Focusing Review

Recent Studies on Online Sample Preconcentration Methods in Capillary Electrophoresis Coupled with Mass Spectrometry

Takayuki KAWAI*1,2,3

1Quantitative Biology Center, RIKEN, Biosystem Bld. E104, 1-3 Yamadaoka, Suita 565-0871, Japan
2Japan Science and Technology Agency, PRESTO, 4-1-8 Honcho, Kawaguchi 332-0012, Japan
3Graduate School of Frontier Biosciences, Osaka University, 1-4 Yamadaoka, Suita 565-0871, Japan

Abstract
This paper reviews recent 5-years application researches using online sample preconcentration (OSP) methods in capillary electrophoresis (CE) coupled with mass spectrometry (MS). CE-MS has excellent analytical features such as high-resolution separation, rapid analysis time, low sample consumption, and small consumption of organic solvents. Recently, low flow-rate interface for electrospray ionization has been developed and zmol-level sensitivity has been achieved. However, application of CE-MS has still been prevented due to small capacity of sample injection volume. OSP methods have been developed to address this issue and up to 5,000-fold sensitivity improvement was obtained so far. Various application has been carried out such as proteomics, metabolomics, and glycomics, and CE-MS attracts much attention as the highest-performance analytical tool for small-volume bioanalysis. Taking account of further progress in CE-MS, smart and practical application of OSP methods will be more important in various analytical fields.

Keywords: Capillary electrophoresis; Mass spectrometry; Online sample preconcentration

1. Introduction
Capillary electrophoresis (CE) is a high-performance separation technique using a capillary as a separation field [1,2]. Typically, fused silica capillary with several tens μm ID is employed and few tens kV of DC voltage is applied for separation of charged molecules. CE has several advantages such as high resolution, short analysis time, and small sample consumption, compared with liquid chromatography (LC) which is generally employed for many separation analyses. Since LC consumes large amount of organic solvents and expensive columns, CE also takes advantages in green chemistry and analytical cost.

Normally, CE employs an optical or other contactless detector such as UV absorbance, laser-induced fluorescence (LIF) [3,4], and capacitively coupled contactless conductivity (C4D) [5,6], not to interfere the electrophoretic separation. On the other hand, mass spectrometry (MS) is quite attractive because of its greater qualification performance providing m/z information. Recent MS technology such as orbitrap [7] and FT-ICR [8] achieves few to sub ppm m/z resolution and we can easily determine the composition formula nowadays. By processing MS/MS or MSn fragmentation results with worldwide database [9], further more comprehensive molecular identification is available. In spite of the great utility, however, hyphenation of CE and MS has been a challenging issue [10,11]. In general, both ends of the capillary must be immersed in background electrolyte (BGE) to conduct high voltage to the capillary, however, the outlet end must be exposed to for MS analysis.

The most employed approach is tandem hyphenation of CE and MS via electrospray ionization (ESI). Typical “sheath type” CE-ESI-MS interface is shown in Fig. 1, which is developed by Agilent Technologies (previously, Hewlett Packard). In this interface, sheath liquid is added to the capillary outlet at a flow rate of typically 1 to 10 μL/min.

*Corresponding author: Takayuki KAWAI
Tel: +81-6-6105-5132; Fax: +81-6-6105-5132
E-mail: takayuki.kawai@riken.jp

Received: 1 January 2017
Accepted: 28 January 2017
J-STAGE Advance Published: 4 February 2017
DOI: 10.15583/jpchrom.2017.001
Pneumatic assistance for stable spray formation is also carried out with nebulizing gas. Recently, miniaturization of this interface attracts much attention [12]. Higher ionization efficiency is obtained as the flow rate at the ESI emitter becomes low [13]. Dovichi’s group has developed a novel CE-MS interface with a tapered glass tip (ending OD less than 10 μm), and reports extreme sensitivity up to 1 zmol at a flow rate of ~20 nL/min in proteome analysis [14]. It would be noteworthy that analytical robustness of tapered structure often decreased in exchange for the high sensitivity, because the smaller ending ID than capillary ID often captures a dust and is easily clogged. Recently, they publish a third generation emitter using a 25 μm-ending ID emitter with a 20 μm-ID capillary to achieve both sensitivity and robustness [15]. This kind of efficient ESI using a small μm-scale emitter and nL/min order flow rate is called "nanoESI" and has been a main stream both in CE-MS and LC-MS, especially in the field of omics bioanalysis.

