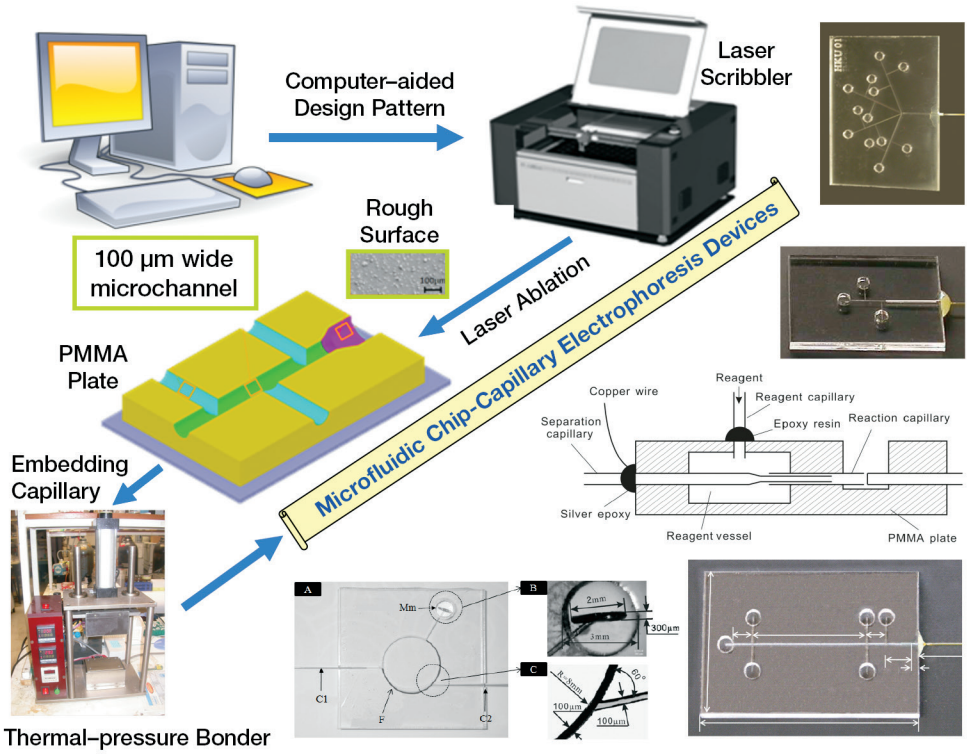
Microfluidic Chip-Capillary Electrophoresis Devices



Edited by Ying Sing Fung

Co-Editors: Qidan Chen, Fuying Du, Wenpeng Guo, Tongmei Ma, Zhou Nie, Hui Sun, Ruige Wu, and Wenfeng Zhao

CRC Press
Taylor & Francis Group

Microfluidic Chip-Capillary Electrophoresis Devices

Microfluidic Chip-Capillary Electrophoresis Devices

Edited by Ying Sing Fung

The University of Hong Kong
Hong Kong, China

Co-Editors: Qidan Chen, Fuying Du, Wenpeng Guo, Tongmei Ma, Zhou Nie, Hui Sun, Ruige Wu, and Wenfeng Zhao

CRC Press
Taylor & Francis Group
Boca Raton London New York

CRC Press is an imprint of the
Taylor & Francis Group, an **informa** business

CRC Press
Taylor & Francis Group
6000 Broken Sound Parkway NW, Suite 300
Boca Raton, FL 33487-2742

Version Date: 20150716

International Standard Book Number-13: 978-1-4822-5165-4 (eBook - PDF)

Visit the Taylor & Francis Web site at
http://www.taylorandfrancis.com

and the CRC Press Web site at
http://www.crcpress.com

Contents

Section III Integration to Enhance Analyte Detection

Section IV Integration to Achieve Intended Application

Section V Summary and Outlook

About the Editors

Dr. Ying Sing Fung received his BSc and MPhil from The University of Hong Kong, Hong Kong SAR, China, in 1975 and 1977, respectively, and PhD from the Imperial College of Science, Technology and Medicine, University of London, United Kingdom, in 1980. He is currently associate professor in the Department of Chemistry, the University of Hong Kong. He has been a member of the editorial board of *Journal of Chemical Education* (Chinese Chemical Society), *Journal of Biochemical and Biophysical Methods* (Elsevier Science, the Netherlands), and also Scientific Advisory Council member for Separation Science (Eclipse, UK).

He was an academic advisory committee member for Asian Conference on Analytical Chemistry, Asia-Pacific International Symposium on Microscale Separation and Analysis, and China-Japan Molten Salt Chemistry and Technology Symposium, and was appointed as guest professor by the following institutions in China: Open Laboratory of Electroanalytical Chemistry (Changchun Institute of Applied Chemistry), Institute of Material Science and Engineering (Jilin University), and Dongguan Institute of Technology.

He was appointed as advisor for various Hong Kong SAR government departments in different capacities such as assessor for the Hong Kong Laboratory Accreditation Scheme for the Innovation and Technology Commission, member of the Working Group on Regulatory Control of Volatile Organic Compounds for the Environmental Protection Department, and consultant on technical matters for the Drainage Services Department, Architectural Services Department, and Corrective Services Department.

He is active in professional societies such as the Council/Executive Board of the Hong Kong Air & Waste Management Association (USA), the Hong Kong Association for the Advancement of Science and Technology, and the Hong Kong Chemical Society, and is technical advisor for industrial and public organizations such as Hong Kong Green Council, Hong Kong Architectural Coating Association, Hong Kong Critical Components Manufacturers Association, and the Hong Kong Metal Finishing Society.

His research interests include microfluidic chip-capillary electrophoresis devices for environmental, biomedical, and food safety application, and chemical sensors and biosensors, based on the piezoelectric quartz crystal technology. He holds 4 patents, and has authored or co-authored 12 books and reviews, more than 130 journal articles, and 290 conference papers. He is the chief editor of this book and also the principal author of Chapters 1, 3, and 17.

Dr. Qidan Chen received her BSc and MSc degree from Jilin University, Changchun, People's Republic of China, in 2005, and PhD from The University

of Hong Kong, Hong Kong SAR, China, in 2010. She is currently associate professor at Zhuhai College, Jilin University. Dr. Chen was appointed as the Honorary Research Associate of the Science Faculty at The Hong Kong University, in 2011. In 2015, she was appointed as a teaching advisory committee member of higher education in Guangdong, People's Republic of China.

Her research interests include the application of nanomaterial in microfluidic-chip capillary electrophoresis (MC-CE) devices and in food testing research, using various international advanced technologies based on semiconductor nanoparticles, microfluidic electrophoresis devices, and laser fabrication techniques, and the development of novel analytical methodology for the determination of toxic environmental pollutants and new analytical methods for the determination of contaminants in food. She is currently a principal investigator of three research grants, the National Natural Science Foundation of China (NSFC) (Grant No. 21307039), foundation for University Excellent Young Teachers Program of Guangdong, and foundation for Distinguished Young Talents in Higher Education of Guangdong (Grant No. 2013LYM_0120). Her research work has led to more than 20 publications in journals such as *Electrophoresis* and presentations at international conference proceedings. She is a co-editor of this book and also principal author of Chapters 10 and 11 of this book.

Dr. Fuying Du received her BSc and MPhil degrees from the Wuhan University, Wuhan, People's Republic of China, in 2003 and 2006, respectively, following by awarding a PhD degree from The University of Hong Kong, Hong Kong SAR, China, in 2012. After graduation, she has taken a postdoctoral position in the Department of Water Quality Engineering, Wuhan University for two years prior to appointment for the current position as a lecturer in the Department of Water Quality Engineering, Wuhan University.

Her research interests include electrochemical sensor and electrochemical biosensor for water analysis, and semiconductor photocatalysts for hydrogen evolution from water splitting. She is a co-editor of this book and also the principal author of Chapters 8 and 9.

Dr. Wenpeng Guo received his BSc from the Sichuan University, Chengdu, People's Republic of China, in 2003, MPhil from the Beijing University of Technology, in 2006, and PhD from The University of Hong Kong, Hong Kong SAR, China, in 2011. He is currently associate professor in The First Affiliated Hospital of Shenzhen University (Shenzhen Second People's Hospital), Shenzhen, People's Republic of China, and also academic advisor to Hubei University of Chinese Medicine, Wuhan. He was appointed as committee member of Guangdong Association of the Integrative Traditional and Western Medicine and as the English editor of *Shenzhen Journal of Integrated Traditional Chinese and Western Medicine*.

