9
Capillary Gel Electrophoresis

Márta Kerékgyártó and András Guttman

9.1
Introduction

The significant impact that high-performance separation tools have made in life sciences during the past few decades suggests the continued improvements with the introduction of new analytical methods offering high and/or special resolving power. In the area of electric field-mediated separations, at present, most laboratories still utilize manual slab gel electrophoresis systems for the analysis of biologically important polymers, a technique that is not only time-consuming and labor intensive but also lacks adequate resolving power and throughput. Capillary gel electrophoresis (CGE) is fast becoming the separation and characterization technique of choice in the bioanalytical field, especially for the analysis of the biopolymers, such as nucleic acids, proteins, and complex carbohydrates. CGE provides a high-resolution and easy-to-use fully automated alternative to slab gel electrophoresis, also offering excellent detection sensitivity as well as rapid separation time for quantitative and qualitative analysis.

In the 1930s, Nobel Laureate Arne Tiselius established most of the important principles of electrophoresis that was based on the size-to-charge ratio of the analyte molecules in the presence of an applied electric field [1]. Later, anticonvective supporting media were used in electrophoresis, such as gels made of starch, polyacrylamide (PA), agarose, and so on. These gels also possessed sieving capabilities, thus polyionic molecules migrated through their networks as a function of their size, instead of their charge-to-mass ratio. Indeed, size separations are very important for the analysis of certain biopolymers like DNA. In the last decade of the twentieth century, a high-performance and fully automated separation technique, capillary electrophoresis (CE), was introduced in the biomedical and clinical research. CE is a family of electric field-mediated separation tools performed in submillimeter i.d. capillaries and/or in micro- and nanofluidic channels, in the modes of capillary zone electrophoresis and associated techniques including CGE, capillary isotachophoresis, capillary isoelectric focusing, micellar electrokinetic capillary chromatography, and capillary electrochromatography. These techniques are capable of separating complex biological

Analytical Separation Science, First Edition. Edited by Jared L. Anderson, Alain Berthod, Verónica Pino Estévez, and Apryll M. Stalcup.

mixtures in just minutes with excellent reproducibility. In case of CGE, the narrow-bore capillaries are filled with cross-linked gels or noncross-linked polymer matrices for the analysis. In addition, existing CGE methods can be readily transferred from the capillary format to electrophoresis microchips (lab-on-a-chip, LOC).

This chapter gives the reader an overview of both the theoretical and practical aspects of CGE, and also discusses the key application areas of nucleic acid, protein, and complex carbohydrate analysis. This also summarizes the latest developments in CGE column technology, including capillary coating and sieving polymers.

9.2 CGE Instrument Design

Figure 3.1 shows the schematic diagram of a typical CGE instrument, including the separation capillary, the high-voltage power supply, and the detection assembly. In CGE, the overall length of the separation capillary is in the range of 10–100 cm (generally 20–100 μm i.d. with 150–360 μm o.d.). Bare fused silica or coated capillaries can be used in single- or multicapillary systems. The inner surface of the separation capillary should be coated (covalent or dynamic) to suppress electroosmotic flow and prevent possible adsorption of the analyte

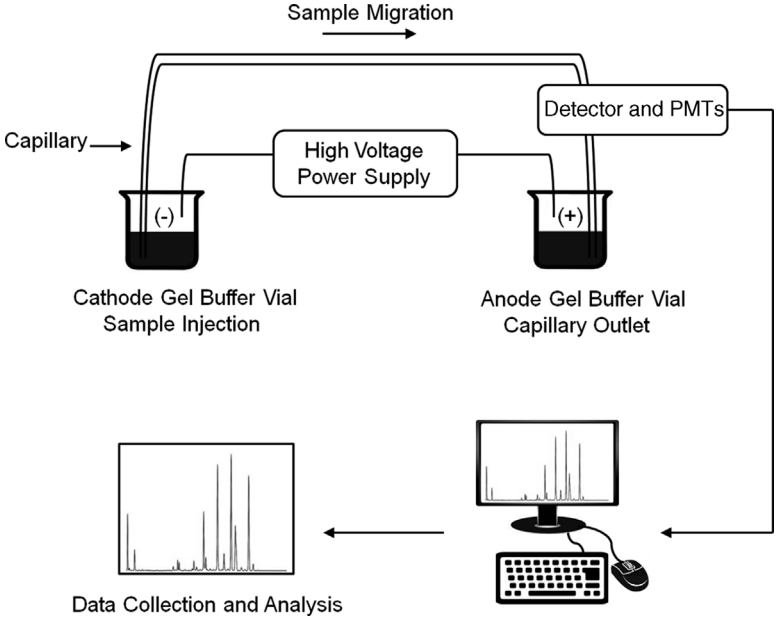

Figure 9.1 Schematic representation of a capillary gel electrophoresis instrument.

molecules. CGE systems mainly utilize electrokinetic injection for sample introduction as the higher viscosity of the gel usually prevents applying pressure injection. The electrokinetic method uses electric field to force the charged analyte molecules into the capillary, while in pressure injection, a sample vial is temporarily pressurized to allow the sample flowing into the capillary. Electrokinetic injection from aqueous samples (with no or little salt) results in large sample intake as the buffer coions do not compete with the sample molecules yielding excellent limits of detection [2]. During the injection process, the electrodes and both ends of the capillary are immersed into the respective sample and outlet buffer vials, and the applied voltage or pressure drives the analyte molecules into the tubing. During the separation process, the sample components migrate within the separation gel-buffer system filled capillary through the detection area. Detection can be achieved by UV/UV-Vis absorbance including scanning diode array, laser- (LIF) or light-emitting diode-induced fluorescence (LEDIF), or other special methods such as electrochemical detection. Most modern CE instruments allow separation voltages up to 30 kV during the analysis, which are orders of magnitudes higher than in traditional slab gel electrophoresis [3].

9.3 Basic Theory of Capillary Gel Electrophoresis

9.3.1 Electrophoretic Mobility

The electrical force (F_e) as defined in Equation 3.1 is the product of the electric field (E) and the net charge of the analyte molecules (Q):

$$F_e = QE. \tag{9.1}$$

When using a polymer matrix in the capillary tubing, a frictional force (F_f) acts in the opposite direction. In Equation 3.2, f is the translational friction coefficient, dt and dx are distance and time increments, respectively.

$$F_f = f\left(\frac{dx}{dt}\right). \tag{9.2}$$

When the electric field is applied, the charged analyte molecules are migrating according to Newton's second law of motion (Equation 3.3), where m is the product of the mass and d^2x/dt^2 is the acceleration, which is equal to the difference of the electrical and frictional forces.

$$m\left(\frac{d^2x}{dt^2}\right) = EQ - f\left(\frac{dx}{dt}\right). \tag{9.3}$$

The analyte molecules migrate with steady-state velocity (v) if the force from the applied electric field strength on the charged solute is counterbalanced by the

frictional force, as shown in Equation 3.4.

$$v = \frac{dx}{dt} = \frac{EQ}{f}. \tag{9.4}$$

The electrophoretic mobility (μ) is calculated as the velocity of per unit field strength, that represents a field normalized velocity:

$$\mu = \frac{v}{E}. \tag{9.5}$$

Different electrophoretic mobilities are due to differences in sample properties such as shape, size, or net charge, all of which influence electromigration. When a polymer matrix is used, the retardation of the analyte molecules is determined by the sieving polymer concentration (P) and its physical interaction with the analyte defined by the retardation coefficient (K_R). In Equation 3.6, μ_0 is the free solution mobility of the analyte, that is, with no polymer matrix in the separation system [4].

$$\mu = \mu_0 \exp(-K_R P). \tag{9.6}$$

In CGE, molecular sieving can be described by the Ogston theory [5] when the hydrodynamic radius of the analyte molecule is in the same range as that of the average pore size of the sieving matrix. Furthermore, the retardation coefficient (K_R) depends on the molecular weight (MW) of the analyte molecule at constant polymer concentration [6] and the mobility of the analyte molecule is a logarithmic function of the MW [7].

$$\mu \sim \exp(-\mathrm{MW}). \tag{9.7}$$

The Ogston theory also considers the migrating analyte molecule as a spherical object with size similar to the gel pores. However, large biomolecules containing flexible chains (e.g., DNA or SDS protein complexes) can migrate through pores of polymer networks smaller than the size of the solute [8]. In this instance, these large biopolymers behave in a "snake-like" motion when migrating through the much smaller gel pores [9–11]. This effect can be described by the reptation model, where the size of the solute (i.e., the chain length, n) is inversely proportional to the mobility:

$$\mu \approx \frac{E}{n}. \tag{9.8}$$

At very high electric field strengths, the biased reptation models are used (Equation 3.9), where b is a function of the size of the sieving polymer, the charge, and the segment length of the migrating molecules.

$$\mu \approx \frac{E}{n} + bE^2. \tag{9.9}$$

According to the Arrhenius equation [12], the viscosity of the gel-buffer exponentially decreases with temperature, where E_a is the activation energy for the viscous flow, R is the universal gas constant, and T is the absolute temperature.

Therefore, the electrophoretic mobility values have to be corrected with the temperature-influenced viscosity change of the solution (1.1% per °C) [13].

$$\eta = \text{constant} \cdot e^{E_a/RT}. \tag{9.10}$$

The electrophoretic mobility (μ) can also be expressed by combining the equations above, and A indicates the collection of all constants:

$$\mu = \frac{Q}{A} \cdot e^{-E_a/RT}. \tag{9.11}$$

9.3.2 Column Efficiency and Resolution

In CGE, the theoretical plate number (N) can be defined by Equation 3.12, where μ is the electrophoretic mobility, l is the effective length of the capillary column, and D is the diffusion coefficient of the migrating molecules in the separation gel-buffer system.

$$N = \mu \frac{E \cdot l}{2D}. \tag{9.12}$$

In addition, resolution (R_s) in CGE can be expressed as

$$R_s = 0.18 \cdot \Delta\mu \sqrt{\frac{E \cdot l}{D \cdot \mu_m}}, \tag{9.13}$$

where resolution (R_s) is calculated from the differences of the electrophoretic mobilities ($\Delta\mu$) between the two peaks [14] and μ_m is the electrophoretic mobility of the analyte molecule of interest. On the other hand, according to Equation 3.14, the limiting factor in the achievement of the high resolution is mainly the so-called Joule heat (Q_j) produced by the applied power ($P = V \times I$) [15], where r is the column radius, L is the total length of the capillary column, and I is the current.

$$Q_j = \frac{P}{r^2 \cdot I \cdot L}. \tag{9.14}$$

Therefore, temperature control is very important to achieve adequate migration reproducibility during CGE. Thus, modern, fully automated CGE instruments are all equipped with some kind of a temperature-control system (liquid or air cooling system) to alleviate Joule heat change-related problems.

