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Chip-Based Capillary Electrophoresis[1)]

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15.1
Introduction

With the advent of the age of integration and miniaturization, the evolutionary step occurred with the implementation of capillary electrophoresis (CE) in one flat planar microchip and resulted in the chip-based CE. Chip-based CE is a typical example of micro total analysis systems, that is, "lab-on-a-chip" [1–5]. The integration means that sample injection, pretreatment, separation, and post-treatment steps are all incorporated onto a single microchip platform with microchannels [6,7]. Therefore, once the samples are loaded and transferred into the injector region, they are separated under a driving force when a high voltage is applied at both ends of the microchannel. The integration was supported by the expectation of a further higher separation capacity and reduced analysis time. The miniaturization not only represents the ability to miniaturize traditional separation systems but also highlights the major advantages of portability, high-speed, low-cost, and minimized solvent and sample consumption [6,7]. The minimum consumption of samples, however, posed tremendous challenges to enhance the sensitivity of detection devices, particularly when compared to traditional CE techniques [8,9]. Fortunately, the major detection methods available for CE are also available for chip-based CE, such as UV-visible absorbance, laser-induced fluorescence (LIF), mass spectrometry (MS), electrochemical (EC) detection, chemiluminescence (CL), and electrochemiluminescence (ECL) [10–12]. Consequently, chip-based CE received significant attention as microplatforms for developing fast, automated, miniaturized, and multiplexed assay devices. Particularly, the chip-based CE also opens many new possibilities for biomedical and pharmaceutical analysis, clinical diagnostics, environmental monitoring, and forensic investigations [12–15].

This chapter will give an overview of the substrate materials, fabrication technologies, surface modification methods, injection methods, and detection techniques, all of which are factors influencing the performance of the chip-based

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CE. Moreover, the applications of the chip-based CE are also summarized concisely. Furthermore, future developments in the related research field, as seen from the authors' perspective, are also discussed.

15.2 Chip-Based CE

15.2.1 Materials and Fabrication of Chip-Based CE

Flexibility, air permeability, electrical conductivity, nonspecific adsorption, solvent compatibility, and optical transparency are main physical characteristics that will affect the performance of the chip-based CE [16]. These factors are intimately ascribed to the substrate material properties, fabrication processes, and surface modification methods. Subsequently, these influencing factors are discussed in detail as follows.

15.2.1.1 Materials for Chip-based CE

The first step of fabricating chip-based CE is selecting a proper substrate material. There are three main factors to consider when choosing a material for a specific CE system: required function, degree of integration, and application. Generally, the generation of heat is a critical and negative factor in electrodriven separations. In the early age of the development of chip-based CE, the first miniaturized device was designed on a silicon chip, where silicon was proposed as a material for the column [17,18]. For a given cross section, heat dissipation is more efficient in rectangular columns. Apart from the better heat dissipation capability [19], monocrystalline silicon can be produced with an excellent precision when appropriate chemical etching procedures are employed. Moreover, silicon has a high elastic modulus (130–180 GPa) and cannot easily be used for making active fluidic components such as valves and pumps. Silicon surface chemistry based on the silanol group (−Si−OH) is well developed, so modification is easily accomplished via silanes [20,21]. However, semiconductor properties of silicon are not well compatible with the high voltages typically applied in CE. It was reported that silicon chips covered by insulating layers of a thermal oxide and a nitride would suffer from electrical breakdown problems that seriously limited the applicable voltages [22].

Moreover, glass and quartz substrates are also the most commonly used chip materials because of their excellent electroosmotic flow (EOF), optical transparency, and silicon chemistry similar to the fused silica capillaries. In addition, a large number of surface modification means are available for these materials [4,7,23–25]. However, it should be pointed out that the fabrication process of the glass or quartz chips using photolithography and wet etching techniques is relatively complex, expensive, time-consuming, and labor-intensive, and sophisticated equipments and facilities are also usually necessary [26,27].

In recent decades, polymeric materials have been increasingly attracting public attention due to their low cost and simple fabrication methods, which allows them to be mass produced. Polydimethylsiloxane (PDMS) has been the dominant alternative to replace silicon and glass [28,29]. Due to the good chemical stability and optical transparency, PDMS is supposed to cooperate with glass chips to construct a closed channel system. One method is to use a flat layer of PDMS to seal glass microchips via irreversible bonding with the glass substrate with patterned microchannels [30]. However, a thin layer of PDMS may lack rigidity, resulting in unwanted distortion of microchannels. Another method is to seal the PDMS structure with a glass substrate to improve the structural rigidity [31]. In addition, PDMS has a good adhesion capacity for a smooth surface, which allows it to form closed channel networks without any specific pretreatments. However, its high hydrophobicity often induces nonspecific adsorption of amino acids and proteins, which diminishes the efficiency of CE. Thus, surface modification of PDMS is absolutely necessary for improving its hydrophilicity and separation performance. Poly(methyl methacrylate) (PMMA) is another popular material for chip-based CE due to its high thermal conductivity, low cost, high dielectric constant, and ease of fabrication [32–35]. Besides the polymeric materials discussed above, other kinds of polymers such as polycarbonate [36,37], polyethylene terephthalate [38–40], thermoset polyester [41,42], cyclo-olefin copolymer [43,44], SU-8 [45], polyfluoropolyether diol methacrylate [46], polystyrene [47], and so on have been reported recently for constructing microchips.

