later, Du *et al.* [18] developed a microfluidic flow injection system with high-throughput nanoliter sample introduction. A capillary sampling probe was used for sample introduction, but the capillary was connected to the chip microchannel and did not need moving for the sample introduction. Sample/buffer solutions were filled in an array of microvials, on the bottom of which slots were fabricated for free passage of the sampling probe. Sample injection was performed by the linear movement of the microvial array. The highest sampling throughput of 1000/h could be obtained with only 0.6 nL sample consumption for each cycle. Similar sample introduction systems were also used for microfluidic sequential injection (SI) analysis [19] and microfluidic CE [20] by the same group. Recently, Kim *et al.* [21] reported a sample introduction method for a well-less CAE chip. Multiple samples were directly injected onto CAE channels from sample loaders using hydrophilic sample bridges.

In our previous work, for the purpose of simplifying the instrument, liquid-core waveguide (LCW) technique was employed to capture the fluorescence emitted from the excited analytes in DNA analysis system [15]. A 5 cm-long Teflon AF-coated fused-silica capillary was used both as the separation channel for CE separation and LCW cell for transmission of emitted fluorescence to the detector. DNA samples of 11 fragments were successfully resolved in 400 s with an LED-excited fluorescence detection combined with a lock-in amplifier technique. However, an SI sample introduction system with a syringe pump, a selective valve and a PTFE tube connected to the separation channel was required, which limited the sample throughput and increased dead space as well as sample consumption. In addition, the detection part did not live up to expectations because the lock-in amplifier used in the work was too large as compared with the other components, which denuded important advantages of microfluidic systems in miniaturization and portability.

In this study, an automated nanoliter sample introduction system with slotted-vial array was combined with a miniaturized CE system slightly modified from our previous work. Continuous sample injection can be achieved in the separation of DNA fragments. This combination resulted in an uninterrupted separation, which is essential to high-throughput analysis. The sensitivity was also improved by using SYTOX Orange as DNA dye and diode laser as light source. PVP was used as sieving matrix possessing the virtue of being replaceable and self-coating. The sampling frequency, the sample/reagent consumption, and the analytical efficiency were also improved in the present system.

2 Materials and methods

2.1 Chemicals

All reagents were of analytical reagent grade unless mentioned otherwise. Deionized water obtained from a Water Pro PS system (LabConco, Kansas, MO, USA) was used throughout. ΦX174-*Hae*III digest DNA Marker (0.5 μg/μL, containing 11 fragments: 72, 118, 194, 234, 271, 281, 310, 603, 872, 1078, and 1353 bp) was purchased from TaKaRa Biotechnology (Dalian, China). DNA sample solutions were prepared fresh by diluting DNA stock solutions with deionized water. SYTOX Orange was obtained from Molecular Probes (Eugene, Salem, USA) and PVP (Av.Mol.Wt.: 1,300,000) from Aldrich Chemical (Milwaukee, WI, USA). PVP powder was added to 1 × TBE (89 mM Tris, 89 mM borate, and 2 mM EDTA) solution to yield 4.0% w/v polymer solution. TBE solution with SYTOX Orange used as running buffer was prepared by adding 5 μL SYTOX Orange solution (0.25 mM) into 1 mL 1 × TBE buffer.

2.2 Automated sample introduction system

The high-throughput auto-sampling system in this work was composed of an array of 24 sample vials fixed on a home-made circular plate (9 cm diameter), which was driven by a stepping motor. These sample vials were made from 0.2 mL microtubes by fabricating 1.5 mm-wide, 2 mm-deep slots on the bottom of the tubes for the passage of the sampling probe, and then horizontally fixed on the circular plate side by side. The sampling probe scanned through the slots by rotating the stepping-motor-driven plate under the computer control.