His research interests include the development of microfluidic chip-capillary electrophoresis devices for biomedical and clinical application and on clinical integrative research of traditional and Western medicine. He holds 18 patents, and has authored or co-authored 2 books and reviews, and more than 15 journal articles. He is a co-editor of this book and also the principal author of Chapters 2, 4, and 5.

Dr. Gloria Kwan-lok SZE received her PhD from The University of Hong Kong (Hong Kong, SAR) in 2009. She took up a postdoctoral research work for the lithium ion battery project funded by the Hong Kong Innovation and Technology Fund for a year. She then worked in the R&D Department at Bureau Veritas HK Ltd. (Hong Kong SAR) in 2010 prior to joining the Government Laboratory in 2012 as a Chemist.

Her research interest is focused on protein characterization using MC-CE devices integrated with Gas-phase Electrophoretic Mobility Molecular Analyzer (GEMMA). She is the author for Chapter 16 of the book entitled "Protein Characterization and Quantitation – Integrating Microfluidic-chip Capillary Electrophoresis (MC-CE) Device with Gas-phase Electrophoretic Mobility Molecular Analyzer (GEMMA)".

Dr. Tongmei Ma received her PhD from The University of Hong Kong, Hong Kong SAR, China, in 2005, and took up a postdoc position in Arizona State University, Tempe, Arizona, after PhD graduation. Since 2009, she has joined the South China University of Technology, Guangzhou, China.

Her research interests include spectral studies on transition metal containing molecules, transition metal clusters, protein molecules, atmospheric pollutants, and others by employing various modern experimental methods combined with theoretical calculations. She is the author of more than 50 journal and conference papers. She is a co-editor of this book and also the principal author of Chapter 16.

Dr. Zhou Nie received his BSc degree in 1998 and Master of Analytical Chemistry in 2003 from Wuhan University, China. He was then worked as a Chemist in SGS OGC Lab (Shenzhen, Guang Dong Province) from 2003 to 2004 for testing petrochemicals and related petroleum products. After working in industry for a year, he took up a PhD study at the Chemistry Department, University of Hong Kong (Hong Kong SAR) and received his PhD degree in 2008.

After graduation he joined SGS R&D Department (Shatin, Hong Kong) from 2009 to 2010. During this period, he had developed and validated new testing methods to meet requirements for environment and safety regulation. From 2010 till present, he works in Amway China (Guangzhou, Guangdong Province, China) focusing on quality control of botanical extracts in health foods for the Chinese market. His current interest is

developing microfluidic chip-capillary electrophoresis devices for on-site quality assessment of Traditional Chinese Medicine and related herbal extract and for determining the binding capacity of bilirubin with human serum proteins. He is a co-editor and principal author of Chapter 12 and 13 of this book.

Dr. Hui Sun received her BSc from the Wuhan University, Wuhan, People's Republic of China, in 1994, and MPhil from the Nankai University, Tianjin, People's Republic of China, in 2002, and PhD from The University of Hong Kong, Hong Kong SAR, China, in 2006. From 2006 to 2010, she worked as research associate for post-doctoral research work in the Department of Chemistry, The University of Hong Kong. She joined the Guangzhou University in 2011. She is currently associate professor in the College of Environmental Science & Engineering, Guangzhou University, Guangzhou, People's Republic of China.

Her research interests include microfluidic chip-capillary electrophoresis devices for environmental, biomedical, and food safety application, and chemical sensors and biosensors based on the piezoelectric quartz crystal technology. She holds 3 patents, and has authored 1 book and more than 50 journal and conference papers. She is a co-editor and principal author of Chapter 6 of this book.

Dr. Ruige Wu received her BSc and MSc, respectively, from Nankai University, Tianjin, People's Republic of China, in 1994, and Peking University, Beijing, People's Republic of China, in 1998. She obtained her PhD on microfluidics applications in analytical chemistry from The University of Hong Kong Hong Kong SAR, China, in 2011. She worked in CapitalBio, Beijing, as a research scientist on microarray technology from 2001 to 2006. She is currently a scientist of Singapore Institute of Manufacturing Technology (SIMTech, A*Star), Singapore.

Her research interests include the applications of microfluidic technology on biomedical and biological areas. She is a co-editor and co-principal author of Chapters 3 and 7 of this book.

Dr. Wenfeng Zhao received her MPhil from Nankai University, Tianjin, People's Republic of China, in 2006, and PhD from The University of Hong Kong, Hong Kong SAR, China, in 2010. She is currently associate professor in the School of Chemical and Engineering, Jiangsu Normal University, Xuzhou, People's Republic of China.

Her research interests include microfluidic chip-capillary electrophoresis devices for biomedical nanoparticles analysis and single molecule detection. She holds a patent, and has authored more than 20 journal and conference papers. She is a co-editor and principal author of Chapters 14 and 15 of this book.

Contributors

Qidan Chen
Department of Chemistry and Pharmacy
Jilin University, Zhuhai College
Zhuhai, People's Republic of China

Fuying Du
Department of Water Quality Engineering
Wuhan University
Wuhan, People's Republic of China

Ying Sing Fung
Department of Chemistry
The University of Hong Kong
Hong Kong SAR, People's Republic of China

Wenpeng Guo
Shenzhen Second People's Hospital
First Affiliated Hospital of Shenzhen University
Shenzhen, People's Republic of China

Gloria Kwan-lok SZE
Government Laboratory
Ho Man Tin Government Offices, Kowloon
Hong Kong SAR, People's Republic of China

Tongmei Ma
School of Chemistry and Chemical Engineering
South China University of Technology
Guangzhou, People's Republic of China

Zhou Nie
Guangzhou Amway (China) Co., Ltd.
Guangzhou, People's Republic of China

Hui Sun
College of Environmental Science & Engineering
Guangzhou University
Guangzhou, People's Republic of China

Ruige Wu
Singapore Institute of Manufacturing Technology
Singapore

Wenfeng Zhao
School of Chemistry and Chemical Engineering
Jiangsu Normal University
Xuzhou, People's Republic of China

Section I

Background

1

Microfluidic Chip-Capillary Electrophoresis: Expanding the Scope of Application for On-Site Analysis of Difficult Samples

Ying Sing Fung
The University of Hong Kong
Hong Kong SAR, People's Republic of China

CONTENTS

1.1 Needs and Approaches for On-Site Analysis of Difficult Samples Containing Interfering Substances

Due to the rising demand for on-site analysis to deliver timely results in the diagnosis of infective agents and treatment of patients under critical conditions, assay of drugs and metabolites at point-of-care, on-site toxic agent detection for home security, and monitoring of environmental pollutants and food contaminants, various methods capable of on-site operation have been developed in the past 20 years. There are two major methods adopted in various analytical methodologies: direct methods, via specific biochemical reactions with target analytes normally present at high levels in a simple sample matrix; and separation methods, via a separation scheme to separate analytes normally present at low levels in complex samples containing interfering substances [1–3].

Direct methods can be divided further into two groups: low sample throughput laboratory-based methods and high sample throughput clinical-based methods. High sample throughput platforms have been developed for routine monitoring of important clinical parameters. However, the automated system is often targeted to a single parameter, and only a limited number of automated analyzers are available for frequently analyzed parameters because of the high development cost for the automated analyzer.

Laboratory-based low sample throughput platforms are developed to meet difficult analyses requiring sample cleanup and analyte enrichment prior to direct determination by specific biochemical reagents. Due to the requirement of expensive instrumentation and supporting facilities, high capital and running costs are often needed. Thus, this approach is not likely to be useful for on-site monitoring applications.

Procedures utilizing various separation schemes are essential in methods for the determination of several analytes in complex samples containing interfering substances, such as the assay of urinary proteins and metabolites [4]. However, for samples with analytes present in widely different concentrations, dilution or preconcentration has to be carried out for the determination of an individual analyte to match its dynamic working-range requirement. For profile analysis, a large number of analytes have to be determined in each assay, thus requiring a large number of dilutions to be carried out for each sample to meet the quantitation requirement for an individual analyte.

