Recent Advances in Trace Bioanalysis by Capillary Electrophoresis

Takayuki KAWAI*,**

*RIKEN Center for Biosystems Dynamics Research, 6-2-4 Furue-dai, Suita, Osaka 565-0874, Japan
**Graduate School of Frontier Biosciences, Osaka University, 1-4 Yamada-oka, Suita, Osaka 565-0871, Japan

Recently, single cell analysis is becoming more and more important to elucidate cellular heterogeneity. Except for nucleic acid that can be amplified by PCR, the required technical level for single cell analysis is extremely high and the appropriate design of sample preparation and a sensitive analytical system is necessary. Capillary/microchip electrophoresis (CE/MCE) can separate biomolecules in nL-scale solution with high resolution, and it is highly compatible with trace samples like a single cell. Coupled with highly sensitive detectors such as laser-induced fluorescence and nano-electrospray ionization-mass spectrometry, zmol level analytes can be detected. For further enhancing sensitivity, online sample preconcentration techniques can be employed. By integrating these high-sensitive techniques, single cell analysis of metabolites, proteins, and lipids have been achieved. This review paper highlights successful research on CE/MCE-based trace bioanalysis in recent 10 years. Firstly, an overview of basic knowledge on CE/MCE including sensitivity enhancement techniques is provided. Applications to trace bioanalysis are then introduced with discussion on current issues and future prospects.

Keywords Capillary electrophoresis (CE), microchip electrophoresis (MCE), single cell analysis, laser-induced fluorescence (LIF), mass spectrometry (MS)

(Received September 3, 2020; Accepted September 29, 2020; Advance Publication Released Online by J-STAGE October 9, 2020)

1 Introduction

In the biological research field, single cell analysis is becoming a new standard. Flow cytometry of fluorescently stained cells is widely employed for visualizing distribution of gene/protein expression among millions of cells.1–3 As DNA and RNA can be easily amplified by PCR and sequenced with a next-generation sequencer, single cell genome and transcriptome analysis is also employed to visualize genetic heterogeneity more comprehensively.4–8 Conversely, other biomolecules such as metabolites, proteins, lipids, and oligosaccharides cannot be basically amplified. Thus, development of a sensitive and lossless analysis system for trace volumes of samples is necessary.

Among various analytical methods, capillary/microchip electrophoresis (CE/MCE) coupled with highly sensitive detectors offers great analytical performance for microscale samples, and has been successfully applied to single cell analysis
even from the early 1990s.9–11 This review article firstly provides an overview of the basic features of CE/MCE that are important for single cell analysis, and then introduces recent (within the last 10 years) application research on trace bioanalysis. Most applications have focused on single cell analysis but a few report on other samples, like laser-microdissected tissue sections. Application research studies are categorized according to the detector and target molecules (metabolites, proteins, and lipids) and introduced one by one.

2 Basic Features of CE/MCE

CE is an electro-driven separation technique using a capillary as the separation field.12–15 A capillary with ID of tens of μm made of fused silica is usually employed for CE, to which DC voltage of tens of kV is applied. Usually, capillary zone electrophoresis (CZE) is employed as the separation mode, where charged analytes are separated based on their size-to-charge ratio. For analytes that cannot be separated with CZE (e.g., uncharged molecules and structural isomers), electrokinetic chromatography (EKC) mode is often utilized.16–19 By adding pseudo-stationary phase (PSP) such as surfactant micelles and/or chiral selectors into the background electrolyte (BGE), analytes can be separated according to the interaction strength with the PSP. Compared with other separation analysis methods like liquid chromatography (LC), CE has advantages in terms of resolution, analysis time, and sample consumption. LC requires a large amount of organic solvents and/or expensive columns, while CE provides advantages in the form of green chemistry and analytical cost.

MCE is another form of CE using a microfluidic device as the separation field.20–23 Although the basic mechanism of electrophoretic separation is the same as CE, MCE usually employs a much shorter microchannel for separation, which can significantly reduce the analysis time to tens of seconds. Compact instrumentation has also been achieved. However, it should be noted that resolution becomes poorer and a complicated multichannel-based injection method is usually required.24 Another advantage of MCE is the connectivity to other microfluidic functions, such as single cell isolation, lysis, chemical labeling, and purification,25–27 which can be integrated to a micro-total analysis system (μTAS) in a single microfluidic device. Of course, system development cost increases according to the complexity of the μTAS design. Depending on the requirements by the sample type, therefore, CE or MCE should be selected.