Paper and toner have become promising materials for producing low-cost and disposable chip-based CE platforms [48]. Paper is a flexible, cellulose-based material that has many advantages: (i) paper is cheap and readily available, (ii) there are various patterns and modification methods available, (iii) the porous structure promotes filtering and separation, (iv) paper has biocompatibility, and (v) the material can be simply disposed by burning or natural degradation. Accordingly, Whitesides and coworkers opened the road both for the fabrication of microfluidic devices and for simultaneous multiplexed detection by demonstrating the feasibility of using paper as substrate [49–52]. Toner is a complex powder composite used in laser printers; it is a powder at the start, becomes a fluid, and ends up as a solid structure to form an image on wax paper or a polyester film. Toner-based devices can be fabricated directly or indirectly using glass, polyester, elastomeric or conductive substrates, and so on [53,54].

15.2.1.2 Fabrication and Surface Modification of Chip-Based CE

For the design of chip-based CE, significant consideration should be taken of both the length and the geometry of the separation channels. A shorter straight channel could lead to rapid separation without significant peak broadening resulting from diffusion. The channel length can also be increased using serpentine geometry, which will result in dispersion of analytes around the turn. Thus, suitable design and proper substrate materials for the chip-based CE are

necessary according to practical requirements, and then can be further advanced for use in the fabrication process.

Glass and Silicon-Based Microchip

In the fabrication of a glass or silicon device, three basic approaches are usually used, which include bulk micromachining, spacer technique, and sacrificial technique [21,55]. The most prevalent fabrication technique is bulk micromachining (see Figure 15.1a), by which micro- and nanofluidic channels are formed by removing material from a wafer and bonding or adhering it to another wafer to encapsulate the channels. Bonding methods suitable for microchip applications can be categorized as direct (including fusion processes) [56,57], anodic [58–60], and adhesive [61]. Several factors must be considered when choosing a bonding process. These factors include the thermal coefficients of expansion of the materials to be bonded, their surface chemistry, temperature limitations resulting from former steps, the presence of any metallic layers as well as cost, throughput, and yield. Another way to precisely control the homogeneity and the depth of the nanochannels is by applying the homogeneity of the thin-film deposition or growth and the selectivity of etching. The thickness of this film is then used as a spacer (shown in Figure 15.1b) [62]. In the sacrificial technique, a cover layer is patterned based on the desired nano- or microfluidic channels, a structural layer is deposited, and then the cover layer is etched away (Figure 15.1c). The wet etching of glass is isotropic and is usually carried out using solutions of hydrofluoric acid. The etch rate is a parabolic function of the HF concentration and is strongly dependent on glass composition; for Corning 7740, the etch rate in 49% concentration, while HF is around 8 μm/min [63]. The sacrificial technique is especially attractive for the fabrication of micro- or nanochannels because the channel scale is greatly dependent on the thickness of the sacrificial layer. In addition, it is also favorable for fabricating multilayered microchips.

Polymer-Based Microchips

Polymers are organic-based, long-chain materials that are favorable for the fabrication of the chip-based CE platforms because they are relatively cost-effective, amenable to mass-production processes (e.g., hot embossing, injection molding, etc.), and adaptable through chemical modification and formulation changes [64–71]. A typical and simple case of a fabrication process for preparing PDMS-based chip is shown in Figure 15.2 [72]. A high-resolution transparency containing the design of the channels, created using a CAD program, is used as a mask in photolithography to produce a positive relief of a photoresist on a silicon wafer (Figure 15.2a). The scale bar gives an indication of the thickness and width of the photoresist. Glass posts are then placed on the wafer to define reservoirs for analyte and buffer solutions (Figure 15.2b). Next, a prepolymer of PDMS is then cast onto the silicon wafer and cured at 65 °C for about 1 h (Figure 15.2c). In Figure 15.2d, the polymer replica with a negative relief of the microchannels is peeled off from the silicon wafer, and then the glass posts are moved away. Then the chip-based CE device can be formed by pasting the