2.3 Microfluidic CE system

The LCW-based CE system modified from our previous work [15] is as shown in Fig. 1. In brief, the separation part was a 7 cm-long (with an effective separation length of

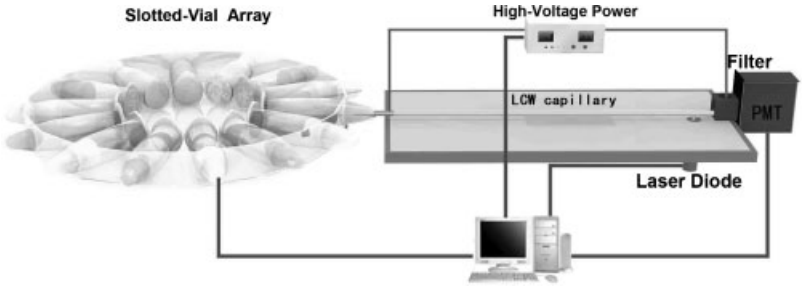

Figure 1. Schematic diagram of the LCW-based miniaturized CE system combined with the nanoliter sample introduction system.

5.5 cm) Teflon AF-coated fused-silica capillary (TSU050375, Polymicro Technologies, Phoenix, AZ, USA), which was used as both the separation channel and LCW detection cell. The inlet terminal of the capillary, with a platinum layer deposited on the outer surface of the capillary, served as both electrode and sample probe, could sweep through the slots of the sample vials. A 532 nm diode laser (Model PGL-XV, 10 mW, Changchun New Industries Optoelectronics Tech., Changchun, China) was used as excitation light source for LIF detection. The laser beam vertically illuminated the detection point on the LCW capillary, and was restricted using a diaphragm with a pinhole of 200 μm diameter fixed immediately to the capillary, without further focusing. The detection point was 15 mm from the LCW capillary outlet. A PMT (Model H5784, Hamamatsu, Japan) directly faced the capillary outlet, and a filter was inserted between the capillary and PMT. The output signal of PMT was recorded by a chart recorder (Model EB1P00, Dahua Instruments, Shanghai, China).

2.4 Procedures

Before use, the LCW capillary was flushed in the sequence: water, 0.2 M HCl, water, 0.1 M NaOH, water, and 1 × TBE buffer, each for 15 min. For DNA fragments separation, the capillary was filled with 4.0% w/v PVP in 1 × TBE solution containing 1.25 μM SYTOX Orange. The outlet reservoir near the PMT was filled with the same solution. The capillary outlet and the platinum electrode were all immersed into the solution of the reservoir while the other electrode and the capillary inlet could scan through the vial slots.

The vials on the plate were filled alternately with 20 μL samples and 100 μL buffer solutions. A home-built HV power supply variable in 0–2000 V range was used for CE separation. Voltages were applied between the electrodes at the outlet and inlet of the capillary. Sample was electrokinetically introduced into the probe capillary when the sampling probe was immersed in the sample solution of the vial. Sample separation was performed by rotating the sample plate to immerse the capillary probe in the buffer solution. A continuous analysis for different samples was achieved by rotating the sample plate to allow the probe sweep through each slot sequentially. The typical program of HV operation set the output voltage to +1.1 kV for 0.5 s during the sample introduction stage and +1.1 kV for 180 s during the separation stage.

Programs written in Labview (National Instruments) were used to control the entire system.

3 Results and discussion

3.1 System overview and considerations

Our group intended to develop an automated, simple and efficient micro-CE system as described previously [15]. It is essential to select a suitable auto-sampler coupled with the miniaturized CE system, especially for high-throughput analysis. The split-flow interfaces were frequently used for achieving continuous and automated sample introduction of microfluidic CE systems [22–26]. In these systems, conventional pumps and valves were usually employed for liquid manipulation. However, the sizes of the pumps and valves are in most cases incompatible with the miniaturization of CE systems, and the sample consumption (typically 1–80 μL) is still relatively large as compared with the amount used for separation. In this work, our former SI sample introduction system was substituted with a high-throughput auto-sampler. This introduction device adopted the principle of Fang's group [18] to realize continuous sample introduction without interruption. Electrokinetic sampling mode is often employed instead of pressure-driven approach, which is not so ideal for transporting viscous liquid. In the present system, the inlet of separation capillary functioned as a sampling probe, and the sample was introduced electrokinetically from the slotted vial into the capillary without resorting to pumps or valves. In order to further decrease the size of the sample introduction system, a circular sample plate was adopted to substitute the linear sample platform [18]. The plate with an array of bottom-slotted vials was driven by a stepping motor. Sample/buffer solutions remained in the vials without leakages, owing to the surface tension when the angular velocity was less than 7.5 rad/s. In this study, an angular velocity of 2.5 rad/s was adopted, which can ensure the time for a step movement between neighboring vials is short enough. Sample change was achieved conveniently by allowing the probe to scan through the slots sequentially.