To deal with the problem of determining trace analytes in complex samples, appropriate sample pretreatment procedures have to be carried out to remove interfering compounds. For example, a high-salt environment in a urine sample can interfere with the determination of urinary metabolites when using mass spectrometry (MS), and it also affects the separation of urinary proteins by capillary electrophoresis (CE) or other separation techniques [5,6].

Pretreatment procedures to clean up complex samples are often tedious and time-consuming. Thus, alternative procedures with online sample pretreatment are needed.

1.2 MC Devices for On-Site Analysis

The emergence and value of microfluidic chip (MC) devices for on-site analysis are indicated by the increasing number of publications under the term "microfluidic chip" since its first appearance in the literature in 1994 (Figure 1.1). The pace of increase was slow at the start (1994–2005) but then showed a rapid increase to present.

The various MC devices developed in the past 20 years are listed in Table 1.1, with a brief description on their modes of operation. Although there are various terms used to describe types of MCs; they generally can be classified into four major types: 1) MC as a general term for any device using microfluidic operation; 2) MC or biochip, which utilizes direct detection by specific biochemical reagents with the analyte for qualitative and semiquantitative analysis; 3) microfluidic chip electrophoresis (MCE) or microchip-CE, which incorporates a separation scheme to handle complex samples for quantitative determination of analytes; and 4) microfluidic chip-capillary

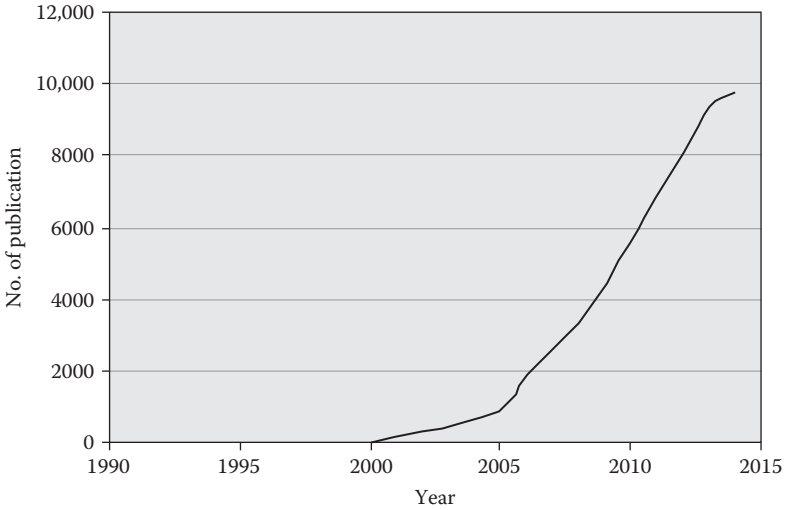


FIGURE 1.1
The cumulative number of publications under microfluidic chip since 1994 to May 2014. (Data from ISI Web of Science.)

TABLE 1.1

Various Microfluidic Chip Devices Developed in the Past 20 Years Since 1994

Microfluidic Chip Device	Year Found	No. of Publications up to 2014[a]	Mode of Operation
Microfluidic chip	1994	9778	Microchip with samples or reagents moved by pumps or electrophoresis with direct analyte detection or analyte separation before detection
Microarray chip/biochip	1994	5364	Samples delivered to specific reagents placed in a given static vial for specific analyte detection
Microfluidic chip electrophoresis or microchip-capillary electrophoresis	1996	2410	Samples or reagents moved by pump or electrophoresis; analyte separated on chip before detection
Microfluidic chip-capillary electrophoresis[b]	1998	90	Samples or reagents moved by electrophoresis in microchip with analyte separated in capillary column; mixing up in terminology with microfluidic chip electrophoresis in literature

[a] Data from ISI Web of Science (1994–2014).
[b] Data from ISI Web of Science (1994–2014) and author's database (2003–2014).

electrophoresis (MC-CE), which integrates a much longer quartz capillary to perform different modes of CE separation and/or incorporates new detection modes to extend the scope of application to difficult samples using on-chip operations, such as sample cleanup, analyte enrichment, high CE separation efficiency, and high detection sensitivity and selectivity for new detection modes. Full definitions of the four forms of microfluidic devices are given in Appendix I.

1.2.1 Microarray Chip/Biochip with Direct Detection

The early form of MC used a pump to transfer samples and reagents to vials fabricated on microchips loaded with chemical or biochemical reagents with specific interaction with the target analytes in the sample for their identification and semiquantitative determination. The number of vials on each chip can reach a few million in the form of a microarray of vials fabricated on the microchip surface, commonly known as a microarray chip or biochip for target sample application. The expensive biochemical reagents needed and the high production costs involved in making microarray chips (a chip can have a few million vials, with each vial containing a different reagent makeup) lead to a high operation cost for using the microarray chip for each assay. In addition, it can only be used once before disposal. As a result

of the difficult-to-avoid production error for filling up a few of the vials with the incorrect reagent makeup, false positives and false negatives have been reported in the use of microarray chips, thus affecting interpretation of the results. The high running cost of the microarray chip eliminates its application for general clinical assays and restricts its application to special areas requiring a lot of information from very precious and limited samples, such as DNA assays for exploratory studies of research subjects.

1.2.2 MCE/Microchip-CE with a Separation Scheme

Although the use of a pump to move microfluids provides a nonbiased movement for all microfluids, it can only deliver microfluids from one position to another at a time by applying differential pressure between the two positions. For an interconnecting microchannel pattern, the control of pressure at different parts of a complex microchannel pattern is complicated and expensive, because many pumps have to be employed. Thus, the pressure-driven channel pattern is limited to a very simple design for each operation. In contrast, the use of electrophoresis as the driving force to move samples and reagents is easy, because only one high-voltage power source is needed. Different potentials can be imposed to different vials fabricated on a microfludic chip by imposing differential voltages at each vial and by switching voltages at a desired program. For complicated operations at an open complex microchannel pattern, high-voltage-driven control of microfluids is highly preferred and frequently used for analytical application.

In addition, separation is required for difficult samples for the determination of analytes at trace levels in an interfering sample matrix, and the procedure can be used for the determination of several related analytes in each assay. As electrophoretic separation can provide both high-separation efficiency for analytes and for moving the samples within the MC to a desirable position, it has been adopted as the driving force for moving microfluids and for analyte separation. MCs using electrophoresis for separation of analytes are known as MCE or microchip-CE. Both MCE and microchip-CE refer to the same MCs using electrophoresis in the separation scheme.

The term microchip-CE can sometimes cause confusion. A considerable number of papers reported in the literature [7–9] do not use a quartz capillary to operate in a given mode of CE for analyte separation. Instead, separation is executed for analyte separation in a segment of microchannel a few micros in length is commercially available MCs. Due to the short separation distance in the MC, only a limited separation can be achieved. Thus, a sample with a simple matrix is often used to show the increase in detection sensitivity for the determination of drugs by using a newly developed electrochemical detector for drugs and associated metabolites [9–11].

Despite the increasing number of publications since 1994 covering many application areas, relatively few reports were found using real samples for testing. This discrepancy may due to 1) interference from the complex

sample matrixes; 2) use of standard, commercially available MCs with fixed channel configuration to test new ideas, such as new detection principle, and use of a standard mixture to illustrate the enhancement in detection sensitivity; and 3) use of MCs that require expensive research instrumentation, making it difficult for other researchers to follow up on previous work for real sample assay.

In summary, for MCs using direct biochemical detection for point-of-care application, sample matrix interference is a major problem that must be tackled to enable the success of MC for real sample application. For MCE and microchip-CE with separation scheme, the relatively short microchannel (a few centimeters in length) fabricated on the microchip for separation is not sufficient for handling complex samples containing interfering substances. The integration of a quartz separation capillary for high-separation efficiency for analyte coupled with MC for sample preparation provides a promising approach for real sample application, with details given in Section 1.2.3.