2.1 Online detection in CE/MCE

For detection, CE/MCE is often coupled with online optical detectors, such as UV absorbance detectors and laser-induced fluorescence (LIF) detectors.28,29 In addition to an optical detection system, CE can also be integrated with a capacitively coupled contactless conductivity detector (C4D).30,31 Such online detectors have great advantages because of the easy, low-cost, and compact instrumentation. However, limits of detection (LODs) of UV and C4D detectors are currently μM level, which is not enough for trace bioanalysis in general.

In contrast, LIF provides great detectability with nM - pM level LOD (equivalent to zmol injection amount). By optimizing the optical configurations, such as laser power/wavelength and emission bandpass filter, sensitivity could be further improved. In the case of DNA analysis, even a single molecule detection was achieved because numerous fluorescent dye molecules are adsorbed into a single DNA molecule.32 In the case of protein analysis, Shi et al. recently achieved LOD of seven antibody molecules by minimizing the background noise produced by Raman scattering (Fig. 1).33 Such great sensitivity is quite suitable for trace bioanalysis and mostly employed as a key detector in many single cell analysis research.

![Illustration of the ultra-sensitive CE-LIF system employed for single cell protein analysis. The cells were stained by BV605 labeled antibody. The fluorescence microscope was adopted to measure the mean fluorescence intensity for selection of experimental conditions. Then, a single cell was drawn into the CE-LIF system for quantification analysis. The evaluated LOD was seven molecules. Reproduced from ref [33] with permission.](image-url)
2-2 Electrochemical detection in CE/MCE
Electrochemical detection (ECD) can be performed on CE/MCE by fabricating a functional electrode around the outlet end of the capillary/microchannel.34-36 Although development of a CE/MCE-ECD system requires delicate fabrication and positioning of the functional electrode,37 it has good sensitivity with nM level LOD and selective detection of the target molecules can be achieved. Several applications to metabolite analysis from single cells have been reported.

2-3 CE/MCE-mass spectrometry
CE/MCE can also be connected to mass spectrometry (MS) usually via electrospray ionization (ESI) platform.38-39 Matrix-assisted laser desorption/ionization (MALDI)-MS can also be coupled, but a fractionation system for spotting separated analytes on a MALDI plate is required.40-41 Technical details in hyphenating CE/MCE and MS are well summarized in other review papers.42,43 Nevertheless, CE/MCE-MS is a quite attractive analytical platform because of its high qualification performance based on the m/z information on the precursor/product ions. Recent high resolution MS technologies, such as orbitrap44 and Fourier transform ion cyclotron resonance (FT-ICR),45 achieve up to sub-ppm m/z resolution, which can easily determine the molecular formula. By searching MS/MS and/or MS*- fragmentation patterns on a worldwide database,46 molecular structure can be further identified.

In CE/MCE-MS, an ESI-MS interface using sheath liquid (organic solvent) has mainly been employed due to its versatile applicability to many biomolecules. As the solvent of BGE is mostly water that is not suitable for stable ESI, the higher sheath flow rate (typically 10 μL/min) provides a more stable ESI signal, but it also decreases sensitivity.47,48 Thus, minimizing the flow rate while keeping the stable ESI is required for trace bioanalysis. Dovichi’s group developed a novel interface for CE-MS using a tapered glass needle (ending OD less than 10 μm, see Fig. 2), and reported extreme sensitivity (up to 1 zmol) at a flow rate of ~20 nL/min in proteome analysis.49 However, analytical robustness seriously decreased due to the tapered structure that is easily clogged by small dust. In order to achieve both sensitivity and robustness, the researchers finally developed a similar emitter but with larger 25 μm-ending ID.50 As for MCE-MS, connecting MCE to MS is much more difficult than CE because the mm-thick substrate of the microchip disturbs the stable formation of capillary in ESI-MS. Among several interface designs,51,52 the approach by Ramsey et al. has been most successful and employs the corner of a rectangular microchip as the emitter end.53 Sheath liquid is supplied from another channel than the separation channel, which are connected near the corner of the microchip.