Since the time for a step movement was less than 0.2 s, no air would be taken into the capillary, which would ensure current stability. The capillary was also employed as channel for both the separation of DNA samples and the transmission of the fluorescence emission, which simplified the structure of the system. The volume of the entire sampling system was significantly smaller than the previous SI sample introduction system. The sample consumption decreased significantly, and the net sample consumption was only 0.3 nL that is 10 000 folds less than the previous result (3 μL) [15].

LED has been employed as the excitation source for fluorimetric detection in previous work [15] due to its advantages of simplicity, low cost, stability, *etc*. However, the sensitivity of this LED-based system, using ethidium bromide as DNA dye, was low, so a lock-in amplifier had to be combined. In order to improve the system sensitivity, a diode laser was adopted as an excitation source, and SYTOX Orange, a high-fluorescence efficiency dye, as the intercalater. These means not only made the system more sensitive but further simplified the entire system and decreased the cost by eliminating the need for an amplifier.

3.2 Sample introduction

In the present system, injection voltage and separation voltage (both 1.1 kV) were kept constant during the continuous analysis to avoid affecting system performance. The effects of different injection times of 0.1, 0.2, 0.5, 1.0, 2.0, and 4.0 s were investigated (Fig. 2). Data for relative fluorescence intensity and theoretical plate number were calculated on 603 bp fragments. When the injection time was 4.0 s, poor resolution of all 11 fragments of the DNA sample was observed, and the peak heights of 872, 1078, and 1353 bp were much lower compared with those of other fragments. This phenomenon may be explained by molecular diffusion and electrokinetic sampling bias effect, resulting in the shorter fragments of DNA sample having more chances to enter in the capillary. Moreover, with the injection time increasing, the sample plug lengthened, which would result in peak broadening and poor separation efficiency. Satisfactory resolution of DNA fragments was observed in the sampling time range of 0.1–2.0 s. The relative fluorescence intensity elevated sharply when the sampling time increased from 0.1 to 0.5 s. With concurrent considerations in relative fluorescence intensity, plate number and the peak height reproducibility, an injection time of 0.5 s was adopted for further studies.

3.3 Sieving matrix

PVP polymer solution features low viscosity and self-coating ability, which hydroxypropylmethylcellulose does not possess. Low viscosity facilitates the capillary refilled with PVP solution, and self-coating ability enables reduction of the electro-osmotic flow and the interaction between DNA and the capillary wall, so the multiple steps and long conditioning time for regenerating the column were avoided [27, 28]. These characteristics of PVP solution make it an ideal medium for high-throughput microfluidic CE analysis. In this work, PVP serving as DNA sizing matrix was filled in the uncoated LCW capillary.

The effect of the PVP concentration was studied in the range 1.5–6.0% with 0.5 s injection time and 150 V/cm field strength using ΦX174-*Hae*III digest DNA sample (Fig. 3). The efficiency of DNA fragments separation increased as the concentration of PVP varied from 1.5 to 5.5% due to the decreased mesh size. When the PVP concentration reached to 6.0%, mesh size might become too small to allow the DNA fragments passing through, so that no signal was