In the “sheath type” interface, however, the total flow rate must be increased by the much faster sheath flow than that inside the capillary, which often causes less efficient ionization. To address this issue, “sheathless type” interfaces have been developed. For conducting high voltage to the capillary outlet, a metal tip, in-capillary electrode, or metal/carbon-deposited capillary were employed [16-19]. These approaches were successful but usually lose robustness because BGE composition is easily changed by electrolysis and affect the separation manner. Bubble formation by the electrolysis is also a serious issue.

Recent approach using a “porous section” is interesting approach [20,21]. Porous section was fabricated by etching the silica wall with hydrofluoric acid to 5-10 μm, where the silica structure becomes porous and can transmit small ions. This thin wall also decreases the OD of the capillary end and contributes to efficient ESI. Because effect of electrolysis can be minimized due to the isolated electrode from the electrolyte in the capillary, efficient and stable separation is available with this interface. This sheathless interface is now called “CESI” and commercially available from Sciex (CESI 8000 Plus, see Fig. 2) and employed for many applications in various fields.

By employing “sheath” or “sheathless” types of CE-MS, other types of ionization modes can be employed such as atmospheric pressure chemical ionization (APCI) [22], atmospheric pressure photoionization (APPI) [23,24], and inductively coupled plasma (ICP) [25-27]. Oppositely, non-liquid ionization like MALDI must be carried out with non-tandem approach: separated samples are fractionated on a metal plate and ionized away from the capillary [28-30].

Electrophoretic separation is carried out to avoid ion suppression and to increase the qualification/quantitation performance. Because non-volatile compounds should not be introduced into MS not to damage the instrument, volatile chemicals are basically employed in CE-MS. Due to this limitation to additives, thus, interaction-based separation modes in CE such as electrokinetic chromatography (EKC), capillary gel electrophoresis (CGE), and affinity CE (ACE) are seriously limited. Basically, capillary zone electrophoresis (CZE) is employed with volatile buffers consisting of formate, acetate, carbonate, and/or ammonium ions. To obtain EKC separation, off-line coupling with MALDI, partial filling approach, coupling with APCI have been also employed [31], but this kind of analysis is unusual due to the limited analytical performance and serious damage-risk to the expensive MS instrument.

As introduced, many types of CE-MS analyses are available with high resolution and rapid analysis time, but...
concentration sensitivity tends to be poor due to the small capacity of the injection volume. To increase the loadable sample volume, online sample preconcentration (OSP) methods have been developed [32]. OSP methods are usually based on reduction of sample migration velocity. General mechanism is shown in Fig. 3. Firstly, large-volume (long plug) sample is introduced to a capillary, where sample matrix (SM) has different components such as pH, conductivity, and/or pseudostationary phase. After voltage application, secondly, fast migrating analytes in SM reduce their velocity after penetrating BGE, so that they accumulate on the SM/BGE boundary. After this accumulation, or preconcentration, the analytes are separated in the BGE with designated separation mode. So far, hundreds of papers have been published with up to 100,000-fold sensitivity increase, however, most research has been carried out in normal CE without MS. Since CE-MS has several restrictions introduced in the previous sections, application of OSP methods to CE-MS must be carried out carefully to obtain effective analytical performance. In this review paper, recent application studies of OSP methods in CE-MS from 2011 are introduced to support better understanding and readers’ future application of OSP methods to CE-MS.