When sheath liquid is employed, the total flow rate must be increased and sensitivity would be more or less decreased. In order to eliminate sheath liquid and minimize the flow rate, sheathless ESI systems have been developed.54-59 Among them, Moini et al. developed a CE sheathless ionization (CESI) system using a conductive thin-walled emitter, which is now commercially available and most widely employed.42,59 The CESI emitter is fabricated by etching the silica wall around the capillary outlet end with hydrofluoric acid until the thickness reaches 5 - 10 μm, where the silica structure becomes porous and can transmit small ions. Based on the minimized flow rate, CESI provides good sensitivity with LOD up to nM (sub-amol) level.60,61 In the case of MCE-MS, application of sheathless ionization has been limited to only one report by Scholl et al.62 because fabrication of the thin-walled conductive segment by hydrofluoric acid etching is difficult in the microfluidic platform.

2-4 Online sample preconcentration techniques
In order to enhance the sensitivity, online sample preconcentration (OSP) methods have been developed for CE/MCE. A large-volume sample is injected to the capillary, which is then electrokinetically focused into a narrow band inside the capillary by the OSP methods. The focused sample was then separated as with normal CE/MCE analysis. Detailed mechanisms and variations of OSP methods are well summarized in other review papers.63-65 Among the many OSP methods, field-amplified sample stacking (FASS) and transient isotachophoresis (tITP) are widely employed,66-70 allowing for up to hundreds-fold sensitivity improvement can be achieved. Although the benefit of OSP methods is clear, a limited number of papers have reported on single cell analysis, probably because of the disadvantages of OSP, such as complicated procedure, decreased separation performance, and many limitations regarding target molecules, sample preparation methods, and separation conditions.

3 Application to Trace Bioanalysis
3-1 CE/MCE-LIF analysis of metabolites
Small biomolecules and drugs are important targets in trace bioanalysis and CE/MCE-LIF has long been employed.71 Since only fluorescently labeled compounds are detected, the design of an analytical system for selective detection of targeted molecules is most important in CE/MCE-LIF of single cells.
The simplest approach is to detect the endogenous molecules by reaction with fluorescent probe. Xu et al. analyzed superoxide activity from single skeletal muscle fibers, which were cultured in a nL-volume microchamber, lysed, and reacted with a fluorescent probe, triphenylphosphonium hydroethidine (TPP-HE). In proportion to the superoxide activity, TPP-HE was converted to a hydroxyl form, which was separated from unreacted TPP-HE by MEKC in the CE-LIF platform. Li et al. developed a BODIPY-based near-infrared fluorescent probe for Na+ and K+ ions. As shown in Fig. 3, normal and cancer cells were incubated with the probe and introduced into a microchannel one by one, which was lysed, and probe-Na+ and probe-K+ conjugates were separately detected in the MCE-LIF platform. It was clearly shown that cancer cells exhibited much higher Na+/K+ ratio than normal cells, which was considered to induce apoptotic volume decrease. Similarly, probe-based single cell analysis was carried out for nitric oxide, thiols, reduced glutathione, formaldehyde, and ATP.

Drug uptake can also be monitored by CE-LIF in the single cell scale. Deng et al. directly aspirated a single K562 cell into the capillary and quantified permeated doxorubicin, an anthracycline antibiotic. Cellular heterogeneity with up to 10-fold difference in uptake amount was observed. However, no other report on drug uptake research by CE/MCE single cell analysis has been reported, because other drugs basically do not have native strong fluorescence unlike doxorubicin. In such a situation, complicated sample pretreatment, including single cell isolation, lysis, chemical labeling, purification, and injection, is required. At least a few μL of sample solution is prepared, which is much larger than the injection volume of normal CE/MCE, seriously decreasing the sensitivity compared with the whole cell injection method. In order to achieve both highly sensitive detection and flexible sample pretreatment, Patel et al. employed an OSP method, large-volume sample stacking (LVSS) for chiral measurement of D-amino acids. Single neurons were collected from Aplysia californica (a giant sea slug), of which amino acids were fluorescently labeled with naphthalene-2,3-dicarboxaldehyde, purified with reversed phase solid microextraction, and a large-volume sample (~1.2 μL) was introduced into the capillary. LVSS increased the sensitivity by...
up to 480-fold and aspartic acid and glutamic acid were optically resolved by cyclodextrin EKC. This work indicated the importance of OSP methods that could increase the flexibility in the single cell analysis workflow.