1.2.3 Integrated MC-CE Device with a Silica Separation Capillary

The first paper incorporating a capillary in the MC was found in 1998 [12] using a silica capillary to collect cells and deliver the contents for reaction with fluorescein on a glass chip with an etched channel for microscopic observation. There are very few papers published on MCs integrated with a separation capillary after 1996, compared with microarray chip/biochip and MCE/microchip-CE development in the subsequent 10 years, possibly because of the additional work involved in incorporating a capillary with a MC. The interest in online integration of an MC with on-chip sample pretreatment capability to a capillary column with high-separation efficiency under electrophoretic run had initially started in Fung's group in 2000, with an aim to solve problems in handling environmental samples containing interfering substances for trace analysis [13]. In view of the slow progress to develop portable devices to deliver urgently needed results on demand and the lack of affordable commercial instrumentation in support of MC devices for on-site analysis, the approach has later been incorporated into devices showing high potential for biomedical application. The advantages gained in integrating CE with MC devices are found to be far beyond the additional work for bonding a quartz capillary to the MC to fabricate the MC-CE device.

CE is a well-established separation technique that provides an efficient separation with a short assay time, reduced reagent consumption, and low cost. Different separation modes, such as isoelectric focusing (IEF), micellar electrokinetic chromatography (MEKC), and capillary zone electrophoresis (CZE), have been established for the separation of molecules with biomedical significance. For example, CZE separates charged analytes

with high resolving power by mass-to-charge ratio (m/z). MEKC provides an effective separating method for both neutral and ionic compounds. IEF produces a pH gradient from the focusing of ampholytes according to their isoelectric points, which can be used to isolate protein fractions for subsequent separation [14,15]. The different separation modes provide flexibility for selecting a desired mode for a given analytical separation. In addition, other sample introduction techniques, such as analyte stacking and isotachophoresis (ITP), have been developed for analyte enrichment and sample cleanup, such as desalting, which can enhance the detection sensitivity.

The integration of a separation capillary with the MC in the MC-CE device has quickened the pace for developing devices for target application in the following ways. First, commercial instrumentation with affordable cost is available from CE manufacturers who have developed CE instrumentation, such as detectors and high-voltage sources, for portable application. Second, various fully developed CE separation modes (MEKC, CZE, and IEF) can be used to assist in the separation of targeted analytes from given samples within the separation capillary. Third, the various analytical enrichment and sample cleanup procedures developed in the microchip can be coupled with CE separation to meet demand for a given task. Fourth, the databases and knowledge accumulated from existing CE separation procedures and buffer systems developed over the years for biomedical, environmental, food safety, security protection, and other areas can be utilized to tackle difficult separation. Fifth, microfluidic techniques developed in MCs, such as mixing, valving, and microflow patterning, can be adopted for on-chip operations prior to CE separation. The integration of two microscale techniques, performing different tasks leads to enhanced capabilities that exceed the use of either technique alone, making it possible to analyze complex real samples for target analytes as shown in Sections II–IV of this book.

The first area of integration of MC-CE devices started in 2000. It incorporated various sample pretreatment procedures, such as sample cleanup and analyte enrichment [13,16,17], with CE technology by using established separation modes for specific biomedical molecules [18–23] and a high-separation efficient CE column to reduce interference from the sample matrix. The second area involved integration with a sensitive and selective detection mode to expand the scope of application to new areas, such as the determination of a non-ultraviolet (UV)-absorptive analyte and nonelectroactive analyte by using optical and electrochemical detectors, respectively.

The success reported in the literature since the mid-2000s using MC-CE devices for the determination of trace analytes in difficult samples covers widely different application areas, including biomedical, environmental, and food safety areas. Wider application was made possible by the availability of affordable facilities and instrumentation at the time to enable the operator to design and fabricate desirable microchannel patterns on MCs for intended application, as ascribed in Section 1.3.

1.3 Fabrication and Control Instrumentation for MC-CE Devices for Intended Application

1.3.1 Instrumentation for Fabrication and Control of MC-CE Devices

There are two major forces driving the development of microchip technology. The first is the need for analyzing small samples for the determination of multi-analytes at low levels, for example, the assay of metabolites in urine. The second is the micromachining capability for making MCs with desired microchannel patterns and electrode vials fabricated at suitable locations to control the movement of samples and analytes for following the procedure of the required analytical task. Personnel with analytical background are mostly chemists by training, and those who are experts in microchip fabrication are engineers, with little chemistry background. This distinction provides a bottleneck for the development of a specific microchip for a target application, as most chemists are often forced to work on a prefabricated chip with standard channel patterns made by engineers, with no consideration for a given analytical task.

The recent advancement in laser fabrication technology driven by industrial prototyping and micromachining allows MC fabrication by computer-aided manufacturing under software control. This technological approach enables the chemist to fabricate a self-designed channel pattern targeting the demand of a given analytical task, leading to a rapid pace of development in recent years of microchip-based methods. The lack of commercially available instrumentation for high-voltage control and sensitive detection for target analytes that are often present in trace levels in complex sample matrixes hinders the pace of development toward real sample application. Recently, there is commercially available instrumentation from Taiwan and Singapore for an economic CO_2 laser ablation unit and programmable high-voltage switching instrumentation. Details are given in Chapters 2 and 3.

The availability of an economic, computer-aided laser ablation unit makes the development of an MC-CE device possible by a chemist to test the desired MC with a self-designed microchannel pattern for analyzing samples with complicated matrixes. The capabilities and advantages for integration of MC with CE for sample pretreatment as well as for the incorporation of sensitive detection modes are discussed in Section 1.3.2, followed by a case study for the application of MC-CE devices to tackle problems in real samples (see Section 1.4).

1.3.2 Fabrication of MC-CE Devices by CO_2 Laser Ablation on PMMA

A commercially available CO_2 laser engraver (V-series, Pinnacle, Great Computer Corporation, Taipei) at an affordable cost was used to fabricate a desired channel pattern onto a poly(methyl methacrylate) (PMMA) polymer surface under the control of CorelDRAW 10 (Corel Corporation, Ottawa,

Ontario) computer software. To assess its capability for repeatable profiling of a microchannel, the fabricated channel was investigated by scanning electron microscopy (SEM); the SEM micrograph in Figure 1.2 shows a cross section of the microchannel produced by CO_2 laser ablation [24]. The profile shows a good match to an overlaid Gaussian curve, with normal distribution of the laser energy for vaporization of PMMA [25]. For laser ablation to a desired depth and width of the microchannels, careful control of the laser power and its velocity drawn across the PMMA surface is required. Details on the effect of laser power and laser speed for fabricating MC-CE devices are given in Chapter 2.

One distinct advantage for laser ablation is the repeatability for fabricating microchannels with a given width and depth, as well as fabricating a specific length of a given segment of the desired microchannel pattern. Thus, one can use different numbers of microchannel segments for mixing of samples and a standard and for calculating the mixing ratio for the on-chip standard addition operation. An example is given below to illustrate the fabrication of an MC-CE device for emergency assay for free bilirubin in the blood of newborn babies. The assay can be carried out at any time of the day by on-chip titration to determine the binding capacity of a given blood sample. Four bilirubin/albumin titrations are needed for each sample with varying mixing ratios between standard bilirubin and the human sera sample to assess its binding capacity. The assay is needed

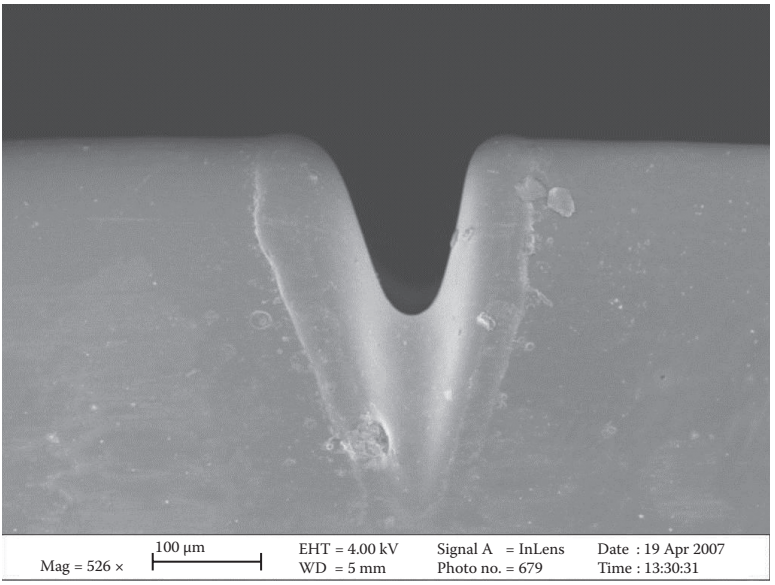


FIGURE 1.2
Cross section of a PMMA microchannel produced by CO_2 laser ablation as shown by the SEM micrograph. (From Nie, Z. and Fung, Y.S. *Electrophoresis* 2008, 29, 1924–1931.)

to be operable by clinical staff because the assay may be urgently needed in the middle of the night. Thus, the MC-CE devices are preloaded with bilirubin at different concentrations for mixing with a specific sera sample injected into the four sample vials for operation to be carried out automatically when the assay is needed.