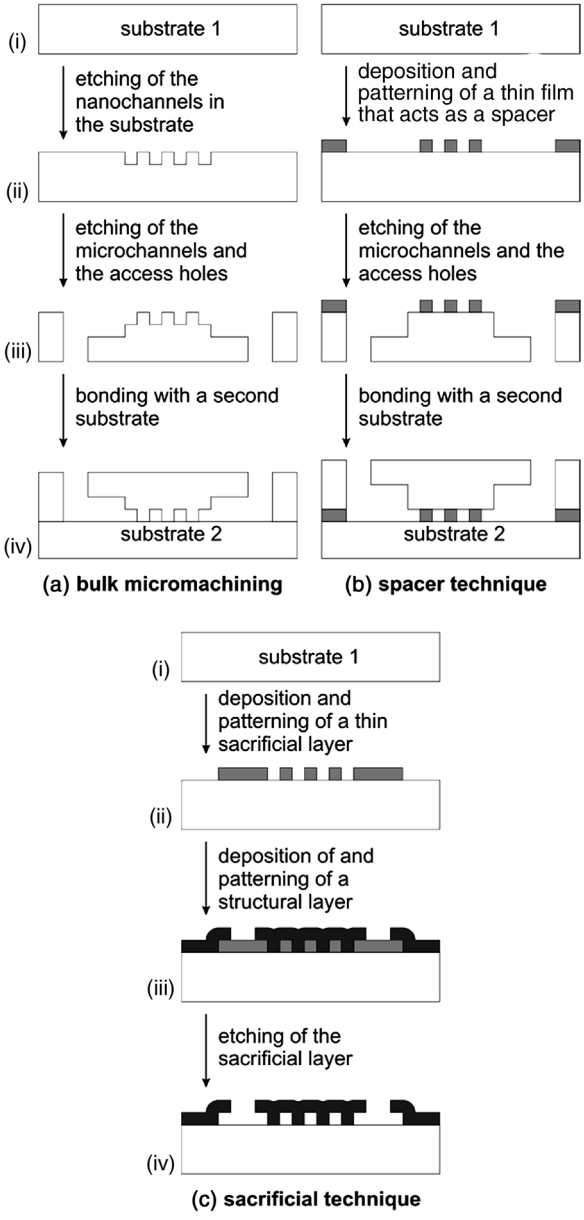

Figure 15.1 Fabrication of planar nanochannels in silicon/glass technology. (a) Bulk micromachining: nanochannels (ii), microchannels, and access holes (iii) are etched in the substrate, then closed by a second substrate (iv). (b) Spacer technique: a thin layer is deposited and patterned (ii), microchannels and access holes (iii) are etched in the substrate, then closed by a second substrate (iv). (c) Sacrificial technique: a thin layer is deposited and patterned (ii), a structural layer is deposited and patterned, then the sacrificial layer is etched away (iv) [55]. Copyright 2008 American Chemical Society.

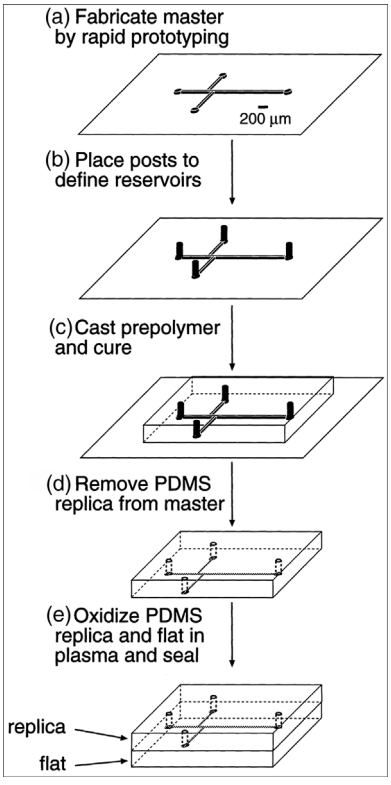

Figure 15.2 Scheme describing the fabrication of enclosed microscopic channels in oxidized PDMS [72]. Copyright 1998 American Chemical Society.

PDMS replica and a flat slab of PDMS or glass using reversible sealing. Reversible sealing between them can also be realized via oxidizing the PDMS replica and the flat slab of PDMS in a plasma discharge for 1 min (Figure 15.2e). In addition, silanol (SiOH) groups can be produced onto the surface of the polymer, which ionize in neutral or basic aqueous solutions and thus confirm the EOF in the channels.

In addition to the method discussed above, soft lithography was also utilized as an effective method for fabricating polymeric microchips, especially PDMS-based and thermoplastic-based ones [47]. Soft lithography involves a nonphotolithographic strategy that is based on self-assembly and replica molding for microfabrication. During this process, an elastomeric stamp with patterned relief structures on its surface is used to generate patterns and structures with feature sizes ranging from 30 nm to 100 µm. It provides a convenient, effective, and low-cost method for the formation and manufacturing of microstructures. As another important microfabrication approach, laser ablation has been widely employed for fabricating polymeric microchips. In the process, a beam of high-energy laser was applied to break bonds in polymer molecules and to remove the

decomposed fragments from the ablation regions. A commercially available laser scriber was usually used to engrave the PMMA substrate [73]. The polymeric microchannels, which are open after the fabrication steps, have to be closed without clogging the channels. Correspondingly, various bonding techniques, including thermal bonding [74,75], solvent-assisted bonding [76], polymerization bonding [77], and microwave-assisted bonding [78], have been developed.