3-2 CE/MCE-ECD analysis of metabolites

Although fabrication of functional electrodes is required, ECD has the advantage of selective and label-free detection. Thus, CE/MCE-ECD has long been employed for targeted single cell analysis.8 He et al. reported ascorbic acid analysis from single rat peritoneal mast cells using the CE-ECD platform. A simple three electrode system using a carbon fiber microdisk bundle electrode as a working electrode was employed, where limit of detection was estimated to be 1.7 μM.83 Wang et al. modified the similar carbon fiber electrode with Pt nanoparticles for enhancing the sensitivity.84 LOD was improved to be 0.5 μM and ascorbic acid was successfully analyzed from single HepG2 liver cancer cells.85 They also achieved detection of reduced glutathione from single HepG2 cells by modifying the working electrode with reduced graphene oxide.82 By immobilizing bio-composite of single walled carbon nanotube, glucose oxidase, and glutaraldehyde linker at a Pd nanoparticle-modified Pt electrode, they also achieved 0.5 μM LOD for glucose as well and successfully detected fmol-level glucose in single MGC-80-3 gastric cancer cells.86 Sha et al. developed a MCE-ECD platform by placing a simple carbon fiber nanoelectrode at the end of the microchannel, which successfully detected dopamine from single pheochromocytoma cells.87

3-3 CE/MCE-MS analysis of metabolites

CE/MCE-MS is the ideal platform for non-target metabolomic profiling, if the sensitivity is enough for single cell analysis. Unfortunately, as the LOD of metabolites in CE/MCE-MS is typically nM level, it is often not enough for analyzing single mammalian cells. Thus, big cells are often targeted. 

Sweedler’s group usually selected Aplysia californica as a neuroscience model animal that has simple neuronal network. Metabolites and peptides were intensively analyzed from single neurons using the CE-MS platform in order to elucidate functions of individual cells.88–92 For improving the ESI efficiency, the researchers employed a 260/130 μm OD/ID metal needle as the ESI emitter, through which 105/40 μm OD/ID fused silica capillary tubing was placed for separation. They also investigated coupling of the single cell CE-MS system with other microscale analysis techniques such as patch clamp and MALDI-MS imaging.93,94 Sampling and CE-MS analysis of a single islet of rat pancreas was successfully performed from the tissue slice, which was firstly utilized for the MALDI-MS imaging.95 It was because MALDI-MS only sacrificed the surface molecules and most of the molecular profiles were well conserved. Combination of these single cell resolution methods is very important to elucidate complicated cellular functions from different analytical aspects.

Recently, Nemes et al. started an analysis of Xenopus laevis embryo.96–100 They isolated each single cell of a 16-cell embryo, and carried out CE-MS metabolome analysis with the same CE-ESI-MS interface as Sweedler’s group. Characteristic small-molecular-activity was found in three blastomeres, which seemed to determine the cell fate and induce the development to neuronal, epidermal, and hindgut tissues.100,101 They also combined the single cell CE-MS techniques with microneedle-based small-volume sampling, which allowed metabolomic monitoring of live embryos without stopping the developmental process. The main reason why such in situ CE-MS analysis could be achieved was the huge size (hundreds of μm in diameter) of the target cells. However, such clever selection of model single cells and analytical application beautifully proved the importance of metabolites for the complicated developmental process.

Liu’s groups reported an MCE-MS platform for single cell analysis.102,103 Intracellular levels of dopamine and glutamic acid were successfully quantified for individual PC-12 neuronal cells by integrating MCE-MS with double nano-electrodes for the efficient cell lysis.103 Kawai et al. reported metabolome analysis of single HeLa cells for the first time by maximizing the CE-MS sensitivity by two techniques.104 Firstly, a novel thin-walled tapered emitter named nanoCESI was developed to improve the sensitivity by up to 800-fold in total.105 Ultra-sensitive sub-pM LOD was achieved for amino acids and around 40 metabolites were identified from single mammalian cells for the first time.