For fabrication of the MC-CE device (Figure 1.3) for the determination of free bilirubin by using the frontal analysis/CE mode, four pairs of double-T injector with a common connection to the inlet of the fused silica separation capillary are fabricated by CO_2 laser with a wavelength at 10.6 μm [24]. All channels are ablated to a standard configuration with 100 μm depth and 150 μm width at the top of all channels. For the four pairs of double-T injectors, identical dimensions are ablated onto a 30 mm × 40 mm × 0.15 mm Slide (PMMA, Ensinger Ltd, Mid Glamorgan). The channel segment with a fixed distance between each T section thus provides an equal volume of test samples. Desirable sera/bilirubin volume ratios can be produced by fabricating channel segments with calculated distance between each T section.

The 3-mm double-T injector is used to introduce a large sample plug, and the 8-mm length channel is used to connect each of the four double-T injectors to the embedded fused silica separation capillary at equal distance. The 50 μm inside diameter and 13-cm capillary (detection window 4 cm from capillary end) with an effective length of 9.8 cm is used for separation. The capillary is sandwiched between two PMMA plates at the intersection of the four double-T injectors.

The PMMA MC with prefabricated channel configuration is bonded by a press with hot plate to the quartz separation capillary under constant pressure and temperature for 15 min at 0.6 MPa and 92°C, respectively.

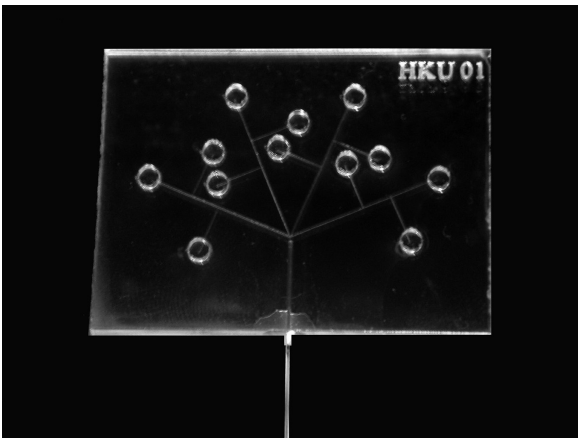


FIGURE 1.3
Intersection between four double-T injectors and the inlet of the separation capillary (60× magnification). (From Nie, Z. and Fung, Y.S. *Electrophoresis* 2008, 29, 1924–1931.)

Satisfactory bonding is shown between the silica capillary and the PMMA plate. After bonding, the MC-CE device is cooled in air to room temperature, washed in distilled water inside an ultrasonic bath, and dried before use.

1.4 Integration of MC-CE Devices for Sample Preparation and Analyte Detection

Integration of the MC-CE device for sample preparation and analyte detection expands its scope to handle complicated sample matrixes, such as determining proteins and metabolites in urine, pesticides in food and environmental samples, and monitoring of drugs and associated metabolites in blood and sera. The simplest integration follows an online format with sequential arrangement for consecutive operations, such as first passing of the sample through the MC for sample cleanup, followed by analyte enrichment by stacking, separation of analytes in the CE column, and finally their quantitation by a detector.

The advantages for sequential operation are as follows: (1) a similarly developed CE procedure can be adopted for operation in MC-CE devices; (2) there is no contamination during operation because the entire procedure is performed in an enclosed MC-CE device; (3) automation of the operation procedure can be done by high-voltage control at electrodes placed in vials fabricated on the MC-CE device; and (4) the MC-CE device can be operated on-site to deliver results on demand.

The disadvantage of the sequential arrangement is that the total analysis time is the sum of the time for all operations to be performed in the procedure. An off-line integration offers an alternative time management. It is more efficient in cost for MC-CE devices for procedures using multiple relative slow sample preparation units to integrate with a fast but expensive detection unit in subsequent operation. An off-line development is necessary to obtain results needed for design of MC-CE devices using online procedures. In summary, the online integration is preferred for on-site analysis. Examples for various forms of integration are given in Sections II and III of this book.

There are two major lines of integration. The first line is based on the lab-on-a-chip approach, such as the incorporation of on-chip sample preparation procedures before analyte separation in the CE separation column. The sample preparation procedures include on-chip dilution, sample cleanup, dual-channel mixing, and online standard addition. The various on-chip operations can be achieved by a controlled manipulation of the microfluids via a preprogrammed high-voltage switching at designated vials, making the MC-CE device a lab-on-a-chip device for field operation. The integration with sample preparation provides the first batch of successful cases to demonstrate the capability of MC-CE devices

using commercially available portable and economic instrumentation for tackling difficult samples for determination of trace levels of analytes.

The second area for integration is the incorporation of new sensitive detection modes on-chip to determine target analytes eluted out from the separation capillary. MCs with desired microchannels can be fabricated to guide the positioning of the microelectrode detector to the desired position at the middle of the exit of the separation capillary, which is known to give the highest detection signal. To enhance the use of quantum dot (QDs)-assisted detection for analytes with no detectable functional groups, the detection zone is guided by the MC to a suitable location within the detection zone of the capillary at the focus of the optical system to reduce a stray-light effect and to lower background noise. Another extremely useful mode to enable the detection of analytes with no detectable functional groups is fabricating a specially designed MC to supply the chemical reagent through the crack of the capillary to react with the analyte after its exit from the separation capillary, making possible the detection of analyte with no detectable function to be detected by sensitive detection mode based on dual-electrode detection as well as the extremely sensitive detection mode based on QDs-assisted laser-induced fluorescence (LIF) detection.

Using a carefully designed MC-CE device with a suitable combination of procedures, such as sample cleanup, analyte enrichment, CE separation mode, and selective and sensitive detection of the analyte eluted out from the capillary column, the procedure developed for the fabricated MC-CE device can be optimized with results to demonstrate its capability to remove interfering substances from difficult sample matrixes for the quantitation of analytes up to the required level for the intended application. Successful cases for integrated MC-CE devices for sample preparation are given in Section 1.4.1 and for integration with new detection modes in Section 1.4.2.

1.4.1 Integrating MC-CE Devices for Sample Preparation

The various MC-CE devices integrated with different sample preparation and analyte enrichment procedures are listed in Table 1.2. The examples listed are cases to handle difficult analytical tasks, such as the determination of minor protein in a high-protein sample, determination of trace levels of protein in urine, and on-chip binding assay incorporating several laboratory operations to be carried out on MC-CE devices to deliver timely results. Commercially available instrumentation is used in the examples listed, such as a UV detector from CE equipment, for portable CE equipment, and high-voltage supply, which can be purchased from CE equipment manufacturers. The only research and developed work required are the design and fabrication of MC-CE device with a desirable microchannel pattern and the optimization of related operation procedures.

Although CE has been well-established in laboratories, it has not been extensively used for online clinical analysis of urinary metabolites

TABLE 1.2

Integrating of Various Sample Preparation Techniques with Microfluidic Chip-Capillary Electrophoresis Devices

Sample	Analyte	Sample Preparation before Capillary Electrophoresis Separation	References
Milk, baby formula, dairy products	Minor proteins	On-chip isoelectric focusing fraction isolation and cleanup On-chip multidimensional separation and analyte enrichment by precolumn stacking	[26–30]
Human sera	Free bilirubin	On-chip titration for binding assay On-chip multisegment mixing	[25,31–33]
Urine	Metabolites	On-chip dilution and standard addition	[16,34–36]
Urine	Trace proteins	On-chip two-dimensional separation and transient isotachophoresis analyte enrichment	[16,37,38]
Ambient air	Carbonyls	Solid phase extraction	[39]
Vegetable	Pesticides	Microextraction and microevaporation	[40,41]

and proteins. One reason is its relatively poor concentration detection limit (cLOD) due to the small volume of the injected sample plug (nanoliter level) and the very short optical path length (micrometers) for absorbance detection. However, the cLOD can be lowered using highly sensitive detection modes such as MS or LIF. Ramautar et al. [42] reviewed CE-MS for its application in metabolomics, covering different capillary coatings, separation modes, data processing methods, and other techniques on intended applications. The use of appropriate sample pretreatment methods is important for improving the detection sensitivity for real samples. For example, solid phase extraction (SPE)-related technique has been employed for selective isolation and enrichment of targeted metabolites, such as nucleosides from urine samples [43]. However, the use of off-line sample pretreatment procedures leads to undesirable lengthening of the analysis time, the requirement for a larger sample volume, and the need of laboratory facilities and environments for the operation of the pretreatment procedures and CE instruments. Thus, the application of CE for routine clinical analysis is currently limited to DNA assays [44,45].