Paper-Based Microchips

An original fabrication for paper-based microchip involved a combination of photoresist and photolithography processes [49]. Then, the surface of the patterned paper was treated with oxygen plasma in order to restore the paper hydrophilicity. Another method involves printing polymeric barriers onto the paper substrate directly, and the polymer solutions are able to penetrate the paper fibers and generate hydrophobic barriers that avoid leakage of aqueous solutions between channels [51]. Fast lithograph and wax printing have also been introduced for fabricating paper-based microfluidics [79,80]. Lu *et al.* [81] assessed three different processes for patterning filter paper using wax: (i) painting wax using a wax pen, (ii) employing an inkjet printer followed by wax painting, and (iii) using a wax printer directly to print the wax. The simplicity, low cost, and speed of the direct printing method make it very attractive for mass production of chip-based CE. However, the successful application of paper-based chips will depend on the availability of suitable surface modification techniques for tailoring the channel wall properties.

15.2.2
Modification of the Microchannels

With the reduction of size of channels, the properties of the microchip materials, including chemical stability, surface chemistry, and optical properties are probably getting more important than originally thought. Generally, motivations for surface modification involve (i) realizing a steady EOF in the sample injection process; (II) reducing the analyte–wall interaction to increase the separation efficiency. For example, the high hydrophobicity of microchips often leads to undesired nonspecific adsorption of samples onto the microchannel surface, which limits their applications in assays of amino acids, proteins, as well as other analytes. Thus, surface modification is sometimes necessary to enhance the separation efficiency [26]. To improve the separation performance, many approaches have been dedicated to the surface modification of microchips. Dynamic coating (physical adsorbed coatings) and permanent coating (chemical grafting) are two main categories of methods for microchip modification [24].

Dynamic coating is typically done using a selected compound as the additive to the background electrolyte. A number of reagents have been used as dynamic coatings in traditional CE in fused-silica capillaries: polymers, charged low molecular weight compounds [82,83], and detergents can also be used in the modification of the chip-based CE. Depending on the charge of the modifying

compounds, the EOF can be suppressed or minimized, or even enhanced. Xu, Li, and Wang [84] described a water-soluble functionalized ionic liquid surfactant, 1-butyl-3-methylimidazolium dodecanesulfonate, for dynamic surface modification of PDMS microchips. This strategy not only moderated the EOF but also reduced the adsorption of fluorescent dyes or proteins onto the microchannel. Most frequently neutral polymers, such as poly(dimethyl acrylamide) [85], poly (hydroxyethyl acrylamide) [86], hydroxyethylcellulose [87], and hydroxypropylmethylcellulose [88] were used to diminish EOF. Many of these polymers can be used simultaneously as both dynamic coating and sieving matrix in DNA sequencing or gel electrophoresis. Cationic detergents including cetyltrimethylammonium bromide, didodecyldimethylammonium bromide as well as polycations such as spermine, polyarginine, polyethyleneimine, chitosan, and nanoparticles can be applied to reverse EOF [89]. To have an EOF of the same direction but independent of pH, anionic detergents such as sodium dodecyl sulfate (SDS) and anionic polymers such as dextran sulfate were employed [90]. Dynamic coating is shown to be particularly attractive for polymer-based microchips because silanization is often not applicable to polymeric materials. A comparison of chemical modification methods shows that dynamic coatings are easier to apply and withstand higher pH values; however, they are not as stable as chemical ones.

A chemical coating, by which chemical compounds (often polymers) are covalently bound to functional groups or immobilized on the microchannel surface, is regarded as a highly effective way for moderating the EOF and reducing the nonspecific adsorption between analytes and the microchannel surface. It typically includes a permanent coating with polymers or small molecules, such as alkylsilane reagents. These two categories overlap somewhat, because the primary step in creating a covalently bound polymer coating is usually the derivatization of the surface with a bifunctional reagent (small molecules) to anchor the coating to the wall [91]. Traditionally, most of the permanent coatings onto the microchannels include one upper polymer layer and one or more intermediate layers that assist in the stabilization of the upper layer on the capillary wall. The chemical coating with polymers for the microchip coating can be divided into the following categories: *in situ* polymerized wall coatings [92], surface-confined living radical polymerization [93], covalent attachment of preformed polymers [94], and covalently bound wall coatings without silanization [91].