9.4 Most Popular Gel Formulations in CGE

In the early1990s, unprecedentedly high resolving power was achieved using narrow bore fused silica capillaries filled with various sieving matrices to

separate biopolymers such as DNA, proteins, and complex carbohydrates. Two types of polymers were used in CGE: cross-linked and noncross-linked. In CGE today, the most commonly used sieving matrices are linear noncross-linked polymers such as linear polyacrylamide (LPA), polyethylene oxide (PEO), polyvinyl alcohol (PVA) polydimethylacrylamide, cellulose derivatives like hydroxypropyl methylcellulose, and polyvinylpyrrolidone (PVP) [16]. The important properties of these sieving matrices are discussed in the following section.

9.4.1 Acrylamide-Based Sieving Polymers

In CGE, the polyacrylamide (PA) based matrix can be used as a cross-linked PA or linear polyacrylamide (LPA) for the separation of biologically important macromolecules. Cross-linked PA gels (chemical gels), have been particularly useful for the analysis of shorter single-stranded DNA (ssDNA) and small proteins by SDS-based CGE [3]. To increase their stability, cross-linked PA gels are covalently attached to the inner surface of the fused silica capillary. These cross-linked gels can only accommodate electrokinetic injection, which results in sharp peaks due to the inherent sample preconcentration at the interface of the gel and the sample buffer, but suffer from biased injection as smaller and highly charged molecules preferably enter the capillary. However, if cross-linked polymers are used as sieving matrices, higher temperatures may cause deterioration of the gel structure (shrinking or bubble formation). Therefore, the use of noncross-linked polymer matrices (namely, physical gels or low-viscosity LPA solutions) became very popular, because they were not sensitive to high temperature and pH and salt concentration changes during the separation process. Furthermore, linear polymer solutions are routinely not covalently linked to the inside wall of the capillary, thus they permit both electrokinetic and pressure injection modes. Noncross-linked gels also enable simple replacement of the separation matrix in the capillary by positive or negative pressure. Such gels were used primarily with coated capillaries for the separation of double-stranded DNA (dsDNA), SDS–protein complexes, and complex carbohydrates [17]. Table 3.1 summarizes the main differences between cross-linked PA and LPA sieving matrices.

9.4.2 Agarose as Sieving Media

Agarose has a larger pore size than PA and is primarily used for the analysis of relatively large DNA fragments [18]. CGE compared to agarose slab gel electrophoresis offers better resolution, especially in the <600 base pair range. In contrast to PA, agarose does not become cross-linked during the gelation process; therefore, the inner surface of the capillary must be coated with a suitable noncharged material (e.g., LPA) to adequately suppress electroosmotic flow. Agarose-gel-filled capillaries were mostly used for the separation of dsDNA samples; however, the effective size range was limited up to ~12 kb [3]. Contrary

Table 9.1 Features of polyacrylamide gels used in CGE.

	Cross-linked polyacrylamide gel (chemical gel)	Noncross-linked polyacrylamide gel (physical gel)
Attachment	Linked to the inside wall of the capillary	Not linked to the inside wall of the capillary
Pore Structure	Well-defined, rigid	Flexible, dynamic, polymer networks with linear/branched structures
Pore Size	Cannot be varied after polymerization	Variable
Viscosity	High viscosity	Low to high viscosity
Heat tolerance	Sensitive	Insensitive
Sample injection mode	Electrokinetic	Electrokinetic and pressure
Application	ssDNA fragments, small proteins	dsDNA fragments, large proteins, complex carbohydrates

to acrylamide-based polymers, agarose-gel-filled capillaries have not been widely used in CGE and not available commercially [3].

9.4.3
Polyethylene Oxide, Polyvinylpyrrolidone, and Other Sieving Matrices

In addition to the most frequently used cross-linked and LPAs, PEO and PVP are also preferred sieving polymers in CGE, providing good separation for biologically important macromolecules. At the beginning of the new millennium, low-viscosity PEO-based sieving matrices were introduced in capillary array electrophoresis for nondenaturing DNA fragment analysis [16]. In 2002, Hu *et al.* used PEO to investigate the protein contents from HT29 human colon adenocarcinoma cells [19]. 21-Hydroxylase deficiency was investigated by Barta *et al.* with a PVP sieving matrix using CGE and primer extension techniques, detecting the most common mutations in the gene [20]. Ganzler *et al.* investigated the separation of SDS–protein complexes using polyethylene glycol and dextran matrices [21,22]. One of the advantages of using polysaccharides as sieving matrix is their low UV absorption. Sieving gel-buffer systems are commercially available for CGE from companies such as Beckman-Coulter (www.beckmancoulter.com), BiOptic Inc. (www.bioptic.com.tw), and Agilent Technologies (www.agilent.com). Beckman-Coulter SDS-MW Gel Buffer has been extensively utilized for quantitative and qualitative analysis of membrane proteins [23], protein biotoxins [24], and antibodies [25]. Another product from the same company has been routinely used for standard N-linked oligosaccharide analysis.

9.5 Capillary Coatings

Special coating techniques were introduced as early as the 1980s in CE to suppress or modify the effects of electroosmotic flow (EOF) and decrease wall absorption of the analytes via dynamic or covalent coatings. To eliminate EOF, Hjerten attached γ-methacryloxypropyl-trimethoxysilane to the capillary wall and then filled the resulting bifunctional reagent activated capillary with an LPA polymerization solution including the catalyst and the initiator [26]. One of the problems with this coating was that linear molecules could not completely cover the capillary wall [22] and thus was not stable for a longer time especially at higher pH values. In 2004, Gao and Liu introduced a cross-linked PA coating to address the issue, and successfully used it for SDS-CGE. Bruin *et al.* attempted to covalently coat fused silica capillaries with the same bifunctional reagent of γ-methacryloxypropyl-trimethoxysilane for the separation of small carbohydrates [27]; however, the Si−O−Si bond was prone to hydrolysis at higher pH values. To alleviate this issue, Cobb, Dolnik, and Novotny developed a more stable direct Si−C bond-based capillary coating to accommodate a wide separation pH range of 2–10 [28]. Covalently, coated capillaries provided rapid separations without the requirement of preseparation equilibration and also reduced sample–wall interactions. Chemically bonding the desired coating substances to capillary walls generally results in more stable coatings as their dynamic counterpart. From the 1990s, in addition to covalently coated capillaries, the so-called noncovalent "dynamic coatings" were introduced. Although dynamic coatings do not completely suppress EOF, they can be easily prepared by simply rinsing the capillary with a polymer solution, detergent, or multivalent ions. Polymers used as dynamic coating materials include polydimethylacrylamide [29], poly-*N*-hydroxyethylacrylamide [30], and epoxy-poly(dimethylacrylamide) [31–33]. Further efforts in CGE to combine the advantages of covalent and dynamic coatings comprised a thin layer of PA dynamically coated to the inner surface of the separation capillary followed by allylamine treatment [34]. Recently, both dynamic and covalently coated capillaries are successfully used in CGE separation with polymer solutions and gels [35,36]. Cross-linked PA coatings are also applied to glass microchips to improve separation efficiency and alleviate adsorption effects [37].

9.6 Detection Options for CGE

9.6.1 UV-Visible Detector

UV-visible detection systems are the most commonly used in CE, including CGE [38]. Two types of light sources are usually employed in UV-Vis detectors:

single line and continuous source. The simplest UV light sources are atomic lamps, which generate strong emission lines at well-defined wavelengths, for example, the low-pressure Hg lamp at 254 nm, the Cd lamp at 229 nm, the Zn lamp at 214 nm, and the As lamp at 200 nm. A medium-pressure Hg lamp is also utilized as a line source providing emission lines at 254, 280, 313, 365, 405, 436, and 546 nm wavelengths. Two special types of sources are available as continuous light sources for UV-Vis absorbance. One of the continuous sources is the deuterium arc lamp, which provides good continuous intensity, ranging from 180 to 350 nm. Lamp noise often caused problems in overall detection performance, but modern deuterium arc lamps have very low noise [39]. The intensity of such light sources steadily decreases, with typical half-lives of approximately 1000 h. The other most frequently used continuous source is the tungsten–halogen lamp, which offers good intensity over part of the UV spectrum and the entire visible range from 280 to 1000 nm. In addition, this type of lamp also features very low noise, short drift, and useful lifetime of 10 000 h. UV-Vis absorbance detection utilizes the so-called "forward optics" design as shown in Figure 3.2a. In this design, a polychromatic light from the source mentioned above is focused onto the entrance slit of a monochromator, which selectively transmits a narrow band of light. The light is conveyed through the capillary to the photodiode detector. In contrast to other liquid-based separation