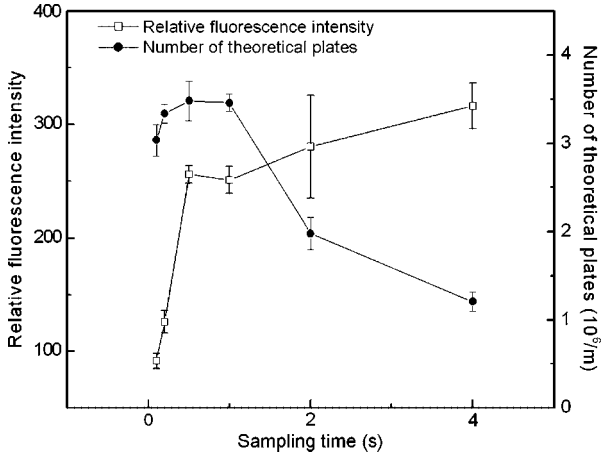

Figure 2. Effects of sampling time on peak height and plate number. Φ × 174-*Hae*III digest DNA Marker, 5 ng/μL; 1 × TBE buffer (pH 8.51); sieving matrix, 4.0% PVP solution; effective separation length, 5.5 cm; applied field strength for both injection and separation, 150 V/cm.

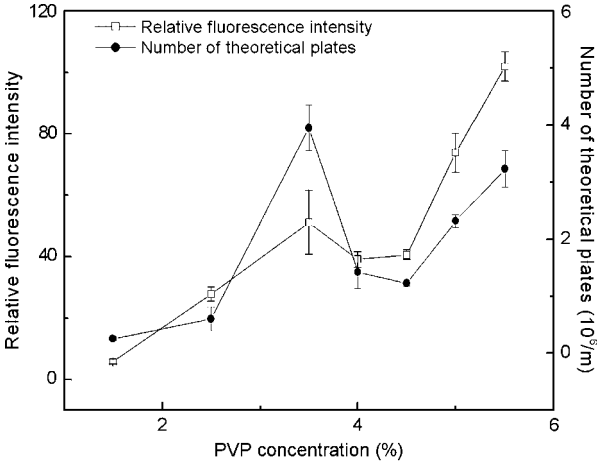

Figure 3. Effects of PVP concentration on peak height and plate number. Sampling time, 0.5 s; other conditions as in Fig. 2.

observed in 10 min after the sample introduction. The migration time would be less as matrix concentration decreased, but the resolution became poor while the concentration dropped below a certain level (<0.5%) presumably due to the disappearance of molecular entanglement. The effect of PVP with two different molecular weights was studied (as shown in Fig. 4). The 4.0% PVP with molecular weight 1 300 000 was adopted on account of its higher separation efficiency. The possible reason is that sieving matrix with high molecular weight may form more entanglement structure.

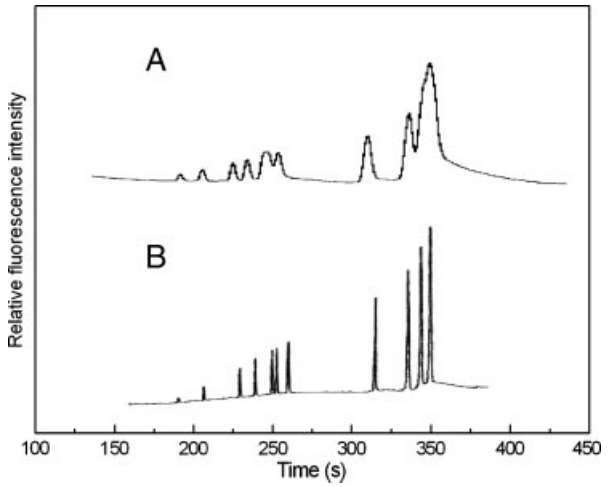

Figure 4. Effects of PVP with different molecular weights on separation efficiency. (A) PVP molecular weight of 360 000; (B) PVP molecular weight of 1 300 000. Sampling time, 0.5 s; other conditions as in Fig. 2.

3.4 Performance of the system

ΦX174-*Hae*III DNA digest sample and DNA Marker DL2000 were used to demonstrate the performance of the system. The reproducibility of the system is illustrated in Fig. 5A showing six replicate sample injections with ΦX174-*Hae*III DNA digest sample at 33/h throughput. The RSD of the peak height of 603 bp fragment was 2.2% ($n = 11$). The capability of continuous automated sample changing is illustrated in Fig. 5B showing electropherograms of different samples introduced sequentially. The electropherogram shows good resolution in the rapid continuous separation of all DNA fragments. The theoretical plate number of 603 bp fragment was 7.3×10^6/m, corresponding to a plate height of 0.14 μm. The limit of detection for 603 bp fragment was 0.4 ng/μL ($S/N = 3$). Gross sample