15.2.3 Sample Injection

Cross-channel injectors and double-T injectors are the most common kinds of integrated injectors for chip-based CE. Cross-channel injectors contain a separation microchannel and a channel that orthogonally intersects the separation microchannel and connects the sample reservoir and sample waste reservoir (Figure 15.3a). In the cross-channel microchip, because an extremely short sample can be introduced into the separation microchannel, the separation could

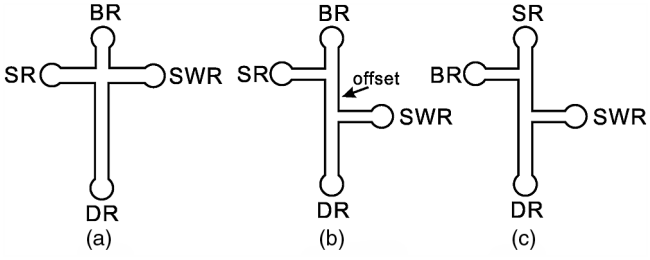

Figure 15.3 Schematic drawings of microchips for chip-based CE: cross-channel injector (a), double-T injector (b), and injector for gated injection (c). BR: buffer reservoir; SR: sample reservoir; DR: detection reservoir; SWR: sample waste reservoir.

have higher efficiencies. To allow a larger volume of the sample solution into a separation channel, the double-T injectors were developed. It can be clearly seen that there is an offset between two arms in the microchip, which allows the sample solution to be loaded into the offset (Figure 15.3b). Accordingly, the sensitivity reduction caused by introducing the buffer is compensated by using double-T injectors.

Various modes have been reported for electrokinetic injection on microfluidic systems, including floating, pinched, and gated modes [95]. The simplest floating injection for chip-based CE is carried out by applying a high voltage to the sample reservoir while the detection reservoir or sample waste reservoir is grounded [96]. In this stage, the sample is introduced directly into the separation channel by electrokinetic injection. Once the sample is introduced into the separation channel, the high voltage is switched to the buffer reservoir and the separation is subsequently initiated [97]. However, the electrokinetic injection may lead to the sample leakage into the main separation channel and reduce the separation efficiency [98,99]. Therefore, improved floating injection was developed by applying back voltages to sample reservoirs and sample waste reservoirs [100,101].

The pinched mode is the most prevalent approach utilized in chip-based CE. A pinching voltage is applied at the buffer reservoir and detection reservoir during sample injection while applying a back voltage to the sample reservoir and sample waste reservoir in the separation phase [102,103]. During the separation stage, all four reservoirs are maintained at exactly the same level of voltage, which prevents the sample zone and hydrodynamic flow from leaking and dilution [104]. However, in the pinched injection, long injection times are required for the analyte to migrate from the sample reservoir to the injection intersection, which limits the analytical speed.

Unlike the pinched injection, the gated injection model is one of the fast injection methods. The sample reservoir is moved from the side branch to the head of the separation channel while the buffer reservoir is moved to the side branch (Figure 15.3c). A gated injection procedure involves a three-step protocol: first, the flow in the chip is established by applying a potential to the buffer reservoir

and the sample reservoir, while the electrodes in the waste reservoir and the detection reservoir are grounded. Based on this method of injection, the analyte flows toward the sample waste reservoir and there is a separate stream of flow from the buffer to detection reservoirs. Then, gated injection was realized by adjusting the voltages of the buffer reservoir and the sample reservoir as a result of which the analyte flow is deflected into the separation channel. Finally, the separation is initiated by switching back the initial values [105]. This protocol allows flow in the separation channel in a continuous sampling mode, but also suffers from an electrokinetic bias [106]. The type of injection methodology that is applied depends on the precision desired, the sample matrix, and the number of voltage sources available.

15.2.4 Detection Techniques

15.2.4.1 Optical Detection

Generally, the chip-based CE can be separated into separation and detection processes. Due to the extremely small volume of samples that are introduced in the separation process, highly sensitive detection methods are required for analyte determination. Optical detection methods have several advantages, such as possessing low detection limits, being isolated from the fluid, and can be used to monitor a wide variety of compounds [107,108]. Several approaches for optical detection have been introduced into chip-based CE devices.

LIF detection remains the most frequently used optical method in chip-based CE systems due to its low detection limits [109]. For LIF, a laser is used for excitation and a photomultiplier or charge coupled device is used for detection. Integration of a light-emitting diode (LED) excitation source and a photodiode detector in a microdevice will yield relatively high sensitive detection of dyes such as rhodamine and fluorescein. For example, Pais *et al.* [110] report a high-sensitivity, disposable lab-on-a-chip with a thin-film organic light-emitting diode excitation source and an organic photodiode detector for on-chip fluorescence analysis. However, since most samples are naturally nonfluorescent, it is necessary to label them using fluorophores, such as rhodamine and fluorescein, which fluoresce in the red and green regions of the spectrum, respectively [26].