3-4 CE-LIF analysis of proteins

As introduced in the metabolites analysis section, CE/MCE-LIF is highly sensitive and has long been employed for single cell protein analysis as well.9 As applications of MCE-LIF were
all carried out for western blotting analysis, they are separately introduced in the next section. As a selective fluorescent probe for labeling proteins, Liu’s group developed activity-based trimodular probes (ABP) which were synthesized by a method based on “click” chemistry.\(^{106,107}\) Their first ABP molecule consists of three segments, binding structure to GB1 subunit of GABA receptor, a photolabile diazirine group, and a fluorescent BODIPY tag.\(^{106}\) As shown in Fig. 5, after incubating single cells with ABP, they were irradiated with UV so that the fluorescent ABP molecule could covalently bind to the receptor proteins. The obtained fluorescent conjugates were then analyzed by CE-LIF. By selectively labeling receptor proteins to a specific chemical structure, function-driven single cell chemical proteomics could be carried out. By controlling the membrane permeability, the researchers also achieved lysosome-targeted labeling.\(^{107}\) Shi et al. reported a single cell immunoassay by developing an ultra-sensitive CE-LIF system (LOD = seven molecules) which eliminated the background signal caused by Raman scattering.\(^{33}\) They labeled single Jurkat cells with BV605-stained antibody to quantify a low copy number (less than 1000) of caspase3 proteins. Allbritton’s group developed another probe-based approach for indirectly evaluating enzymatic activity from single cells.\(^{108-113}\) They microinjected fluorescently labeled phosphorylated peptides into single A431 epidermoid carcinoma cells, which were lysed after 60 s incubation and analyzed by CE-LIF for quantifying the dephosphorylation activity of protein tyrosine phosphatases.\(^{113}\)

3·5 MCE-LIF for single cell western blotting

Western blotting is a very powerful quantification tool in targeted protein analysis and miniaturization has been investigated for single cell analysis.\(^{114-121}\) Most successful examples were reported by Herr’s group. An open-microfluidic device made from polyacrylamide gel decorated with benzophenone was developed, which is like an integrated miniaturized SDS-PAGE gel. As shown in Fig. 6, single cells were introduced into the fabricated microchambers, lysed, and were finally separated by MCE. The separated proteins inside the microgel device were immobilized by UV irradiation via the benzophenone group, followed by diffusion-based staining with fluorescently labeled antibody. Single-cell differentiation of rat neural stem cells and responses to mitogen stimulation were successfully detected. Amazing analytical performance of multiplexed analysis of 11 proteins per single cell, LOD of 30000 molecules, and throughput of 2000 cells per 4 h were achieved.\(^{118}\) This system is now commercially available and the single cell western blotting technique has become more popular in the biological research field.
3.6 CE/MCE-MS analysis of proteins

MS is a very powerful tool in proteome research. LC-MS-based shotgun proteomics is predominant, but CE-MS has also attracted attention as a complementary proteomic analysis tool to LC-MS. Single cell protein analysis with CE-MS has only been carried out by Nemes’s group using *Xenopus* and zebrafish embryos. As with metabolites analysis, for example, single cells were isolated from a 16-cell *Xenopus* embryo, proteins were lysed and digested with trypsin, and prepared peptides were analyzed by CE-MS (Fig. 7). Significant technical improvement was not observed in these applications, however, proteomic heterogeneity of single cells in the developmental stage was clearly shown for the first time and the biological impact of these research studies was very high.

The MCE-MS platform was employed for protein analysis by Ramsey’s group. By cleverly regulating the microflow in the same design of the microchip as reported in 2008, they introduced single red blood cells into the separation channel with lysis buffer. After the cell lysis, proteins were separated and detected with ESI-MS. They achieved detection of α and β subunits of hemoglobin with high throughput (12 cells/min). Although the next most abundant protein carbonic anhydrase I (∼7 amol/cell) could not be detected, this work indicated the potential of MCE-MS for the top-down proteomics in the single cell scale.

As *in vivo* trace bioanalysis, CE-MS proteome analysis has also been applied to tissue microsections collected via the microdissection process. For example, Ligat et al. collected cancer cells from early and late stages of pancreatic cancer tissue slices with a laser microdissection system, which were analyzed by CE-MS after tryptic digestion for shotgun proteomic profiling. Several biomarkers were detected from early stage cancer cells and it would lead to a new blood diagnosis tool for detecting early stage pancreas cancer. It should be noted that laser microdissection usually requires staining to clearly visualize the tissue microstructures, which would flush out most small molecules. Thus, application of microdissection-based sampling has so far been limited to protein and gene analysis.