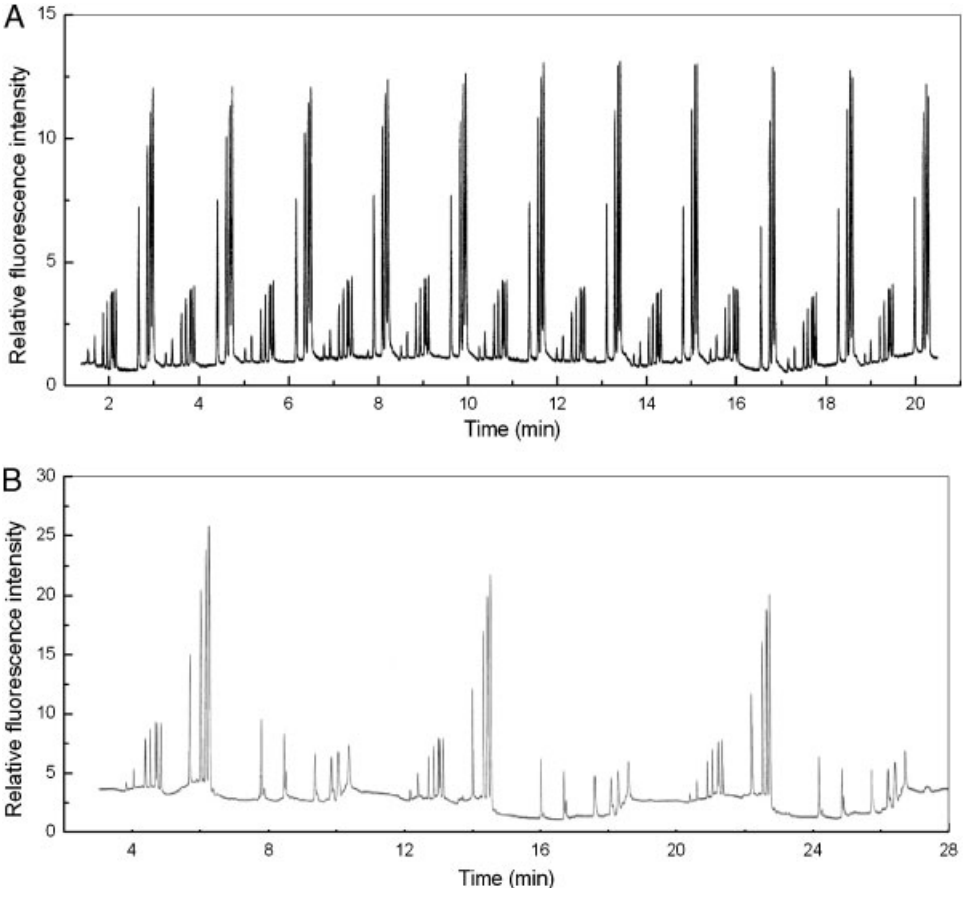

Figure 5. (A) Electropherogram of repetitively introducing 5 ng/μL ΦX174-*Hae*III digest DNA Marker for 11 times. (B) Electropherogram of alternatingly introducing 5 ng/μL ΦX174-*Hae*III digest DNA Marker and 5 ng/μL DL2000 DNA Marker for three cycles. Sampling time, 0.5 s; other conditions as in Fig. 2.

consumption was 0.3 nL, and the remaining sample in the slotted vial may be recovered for further use. The above results showed better performance of the present system compared with those results reported previously [15].

4 Concluding remarks

In this study, the LCW-based micro-CE system was combined with an auto-sampler for continuous DNA separation, based on which an automated high-throughput analysis system was developed. In order to improve the sensitivity and efficiency, diode laser and SYTOX Orange, as well as the readily changeable and self-coating PVP matrix, were employed. The entire system was improved in the degree of simplicity, sensitivity, throughput, reproducibility, and sample economy. The system developed in this work demonstrated future potentials in producing a portable instrument for routine analysis. This simple but efficient system could not only be applied to DNA analysis, but also be modified for the separation and detection of RNA, peptide, protein, lipid, fatty acid, and single cell.