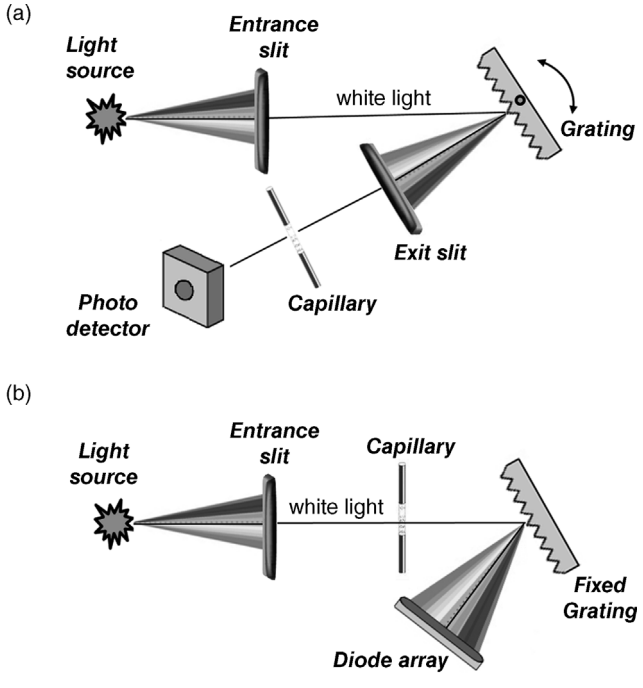

Figure 9.2 Design of forward optics (a) of a conventional optical detector and reverse optics (b) for diode-array detector.

techniques, the detection sensitivity in UV-Vis absorbance detection is limited, because the path length is a direct function of the capillary diameter, that is, rather short. If the sample components do not possess UV absorbing features, indirect UV detection can be utilized, which is universal, can be applied nonselectively, and is useful for the simultaneous detection of absorbing and nonabsorbing analytes. The disadvantages of indirect UV detection are that these detectors cannot be used for additional identify confirmation and analytes can exhibit as positive and negative peaks [40].

9.6.2 Diode Array Detector

Multiwavelength detectors, rather known as diode array detectors (DAD), utilize a "reverse optics" design. The first diode array detector for CE was described for on-capillary UV detection in 1989 [41]. In this detection design, a polychromatic light from the source (e.g., deuterium lamps) was first transported through the capillary column, after which it was focused on the entrance slit of a monochromator. This monochromator contained a diffraction grating, which diffused the light onto an array system consisting of numerous photodiodes. Each diode measured a narrow band of the spectrum as shown in Figure 3.2b. The bandwidth of the light detected by a diode was related to the size of the monochromator entrance slit and to the size of the diode. The number of individual diodes in the array system defined the wavelength resolution of the detector. Software can automatically calculate the maximum absorbance in DADs, and the data can be visualized in a three-dimensional form. Using a diode-array detector, a whole wavelength range can be utilized; thus all light-absorbing components of the sample can be detected within a single analysis. Contrary to single-wavelength UV-Vis absorbance detection, diode-array detectors provide spectral analysis of each sample component along with qualitative information [40].

9.6.3 Laser-Induced Fluorescence Detector

Laser-induced fluorescence (LIF) detection is one of the most sensitive online detection schemes for CE, capable of even single-molecule detection, but most often requires chemical derivatization. For the analysis of proteins, mostly fluorescein isothiocyanate (FITC) [42], naphthalene-2,3-dicarboxaldehyde (NDA) [43], and 3-(2-furoyl)quinoline-2-carboxaldehyde (FQ) are used [19]. Several charged fluorescent reagents are commercially available for carbohydrate analysis such as 8-aminonaphthalene-1,3,6-trisulfonic acid (ANTS) [44], 5-amino-2-napthalenesulfonic acid, 7 aminonaphthalene-1,3-disulfonic acid, 2-aminonaphthalene-1-sulfonic acid [45], and 8-aminopyrene-1,3,6-trisulfonic acid (APTS) [46,47]. In LIF detection, there are two main types of lasers that are usually utilized: (1) continuous wavelength and pulsed ones. Frequently used lasers are helium–neon that emit light at 543 nm, 594 nm, 604 nm, 612 nm and 633 nm;

helium–cadmium laser, which produces radiation at 325 nm, 354 nm and 442 nm; and argon–ion laser (air cooled), which emits at 457 nm, 472 nm, 476 nm, 488 nm, 496 nm, 501 nm, and 514 nm. Fluorophores used for the analysis of nucleic acids with LIF are fluorescent intercalator dyes (e.g., monomeric dyes: ethidium bromide, dimeric dyes: ethidium homodimer, TOTO, YOYO) and covalently bound dyes such as fluorescein and rhodamine derivatives. In CGE-LIF detection, several fluorescent DNA labeling dyes, such as SYBR Green I [48], Quant-iT OliGreen ssDNA reagent [49], YOYO-1 [50], YO-PRO-1 [51] were utilized for routine DNA analysis. For the investigation of dsDNA, a monomeric dye SYBR Green I has been used in conjugation with laser-induced fluorescence detection. In LIF detection, the light from the laser is focused on the detection window close to the outlet end of the separation capillary. The fluorescent signal is produced by laser illumination and the emitted light is measured as shown in Figure 3.3. Laser-quality mirrors and lenses are used to direct the beam toward the window on the capillary column. The use of these mirrors with good reflectance are not usually a limiting factor in LIF detection, because most of the lasers have more power than necessary to generate the fluorescent signal. On the other hand, there are some drawbacks in using these mirrors, that is, the long path of the rays from the laser source to capillary and small mechanical

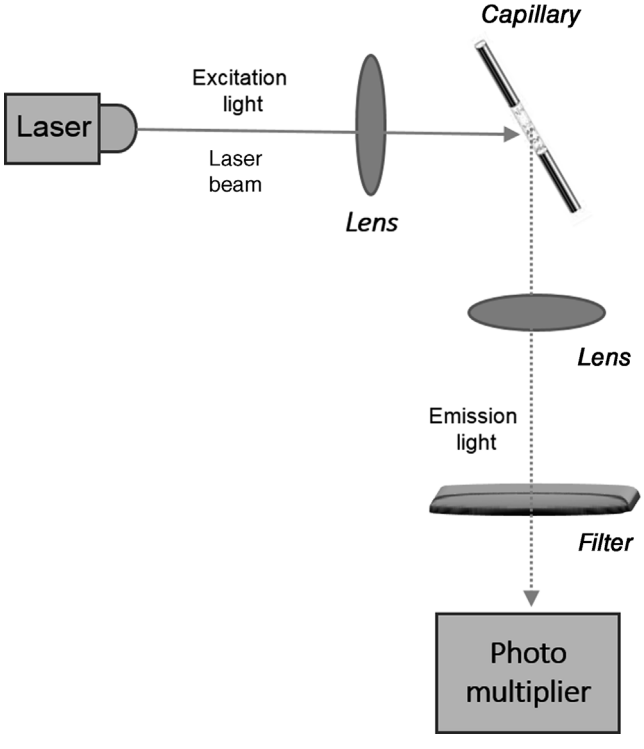

Figure 9.3 Laser-induced fluorescence detection in capillary gel electrophoresis.

vibrations of the system causing noise [52]. Most of these limiting factors can be avoided, if optical fibers or a ball lens design is used, which collect the light from the laser source and focus it onto the capillary [53]. To achieve high sensitivity with low light losses and/or scatter, the use of off-capillary sheath flow arrangement was recommended by Dovichi *et al.* [54]. In LIF detection, very low light can be measured using devices such as photomultiplier tubes, avalanche photodiodes, or image detectors like charge coupled devices and intensified charge coupled devices [52].

9.6.3.1 Light-Emitting Diode-Induced Fluorescence Detection

For high detection sensitivity, a strong light source is required to obtain a good signal in fluorescent detection. For many years, lasers were exclusively used for this purpose, but lately LEDIF detections have emerged [55]. New-generation LEDs offer an alternative light source to lasers for fluorescent detection. LEDs are small solid-state light sources, which are less expensive than lasers and consume very low energy [56]. Some labeling dyes are routinely used in CGE-LEDIF, such as 5-carboxy-tetramethylrhodamine *N*-succinimidyl ester, 3-(2-furoyl)quinoline-2-carboxaldehyde (FQ), naphthalene-2,3-dialdehyde (NDA), and fluorescein isothiocyanate (FITC) for the analysis of amino acids and proteins [57]. 8-aminopyrene-1,3,6-trisulfonate (APTS, λ_{ex} 420 nm and λ_{em} 500 nm) and 8-aminonaphtalene-1,3,6-trisulfonate (ANTS, λ_{ex} 356 nm and λ_{em} 512 nm) are routinely used in CGE-LEDIF-based glycan profiling experiments [58]. In LED detection, carboxyfluorescein dyes such as fluorescein, 6-carboxyfluorescein and rhodamine B were utilized for the analysis of single-stranded DNA oligonucleotides [59]. One of the main advantages of these types of light sources is their capability to work in the visible or near-infrared region, that is, above 635 nm. LEDs have also been utilized from 390 nm to 440 nm in a few cases (e.g., a ultraviolet-emitting laser diode based on gallium nitride [60]). LEDs can be used in integrated optical detectors in microchip designs [40].

9.7 Capillary Gel Electrophoresis of Nucleic Acids

At present, most bioanalytical laboratories still utilize manual PA/agarose slab gel electrophoresis for the size separation of nucleic acids. The use of these tools are time-consuming and labor intensive, also requiring improvements in terms of resolving power [61]. CGE is a latest emerging technique used in molecular biology and biotechnology industry laboratories, especially advantageous for analyzing nucleic acid as it offers an easy-to-use, fully automated, and reliable approach with rapid separations, high sensitivity, excellent resolution, and ruggedness [62]. Large-scale and high-throughput analysis of nucleic acids by CGE include genotyping [63], polymerase chain reaction (PCR) product sizing [64], DNA restriction fragment analysis [65], and even DNA sequencing [61]. To

describe DNA motion during electrophoresis separation in a gel matrix or entangled polymer network, three different theories can be applied: the Ogston, the reptation, and the biased reptation models (as discussed in Section 3.3.1).