3.7 Single cell lipid analysis

Lipids have important functions in cell proliferation, differentiation, migration, and programmed cell death, and are important targets in bioanalysis. Although CE/MCE platform has been expected to achieve single cell lipid analysis, no paper has reported detection of endogenous cellular lipids. Instead, lipid metabolism has been analyzed in the single cell scale by injecting fluorescently labeled artificial probes. Dovichi’s group labeled two different types of glycosphingolipids with TMR and BODIPY fluorophores and injected them into rat cerebella neurons. After incubation, each single neuron was analyzed with CE-LIF to detect catabolized lipids. Different glycosylation patterns were clearly observed for the two glycosphingolipids simultaneously. For further progress of single cell lipid analysis, effective sample preparation method including fluorescent labeling with minimal sample loss by surface adsorption is strongly required.

4 Conclusions

CE/MCE-based trace bioanalysis of metabolites, proteins, and lipids has been successfully carried out. The biological implications of cellular heterogeneity are being gradually elucidated by these cutting-edge technologies, which have attracted attention in the biological research field. However, highly variable methodologies have been developed with different types of detectors, samples, and probes, indicating the trace bioanalysis system is still far from standardization. For example, most analyses have been carried out with a manual sample injection process and cannot be automated. Poor flexibility in sample pretreatment is also a serious issue. When a single cell is prepared with the conventional pretreatment process, analytes are diluted, contaminated, and lost via surface adsorption, hindering application of CE/MCE to trace bioanalysis. OSP methods can address the dilution issue by allowing large-volume sample injection, but actual application examples are still limited. As single cell glycome analysis has never been achieved, CE/MCE is expected to contribute to advancements in this area. It is also expected that more applications will be carried out with *in vivo* samples.
collected with the microdissection technique. Especially, a sampling method without staining is required for analyzing small molecules. CE/MCE-based trace bioanalysis is still on the road to maturation, but it has tremendous potential for not only radically promoting advancements in basic biological research, but also in medical and pharmaceutical research and development.

5 Acknowledgements

This work was partly supported by the AMED Project for utilizing glycans in the development of innovative drug discovery technologies and the Japan Society for the Promotion of Science KAKENHI (16K14930, 19H02567, and 19H05545).