The authors are indebted to the financial supports from National Natural Science Foundations of China (20527003, 20675012), National Basic Research Program of China (2007CB714503), and Key Laboratory for Precision and Non-traditional Machining of Ministry of Education, Dalian University of Technology (JMTZ200602).

The authors have declared no conflict of interest.

5 References

[1] Smith, L. M., *Nature* 1991, *349*, 812–813.

[2] Quigley, W. W. C., Dovichi, N. J., *Anal. Chem.* 2004, *76*, 4645–4658.

[3] Babu, C. V. S., Song, E. J., Babar, S. M., Wi, M. H., Yoo, Y. S., *Electrophoresis* 2006, *27*, 97–110.

[4] Song, E. J., Babar, S. M., Oh, E., Hasan, M. N. *et al.*, *Electrophoresis* 2008, *29*, 129–142.

[5] Krylov, S. N., Dovichi, N. J., *Anal. Chem.* 2000, *72*, 111–128.

[6] Carrilho, E., *Electrophoresis* 2000, *21*, 55–65.

[7] Mathies, R. A., Huang, X. H., *Nature* 1992, *359*, 167–169.

[8] Bruin, G. J. M., *Electrophoresis* 2000, *21*, 3931–3951.

[9] Cao, X. D., Fang, Q., Fang, Z. L., *Anal. Chim. Acta* 2004, *513*, 473–479.

[10] Righetti, P. G., Gelfi, C., D'Acunto, M. R., *Electrophoresis* 2002, *23*, 1361–1374.

[11] Behr, S., Mätzig, M., Levin, A., Eickhoff, H., Heller, C., *Electrophoresis* 1999, *20*, 1492–1507.

[12] Liu, S. R., Guttman, A., *Trends Anal. Chem.* 2004, *23*, 422–431.

[13] Lin, Y. W., Chang, H. T., *J. Chromatogr. A* 2005, *1073*, 191–199.

[14] Wu, D. P., Qin, J. H., Lin, B. C., *J. Chromatogr. A* 2008, *1184*, 542–559.

[15] Wang, S. L., Fan, X. F., Xu, Z. R., Fang, Z. L., *Electrophoresis* 2005, *26*, 3602–3608.

[16] Smith, E. M., Xu, H., Ewing, A. G., *Electrophoresis* 2001, *22*, 363–370.

[17] Zhang, B. L., Foret, F., Karger, B. L., *Anal. Chem.* 2001, *73*, 2675–2681.

[18] Du, W. B., Fang, Q., He, Q. H., Fang, Z. L., *Anal. Chem.* 2005, *77*, 1330–1337.

[19] Du, W. B., Fang, Q., Fang, Z. L., *Anal. Chem.* 2006, *78*, 6404–6410.

[20] He, Q. H., Fang, Q., Du, W. B., Huang, Y. Z., Fang, Z. L., *Analyst* 2005, *130*, 1052–1058.

[21] Kim, Y. H., Yang, I., Park, S. R., *Anal. Chem.* 2007, *79*, 9205–9210.

[22] Fang, Q., Wang, F. R., Wang, S. L., Liu, S. S., Fang, Z. L., *Anal. Chim. Acta* 1999, *390*, 27–37.

[23] Fu, C. G., Fang, Z. L., *Anal. Chim. Acta* 2000, *422*, 71–79.

[24] Huang, X. J., Pu, Q. S., Fang, Z. L., *Analyst* 2001, *126*, 281–284.

[25] Wang, S. L., Huang, X. J., Fang, Z. L., Dasgupta, P. K., *Anal. Chem.* 2001, *73*, 4545–4549.

[26] Attiya, S., Jemere, A. B., Tang, T., Fitzpartrick, G. *et al.*, *Electrophoresis* 2001, *22*, 318–327.

[27] Gao, Q. F., Yeung, E. S., *Anal. Chem.* 1998, *70*, 1382–1388.

[28] Erickson, D., Liu, X. Z., Venditti, R., Li, D. Q., Krull, U. J., *Anal. Chem.* 2005, *77*, 4000–4007.