One of the most frequently used techniques in the nucleic acid field is the analysis of single nucleotide polymorphism (SNP), which can be detected by regular CGE format [66] as Barta *et al.* reported on high-resolution SNP analysis of the 21-hydroxylase gene [20]. In their work, they employed a primer extension technique utilizing a 422-bp fragment of the CYP21 gene containing the SNP. The site was amplified by PCR followed by separation of the two possible primer extension products (mutant and wild-type) and the Cy5-labeled primer by CGE in 90 s. Another CGE approach is constant denaturant capillary electrophoresis (CDCE), which permits high-resolution separation of single-base variations based on their differential melting temperatures for applications such as mutation analysis and single-nucleotide polymorphism discovery [67]. Xue *et al.* used the CDGE for pooled blood samples to identify SNPs in Scnn1a and Scnn1b genes, and utilized a two-point LIF detection setup to improve mutation identification [68]. Allele-specific PCR is another very popular genotyping method, also referred to as the amplification refractory mutation system [69]. This amplification method is based on the utilization of allele-specific primers to hybridize at the 3′-end of the SNP site, followed by amplification using a special DNA polymerase enzyme lacking 3′-exonuclease activity, thus amplification can be carried out only in the case where the primer perfectly matches with the template. Szántai *et al.* introduced a novel haplotyping technique to analyze two SNPs (-616CG and -521CT) in the Dopamine D4 receptor gene by CGE. Our laboratory reported a successful haplotyping of two adjacent miRNA-binding SNPs in the Wolframin gene by combining allele-specific amplification and rapid CGE analysis with LEDIF detection.

DNA sequencing by CGE became another mature field in a remarkably short period of time. In the 1980s, the first paper by Guttman reported on high-resolution separation of DNA fragments suggesting that CGE can solve the DNA sequencing problem [61,70]. Later, multicapillary array instrumentation was introduced by Mathies and coworkers for high throughput analysis of DNA sequencing fragments [38]. Because of the capillary bundle, these array systems also required sensitive detection design. Dovichi and coworkers developed a high-sensitivity postcolumn laser-induced fluorescence detector based on a sheath flow cuvette to decrease background signal originating from the light scatter of the gel-filled capillary [71]. Currently, in spite of the advent of second- and third-generation DNA sequencing techniques, fully automated, multicapillary DNA sequencing tools are still utilized to investigate hundreds of DNA samples with high read lengths [72]. Microfabricated capillary arrays provide new opportunities in multiplexing DNA analysis. Easley *et al.* described a fully integrated microfluidic genetic analysis device with sample-in-answer-out capability [73]. In addition, numerous experiments have been carried out in nanoslits, nanochannels, channels with nanosized entropic traps and pillars to investigate DNA fragments [74].

9.8
Protein Separation by Capillary Gel Electrophoresis

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) is another very frequently used tool in biological research laboratories for size separation and purity check of proteins [22]. Traditional SDS-PAGE requires time-consuming and labor-intensive manual steps, is poorly automated, and also lacks adequate resolving power in one-dimensional format. Capillary SDS gel electrophoresis (also referred to as CE-SDS) provides many advantages over classical SDS-PAGE by offering fully automated operation, on-column detection with high-sensitivity, excellent resolution and capability of accurate protein quantification, and molecular mass assessment [57,75]. The first papers on SDS-CGE were published as early as in the 1980s by applying agarose [76] and cross-linked PA [77] as sieving polymers. These gels were directly filled into the capillary columns prior to analysis, either as hot solutions (agarose) or as polymerization reaction mixtures (crosslinked PA). In the early 1990s, LPA substituted cross-linked PA, but the polymerization process was still accomplished inside the capillary [21]. The life span of these capillaries were limited (<10 runs), with poor run-to-run reproducibility. Later, water-soluble and replaceable linear and/or slightly branched polymers were also introduced such as low concentration LPA [21,78,79], cross-linked PA with very low cross-linker concentration [80–82], PEO [83], polyethylene glycol [21], dextran [21], and pullulan (hexosyl polymer) [84,85] as sieving matrices for CGE separation. Currently, SDS-CGE is widely used in the biopharmaceutical industry during quality control of therapeutic proteins such as recombinant monoclonal antibodies [86–89] as shown in Figure 3.4. SDS-CGE was successfully coupled to matrix-assisted laser desorption ionization (MALDI) MS by utilizing a moving poly(tetrafluoroethylene) membrane past the outlet end of the capillary [90] to continuously collect fractions for MS analysis. CGE-MALDI-MS has also been utilized for the quality control of monoclonal antibodies in the biopharmaceutical industry [91].

9.9
Capillary Gel Electrophoresis of Complex Carbohydrates

Glycosylation is one of the most common and structurally diverse post-translational modifications of proteins, which plays crucial roles in different biochemical processes, including protein stability, folding, localization, and cellular communications [92]. Numerous changes in biological activities are often the result of alterations in glycosylation, through site occupancy changes either on the polypeptide chain or in the variation of the oligosaccharide structures at a particular site on a protein (microheterogeneity). Aberrant glycosylation has been found in many diseases including immune deficiencies, cardiovascular syndromes, and cancer [93]. To separate glycans with high selectivity presents yet another challenge in the analysis of carbohydrate structures derived from

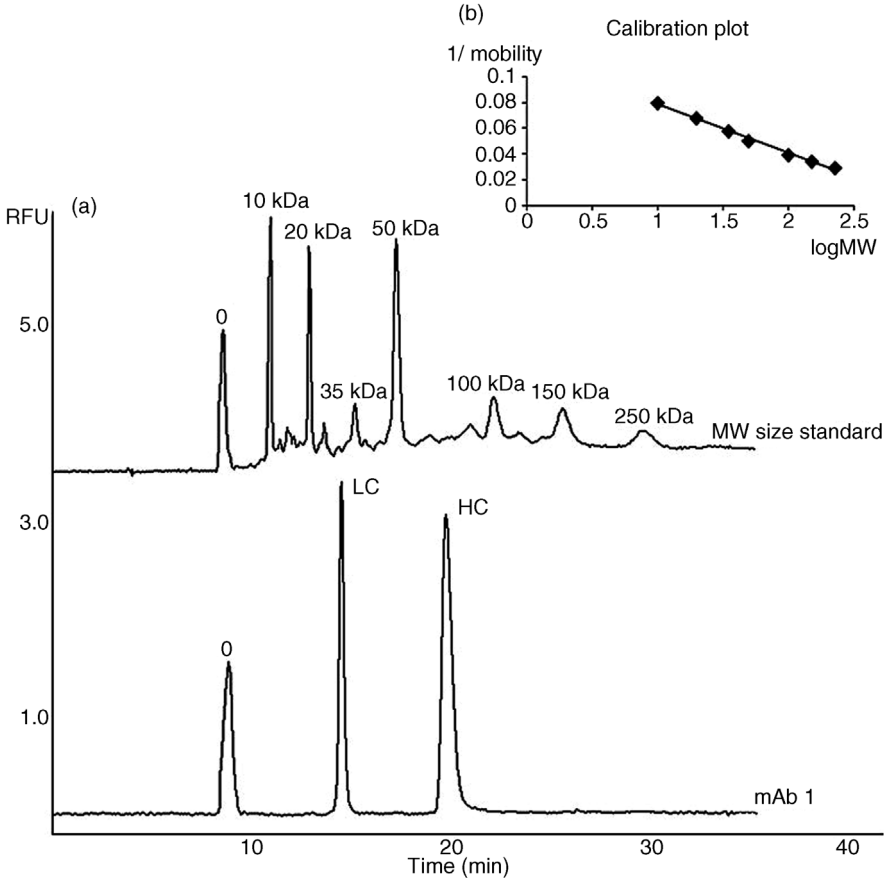

Figure 9.4 High-throughput analysis of a therapeutic monoclonal antibody by multicapillary SDS gel electrophoresis. (a) Upper trace: covalently fluorophore tagged molecular mass ladder; lower trace: reduced mAb sample (0 remaining labeling dye, LC and HC represent the light and the heavy chains of mAb). (b) The calibration plot for molecular mass assessment in the 10–250 kDa mass range. (Reproduced with permission from Ref. [88].)

glycoproteins, because most native glycans lack fluorophore or chromophore features in their structures that would enable their UV-Vis or LIF detection. A great advantage of CGE in glycan separation is the ability to distinguish both linkage and positional isomers [94,95]. CGE-LIF also offers carbohydrate sequencing options by top–down digestion and bottom–up identification using a series of sugar-specific exoglycosidase as shown in Figure 3.5. Typically, the carbohydrate moiety of a glycoprotein is enzymatically removed with endoglycosidases such as PNGase F, endo-H, and so on. The liberated glycans are then subject to fluorescent labeling for CGE, in most instances using 8-aminopyrene-1,3,6-trisulfonic acid (APTS). For glycan labeling other charged fluorescent reagents were also discussed in Section 3.6.3. Other tags for aldose labeling with

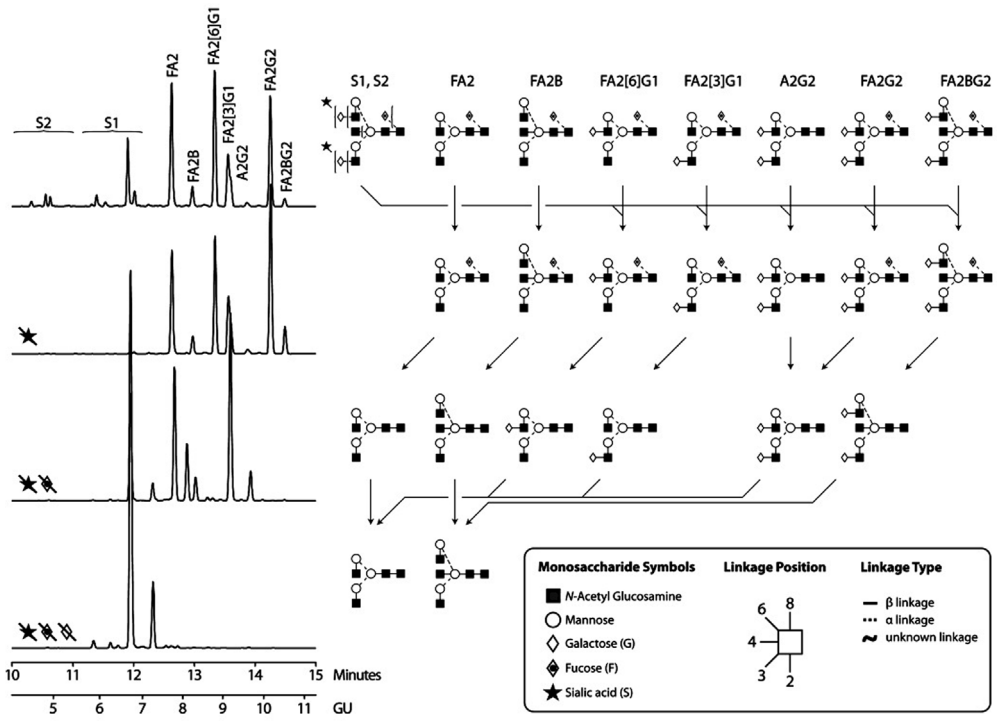

Figure 9.5 Overview of the experimental strategy for exoglycosidase digestion-based carbohydrate sequencing of IgG *N*-glycan structures by CGE-LIF. (Reproduced with permission from Ref. [98].)