6 References

1. A. Adan, G. Alizada, Y. Kiraz, Y. Baran, and A. Nalbant, Crit. Rev. Biotechnol., 2017, 37, 163.
2. D. Aebisher, D. Bartusik, and J. Tabarkiewicz, Biomed. Pharmacother., 2017, 85, 434.
3. Y. Gong, N. Fan, Y. Yang, B. Peng, and H. Jiang, Electrophoresis, 2019, 40, 1212.
4. A. Yasen, A. Aini, W. Li, H. Wang, B. Ran, T. Tuxun, Y. Maimaitimijiati, Y. Shao, T. Aji, and H. Wen, Infect. Genet. Evol., 2020, 104198.
5. W. Chen, S. Li, A. S. Kulkarni, L. Huang, J. Cao, K. Qian, and J. Wu, Biotechnol. J., 2020, 15, 1900262.
6. C. Zhu, M. Yu, H. Huang, J. Juric, A. Abnousi, R. Hu, J. Lucero, M. M. Behrens, M. Hu, and B. Ren, Nat. Struct. Mol. Biol., 2019, 26, 1063.
7. A. Kulkarni, A. G. Anderson, D. P. Merullo, and G. Konopka, Curr. Opin. Biotechnol., 2019, 58, 129.
8. J. G. Camp, R. Platt, and B. Treutlein, Science, 2019, 365, 1401.
9. T. T. Lee and E. S. Yeung, Anal. Chem., 1992, 64, 3045.
10. H. K. Kristensen, Y. Y. Lau, and A. G. Ewing, J. Neurosci. Methods, 1994, 51, 183.
11. J. A. Jankowski, S. Tracht, and J. V. Sweedler, TrAC, Trends Anal. Chem., 1995, 14, 170.
12. C. A. Monnig and R. T. Kennedy, Anal. Chem., 1994, 66, 2808R.
13. J. W. Jorgenson and K. D. Lukacs, Anal. Chem., 1981, 53, 1298.
14. J. S. Aulakh, R. Kaur, and A. K. Malik, in "Capillary Electrophoresis: Methods and Protocols" ed. P. Schmitt-Kopplin, 2016, Springer, NY, 197.
15. H. H. See and N. A. Ali, in "Encyclopedia of Analytical Science", ed. P. Worsfold, C. Poole, A. Townsend, and M. Miró, 3rd ed., 2019, Academic Press, Oxford, 328.
16. S. Terabe, K. Otsuka, and T. Ando, Anal. Chem., 1985, 57, 834.
17. S. Terabe, K. Otsuka, K. Ishikawa, A. Tsuchiya, and T. Ando, Anal. Chem., 1984, 56, 111.
18. M. E. Swartz, J. R. Marzoe, E. R. Grover, and P. R. Brown, Anal. Biochem., 1995, 231, 65.
19. S. Terabe, M. Shibata, and Y. Miyashita, J. Chromatogr. A, 1989, 480, 403.
20. S. C. Jacobson, R. Hergenroder, L. B. Koutny, and J. M. Ramsey, Anal. Chem., 1994, 66, 1114.
21. S. C. Jacobson, L. B. Koutny, R. Hergenroder, A. W. Moore, and J. M. Ramsey, Anal. Chem., 1994, 66, 3472.
22. S. C. Jacobson, R. Hergenroder, A. W. Moore, Jr., and J. M. Ramsey, Anal. Chem., 1994, 66, 4127.
23. B. Ebru, Curr. Pharm. Anal., 2019, 15, 109.
24. M. Zhang, S. C. Phung, P. Smejkal, R. M. Gujtt, and M. C. Breadmore, Trends Environ. Anal. Chem., 2018, 18, 1.
25. Á. Ríos, Á. Ríos, M. Zougagh, and M. Zougagh, TrAC, Trends Anal. Chem., 2013, 43, 174.
26. L. Ferey and N. Deluainay, Sep. Purif. Rev., 2016, 45, 193.
27. H. Cong, X. Xu, B. Yu, H. Yuan, Q. Peng, and C. Tian, J. Micromech. Microeng., 2015, 25, 053001.
28. E. Gassmann, J. E. Kuo, and R. N. Zare, Science, 1985, 230, 813.
29. E. Ban and E. J. Song, J. Chromatogr. B, 2013, 929, 180.
30. A. J. Zemann, E. Schnell, D. Volgger, and G. K. Bonn, Anal. Chem., 1998, 70, 563.
31. A. A. Elbashir and H. Y. Aboul-Enine, Biomed. Chromatogr., 2014, 28, 1502.
32. T. Kaneta, Chem. Rec., 2019, 19, 452.
33. M. Shi, X. Geng, C. Wang, and Y. Guan, Anal. Chem., 2019, 91, 11493.
34. J. J. P. Mark, R. Scholz, and F.-M. Matysik, J. Chromatogr. A, 2012, 1267, 45.
35. Y. Du and E. Wang, J. Sep. Sci., 2007, 30, 875.
36. H. Yu, X. Xu, J. Sun, and T. You, Cent. Eur. J. Chem., 2012, 10, 639.
37. L. García-Carmona, A. Martín, T. Sierra, M. C. González, and A. Escarpa, Electrophoresis, 2017, 38, 80.
38. A. Týová, V. Ledvina, and K. Klepářík, Electrophoresis, 2017, 38, 115.
39. G. Bonvin, J. Schappler, and S. Rudaz, J. Chromatogr. A, 2012, 1267, 17.
40. H. K. Musyimi, D. A. Narcisse, X. Zhang, W. Strijewski, S. A. Soper, and K. K. Murray, Anal. Chem., 2004, 76, 5968.
41. A. Amini, S. J. Dormady, L. Riggs, and F. E. Regnier, J. Chromatogr. A, 2000, 894, 345.
42. W. Zhang, T. Hankemeier, and R. Ramautar, Curr. Opin. Biotechnol., 2017, 43, 1.
43. A. Stolz, K. Joos, O. Höcker, J. Römer, J. Schlecht, and C. Neussiß, Electrophoresis, 2019, 40, 79.
44. R. H. Perry, R. G. Cooks, and R. J. Noll, Mass Spectrom. Rev., 2008, 27, 661.
45. E. N. Nikolaev, Y. I. Kostyukevich, and G. N. Vladimirov, Mass Spectrom. Rev., 2016, 35, 219.
46. S. Na and E. Pack, Mass Spectrom. Rev., 2015, 34, 133.
47. C. C. Liu, J. Zhang, and N. J. Duchivi, Rapid Commun. Mass Spectrom., 2005, 19, 187.
48. A. El-Faramawy, K. W. M. Sio, and B. A. Thomson, J. Am. Soc. Mass Spectrom., 2005, 16, 1702.
49. L. Sun, G. Zhu, Y. Zhao, X. Yan, S. Mou, and N. J. Duchivi, Angew. Chem. Int. Ed., 2013, 52, 13661.
50. L. Sun, G. Zhu, Z. Zhang, S. Mou, and N. J. Duchivi, J. Proteome Res., 2015, 14, 2321.
51. X. Feng, B.-F. Liu, J. Li, and X. Liu, Mass Spectrom. Rev., 2015, 34, 535.
52. F. Kitagawa and K. Otsuka, J. Pharm. Biomed. Anal., 2011, 53, 688.
53. J. S. Mellors, V. Gorbounov, R. S. Ramsey, and J. M. Ramsey, Anal. Chem., 2008, 80, 6881.
54. J. H. Wahl, D. C. Gale, and R. D. Smith, J. Chromatogr A, 1994, 659, 217.
55. G. A. Valaskovic and F. W. Mclafferty, J. Am. Soc. Mass Spectrom., 1996, 7, 1270.
56. G. A. Valaskovic and F. W. Mclafferty, Rapid Commun. Mass Spectrom., 1996, 10, 825.
57. P. Cao and M. Moini, J. Am. Soc. Mass Spectrom., 1998, 9,
Nemes, Anal. Chem., 2019, 91, 4797.