2. Field-amplified sample stacking and related techniques

One of the most basic OSP methods in CE is field-amplified sample stacking (FASS), which is employed for charged analytes [33,34]. In FASS, analytes are dissolved in lower conductivity (σ) SM and injected as a long plug between BGE zones with higher σ in the capillary. After voltage is applied, the electric field strength (E) in the SM (E_{SM}) is amplified compared to E_{BGE} according to the different σ. Hence, the analytes electrophoretically migrate faster in the SM. After penetrating into the BGE with the reduced E, the velocity of the analyte becomes so slow to be accumulated around the SM/BGE boundary, or concentrated. After the sample concentration, the focused analytes are separated by CZE in the BGE. As only tens-fold sensitivity increase is usually available due to limited injection volume, similar techniques such as field-amplified sample injection (FASI) and large-volume sample stacking (LVSS) have been developed to increase the loadable volume [35-40].

As FASS can be effective just by using a low σ sample, it has been mostly employed among all the OSP methods in CE-MS. One of the most popular applications is proteome analysis [41-44]. Dovichi’s group published several papers on FASS-CE-nanoESI-MS for high performance bottom-up proteome analysis [41,42]. They identify 167 proteins from 1 ng E. coli proteins and 100 phosphorylated proteins from only 2 ng proteins from MCF-10A cell line. Li et al. reports high-sensitive top-down proteomics [44] using fractionation by gel-eluted liquid fraction entrapment electrophoresis (GELFrEE) and analysis by FASS-CE-nanoESI-MS. They identified 30 proteins in the mass range between 30-80 kDa from Pseudomonas aeruginosa. Metabolome analysis is also important application [45-49]. Moreno-Gonzalez et al. reports FASS-CE-ESI-MS of nine aminoglycosides in honey and 5-fold sensitivity increase was achieved [47]. Beutner et al. reports two dimensional separation of ion chromatography (IC) and CE coupled with ESI-MS [48]. Since unnecessary salts are removed by the suppressor in IC, hyphenation of IC and CE via FASS is quite an effective approach. Ito et al. employed FASS-CESI-MS to structurally characterize four kinds of pyridylaminated oligosaccharide from neutral glycosphingolipids [50]. They achieved totally 20,000-fold sensitivity increase including 125-fold improvement by FASS compared with conventional CE-MS approach. FASS was also employed for peptide analysis [51] and environmental analysis [52] where up to 330-fold sensitivity increase was achieved.

FASS-related techniques such as FASI and LVSS has also been applied to CE-MS [53-61]. Ye et al. reports combination of reverse-phase solid-phase extraction (SPE) and FASI-CE-ESI-MS for brain-gut peptides [53]. Chen et al. also reports coupling of SPE with FASI-CE-ICP-MS for toxic mercury and its methyl and ethyl forms [55]. As with two-dimensional separation by IC-CE, SPE effectively removes unnecessary salts so that efficient hyphenation with FASI was achieved with up to 100-fold sensitivity increase. Wang et al. developed novel interface system for counter-flow-assisted FASI as shown in Fig. 4 [58]. By fabricating a PDMS-made cross channel, they supply both sheath liquid and counter-flow to the separation capillary. To enhance the preconcentration factor, furthermore, dynamic pH junction (introduced in the next chapter) was also applied. With this smart microchip, they achieved up to 1,500-fold sensitivity increase in peptide analysis. One of the
significant benefits of FASS-related techniques is its compatibility with other OSP methods because FASS only requires low σ condition for preconcentration. Reza et al. reports FASI-CE-MALDI-MS for high-sensitive protein analysis. They achieved great limit of detection (LOD) of 5-10 nM with FASI-based preconcentration by up to 4800-fold. It should be noted that FASI can introduce extremely large-volume sample from the inlet reservoir and provide much higher sensitivity increase compared to normal FASS.

As with FASS, Sweedler’s group applied LVSS for the analysis of endogenous nucleotides [61]. Up to 200-fold sensitivity increase was obtained and even a single cell analysis was achieved. Unlike FASI by electrokinetic injection, LVSS is carried out by pressure injection and usually does not accompany discrimination of injected sample amount, which provides further more reliable quantitation than that by FASI.