2,3-naphthalenediamine to produce highly fluorescent naphthimidazole derivatives were also attempted by Kuo *et al.* [96]. Kerékgyártó and Guttman reported on the establishment of an 8-aminonaphthalene-1,3,6-trisulfonic acid (ANTS)-labeled *N*-glycan database for rapid (<200 s) CGE-LEDIF-based glycan profiling [97]. CGE-LIF is an excellent orthogonal tool to HILIC-LIF for the analysis of glycans based on its different separation mechanism [98]. CGE can also be used in conjugation with MS for the analysis of carbohydrates for glycoprofiling of biotherapeutics [99–101]. Another advantage of CGE-based tools is the option of easy multiplexing up to 12, 48, or even 96 capillaries for high-throughput applications [102,103].

9.10 Future Trends: Miniaturization, Lab-On-a-Chip systems

Microfabricated lab-on-a-chip (LOC) systems have been developed with the aim to integrate multiple analytical processes on a monolithic chip platform including sample pretreatment, solution distribution/mixing, separation, detection, and

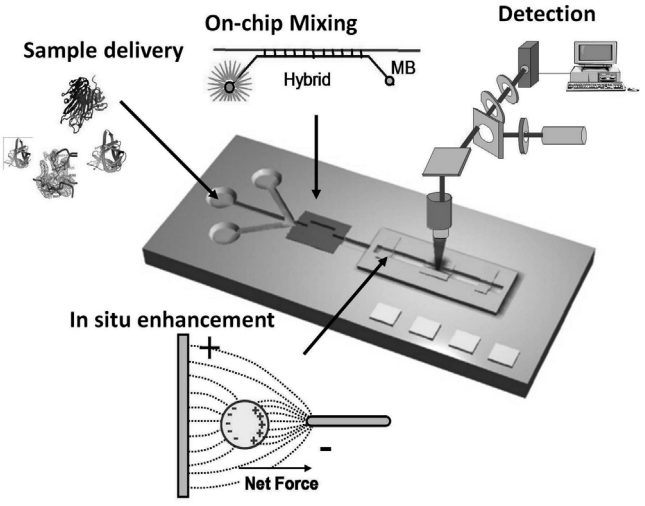

Figure 9.6 Lab-on-a-chip electrophoresis platform. (Reproduced with permission from Ref. [128].)

so on, as shown in Figure 3.6 [104]. The major objective of the implementation of microchips or microfluidic analysis systems is to utilize standard laboratory processes in a miniaturized format. Because of the short length of the separation channels, microchip CGE is very fast, separations take place from a few seconds to a few minutes [22]. Capillary electrophoresis microchips employ channels etched into planar substrates using common microfabrication tools developed for the semiconductor industry using photolithography and chemical etching. In such microchips, well-defined amounts of samples can be introduced with simple cross or double T-injector structures [105]. The separated molecules are typically detected by confocal microscopy with laser-induced fluorescence. These features make CE microchips attractive technology platforms for next-generation instrumentation. Microfabricated devices have already been used for the analysis of biomolecules such as DNA fragments [106,107], DNA sequencing [108], PCR product sizing [109], genotyping [110], cell and tissue studies [111], protein separation [22,112,113], carbohydrate profiling [114], and environmental monitoring [115]. Another approach of miniaturization is ultrathin-layer gel electrophoresis, which is a combination of CGE and slab gel electrophoresis, offering multilane separation to allow rapid and efficient analysis of biopolymers [116]. In addition, miniaturization also provides the integration option with applications of micromachined chromatographic phase techniques such as high-performance micro and nanovolume LC [117].

Several microfluidic strategies have emerged from the effective integration of multiple microfluidic components toward fully automated lab-on-a-chip systems for sophisticated biomedical analyses [118]. Rech *et al.* reported compact four-color microchip electrophoresis (ME)-based genotyping instruments [119]. Rhazi *et al.* utilized a commercial microfluidic protein assay system for

quantitative and qualitative analyses of high MW glutenin subunits [120] with comparable sensitivity and resolution to conventional RP-HPLC, but at a timescale approximately 100 times faster. Tunable thick polymer coatings were introduced for on-chip electrophoretic protein and peptide separation by He *et al.* [121]. In the clinical analysis field, ME technology is used for miniaturized bioanalytical devices [122]. Zhang *et al.* reported on the establishment of a microfluidic bead-based immune sensor for the detection of a cancer biomarker, α-fetoprotein, using multienzyme nanoparticle amplification and quantum dot labeling [123]. Qian *et al.* demonstrated that PDMS/glass microchip CE is a valuable technique for rapid and reliable investigation of high-density lipoprotein subclasses in clinical samples [124]. Kamruzzaman *et al.* introduced a sensitive chemiluminescence method for microchip identification of vitamin B1 [125]. Commercially available microchip gel electrophoresis instruments are on the market and frequently utilized in the pharmaceutical industry for the investigation of antibody cell culture samples [126]. Primack *et al.* [127] reported on the establishment of high-throughput antibody glycan profiling during cell culture expression, including clone selection and cell culture process optimization. With this method, the relative levels of high mannose, fucosylated and galactosylated glycoforms in the Fc domain were measured by microchip CE for hundreds of crude cell culture samples in less than 1 min. As with CE, even more selective and specific detection of analytes is possible by coupling ME to mass spectrometry (MS). The most common ionization interfaces for MS are electrospray ionization (ESI) and MALDI [121]. The main advantage of ME-ESI-MS is the low flow rate, which results in seamless interfacing without disrupting the electrophoretic separation.

Abbreviations

CE	capillary electrophoresis
CGE	capillary gel electrophoresis
LIF	laser-induced fluorescence
LEDIF	light-emitting diode-induced fluorescence
PA	polyacrylamide
CPA	cross-linked polyacrylamide
LPA	linear polyacrylamide
PEO	polyethylene oxide
PVP	polyvinylpyrrolidone
ssDNA	single-stranded deoxyribonucleic acid
dsDNA	double-stranded deoxyribonucleic acid
EOF	electroosmotic flow
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
PCR	polymerase chain reaction
SNP	single nucleotide polymorphism
APTS	8-aminopyrene-1,3,6-trisulfonic acid

ANTS	8-aminonaphthalene-1,3,6-trisulfonic acid
LOC	lab-on-a-chip
DAD	diode array detector
CDCE	constant denaturant capillary electrophoresis
ME	microchip electrophoresis

Acknowledgments

This work was supported by the European Union and the State of Hungary, cofinanced by the European Social Fund in the framework of TÁMOP 4.2.4.A/2-11-1-2012-0001 "National Excellence Program." AG acknowledges the support of the MTA-PE Translational Glycomics program (#97101).

References

1 Tiselius, A. (1937) A new apparatus for electrophoretic analysis of colloidal mixtures. *Trans. Faraday Soc.*, **33**, 524–531.
2 Kerekgyarto, M., Nemeth, N., Kerekes, T., Ronai, Z., and Guttman, A. (2013) Ultrafast haplotyping of putative microRNA-binding sites in the WFS1 gene by multiplex polymerase chain reaction and capillary gel electrophoresis. *J. Chromatogr. A*, **1286**, 229–234.
3 Guttman, A. and Schwartz, H.E. (1998) Separation of DNA by capillary electrophoresis, in *Capillary Electrophoresis, Theory and Practice*, 2nd edn (ed. P. Camilleri), CRC Press, Boca Raton, pp. 397–441.
4 Andrews, A.T. (1986) *Electrophoresis: Theory, Techniques, and Biochemical and Clinical Applications*, 2nd edn, Clarenden Press, Oxford University Press, Oxford.
5 Ogston, A.G. (1985) The spaces in a uniform random suspension of fibres. *Trans. Faraday Soc.*, (54), 1754–1757.
6 Grossman, P.D., Menchen, S., and Hershey, D. (1992) Quantitative analysis of DNA-sequencing electrophoresis. *Genet. Anal. Tech. Appl.*, **9** (1), 9–16.
7 Ferguson, K.A. (1964) Starch-gel electrophoresis: application to the classification of pituitary proteins and polypeptides. *Metabolis* (13 SUPPL), 985–1002.
8 Lumpkin, O.J., Dejardin, P., and Zimm, B.H. (1985) Theory of gel electrophoresis of DNA. *Biopolymers*, **24** (8), 1573–1593.
9 de Gennes, P.G. (1979) *Scaling Concepts in Polymer Physics*, Cornell University Press, Ithaca, N.Y.
10 Slater, G.W. and Noolandi, J. (1989) The biased reptation model of DNA Gel-electrophoresis: mobility vs molecular-size and gel concentration. *Biopolymers*, **28** (10), 1781–1791.
11 Viovy, J.L. and Duke, T. (1993) DNA electrophoresis in polymer-solutions: ogston sieving, reptation and constraint release. *Electrophoresis*, **14** (4), 322–329.
12 Arrhenius, S. (1989) Über die Reaktionsgeschwindigkeit bei der inversion von rohrzucker durch Säuren. *Z. Physik. Chem.*, **4**, 226.
13 Szoke, M., Sasvari-Szekely, M., and Guttman, A. (1999) Ultra-thin-layer agarose gel electrophoresis. I. Effect of the gel concentration and temperature on the separation of DNA fragments. *J. Chromatogr. A*, **830** (2), 465–471.
14 Karger, B.L., Cohen, A.S., and Guttman, A. (1989) High-performance capillary electrophoresis in the biological sciences. *J. Chromatogr.*, **492**, 585–614.
15 Nelson, R.J., Paulus, A., Cohen, A.S., Guttman, A., and Karger, B.L. (1989) Use of peltier thermoelectric devices to control column temperature in high-performance capillary electrophoresis. *J. Chromatogr.*, **480**, 111–127.