Various MC-CE devices listed in Table 1.2 have integrated analyte enrichment procedure on-chip for sample preparation and are used to determine analytes presented at low levels in difficult samples, such as the determination of urinary proteins and assay of minor proteins in milk, baby formula, and dairy products. The MC-CE devices for the aforementioned applications are all using UV-visible detectors available commercially from CE manufacturers. The results from using MC-CE devices integrating with on-chip analyte enrichment procedures with UV-visible detection are found to be sufficient to meet the required detection sensitivity. The outcome demonstrates that the on-chip integration of sample preparation techniques

in the MC-CE devices to match the need of the user is essential for the success the MC-CE device for the intended application. The use of commercially available standard MC with double-T configuration has a very limited scope of application for real sample analysis. The approach for fabricating specific MC-CE devices with target for intended application provides the most promising path for the proof-of-concept stage to demonstrate the capability of the device fabricated to meet the required the specifications for real sample application prior to its commercialization.

1.4.2 Integrating MC-CE Devices to Enhance Detection Sensitivity and Selectivity for Analyte Detection

Most of the work reported by MC-CE devices are using UV detectors commercially available from CE instrument manufacturers as received. The areas covered include urine analysis of metabolites by UV absorbance detection [16,17,46–48]. The capillary column can be easily integrated with PMMA chips by thermal bonding to enable sample pretreatment and analyte preconcentration to be carried out on a PMMA chip with a desired microchannel pattern [16,17] prior to CE separation and UV detection directly using commercially available CE equipment. With the recent availability of other sensitive detectors at an affordable cost from CE manufacturers, such as LIF detectors based on semiconductor laser and other high-sensitivity optical detectors, including fluorescence detection [49–51], bioluminescence [52], and electrochemiluminescence (ECL) detection [53], the scope of application for MC-CE devices is expected to expand rapidly in near future.

The various sensitive detection modes integrated with MC-CE devices are shown in Table 1.3, including sensitive detection mode based on LIF, electrochemical detection (ECD) (dual-electrode detection), and the mass-based gas-phase electrophoretic mobility molecular analyzer (GEMMA). Details on the two commonly available highly sensitive detection modes, QDs-assisted LIF detection and the dual-electrode detection are given in Section 1.4.2.1.

1.4.2.1 ECD/Dual-Electrode Detection

ECD is commonly used for MC-CE detection, particularly in early publications [58–63]. It is mainly due to the easy integration of miniaturized ECD mode online with MC-CE for detection after separation. ECD is sensitive, portable, and low in capital and running costs. The problem involving the use of ECD as a detector for MC is that the detection sensitivity is critically dependent on the positioning of the microelectrode to the separation capillary for detecting exiting analytes. In addition, easy replacement of a fouled microelectrode and plugged separation capillary are required during the operation for assay of metabolites in urine.

Du and Fung [46] have developed a dual opposite carbon microdisk electrode (DOCME) detection cell using microchannels fabricated on a PMMA

TABLE 1.3

Integrated Microfluidic Chip-Capillary Electrophoresis Device with Sensitive and Selective Detection Modes

Detection Method	Sample	Analyte	Special Feature	References
Electrochemical Detector				
Opposite dual microelectrodes	Wine	Polyphenols	Improve detection selectivity	[46–48]
Serial dual microelectrodes	Urine	Metabolites, proteins	Direct detection of electroactive analytes	[49]
	Tear fluid	Amino acids	Indirect detection of nonelectroactive analytes	[50]
Electrochemiluminescence	Urine	Racemic drugs	High detection sensitivity	[51]
LIF Detector				
QD-mediated LIF detection	Potato chips	Acrylamide	Indirect detection of analyte without detectable groups	[52,53]
Immobilized QD/LIF detection	Vegetables	Organophosphorus pesticides	High detection sensitivity High detection selectivity	[54,55]
Mass-Based Detector				
GEMMA	Milk	Proteins	Analyte identification	[56,57]

Note: LIF, laser-induced fluorescence; QD, quantum dot; GEMMA, gas-phase electrophoretic mobility molecular analyzer.

chip to guide the positioning of two carbon microdisk electrodes placed at opposite sides of the exit of the separation capillary for the determination of polyphenols in wine. Different constant potentials were imposed on the two electrodes placed in an identical environment facing the exit of eluted analytes with a lower potential to detect the background current and a working potential to detect analytes separated by CE. The use of the current ratio of two identical and opposite microelectrodes held at different potentials has demonstrated its capability to differentiate analytes from electroactive impurities coeluted from the CE column at the same migration time. The setup and fabrication of DOCME are given in Chapter 8. As both the separation capillary and DOCME are placed in positions guided by microchannels fabricated onto the PMMA chip, repeatable replacement for fouled DOCME and plugged separation capillary can be easily performed. Details on the fabrication and performance of the DOCME detector are given in Chapter 8.

1.4.2.2 QD-Assisted Detection

QDs were utilized to enhance the detection sensitivity for fluorescence detection [52,54]. Chen and Fung [54] have applied QDs to assist the detection of acrylamide in potato crisp and organophosphorus pesticides (OPs) in vegetables, because both of them do not possess desirable functional groups for UV and ECD. The former provides an indirect detection and the latter a more sensitive direct detection. Using the MC-CE device with QD-assisted LIF detection, a selective method using a simple procedure is developed for the rapid determination of four OPs in vegetable samples: mevinphos, phosalone, methidathion, and diazinon. CdTe/Cds core-shell QDs were immobilized at the inside surface of the separation capillary for selective binding of OPs with enhanced fluorescence signal. For the four OPs investigated by the fabricated MC-CE device, working ranges from 0.1 to 30 mg/kg and cLODs from 50 to 180 μg/kg had been achieved. The total assay time of 12 min is highly desirable compared to the lengthy solvent extraction and time-consuming SPE step for sample preparation, which takes hours to perform. MC-CE devices provide a promising highly sensitive detection mode based on QDs-assisted LIF indirect detection to determine metabolites present at low levels in complex urine samples with no strong detectable functional groups. Details are given in Chapters 10 and 11.

1.5 Application of MC-CE Devices

Noticeable advancement of MC-CE devices has been made in the past 10 years, in particular, for their success demonstrated for assay of proteins and metabolites in complex urine samples. Based on the integration of MC,

with enrichment and cleanup capability, to CE, with matured separation and detection modes, the scope of application of MC-CE devices is found exceeding the use of either technique alone. For example, despite using the less sensitive UV detector in MC-CE compared to the use of LIF detectors in commercially available MCE devices, the detection limits of four urinary proteins investigated by the MC-CE device developed have shown to deliver a better performance, reflecting the beneficial effect of desalting and analyte enrichment carried out on-chip prior to CE separation.