3. Dynamic pH junction

Dynamic pH junction (PHJ) developed by Britz-McKibbin and Chen is a sample stacking technique based on the mobility change by pH shift [62]. In PHJ, SM/BGE with low/high pH are usually employed, respectively, mainly for the analysis of amphoteric analytes. After the voltage application, the analytes migrating toward cathode penetrate into high pH zone, where they acquire negative charge by the pH increase to start migration toward the anode. Hence, the analytes are swept by the dynamically moving pH junction so that the sample band is sharpened. After the concentration, they are separated by CZE in the high pH BGE.

According to the preference of PHJ, proteins, peptides, and amino acids are mostly analyzed which have moderate pKa or pl values [63-73]. As with FASS, Dovichi’s group intensively reports ultra-sensitive proteome analysis by PHJ-CZE-nanoESI-MS [63-67]. Final version of their development is hyphenated system consisting of online tryptic digestion reactor, strong cation exchange (SCX) microcolumn, and PHJ-CZE-nanoESI-MS [64]. The introduced proteins are digested on the micro-reactor, captured and fractionated by SCX, and finally concentrated/separated/detected by CE-MS (Fig. 5). By using this great set-up, they achieved Nearly 1000 protein identifications from 50 ng of Xenopus laevis, which is truly most sensitive proteome analysis system in the world. Rodrigues et al. reported PHJ-CE-ESI-MS for 27 amino acids and achieved six-fold sensitivity increase [69]. Metabolome analysis is also performed via PHJ-CE-MS because migration rate of organic acids and amines can be regulated by pH change [74-76]. Yu et al. reports PHJ-CZE-ESI-MS of 13 organic acids and 20 amino acids from urine, and up to seven-fold sensitivity increase was obtained [74]. Kohler et al. also reported sensitive drug analysis from urine via PHJ-CE-ESI-MS, but similarly limited sensitivity improvement less than 10-fold was obtained [77]. This is because each analyte has different condition for optimized preconcentration according to their pKa or pl values, and there is usually no good comparable condition in.
case analyzing many analytes. This is why full focusing capacity by up to hundreds-fold is not available in analyzing metabolites, especially amino acids. Oppositely in proteome analysis, good enrichment was achieved, because SCX pretreatment can fractionate samples with similar $pK_a$ values, which is quite compatible with PHJ. Therefore, smart approach for sample pretreatment is mostly important in practical application of PHJ.

4. Transient isotachophoresis and normal isotachophoresis

Isotachophoresis (ITP) is not only a separation mode in CE but also an OSP method [78]. A brief explanation of ITP is provided here in a case of anion analysis. Sample is injected between leading/terminal electrolytes (LE/TE) containing anionic components with larger/smaller electrophoretic mobility than those of the analytes, respectively. After voltage application, each analyte migrates with its unique velocity to be separated into spatially continuous zones between LE and TE, in the order of faster electrophoretic migration. The electric field strength in each zone is automatically changed according to Kohlrusch regulating function [79] so that the migration rate of each species becomes identical to keep the electric neutrality of each zone. Since the electric field strength is determined only by the electrophoretic mobility of the analyte, the bandwidth of each zone is automatically changed to a certain sample concentration with determined electric field strength. The lower the original sample concentration is, therefore, the relatively higher concentration efficiency is provided. The concentrated analytes are finally detected as sequential trapezoidal peaks.

To obtain separated peaks, Foret et al. have developed transient ITP (tITP) [80,81] which consists of CZE separation after the ITP concentration. In a typical case, sample and LE are introduced into the capillary as plugs between the TE as the BGE. After ITP concentration between LE and TE, the TE in front of the LE plug penetrates into the LE zone and then into sample zone, where ITP condition is broken and sample are separated by CZE.