16 Guttman, A. (2003) Gel and polymer-solution mediated separation of biopolymers by capillary electrophoresis. *J. Chromatogr. Sci.*, **41** (9), 449–459.
17 Ettre, L.S. and Guttman, A. (2004) The evolution of capillary gel electrophoresis: from proteins to DNA sequencing. *LCGC North Am.*, **22** (9), 896–904.
18 Stellwagen, N.C. and Stellwagen, E. (2009) Effect of the matrix on DNA electrophoretic mobility. *J. Chromatogr. A*, **1216** (10), 1917–1929.
19 Hu, S., Jiang, J., Cook, L.M., Richards, D.P., Horlick, L., Wong, B., and Dovichi, N.J. (2002) Capillary sodium dodecyl sulfate-DALT electrophoresis with laser-induced fluorescence detection for size-based analysis of proteins in human colon cancer cells. *Electrophoresis*, **23** (18), 3136–3142.
20 Barta, C., Ronai, Z., Sasvari-Szekely, M., and Guttman, A. (2001) Rapid single nucleotide polymorphism analysis by primer extension and capillary electrophoresis using polyvinyl pyrrolidone matrix. *Electrophoresis*, **22** (4), 779–782.
21 Ganzler, K., Greve, K.S., Cohen, A.S., Karger, B.L., Guttman, A., and Cooke, N.C. (1992) High-performance capillary electrophoresis of SDS-protein complexes using UV-transparent polymer networks. *Anal. Chem.*, **64** (22), 2665–2671.
22 Zhu, Z., Lu, J.J., and Liu, S. (2012) Protein separation by capillary gel electrophoresis: a review. *Anal. Chim. Acta*, **709**, 21–31.
23 Lin, C., Cotton, F., Boutique, C., Dhermy, D., Vertongen, F., and Gulbis, B. (2000) Capillary gel electrophoresis: separation of major erythrocyte membrane proteins. *J. Chromatogr. B*, **742** (2), 411–419.
24 Fruetel, J.A., Renzi, R.F., Vandernoot, V.A., Stamps, J., Horn, B.A., West, J.A., Ferko, S., Crocker, R., Bailey, C.G., Arnold, D. *et al.* (2005) Microchip separations of protein biotoxins using an integrated hand-held device. *Electrophoresis*, **26** (6), 1144–1154.
25 Zhang, J., Burman, S., Gunturi, S., and Foley, J.P. (2010) Method development and validation of capillary sodium dodecyl sulfate gel electrophoresis for the characterization of a monoclonal antibody. *J. Pharm. Biomed. Anal.*, **53** (5), 1236–1243.
26 Hjerten, S. (1985) High-performance electrophoresis: elimination of electroendosmosis and solute adsorption. *J. Chromatogr.*, **347** (2), 191–198.
27 Bruin, G.J., Huisden, R., Kraak, J.C., and Poppe, H. (1989) Performance of carbohydrate-modified fused-silica capillaries for the separation of proteins by zone electrophoresis. *J. Chromatogr.*, **480**, 339–349.
28 Cobb, K.A., Dolnik, V., and Novotny, M. (1990) Electrophoretic separations of proteins in capillaries with hydrolytically stable surface structures. *Anal. Chem.*, **62** (22), 2478–2483.
29 Madabhushi, R.S. (1998) Separation of 4-color DNA sequencing extension products in noncovalently coated capillaries using low viscosity polymer solutions. *Electrophoresis*, **19** (2), 224–230.
30 Albarghouthi, M.N., Stein, T.M., and Barron, A.E. (2003) Poly-*N*-hydroxyethylacrylamide as a novel, adsorbed coating for protein separation by capillary electrophoresis. *Electrophoresis*, **24** (7–8), 1166–1175.
31 Chiari, M., Cretich, M., Damin, F., Ceriotti, L., and Consonni, R. (2000) New adsorbed coatings for capillary electrophoresis. *Electrophoresis*, **21** (5), 909–916.
32 Chiari, M., Cretich, M., Desperati, V., Marinzi, C., Galbusera, C., and De Lorenzi, E. (2000) Evaluation of new adsorbed coatings in chiral capillary electrophoresis and the partial filling technique. *Electrophoresis*, **21** (12), 2343–2351.
33 Cretich, M., Stastna, M., Chrambach, A., and Chiari, M. (2002) Decreased protein peak asymmetry and width due to static capillary coating with hydrophilic derivatives of polydimethylacrylamide. *Electrophoresis*, **23** (14), 2274–2278.
34 Shieh, C.H. (1995) Coated capillary columns and electrophoretic separation methods for their Use. Beckman

Instruments, Inc., U.S.A. Patent Application No. US5462646.
35 Horvath, J. and Dolnik, V. (2001) Polymer wall coatings for capillary electrophoresis. *Electrophoresis*, **22** (4), 644–655.
36 Righetti, P.G., Gelfi, C., Verzola, B., and Castelletti, L. (2001) The state of the art of dynamic coatings. *Electrophoresis*, **22** (4), 603–611.
37 Lu, J.J. and Liu, S. (2006) A robust cross-linked polyacrylamide coating for microchip electrophoresis of dsDNA fragments. *Electrophoresis*, **27** (19), 3764–3771.
38 Huang, X.C., Quesada, M.A., and Mathies, R.A. (1992) DNA sequencing using capillary array electrophoresis. *Anal. Chem.*, **64** (18), 2149–2154.
39 Wani, I.A. (2014) Nanomaterials, novel preparation routes, and characterizations, in *Nanotechnology Applications for Improvements in Energy Efficiency and Environmental Management* (eds M.A. Shah, M.A. Bhat, and J. Paulo Davim), Research Essentials Collection, Hershey, p. 478.
40 Crego, A.L. and Marina, M.L. (2005) UV-Vis absorbance detection in capillary electrophoresis, in *Analysis and Detection by Capillary Electrophoresis* (eds M.L. Marina, A. Ríos, C.-L. Mancha, and M. Valcárcel), Elsevier Science, Oxford, pp. 225–304.
41 Kobayashi, S., Ueda, T., and Kikumoto, M. (1989) Photodiode array detection in high-performance capillary electrophoresis. *J. Chromatogr.*, **480**, 179–184.
42 Liu, Y., Wang, R., Gao, L., Jia, Z.P., Xie, H., Zhang, J.L., Ma, J., Zhang, A.M., and Xie, X.H. (2011) Capillary electrophoresis detection of lung cancer and adjacent normal tissue differences in protein mixture. *Acta Chim. Sinica*, **69** (5), 543–547.
43 Ye, M., Hu, S., Quigley, W.W., and Dovichi, N.J. (2004) Post-column fluorescence derivatization of proteins and peptides in capillary electrophoresis with a sheath flow reactor and 488 nm argon ion laser excitation. *J. Chromatogr. A*, **1022** (1–2), 201–206.
44 Guttman, A., Cooke, N., and Starr, C.M. (1994) Capillary electrophoresis separation of oligosaccharides. I. Effect of operational variables. *Electrophoresis*, **15** (12), 1518–1522.
45 ElRassi, Z. and Mechref, Y. (1996) Recent advances in capillary electrophoresis of carbohydrates. *Electrophoresis*, **17** (2), 275–301.
46 Guttman, A. (1996) High-resolution carbohydrate profiling by capillary gel electrophoresis. *Nature*, **380** (6573), 461–462.
47 Guttman, A., Chen, F.T., Evangelista, R.A., and Cooke, N. (1996) High-resolution capillary gel electrophoresis of reducing oligosaccharides labeled with 1-aminopyrene-3,6,8-trisulfonate. *Anal. Biochem.*, **233** (2), 234–242.
48 Ulvik, A., Refsum, H., Kluijtmans, L.A., and Ueland, P.M. (1997) C677T mutation of methylenetetrahydrofolate reductase gene determined in blood or plasma by multiple-injection capillary electrophoresis and laser-induced fluorescence detection. *Clin. Chem.*, **43** (2), 267–272.
49 Reyderman, L. and Stavchansky, S. (1996) Determination of single-stranded oligodeoxynucleotides by capillary gel electrophoresis with laser induced fluorescence and on column derivatization. *J. Chromatogr. A*, **755** (2), 271–280.
50 Fasco, M.J., Treanor, C.P., Spivack, S., Figge, H.L., and Kaminsky, L.S. (1995) Quantitative RNA-polymerase chain reaction-DNA analysis by capillary electrophoresis and laser-induced fluorescence. *Anal. Biochem.*, **224** (1), 140–147.
51 Butler, J.M., Wilson, M.R., and Reeder, D.J. (1998) Rapid mitochondrial DNA typing using restriction enzyme digestion of polymerase chain reaction amplicons followed by capillary electrophoresis separation with laser-induced fluorescence detection. *Electrophoresis*, **19** (1), 119–124.
52 Veledo, M.T., Lara-Quintanar, P., de Frutos, M., and Diez-Masa, J.C. (2005) Fluorescence detection in capillary electrophoresis, in *Analysis and Detection*

by Capillary Electrophoresis (eds M.L. Marina, A. Ríos, C.-L. Mancha, and M. Valcárcel), Elsevier Science, Oxford, pp. 305–374.