The application of MC-CE devices developed for biomedical, food, environmental, and pharmaceutical or herbal medicine analysis is listed in Table 1.4. MC-CE has been shown to cover a wide range of application areas with results from the integration of these complementary techniques by the MC-CE device with target on the intended application. Compared to existing methods, MC-CE devices offer similar analytical performance. In addition, the flexibility of the MC-CE device for handling difficult urine samples using on-chip sample dilution up to 500-fold and online standard addition to handle difficult sample matrixes is much preferred to commercially available MCE chips. For the determination of drugs and associated metabolites, the separation of chiral drugs has been shown to be achievable in urine with the incorporation of a highly efficient separation capillary with sufficient column length in the MC-CE device [51]. Coupling with on-column sensitive ECL detection, the chiral compounds separated can be detected at required concentrations. The on-chip integration of sensitive detection modes such as

TABLE 1.4

Application of Microfluidic Chip-Capillary Electrophoresis Devices in Biomedical, Food, Environmental, and Pharmaceutical Areas

Application Area	Analyte and Sample	References
Medical assay	General	[49,64–72]
	Diagnostic application	[31,40,44,73–76]
	Bilirubin binding assay	[24,32,33,53]
	General metabolite assay	[17,36,77,78]
	Urine metabolites and biomarkers	[34,35,79,80]
	Urinary protein	[16,37]
Food analysis	General protein analysis	[28,38,56,57,81–84]
	Proteins in milk and dairy products	[26,27,85]
	Food quality assessment	[30,86,87]
	Polyphenols in wine	[46,47]
	Acrylamide in potato crisps	[52]
Environment analysis	Pesticides in vegetables	[40,44,54,55,85]
	Carbonyl in air	[39,68]
Biological analysis	Organelles in cell extract	[88–90]
	Amino acids, proteins in biofluids	[50,91]
Pharmaceutical analysis	Active Ingredients in herbal medicine	[51,92,93]

ECL, dual-microelectrode detection, and QDs-assisted LIF detection provides additional drive for further development of MC-CE devices to explore uncharted areas for determining ultratrace levels of metabolites in complex urine samples.

1.6 Aim and Outline of This Book

This book aims to introduce readers to the methodology and benefits gained by integration of two complementary technologies, MC and CE, with results to demonstrate their capability to expand the scope of application to tackle difficult analytical tasks for the determination trace levels of analyte in samples containing potential interfering substances.

This integration is made possible by the availability of affordable equipment for computer-aided fabrication of MC using a CO_2 laser, as it enables the user to design microchannel patterns to solve the problem for difficult samples in an intended application area. With the user fabricating his or her own MC, with application built into the design, and coupled with the use of a highly efficient CE separation and sensitive detection mode, the pace for commercialization of MC-based devices will be quicker, as the device is designed and tested with the need of the market in mind right at the beginning. With this in consideration, this book is divided into the following sections, with key points shown.

Section I Background

- To present a brief account of the historical development of MC, MCE, Microchip-CE, and MC-CE devices
- To highlight the recent development of instrumentation and facilities which makes possible the fabrication of MC-CE devices with designed microchannel patterns by the user for the intended application
- To illustrate the integration of MC with CE for expanding the scope of application in MC-CE devices and give a brief survey on the success made in various application areas

Section II Integration to Improve Sample Preparation and Cleanup

- To explain the need for integrating sample preparation in MC-to-CE separation for real sample analysis
- To give a brief description on the operations for sample cleanup and analyte enrichment by integrated MC-CE devices for the intended application

- To illustrate the salient features and successes of MC-CE devices fabricated to handle difficult samples in demanding application areas as shown in each chapter

Section III Integration to Enhance Analyte Detection

- To introduce the principle and methodology for integrating new detection modes in MC-CE devices
- To explain the operation and benefits gained by integration of sensitive and selective analyte detection modes in MC-CE devices
- To highlight the special features and advantages gained by special MC-CE devices fabricated to determine analytes not possible to be detected by conventional detection modes as shown in each chapter

Section IV Integration to Achieve Intended Application

- To present an overview on integrating MC-to-CE for intended application in biomedical, environmental, and food safety areas
- To explain the design of MC-CE devices and how they can solve the problems for tackling difficult samples for determination of trace levels of analyte in complex sample matrixes containing interfering substances
- To discuss the optimization of the operation procedure for the MC-CE devices fabricated and the successes achieved by MC-CE devices fabricated for intended application

Section V Summary and Outlook

- To summarize on the achievement made by MC-CE devices to date
- To discuss the obstacles to be overcome for commercialization of MC-CE devices for intended application
- To highlight the path to be taken and tasks to be performed for future development of MC-CE devices

References

1. Assadi, F.K. Quantitation of microalbuminuria using random urine samples. *Pediatr Nephrol* 2002, 17, 107–110.
2. Ebbels, T.M., Holmes, E., Lindon, J.C. and Nicholson, J.K. Evaluation of metabolic variation in normal rat strains from a statistical analysis of ^{1}H NMR spectra of urine. *J Pharmaceut Biomed Anal* 2004, 36, 823–833.

3. Iadarola, P., Cetta, G., Luisetti, M., Annovazzi, L., Casado, B., Baraniuk, J., Zanone, C. and Viglio, S. Micellar electrokinetic chromatographic and capillary zone electrophoretic methods for screening urinary biomarkers of human disorders: A critical review of the state-of-the-art. *Electrophoresis* 2005, 26, 752–766.
4. German, J.B., Hammock, B.D. and Watkins, S.M. Metabolomics: Building on a century of biochemistry to guide human health. *Metabolomics* 2005, 1, 3–9.
5. Issaq, H.J., Abbott, E. and Veenstra, T.D. Utility of separation science in metabolomic studies. *J Sep Sci* 2008, 31, 1936–1947.
6. Wood, S.L., Knowles, M.A., Thompson, D., Selby, P.J. and Banks, R.E. Proteomic studies of urinary biomarkers for prostate, bladder and kidney cancers. *Nat Rev Urol* 2013, 10, 206–218.
7. Chandra, P., Zaidi, S.A., Noh, H.B., and Shim, Y.B. Separation and simultaneous detection of anticancer drugs in a microfluidic device with an amperometric biosensor. *Biosens Bioelectron* 2011, 28, 326–332.
8. Zhao, X., You, T., Qiu, H., Yan, J.L., Yang, X.R. and Wang, E.K. Electrochemiluminescence detection with integrated indium tin oxide electrode on electrophoretic microchip for direct bioanalysis of lincomycin in the urine. *J Chromatogr* 2004, 810, 137–142.
9. Zhang, Q.L., Lian, H.Z., Wang, W.H., and Chen, H.Y. Separation of caffeine and theophylline in poly(dimethylsiloxane) microchannel electrophoresis with electrochemical detection. *J Chromatogr A* 2005, 1098, 172–176.
10. Zhang, Q.L., Xu, J.J., Li, X.Y., Lian, H.Z. and Chen, H.Y. Determination of morphine and codeine in urine using poly(dimethylsiloxane) microchip electrophoresis with electrochemical detection. *J Pharmaceut Biomed Anal* 2007, 43, 237–242.
11. Ding, Y., Qi, Y. and Suo, X. Rapid determination of beta(2)-agonists in urine samples by microchip micellar electrokinetic chromatography with pulsed electrochemical detection. *Anal Methods* 2013, 5, 2623–2629.
12. Ocvirk, G., Tang, T. and Harrison, D.J. Optimization of confocal epifluorescence microscopy for microchip-based miniaturized total analysis systems. *Analyst* 1998, 123, 1429–1434.
13. Fung, Y.S., Capillary electrophoresis for environmental analysis. *Abstract of 4th Chinese Capillary Electrophoresis and Related Microscale Techniques*, Guangzhou, China, June 11–13, 2000, p. 18.
14. Kuhn, R. and Hoffstetter-Kuhn, S. *Capillary Electrophoresis: Principles and Practice.* Springer-Verlag, Berlin, 1993, pp. 5–36.
15. Wehr, T., Rodríguez-Díaz, R. and Zhu, M. *Capillary Electrophoresis of Proteins*. Marcel Dekker, New York, 1999, pp. 131–233.
16. Wu, R., Yeung, W.S.B. and Fung, Y.S. 2D t-ITP/CZE determination of clinical urinary proteins using microfluidic-chip capillary electrophoresis device. *Electrophoresis* 2011, 32, 3406–3414.
17. Guo, W.P., Lau, K.M. and Fung, Y.S. Microfluidic chip-capillary electrophoresis for two orders extension of adjustable upper working range for profiling of inorganic and organic anions in urine. *Electrophoresis* 2010, 31, 3044–3052.
18. Liu, Y.J., Foote, R.S., Jacobson, S.C., Ramsey, R.S. and Ramsey, J.M. Electrophoretic separation of proteins on a microchip with noncovalent, postcolumn labeling. *Anal Chem* 2000, 72, 4608–4613.
19. Lacher, N.A., de Rooij, N.F., Verpoorte, E. and Lunte, S.M. Comparison of the performance characteristics of poly(dimethylsiloxane) and Pyrex microchip electrophoresis devices for peptide separations. *J Chromatogr A* 2003, 1004, 225–235.