For the application to CE-MS, tITP is more often employed because tITP provides separated peaks which are easily quantified. As with FASS and PHJ, major application field of tITP-CE-MS includes proteomics [82-88]. Wang et al. reported combination of reverse-phase solid phase microextraction with tITP-CZE-nanoESI-MS [82]. They succeeded in identification of up to 370 proteins from 50 ng of *Xenopus laevis* proteins. As with FASS, tITP becomes more efficient for low conductivity sample, thus, SPME and tITP were a good coupling for high sensitive proteome analysis. Heemskerk et al. also employed tITP for concentrating phosphorylated proteins, where sample injection volume could be increased up to 37% of the total capillary length (250 nL) [83]. By employing ultra-low flow rate condition (less than 10 nL/min) in CESI-MS, they identified 12 phosphopeptides which is greater than the identified number obtained in LC-MS of 50 ng proteins. Glycoprotein analysis is also carried out with tITP-CE-MS [89,90]. Due to large-volume injection up to 25% of the whole capillary, high-sensitive sequencing and structural characterization of glycoforms were successfully carried out. Metabolome analyses with tITP-CE-MS have also been reported [91-96]. Ramautar et al. reported tITP-ESI-MS of urine metabolites [92], and up to 30-fold sensitivity improvement was achieved, where more than 1300 molecular features could be observed whereas conventional sheath-type CE-MS only provided 300 molecular features (Fig. 6).

Although the number is limited, normal ITP is also sometimes employed [97-101]. Vio et al. combined ITP with ICP-MS for analyzing 13 lanthanides to evaluate MOX fuel [100]. Up to 100-fold sensitivity improvement was achieved and quantitation potential by measuring the peak width was indicated. Although ITP provides sequentially segmented elution behavior which are often difficult to quantify via optical detection, MS provides $m/z$-based separated information so that quantification is much easier. Unlike CZE separation, theoretically, accurate quantification is obtained by measuring the band width in ITP, which would be a great advantage in non-target quantitative omics research in the future.
5. Sweeping

One of the most popular concentration techniques using molecular interaction is sweeping, which was developed by Quirino and Terabe [102]. In a typical sweeping process, neutral and hydrophobic analytes dissolved in a SM are introduced as a long plug between the BGE zones containing pseudo-stationary phase (PSP, usually charged micelles). Instead, the PSP migrates and penetrates into the SM zone, where the micelle uptakes the analytes and “sweep” them onto the BGE/SM boundary. After the concentration is finished, analytes are separated by EKC in the BGE.

As indicated in chapter 1, non-volatile PSP is harmful to MS instruments so that the application of sweeping to CE-MS is quite limited. Only a few numbers of papers have been published from Quirino’s group [57,103,104]. Although contribution to the actual biological discovery has been published from Quirino’s group [57,103,104].

5. Conclusions

As introduced, coupling of OSP methods with CE-MS has achieved great sensitivity in several omics researches. Although contribution to the actual biological discovery has been still limited, there is no doubt that CE-MS is becoming a next generation bioanalytical tool. One of the most important strategies for the further progress of CE-MS is on how to employ OSP methods. OSP methods provides excellent improvement in sensitivity but it is usually limited only when ideal condition is provided (i.e., sample does not contain any unnecessary salts). To achieve truly practical analysis, thus, smart construction of whole analytical system including sample collection and pretreatment is of more importance. For example, LVSS provides high sensitivity and resolution via a simple procedure (full-capillary injection and constant voltage application) [35-40], where the optimization process can be minimized. It is suitable for the total system development and therefore, further deeper understanding of OSP methods are required.

Acknowledgments

This author expresses his gratitude to Prof. Koji Otsuka (Kyoto Univ.), Prof. Yoshinobu Baba (Nagoya Univ.), Prof. Jonathan V Sweeney (Univ. Illinois), Prof. Hiroki R Ueda (Univ. Tokyo), Assoc. Prof. Fumihiko Kitagawa (Hirosaki Univ.), Dr. Yoshihisa Hagihara (AIST), Dr. Hidenori Nagai (AIST), Dr. Yo Tanka (RIKEN), Assist. Prof. Kenji Sueyoshi (Osaka Pref. Univ.), and all the other collaborators for their intensive supervision and support for this work. This author also appreciates Society for Chromatographic Sciences for awarding the Encouragement Award in 2016 and gave him the opportunity to publish this focusing review.