53 Bruno, A.E., Gassmann, E., Pericles, N., and Anton, K. (1989) On-column capillary flow cell utilizing optical waveguide for chromatographic applications. *Anal. Chem.*, **61**, 876–883.

54 Dovichi, N.J., Martin, J.C., Jett, J.H., and Keller, R.A. (1983) Attogram detection limit for aqueous dye samples by laser-induced fluorescence. *Supramol. Sci.*, **219** (4586), 845–847.

55 Swinney, K. and Bornhop, D.J. (2000) Detection in capillary electrophoresis. *Electrophoresis*, **21** (7), 1239–1250.

56 Malik, A.K. and Faubel, W. (2000) Photothermal and light emitting diodes as detectors for trace detection in capillary electrophoresis. *Chem. Soc. Rev.*, **29** (4), 275–282.

57 Rodat-Boutonnet, A., Naccache, P., Morin, A., Fabre, J., Feurer, B., and Couderc, F. (2012) A comparative study of LED-induced fluorescence and laser-induced fluorescence in SDS-CGE: application to the analysis of antibodies. *Electrophoresis*, **33** (12), 1709–1714.

58 Ruhaak, L.R., Zauner, G., Huhn, C., Bruggink, C., Deelder, A.M., and Wuhrer, M. (2010) Glycan labeling strategies and their use in identification and quantification. *Anal. Bioanal. Chem.*, **397** (8), 3457–3481.

59 Hurth, C., Lenigk, R., and Zenhausern, F. (2008) A compact LED-based module for DNA capillary electrophoresis. *Appl. Phys. B*, **93** (2–3), 693–699.

60 Nakamura, S. and Kaenders, W. (1999) Market-ready blue diodes excite spectroscopists. *Laser Focus World*, **35** (4), 69–75.

61 Karger, B.L. and Guttman, A. (2009) DNA sequencing by CE. *Electrophoresis*, (30 Suppl 1), S196–S202.

62 Kerekgyarto, M., Kerekes, T., Tsai, E., Amirkhanian, V.D., and Guttman, A. (2012) Light-emitting diode induced fluorescence (LED-IF) detection design for a pen-shaped cartridge based single capillary electrophoresis system. *Electrophoresis*, **33** (17), 2752–2758.

63 Pan, T.Y., Wang, C.C., Shih, C.J., Wu, H.F., Chiou, S.S., and Wu, S.M. (2014) Genotyping of intron 22 inversion of factor VIII gene for diagnosis of hemophilia A by inverse-shifting polymerase chain reaction and capillary electrophoresis. *Anal. Bioanal. Chem.*, **406** (22), 5447–5454.

64 Monstein, H.J., Tarnberg, M., Persis, S., and Johansson, A.G. (2014) Comparison of a capillary gel electrophoresis-based multiplex PCR assay and ribosomal intergenic transcribed spacer-2 amplicon sequencing for identification of clinically important Candida species. *J. Microbiol. Methods*, **96**, 81–83.

65 Wang, R., Xie, H., Xu, Y.B., Jia, Z.P., Meng, X.D., Zhang, J.H., Ma, J., Wang, J., and Wang, X.H. (2012) Study on detection of mutation DNA fragment in gastric cancer by restriction endonuclease fingerprinting with capillary electrophoresis. *Biomed. Chromatogr.*, **26** (3), 393–399.

66 Choi, H. and Kim, Y. (2003) Capillary electrophoresis of single-stranded DNA. *B. Kor. Chem. Soc.*, **24** (7), 943–947.

67 Bjorheim, J. and Ekstrom, P.O. (2005) Review of denaturant capillary electrophoresis in DNA variation analysis. *Electrophoresis*, **26** (13), 2520–2530.

68 Xue, M.Z., Bonny, O., Morgenthaler, S., Bochud, M., Mooser, V., Thilly, W.G., Schild, L., and Leong-Morgenthaler, P.M. (2002) Use of constant denaturant capillary electrophoresis of pooled blood samples to identify single-nucleotide polymorphisms in the genes (Scnn1a and Scnn1b) encoding the alpha and beta subunits of the epithelial sodium channel. *Clin. Chem.*, **48** (5), 718–728.

69 Szántai, E., Ronai, Z., Sasvari-Szekely, M., Bonn, G., and Guttman, A. (2006) Multicapillary electrophoresis analysis of single-nucleotide sequence variations in the deoxycytidine kinase gene. *Clin. Chem.*, **52** (9), 1756–1762.

70 Guttman, A. and Cooke, N. (1991) Capillary gel affinity electrophoresis of DNA fragments. *Anal. Chem.*, **63** (18), 2038–2042.

71 Swerdlow, H., Wu, S.L., Harke, H., and Dovichi, N.J. (1990) Capillary gel electrophoresis for DNA sequencing. Laser-induced fluorescence detection with the sheath flow cuvette. *J. Chromatogr.*, **516** (1), 61–67.

72 Kircher, M. and Kelso, J. (2010) High-throughput DNA sequencing – concepts and limitations. *Bioessays*, **32** (6), 524–536.

73 Easley, C.J., Karlinsey, J.M., Bienvenue, J.M., Legendre, L.A., Roper, M.G., Feldman, S.H., Hughes, M.A., Hewlett, E.L., Merkel, T.J., Ferrance, J.P. *et al.* (2006) A fully integrated microfluidic genetic analysis system with sample-in-answer-out capability. *Proc. Natl. Acad. Sci. USA*, **103** (51), 19272–19277.

74 Salieb-Beugelaar, G.B., Dorfman, K.D., van den Berg, A., and Eijkel, J.C. (2009) Electrophoretic separation of DNA in gels and nanostructures. *Lab. Chip*, **9** (17), 2508–2523.

75 Guttman, A. and Nolan, J. (1994) Comparison of the separation of proteins by sodium dodecyl sulfate-slab gel electrophoresis and capillary sodium dodecyl sulfate-gel electrophoresis. *Anal. Biochem.*, **221** (2), 285–289.

76 Hjerten, S. (1983) High-performance electrophoresis: the electrophoretic counterpart of high-performance liquid chromatography. *J. Chromatogr. A*, **270**, 1–6.

77 Cohen, A.S. and Karger, B.L. (1987) High-performance sodium dodecyl sulfate polyacrylamide gel capillary electrophoresis of peptides and proteins. *J. Chromatogr.*, **397**, 409–417.

78 Okada, H., Kaji, N., Tokeshi, M., and Baba, Y. (2008) Poly(methylmethacrylate) microchip electrophoresis of proteins using linear-poly(acrylamide) solutions as separation matrix. *Anal. Sci.*, **24** (3), 321–325.

79 Widhalm, A., Schwer, C., Blaas, D., and Kenndler, E. (1991) Capillary zone electrophoresis with a linear, non-cross-linked polyacrylamide-gel – separation of proteins according to molecular mass. *J. Chromatogr.*, **549** (1–2), 446–451.

80 Han, J. and Singh, A.K. (2004) Rapid protein separations in ultra-short microchannels: microchip sodium dodecyl sulfate-polyacrylamide gel electrophoresis and isoelectric focusing. *J. Chromatogr. A*, **1049** (1–2), 205–209.

81 Hatch, A.V., Herr, A.E., Throckmorton, D.J., Brennan, J.S., and Singh, A.K. (2006) Integrated preconcentration SDS-PAGE of proteins in microchips using photopatterned cross-linked polyacrylamide gels. *Anal. Chem.*, **78** (14), 4976–4984.

82 Lo, C.T., Throckmorton, D.J., Singh, A.K., and Herr, A.E. (2008) Photopolymerized diffusion-defined polyacrylamide gradient gels for on-chip protein sizing. *Lab Chip*, **8** (8), 1273–1279.

83 Verhelst, V., Mollie, J.P., and Campeol, F. (1997) Capillary zone electrophoretic determination of ionic impurities in silicone products used for electronic applications. *J. Chromatogr. A*, **770** (1–2), 337–344.

84 Griebel, A., Rund, S., Schonfeld, F., Dorner, W., Konrad, R., and Hardt, S. (2004) Integrated polymer chip for two-dimensional capillary gel electrophoresis. *Lab Chip*, **4** (1), 18–23.

85 Michels, D.A., Hu, S., Dambrowitz, K.A., Eggertson, M.J., Lauterbach, K., and Dovichi, N.J. (2004) Capillary sieving electrophoresis-micellar electrokinetic chromatography fully automated two-dimensional capillary electrophoresis analysis of *Deinococcus radiodurans* protein homogenate. *Electrophoresis*, **25** (18–19), 3098–3105.

86 Salas-Solano, O., Tomlinson, B., Du, S., Parker, M., Strahan, A., and Ma, S. (2006) Optimization and validation of a quantitative capillary electrophoresis sodium dodecyl sulfate method for quality control and stability monitoring of monoclonal antibodies. *Anal. Chem.*, **78** (18), 6583–6594.