CL detection is another common optical detection method, which is used to detect the production of light through a chemical reaction. CL detection has the advantage of not requiring an excitation source that raises background fluorescence. CL methods require very sensitive detectors and have been used for both off- and on-chip formats. For example, Zhao *et al.* [111] integrated cell injection/loading, cytolysis, electrophoretic separation, and CL detection on a simple cross-microfluidic chip for the determination of intracellular sulfydryl compounds, and the selective CL detection was achieved by employing the luminol–$Na_2S_2O_8$ reaction. Under the CL conditions selected, many compounds in biological systems such as amino acids, biogenic amines, peptides, and proteins naturally did not produce any CL signal, which further ensure a high selectivity of

the proposed chip-based CE–CL assays. Harrison and coworker [112] developed the horseradish peroxidase catalyzed reaction of luminol with peroxide as a post-separation detection scheme for chip-based CE analysis. It should be pointed out that CL measurements are strongly affected by experimental factors, including temperature, pH, ionic strength, and solvent and solution composition; all these conditions should be optimized in practical applications [113].

UV absorption is the most common detection method in traditional CE systems because of its ability to directly detect a wide range of analytes without any additional derivatization steps. However, in chip-based CE systems, the sensitivity is limited by the short optical path length across the separation channel and thus absorbance detection is much less common. To improve the sensitivity, the path length is increased by employing a U- or Z-shaped detection cell, making it possible to measure the absorbance along the length of the detection cell [114,115]. Recently, UV absorption was used as a feasible approach for detecting peptides, as peptide bonds have a relatively strong absorption of UV radiation (185–220 nm) [116,117]. Simultaneous label-free detection of UV absorbance and native UV-excited fluorescence in an electrophoresis microchip was designed by Kutter and coworkers [118]. It was shown that serotonin, tryptophan, propranolol, and acetaminophen could all be detected in the micromolar range using absorbance detection.

15.2.4.2 MS Detection

MS detection techniques have enormous advantages in terms of sensitivity, accessible mass range, and structural and molecular weight information. Combination of MS with chip-based CE could help achieve a high separation efficiency in the analysis of complex analytes associated with the field of proteomics [119,120]. Electrospray ionization (ESI) and matrix-assisted laser desorption/ionization are two popular types of ionization interfaces for MS instruments. However, ESI interfaces are widely applied in chip-based CE, due to their simple structure and compatibility with ionizing analytes dissolved in a liquid phase [121]. Several applications of chip-based CE-MS to the analysis of small organic compounds, including peptides, have been demonstrated [122–124]. However, the analysis of proteins is difficult, since the silanol groups on the microchannel surface are dissociated and negatively charged when the pH>2; proteins are easily trapped by the negatively charged surface. Thus, some modification has to be applied to the system to analyze proteins [125]. For example, Akashi *et al.* [126] coated the microchannel surface of the microchip with a basic polymer, polyE-323, for the separation of basic proteins, such as DNA-binding proteins.

15.2.4.3 EC Detection

Although the above-mentioned detection techniques such as LIF and MS provide many advantages such as high sensitivity or structural recognition, the detection systems have a relatively larger size and are more expensive, which is not in accordance with the miniaturization and cost-effectiveness of chip-based

CE devices. EC detection offers an attractive approach to address this issue [127]. EC detection can analyze compounds without derivatization, and the sensitivity is comparable to that of LIF detection. In addition, electrodes can be directly located onto the microchip to form a fully integrated system [12,14]. There are mainly three general modes of EC detections: amperometric detection, conductivity detection, and potentiometric detection.

Amperometric detection is accomplished by applying a constant potential to the working electrode and measuring the resulting current [3,11]. Both a traditional three-electrode setup and a two-electrode configuration are available for amperometric detection [128–130]. This detection is attractive due to its high sensitivity and performance and has been used in many applications. For example, Wang *et al.* [131] described the simultaneous detection of glucose, ascorbic acid, acetaminophen, and uric acid using a downstream gold-coated thick-film amperometric detector at different migration times. Another PDMS/glass microchip that utilizes amperometric detection via an off-chip platinum working electrode for detecting uric acid in urine was developed by Henry and Fanguy [132].

Conductivity detection measures the conductance of a solution, and the response is proportional to the concentration of the analyte ions [9]. Conductivity detection involves a two-electrode system in which electrodes are either in direct contact with the background electrolyte solution (contact) or are externally and capacitively coupled to the solution (contactless). An alternating current (AC) potential is applied across the detection electrodes, which eliminates faradic reactions, and the signal due to the conductivity of the bulk solution (ac-current) is measured. As the intensity of detection depends solely on a difference in conductivity between the bulk solution and the analyte zones, the selection of the electrolyte solution becomes an important factor [11,133]. In addition, the availability of this instrument prompted a number of publications that were concerned with the development of different applications [134,135]. For example, Kuban and Hauser demonstrated the detection of inorganic ions in clinical samples in less than 90 s using chip-based CE by employing capacitively coupled contactless conductivity detection [135].