20. Bousse, L., Mouradian, S., Minalla, A., Yee, H., Williams, K. and Dubrow, R. Protein sizing on a microchip. *Anal Chem* 2001, 73, 1207–1212.
21. Jin, L.J., Giordano, B.C. and Landers, J.P. Dynamic labeling during capillary or microchip electrophoresis for laser-induced fluorescence detection of protein-SDS complexes without pre- or postcolumn. labeling *Anal Chem* 2001, 73, 4994–4999.
22. Giordano, B., Jin, L., Couch, A.J., Ferrance, J.P. and Landers, J.P. Microchip laser-induced fluorescence detection of proteins at submicrogram per milliliter levels mediated by dynamic Labeling under pseudonative conditions. *Anal Chem* 2004, 76, 4705–4714.
23. Hatch, A.V., Herr, A.E., Throckmorton, D.J., Brennan, J.S. and Singh, A.K. Integrated preconcentration SDS-PAGE of proteins in microchips using photopatterned cross-linked polyacrylamide gels. *Anal Chem* 2006, 78, 4976–4984.
24. Nie, Z. and Fung, Y.S. Microchip Capillary Electrophoresis for frontal analysis of free bilirubin and study of its interaction with-human serum albumin. *Electrophoresis* 2008, 29, 1924–1931.
25. Klank, H., Kutter, J.P. and Geschke, O. CO_2-laser micromachining and back-end processing for rapid production of PMMA-based microfluidic systems. *Lab Chip* 2002, 2, 242–246.
26. Wu, R.G., Fung, Y.S. and Yeung, W.S.B. Determination of lactoferrin and β-lactoglobulin in dairy products by microfluidic-chip capillary electrophoresis. *Abstract P4-10, 9th Asia-Pacific International Symposium on Microscale Separation and Analysis (APCE 2009) and 1st Asian-Pacific International Symposium on Lab-on-Chip (APLOC2009)*, Shanghai, China, October 28–31, 2009, p. 238.
27. Wu, R.G., Fung, Y.S. and Yeung, W.S.B. Determination of lactoferrin and immunoglobulin G in baby formula by microfluidic-chip capillary electrophoresis. *Abstract PB8, 10th Asian-Pacific International Symposium on Microscale Separations and Analysis (APCE 2010)*, Hong Kong, China, December 10–13, 2010, p. 99.
28. Wu, R.G., Fung, Y.S. and Yeung, W.S.B. Microfluidic chip-capillary electrophoresis for separation and determination of low abundance proteins. *Abstract, Microfluidic and Nanofluidic Devices for Chemical and Biochemical Experimentation Symposium, 2010 International Chemical Congress of Pacific Basin Societies (Pacifichem 2010)*, Honolulu, HI, December 15–20, 2010, TECH Paper 547.
29. Wu, R.G., Wang, Z.P., Zhao, W.F., Yeung, W.S.B. and Fung, Y.S. Multi-dimension microchip-capillary electrophoresis device for determination of functional proteins in infant milk formula. *J Chromatogr A* 2013, 1304, 220–226.
30. Wu, R.G., Wang, Z., Fung, Y.S., Seah, D.Y. P. and Yeung, W.S.B. Assessment of adulteration of soybean proteins in dairy products by 2D microchip-CE device. *Electrophoresis* 2014, 35, 1728–1734.
31. Nie, Z. and Fung, Y.S. Determination of binding capacity of albumin for bilirubin by insitu titration at a circular ferrofluid driven micromixer in microfluidic chip-capillary electrophoresis. *Abstract P01-05, 8th Asia-Pacific International Symposium on Microscale Separation and Analysis (APCE 2008)*, Kaohsiung, Taiwan, November 2–5, 2008, p. 99.
32. Nie, Z., Sun, H. and Fung, Y.S. Determination of binding capacity of albumin for bilirubin by microfluidic chip-capillary electrophoresis based on insitu titration at a circular ferrofluid driven micromixer. *Abstract P2-02, 9th Asia-Pacific International Symposium on Microscale Separation and Analysis (APCE 2009) and 1st Asian-Pacific International Symposium on Lab-on-Chip (APLOC2009)*, Shanghai, China, October 28–31, 2009, p. 155.

33. Sun, H. and Nie, Z., Fung, Y.S. Determination of free bilirubin and its binding capacity by albumin using a microfluidic chip-capillary electrophoresis device with a multi-segment circular-ferrofluid driven micromixing injection. *Electrophoresis* 2010, 31, 3061–3069.
34. Guo, W.P. and Fung, Y.S. Microfluidic chip-capillary electrophoresis with adjustable on-chip sample dilution for profiling of urinary markers. *Proceedings, 14th International Conference on Miniaturized Systems for Chemistry and Life Sciences (μTAS 2010)*, Groningen, The Netherlands, October 3–7, 2010, Paper W11A, pp. 1481–1483.
35. Guo, W.P. and Fung, Y.S. Microfluidic chip-capillary electrophoresis with adjustable on-chip sample dilution for profiling of urinary markers. *Abstract PB11, 10th Asian-Pacific International Symposium on Microscale Separations and Analysis (APCE 2010)*, Hong Kong, China, December 10–13, 2010, p. 102.
36. Guo, W.P. and Fung, Y.S. Microfluidic chip-capillary electrophoresis with dynamic multi-segment standards addition for rapidly identifying nephrolithiasis markers in urine. *Electrophoresis* 2011, 32, 3437–3445.
37. Wu, R.G., Fung, Y.S. and Yeung, W.S.B. Microfluidic-chip capillary electrophoresis for determination of clinical urinary proteins. *Abstract PB9, 10th Asian-Pacific International Symposium on Microscale Separations and Analysis (APCE 2010)*, Hong Kong, China, December 10–13, 2010, p. 100.
38. Wu, R.G., Fung, Y.S. and Yeung, W.S.B. Microfluidic-chip capillary electrophoresis for analysis of clinical urinary proteins. *Abstract P136, 25th International Symposium on Microscale Bioseparations (MSB 2010)*, Prague, Czech Republic, March 21–25, 2010, p. 129.
39. Sun, H. and Fung, Y.S. Integrating micellar electrokinetic capillary chromatography with molecular imprinting polymer-solid phase extraction for ambient carbonyl determination. *Abstract, the 12th Asian-Pacific International Symposium on Microscale Separations and Analysis (APCE 2012)*, Singapore, December 16–19, 2012, SICC7-A-0240, p. 1.
40. Chen, Q.D. and Fung, Y.S. Determination of organophosphate pesticides in vegetables by integrating microextraction with microfluidic-chip capillary electrophoresis. *Abstract P4-01, 9th Asia-Pacific International Symposium on Microscale Separation and Analysis (APCE 2009) and 1st Asian-Pacific International Symposium on Lab-on-Chip (APLOC2009)*, Shanghai, China, October 28–31, 2009, p. 226.
41. Chen, Q.D., Ma, T.M., Du, F.Y. and Fung, Y.S. Microchip-CE device with on-chip micro-evaporator for determination of organophosphorus pesticides in vegetable. *Abstract, the 12th Asian-Pacific International Symposium on Microscale Separations and Analysis (APCE 2012)*, Singapore, December 16–19, 2012, SICC7-A-0232, p. 1.
42. Ramautar, R., Somsen, G.W. and de Jong, G.J. CE-MS in metabolomics. *Electrophoresis* 2009, 30, 276–291.
43. La, S., Cho, J., Kim, J. and Kim, K.R. Capillary electrophoretic profiling and pattern recognition analysis of urinary nucleosides from thyroid cancer patients. *Anal Chim Acta* 2003, 486, 171–182.
44. Qin, J., Fung, Y.S. and Lin, B.C. DNA diagnosis by capillary electrophoresis and microfabricated electrophoretic devices. *Expert Rev Mol Diagn* 2003, 3, 387–394.
45. Chang, P.L., Kuo, I.T., Chiu, T.C. and Chang, H.T. Fast and sensitive diagnosis of thalassemia by capillary electrophoresis. *Anal Bioanal Chem* 2004, 379, 404–410.