87 Szekely, A., Szekrenyes, A., Kerekgyarto, M., Balogh, A., Kadas, J., Lazar, J., Guttman, A., Kurucz, I., and Takacs, L. (2014) Multicapillary SDS-gel electrophoresis for the analysis of fluorescently labeled mAb preparations: a high throughput quality control process for the production of QuantiPlasma and

PlasmaScan mAb libraries. *Electrophoresis*, **35** (15), 2155–2162.
88 Szekrenyes, A., Roth, U., Kerekgyarto, M., Szekely, A., Kurucz, I., Kowalewski, K., and Guttman, A. (2012) High-throughput analysis of therapeutic and diagnostic monoclonal antibodies by multicapillary SDS gel electrophoresis in conjunction with covalent fluorescent labeling. *Anal. Bioanal. Chem.*, **404** (5), 1485–1494.
89 Tous, G.I., Wei, Z., Feng, J., Bilbulian, S., Bowen, S., Smith, J., Strouse, R., McGeehan, P., Casas-Finet, J., and Schenerman, M.A. (2005) Characterization of a novel modification to monoclonal antibodies: thioether cross-link of heavy and light chains. *Anal. Chem.*, **77** (9), 2675–2682.
90 Lu, J.J., Zhu, Z., Wang, W., and Liu, S. (2011) Coupling sodium dodecyl sulfate-capillary polyacrylamide gel electrophoresis with matrix-assisted laser desorption ionization time-of-flight mass spectrometry via a poly (tetrafluoroethylene) membrane. *Anal. Chem.*, **83** (5), 1784–1790.
91 Haselberg, R., de Jong, G.J., and Somsen, G.W. (2013) CE-MS for the analysis of intact proteins 2010–2012. *Electrophoresis*, **34** (1), 99–112.
92 Dwek, R.A. (1996) Glycobiology: toward understanding the function of sugars. *Chem. Rev.*, **96** (2), 683–720.
93 Lowe, J.B. and Marth, J.D. (2003) A genetic approach to mammalian glycan function. *Annu. Rev. Biochem.*, **72**, 643–691.
94 Guttman, A. (2013) Capillary electrophoresis in the N-glycosylation analysis of biopharmaceuticals. *Trends Anal. Chem.*, **48**, 132–143.
95 Mittermayr, S., Bones, J., and Guttman, A. (2013) Unraveling the glyco-puzzle: glycan structure identification by capillary electrophoresis. *Anal. Chem.*, **85** (9), 4228–4238.
96 Kuo, C.Y., Wang, S.H., Lin, C., Liao, S.K., Hung, W.T., Fang, J.M., and Yang, W.B. (2012) Application of 2,3-naphthalenediamine in labeling natural carbohydrates for capillary electrophoresis. *Molecules*, **17** (6), 7387–7400.
97 Kerékgyártó, M. and Guttman, A. (2014) Toward the generation of an aminonaphthalene trisulfonate labeled N-glycan database for capillary gel electrophoresis analysis of carbohydrates. *Electrophoresis*, **35** (15), 2222–2228.
98 Mittermayr, S., Bones, J., Doherty, M., Guttman, A., and Rudd, P.M. (2011) Multiplexed analytical glycomics: rapid and confident IgG N-glycan structural elucidation. *J. Proteome Res.*, **10** (8), 3820–3829.
99 Haselberg, R., de Jong, G.J., and Somsen, G.W. (2013) Low-flow sheathless capillary electrophoresis-mass spectrometry for sensitive glycoform profiling of intact pharmaceutical proteins. *Anal. Chem.*, **85** (4), 2289–2296.
100 Liu, Y., Salas-Solano, O., and Gennaro, L.A. (2009) Investigation of sample preparation artifacts formed during the enzymatic release of N-linked glycans prior to analysis by capillary electrophoresis. *Anal. Chem.*, **81** (16), 6823–6829.
101 Thakur, D., Rejtar, T., Karger, B.L., Washburn, N.J., Bosques, C.J., Gunay, N.S., Shriver, Z., and Venkataraman, G. (2009) Profiling the glycoforms of the intact alpha subunit of recombinant human chorionic gonadotropin by high-resolution capillary electrophoresis-mass spectrometry. *Anal. Chem.*, **81** (21), 8900–8907.
102 Laroy, W., Contreras, R., and Callewaert, N. (2006) Glycome mapping on DNA sequencing equipment. *Nat. Protoc.*, **1** (1), 397–405.
103 Ruhaak, L.R., Hennig, R., Huhn, C., Borowiak, M., Dolhain, R.J., Deelder, A.M., Rapp, E., and Wuhrer, M. (2010) Optimized workflow for preparation of APTS-labeled N-glycans allowing high-throughput analysis of human plasma glycomes using 48-channel multiplexed CGE-LIF. *J. Proteome Res.*, **9** (12), 6655–6664.
104 Breadmore, M.C. (2012) Capillary and microchip electrophoresis: challenging the common conceptions. *J. Chromatogr. A*, **1221**, 42–55.
105 Effenhauser, C.S., Bruin, G.J., and Paulus, A. (1997) Integrated chip-based capillary

electrophoresis. *Electrophoresis*, **18** (12–13), 2203–2213.

106 Jang, Y.C., Jha, S.K., Chand, R., Islam, K., and Kim, Y.S. (2011) Capillary electrophoresis microchip for direct amperometric detection of DNA fragments. *Electrophoresis*, **32** (8), 913–919.

107 Shi, N. and Ugaz, V.M. (2014) Using microchip gel electrophoresis to probe DNA–drug binding interactions. *Methods Mol. Biol.*, **1094**, 13–24.

108 Meltzer, R.H., Krogmeier, J.R., Kwok, L.W., Allen, R., Crane, B., Griffis, J.W., Knaian, L., Kojanian, N., Malkin, G., Nahas, M.K. *et al.* (2011) A lab-on-chip for biothreat detection using single-molecule DNA mapping. *Lab Chip*, **11** (5), 863–873.

109 Liu, P., Li, X., Greenspoon, S.A., Scherer, J.R., and Mathies, R.A. (2011) Integrated DNA purification, PCR, sample cleanup, and capillary electrophoresis microchip for forensic human identification. *Lab Chip*, **11** (6), 1041–1048.

110 Barta, C., Ronai, Z., Nemoda, Z., Szekely, A., Kovacs, E., Sasvari-Szekely, M., and Guttman, A. (2001) Analysis of dopamine D4 receptor gene polymorphism using microchip electrophoresis. *J. Chromatogr. A*, **924** (1–2), 285–290.

111 Nie, F.Q., Yamada, M., Kobayashi, J., Yamato, M., Kikuchi, A., and Okano, T. (2007) On-chip cell migration assay using microfluidic channels. *Biomaterials*, **28** (27), 4017–4022.

112 Nagata, H., Tabuchi, M., Hirano, K., and Baba, Y. (2005) High-speed separation of proteins by microchip electrophoresis using a polyethylene glycol-coated plastic chip with a sodium dodecyl sulfate-linear polyacrylamide solution. *Electrophoresis*, **26** (14), 2687–2691.

113 Spisak, S., Tulassay, Z., Molnar, B., and Guttman, A. (2007) Protein microchips in biomedicine and biomarker discovery. *Electrophoresis*, **28** (23), 4261–4273.

114 Donczo, B., Kerekgyarto, J., Szurmai, Z., and Guttman, A. (2014) Glycan microarrays: new angles and new strategies. *Analyst*, **139** (11), 2650–2657.

115 Ge, R., Allen, R.W., Aldous, L., Bown, M.R., Doy, N., Hardacre, C., MacInnes, J.M., McHale, G., and Newton, M.I. (2009) Evaluation of a microfluidic device for the electrochemical determination of halide content in ionic liquids. *Anal. Chem.*, **81** (4), 1628–1637.

116 Guttman, A. and Ronai, Z. (2000) Ultrathin-layer gel electrophoresis of biopolymers. *Electrophoresis*, **21** (18), 3952–3964.

117 He, B., Tait, N., and Regnier, F. (1998) Fabrication of nanocolumns for liquid chromatography. *Anal. Chem.*, **70** (18), 3790–3797.

118 Mark, D., Haeberle, S., Roth, G., von Stetten, F., and Zengerle, R. (2010) Microfluidic lab-on-a-chip platforms: requirements, characteristics and applications. *Chem. Soc. Rev.*, **39** (3), 1153–1182.

119 Rech, I., Marangoni, S., Gulinatti, A., Ghioni, M., and Cova, S. (2010) Portable genotyping system: four-colour microchip electrophoresis. *Sensor. Actuat. B*, **143** (2), 583–589.

120 Rhazi, L., Bodard, A.L., Fathollahi, B., and Aussenac, T. (2009) High throughput microchip-based separation and quantitation of high-molecular-weight glutenin subunits. *J. Cereal Sci.*, **49** (2), 272–277.

121 He, X.W., Chen, Q.S., Zhang, Y.D., and Lin, J.M. (2014) Recent advances in microchip-mass spectrometry for biological analysis. *Trends Anal. Chem.*, **53**, 84–97.

122 Rios, A., Zougagh, M., and Avila, M. (2012) Miniaturization through lab-on-a-chip: utopia or reality for routine laboratories? A review. *Anal. Chim. Acta*, **740**, 1–11.

123 Zhang, H., Liu, L., Fu, X., and Zhu, Z. (2013) Microfluidic beads-based immunosensor for sensitive detection of cancer biomarker proteins using multienzyme-nanoparticle amplification and quantum dots labels. *Biosens. Bioelectron.*, **42**, 23–30.

124 Wang, H., Wang, D., Wang, J., Gu, J., Han, C., Jin, Q., Xu, B., He, C., Cao, L., Wang, Y. *et al.* (2009) Application of poly (dimethylsiloxane)/glass microchip for fast electrophoretic separation of serum

small, dense low-density lipoprotein. *J. Chromatogr. A*, **1216** (35), 6343–6347.

125 Kamruzzaman, M., Alam, A.M., Kim, K.M., Lee, S.H., Kim, Y.H., Kabir, A.N., Kim, G.M., and Dang, T.D. (2013) Chemiluminescence microfluidic system of gold nanoparticles enhanced luminol-silver nitrate for the determination of vitamin B12. *Biomed. Microdevices*, **15** (1), 195–202.

126 Han, H., Livingston, E., and Chen, X. (2011) High throughput profiling of charge heterogeneity in antibodies by microchip electrophoresis. *Anal. Chem.*, **83** (21), 8184–8191.

127 Primack, J., Flynn, G.C., and Pan, H. (2011) A high-throughput microchip-based glycan screening assay for antibody cell culture samples. *Electrophoresis*, **32** (10), 1129–1132.

128 Sin, M.L.Y., Gao, J., Liao, J.C., and Wong, P.K. (2011) System integration – a major step toward lab on a chip. *J. Biol. Eng.*, **5** (6), 1–21.