Potentiometric detection is established based on the theory that the potential of an ion-selective electrode is relative to a reference electrode and the charge separation generates a potential between the working and the reference electrodes that depends on the type of ion and its concentration. When it is combined with separation on chip-based CE, potentiometric detection is hard to use for the detection of multiple analytes. The ion-selective membrane must be semipermeable to more than one ionic species but not highly permeable to the background buffer ion. Therefore, reports on chip-based CE with potentiometric detection were rarely published [9,13].

In addition to the approaches mentioned above, ECL detection emerged as a new alternative detection method for chip-based CE. ECL provides a means of converting electrical energy into radiative energy. When a voltage is applied to an electrode, the activated reagents are formed at electrode surfaces via electron transfer reactions. Meanwhile, a photon of light is generated when the excited

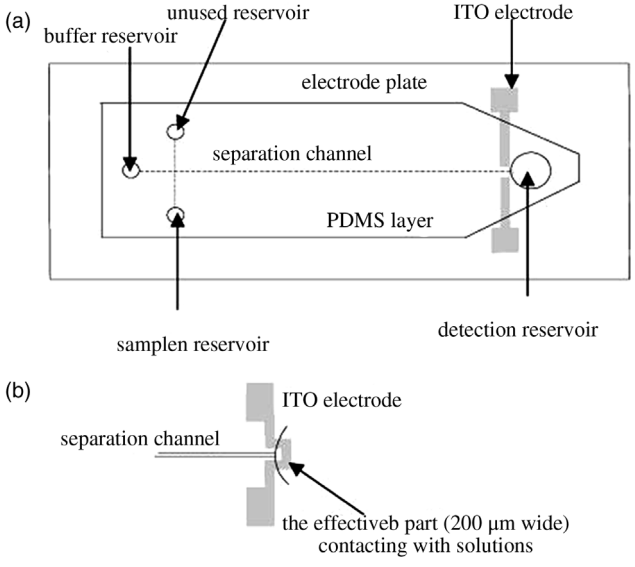

Figure 15.4 Schematic illustration of a microchip CE-ECL device. (a) Top view of the PDMS layer and electrode plate. (b) An enlargement of the detection region. Distance between the separation channel outlet and the ITO electrode, 30 μm [139]. Copyright 2003 American Chemical Society.

molecule decays to the ground state. ECL detection has been proven to be a powerful analytical tool combining the simplicity of electrochemical detection and the inherent sensitivity and wide linear range of CL detection [136]. Among the ECL systems, luminol–H_2O_2 and $Ru(bpy)_3^{2+}$ ECL were the most widely used reagents in fundamental studies and commercial applications [137]. They were characterized as efficient ECL reagents because of their strong luminescence, good solubility in aqueous solvents at room temperature, and fast electron transfer reaction at an easily attainable potential [138]. Wang's group [139] was the first to integrated an indium tin oxide (ITO) electrode-based $Ru(bpy)_3^{2+}$ ECL detector onto a chip-based CE. The chip-based CE–ECL system consisted of a PDMS layer containing separation and injection channels and an electrode plate with an ITO electrode fabricated using a photolithographic method (Figure 15.4). In addition, they developed a simultaneous EC and ECL detection scheme for both chip-based CE and conventional CE. In this dual detection scheme, $Ru(bpy)_3^{2+}$ was used both as an ECL reagent and as a catalyst (in the formation of $Ru(bpy)_3^{2+}$) for the EC detection. The results indicated that this dual EC and ECL detection strategy could provide a simple and convenient detection method for analysis of more kinds of analytes in CE separation than the single EC or ECL detection separately [140]. However, compared to microchip EC detection, the applications of ECL detection coupled to chip-based CE are very limited at present. Further work should be undertaken on integrated chip-based CE–ECL

systems offering high reproducibility at comparatively low cost and, importantly, to widen the applications by the use of efficient ECL probes.