References

[1] Monnig, C. A.; Kennedy, R. T. Anal. Chem. 1994, 66, 280R-314R.
[2] Jorgenson, J. W.; Lukacs, K. D. Anal. Chem. 1981, 53, 1298-1302.
[3] Gassmann, E.; Kuo, J. E.; Zare, R. N. Science 1985, 230, 813-814.
[4] Ban, E.; Song, E. J. J. Chromatogr. B 2013, 929, 180-186.
[5] Zemann, A. J.; Schnell, E.; Volgger, D.; Bonn, G. K. Anal. Chem. 1998, 70, 563-567.
[6] Elbashir, A. A.; Aboul-Enein, H. Y. Biomed. Chromatogr. 2014, 28, 1502-1506.
[7] Perry, R. H.; Cooks, R. G.; Noll, R. J. Mass Spectrom. Rev. 2008, 27, 661-699.
[8] Nikolaev, E. N.; Kostyukevich, Y. I.; Vladimirov, G. N. Mass Spectrom. Rev. 2016, 35, 219-258.
[9] Na, S.; Paek, E. Mass Spectrom. Rev. 2015, 34, 133-147.
[10] Týčová, A.; Ledvina, V.; Klepářík, K. Electrophoresis 2017, 38, 115-134.
[11] Bonvin, G.; Schappler, J.; Rudaz, S. J. Chromatogr. A 2012, 1267, 17-31.
[12] Liu, C. C.; Zhang, J.; Dovichi, N. J. Rapid Commun. Mass Spectrom. 2005, 19, 187-192.
[13] El-Faramawy, A.; Siu, K. W. M.; Thomson, B. A. J. Am. Soc. Mass Spectrom. 2005, 16, 1702-1707.
[14] Sun, L.; Zhu, G.; Zhao, Y.; Yan, X.; Mou, S.; Dovichi, N. J. Angew. Chem. Int. Ed. 2013, 52, 13661-13664.
[15] Sun, L.; Zhu, G.; Zhang, Z.; Mou, S.; Dovichi, N. J. Proteome Res. 2015, 14, 2312-2321.
[16] Cao, P.; Moini, M. J. Am. Soc. Mass Spectrom. 1998, 9, 1081-1088.
[17] Valaskovic, G. A.; Mclaflery, F. W. J. Am. Soc. Mass Spectrom. 1996, 7, 1270-1272.
[18] Valaskovic, G. A.; Mclaflery, F. W. Rapid Commun. Mass Spectrom. 1996, 10, 825-828.
[19] Wahl, J. H.; Gale, D. C.; Smith, R. D. J. Chromatogr. A 1994, 659, 217-222.
[20] Moini, M. Anal. Chem. 2007, 79, 4241-4246.
[21] Janini, G. M.; Conrads, T. P.; Wilkens, K. L.; Issaq, H. J.; Veenstra, T. D. Anal. Chem. 2003, 75, 1615-1619.
[22] Tanaka, Y.; Otsuka, K.; Terabe, S. J. Pharm. Biomed. Anal. 2003, 30, 1889-1895.
[23] Axén, J.; Malmström, D.; Axelsson, B.-O.; Petersson, P.; Sjöberg, P. J. Rapid Commun. Mass Spectrom. 2010, 24, 1260-1264.
[24] Mol, R.; De Jong, G. J.; Somsen, G. W. Electrophoresis 2005, 26, 146-154.
[25] Michalke, B. Electrophoresis 2005, 26, 1584-1597.
[26] Tangen, A.; Lund, W. J. Chromatogr. A 2000, 891, 129-138.
[27] Stewart, I. I.; Olesik, J. W. J. Chromatogr. A 2000,
[77] Kohler, I.; Schappler, J.; Rudaz, S. Anal. Chim. Acta 2013, 780, 101-109.