123. C. Lombard-Banek, S. Reddy, S. A. Moody, and P. Nemes, Mol. Cell. Proteomics, 2016, 15, 2756.

124. C. Lombard-Banek, S. A. Moody, and P. Nemes, Angew. Chem. Int. Ed., 2016, 55, 2454.

125. C. Lombard-Banek, S. A. Moody, and P. Nemes, Front. Cell. Dev. Biol., 2016, 4.

126. J. S. Mellors, K. Jorabchi, L. M. Smith, and J. M. Ramsey, Anal. Chem., 2010, 82, 967.

127. L. Ligat, N. Saint-Laurent, A. El-Mrani, V. Gigoux, T. Al Saati, R. Tomasini, J. Nigri, S. Dejean, F. Pont, R. Baer, J. Guillermet-Guibert, P. Cordelier, F. Lopez, and M. Dufresne, Br. J. Cancer, 2015, 113, 1590.

128. X. Fang, C. Wang, B. M. Balgley, K. Zhao, W. Wang, F. He, R. J. Weil, and C. S. Lee, J. Proteome Res., 2012, 11, 3937.

129. Y. A. Hannun and L. M. Obeid, Nat. Rev. Mol. Cell Biol., 2018, 19, 175.

130. A. Proctor and N. L. Allbritton, Analyst, 2019, 144, 961.

131. A. Proctor, C. E. Sims, and N. L. Allbritton, J. Chromatogr. A, 2017, 1523, 97.

132. A. J. Dickinson, S. A. Hunsucker, P. M. Armistead, and N. L. Allbritton, Anal. Bioanal. Chem., 2014, 406, 7027.

133. K. Wang, D. Jiang, C. E. Sims, and N. L. Allbritton, J. Chromatogr. B, 2012, 907, 79.

134. R. B. Keithley, M. P. Metzinger, A. M. Rosado, and N. J. Dovichi, Talanta, 2013, 111, 206.

135. D. C. Essaka, J. Prendergast, R. B. Keithley, M. M. Palcic, O. Hindsgaul, R. L. Schnaar, and N. J. Dovichi, Anal. Chem., 2012, 84, 2799.

136. D. C. Essaka, J. Prendergast, R. B. Keithley, O. Hindsgaul, M. M. Palcic, R. L. Schnaar, and N. J. Dovichi, Neurochem. Res., 2012, 37, 1308.