15.3 Applications

Chip-based CE has been employed in numerous applications, mainly focusing on the separation and detection of inorganic and small organic molecules [106,141]. For example, Pumera *et al.* [142] demonstrated the efficiency of the PMMA-based microchip and its detection configuration by separating and detecting the cations of potassium, sodium, barium, and lithium and the anions of chloride, sulfate, fluoride, acetate, and phosphate. The separation of a mixture of anions was performed in the anodic mode using 20 mM MES/His (pH 6.1) as the electrophoretic medium. The response was linear (over the 20 μM–7 mM range) and reproducible (RSD = 3.4–4.9%, $n = 10$), with detection limits of 2.8 and 6.4 μM (for potassium and chloride, respectively). An ionic-explosive microchip system for separating and detecting inorganic explosive residues, based on the coupling of a chip-based CE with a contactless conductivity detector was described by Wang *et al.* [70]. Meanwhile, many direct bioanalytical applications of CE–EC microchips have focused on the separation and detection of catecholamine neurotransmitters [12]. Ding *et al.* [143] analyzed five aminoglycoside antibiotics, namely, spectinomycin, streptomycin, amikacin, paromomycin, and neomycin, by employing chip-based CE with amperometric detection. Under the optimum conditions, linear relationships between the signal and the concentration were obtained in the 4.9–316.8 μM range. This alternative method is rapid, sensitive, and portable and enables the analysis of aminoglycoside antibiotics in a milk sample.

Amino acids have been investigated using chip-based CE due to their clinical and biological importance, which was reviewed by Ou *et al.* [26]. For instance, Dossi *et al.* [144] described a mixed valent ruthenium oxide/hexacyanoruthenate polymeric film that was electrochemically deposited onto glassy carbon electrodes and was proposed for the detection of biogenic amines and their amino acid precursors. The ability of this ruthenium coating to electrocatalyze the oxidation of aliphatic and heterocyclic amines, as well as their amino acid precursors, was checked by using ethanolamine, tryptamine, and tryptophane as prototype compounds.

In addition, biomacromolecules such as peptides and proteins have always been an important part of biological analysis and a chip-based CE with a rapid separation speed has been demonstrated and summarized by Kašička [117]. Endonuclease V (EndoV) is an enzyme that clips a double-stranded DNA molecule containing mismatched base pairs (i.e., heteroduplexes) on the 3′ side of the mismatch. Unfortunately, EndoV can also nick double-stranded DNA at matched sites, which can generate false positive signals when attempting to transduce the presence and location of mismatches. Kotani *et al.* [145] reported

a high-sensitivity mutation scanning assay using thermostable EndoV and DNA ligase for the detection of sporadic mutations. The products of the mutation scanning assay were separated using chip-based CE and detected using a dual-color LIF detector. The entire separation required 7 min.

Moreover, single-cell analysis is an important issue in biology, because seemingly identical cells are often quite heterogeneous in their chemical composition and biological activity. Zhao *et al.* [111] described an analytical method based on chip-based CE and CL detection for the determination of intracellular sulfydryl compounds. Sulfydryl compounds including cysteine (Cys), glutathione (GSH), and hemoglobin (Hb) were selected as the test compounds. The chip-based CE separation was completed within 120 s. In addition, this method was applied to analyze individual red blood cells collected from both healthy people and cancer patients. It was found that the average intracellular contents of Cys, GSH, and Hb were in the range of 26–43 amol per cell, 128–323 amol per cell, and 522–667 amol per cell, respectively, for cancer patients, compared to 579–609 amol Hb per cell and not detectable Cys and GSH for healthy subjects. Accordingly, by using different hyphenated detectors, or by integration with immunoassay, PCR/ligation detection reaction, and related technologies, chip-based CE can be constructed for application over diverse platforms used in genomics, proteomics, and metabolomics study for the early diagnosis of cancer [14].

15.4 Conclusions and Outlook

As discussed above, various applications of chip-based CE devices have indicated that these methods are extremely powerful tools for microseparations and detections of various analytes. However, the increasing demands for their development put forward higher requirements: (a) ultrasensitivity to the limited volume samples. Increasing the sensitivity of chip-based CE devices always involves concentration approaches based on electrophoretic phenomena that are broadly discussed as "stacking," while those involving partitioning onto or into a distinct phase are considered as "extraction." The past decade has experienced the growth in the combination of multiple complementary stacking and extraction methods to improve the performance in sensitivity. Maximizing sensitivity achieved while minimizing the time required remains another major challenge in this area. (b) High separation efficiency with limited microchannel length. Microchip fabrication allows for easy access to multiplexed liquid-phase separation compartments with dimensions in the low micrometer range. Therefore, a microchannel wall coating is becoming essential for the fabrication of microchip devices. There were several applications of chip-based CE where using a wall coating successfully separated complex mixtures. Disposable low-cost chips may be used more and more frequently and various coatings are likely to allow high-resolution separations with short analysis times. (c) Good reproducibility for

large-scale manufacture and applications. As the high sensitivity of chip-based CE devices is vulnerable to the influence of small sample volume, poor repeatability will lead to unexpected measuring errors. Therefore, precise control of fine microchannel design, machining, and modification is still a matter of urgency. In the coming years, the development of chip-based CE may revolutionize many industries, such as drug analysis, resequencing for pharmacogenetics, proteomics, and clinical diagnostics.

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