[78] Malá, Z.; Gebauer, P.; Boček, P. Electrophoresis 2015, 36, 2-14.

[79] Hruška, V.; Gaš, B. Electrophoresis 2007, 28, 3-14.

[80] Foret, F.; Szoko, E.; Karger, B. L. J. Chromatogr. A 1992, 608, 3-12.

[81] Timerbaev, A. R.; Hirokawa, T. Electrophoresis 2006, 27, 323-340.

[82] Wang, Y.; Fonslow, B. R.; Wong, C. C. L.; Nakorchevsky, A.; Yates, J. R. Anal. Chem. 2012, 84, 8505-8513.

[83] Heemskerk, A. a. M.; Busnel, J.-M.; Schoenmaker, B.; Derks, R. J. E.; Klychnikov, O.; Hensbergen, P. J.; Deelder, A. M.; Mayboroda, O. A. Anal. Chem. 2012, 84, 4552-4559.

[84] Staňová, A.; Marák, J.; Rezeli, M.; Páger, C.; Kilár, F.; Kaniansky, D. J. Chromatogr. A 2011, 1218, 8701-8707.

[85] Medina-Casanellas, S.; Benavente, F.; Barbosa, J.; Sanz-Nebot, V. Electrophoresis 2011, 32, 1750-1759.

[86] Guo, X.; Fillmore, T. L.; Gao, Y.; Tang, K. Anal. Chem. 2016, 88, 4418-4425.

[87] Wang, C.; Lee, C. S.; Smith, R. D.; Tang, K. Anal. Chem. 2012, 84, 10395-10403.

[88] Wang, C.; Lee, C. S.; Smith, R. D.; Tang, K. Anal. Chem. 2013, 85, 7308-7315.

[89] Gahoual, R.; Busnel, J.-M.; Beck, A.; François, Y.-N.; Leize-Wagner, E. Anal. Chem. 2014, 86, 9074-9081.

[90] Gahoual, R.; Biacchi, M.; Chicher, J.; Kuhn, L.; Hammann, P.; Beck, A.; Leize-Wagner, E.; François, Y. N. mAbs 2014, 6, 1464-1473.

[91] Piešťanský, J.; Maráková, K.; Veizerová, L.; Galba, J.; Mikuš, P. Anal. Chim. Acta 2014, 826, 84-93.

[92] Ramautar, R.; Busnel, J.-M.; Deelder, A. M.; Mayboroda, O. A. Anal. Chem. 2012, 84, 885-892.

[93] Malá, Z.; Gebauer, P.; Boček, P. Anal. Chim. Acta 2011, 707, 1-6.

[94] Malá, Z.; Pantůčková, P.; Gebauer, P.; Boček, P. Electrophoresis 2013, 34, 777-784.

[95] Kler, P. A.; Huhn, C. Anal. Bioanal. Chem. 2014, 406, 7163-7174.

[96] Piešťanský, J.; Maráková, K.; Kovaľ, M.; Mikuš, P. J. Chromatogr. A 2014, 1358, 285-292.

[97] Gebauer, P.; Malá, Z.; Boček, P. Electrophoresis 2014, 35, 746-754.

[98] Malá, Z.; Gebauer, P.; Boček, P. Electrophoresis 2013, 34, 3072-3078.

[99] Gebauer, P.; Malá, Z.; Boček, P. Electrophoresis 2013, 34, 3245-3251.

[100] Vio, L.; Crétier, G.; Chartier, F.; Geertsen, V.; Gourgiotis, A.; Isnard, H.; Rocca, J.-L. Talanta 2012, 99, 586-593.

[101] Marák, J.; Staňová, A.; Gajdoštinová, S.; Škultéty, L. U.; Kaniansky, D. Electrophoresis 2011, 32, 1273-1281.

[102] Quirino, J. P.; Terabe, S. Science 1998, 282, 465-468.

[103] Quirino, J. P.; Haddad, P. R. J. Sep. Sci. 2011, 34, 2872-2878.

[104] Quirino, J. P. Electrophoresis 2011, 32, 665-668.