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The separation of biomolecules using capillary electrochromatography

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The unique properties of capillary electrochromatography such as high performance, high selectivity, minimum consumption of both reagents and samples, and good compatibility with mass spectrometry make this technique an attractive one for the analysis of biomolecules including peptides, proteins, carbohydrates, nucleosides and nucleotides. Irreversible adsorption between the biomolecules and the charged packing surface leads to a lack of reproducibility and serious peak tailing, so various approaches have been taken to overcome this and to improve the technique for future challenges.

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Abbreviations

CE	capillary electrophoresis
CEC	capillary electrochromatography
COMOSS	collocate monolith support structure
EOF	electro-osmotic flow
HPLC	high-performance liquid chromatography
MS	mass spectrometry
OT-CEC	open-tubular CEC
PC-CEC	packed-column CEC
PDMS	polydimethyl siloxane
PMP	phenyl-3-methyl-5-pyrazoloe
SCX	strong cation-exchange packing

Introduction

The great progress in the life sciences, such as the decoding of the human genome, provides a challenge for analytical chemistry to develop very efficient and selective analytical methods and materials. The micro-separation technology of capillary electrochromatography (CEC) has been developed rapidly in recent years as it has the advantages of the high selectivity of high-performance liquid chromatography (HPLC) and the high efficiency of capillary electrophoresis (CE). Moreover, minimum consumption of both reagents and samples and good compatibility with mass spectrometry (MS) make it an attractive alternative technology.

According to the stationary phase used, CEC can be classified into two main types: open-tubular CEC (OT-CEC) and packed-column CEC (PC-CEC). In OT-CEC the stationary phase is attached to the inner wall of the capillary, whereas in PC-CEC the capillary is packed with solid material confined between two frits. Recently developed monolithic or continuous-bed columns may be considered as the third type of CEC.

In its early stages, CEC was mainly used for the separation of neutral compounds such as polyaromatic hydrocarbons and less polar compounds. With the development of CEC column technology and improvement in the instrumentation, the application of CEC to separating biomolecules received more and more attention. Krull *et al.* [1] gave an extensive review of CEC applications to biopolymers based upon the CEC literature available until 1999, so this review will focus mainly on the development of CEC for separating biomolecules since 2000.

Capillary electrochromatography of peptides and proteins

The general problem of irreversible adsorption of peptides and proteins to naked silica capillary walls or to packing materials of silica-based systems hinders the ready application of CEC to separating biomolecules. These interactions are caused by electrostatic effects mediated by negatively charged silanol groups and positively charged functionalities within the biomolecule structure, resulting in a lack of reproducibility of the elution time or the efficiency. Several contributions have been made to resolve this problem using different CEC modes.

Open-tubular capillary electrochromatography of peptides and proteins

In order to eliminate undesired adsorption, modifiers with large steric groups were bonded or adsorbed on the capillary wall. Pesek and coworkers [2,3] modified the wall of etched capillaries with n-octadecyl and cholesterol groups, so that the large n-octadecyl and cholesterol groups impeded the contact between the negatively charged silanols and the positively charged peptides. Batch-to-batch reproducibility for both capillaries was excellent, as characterized by the selectivity and peak shape behavior of two basic small molecules and two proteins. Charvatova and coworkers [4,5] used porphyrin derivatives as the inner wall modifiers to separate peptides that contain aromatic amino acids and oligopeptides. Recently, a DNA aptamer that forms a G-quartet conformation was covalently attached to a capillary surface to

separate two forms of bovine β-lactoglobulin that differ by only two amino residues [6].

A second class of compounds, polymeric modifiers, has been employed to enhance performance in CEC. The porous layer was highly cross-linked by *in situ* polymerization of vinylbenzyl chloride and divinylbenzene and grafted to the inner wall of silica capillaries [7]. The chloromethyl functionalities at the surface of the porous polymeric support layer were then reacted with *N*,*N*-dimethyldodecylamine to obtain a positively charged chromatographic surface with fixed C_{12} alkyl chains. Several basic proteins and peptides were separated on such porous-layer open tubular capillaries (PLOT), which showed superior performance to the corresponding capillary zone electrophoresis under the same experimental conditions. Xu and Regnier [8] reported protein separations in the OT-CEC mode using columns prepared with the immobilization of poly(aspartic acid). Column efficiency in the electrokinetic elution mode was 10–100 times higher than that in HPLC. Moreover, protein mixtures could be separated in the isocratic elution mode in OT-CEC with resolutions approaching that achieved in the gradient elution mode by HPLC.

OT-CEC also can be performed in the dynamically modified CEC mode [9]. The separation of impurities from two synthetic peptides was carried out in OT-CEC by adsorbing fluorosurfactants (anionic and zwitterionic) to the inner wall of the capillary [10].

Packed column capillary electrochromatography of peptides and proteins

The most widely used columns in CEC are packed with alkyl silica stationary phases. Several methods can be chosen to avoid undesired adsorption between basic compounds and silanols on the packing surface. Unger and coworkers [11–13] studied the retention behavior of linear and cyclic peptides in PC-CEC on a variety of n-alkyl silica reversed-phase sorbents and mixed-mode phases containing both strong cation-exchange (sulfonic acid) and n-alkyl groups. Electrostatic interactions between the sorbents and the charged peptides could be suppressed if buffers with high salt concentrations and low pH values were used. Eluents with high ionic strength, however, result in serious Joule heating and bubble formation, and low pH eluents favor low electro-osmotic flow (EOF) velocity and long elution time.

Ion-exchangers have been introduced for CEC separations of peptides and proteins as well. Ten peptides were baseline separated on a strong cation-exchange (SCX) stationary phase within 3.5 min by Ye *et al.* [14]. Theoretical plates for small peptides of up to 460 000 plates/m were obtained and a wide range of pH could be applied while keeping high EOF velocity. Basic peptides cannot, however, be eluted from SCX stationary phases because of the strong electrostatic interaction between the basic peptides and the sulfonic groups on the surface of the SCX stationary phase. In order to decrease the strong electrostatic interactions, tentacular weak cation-exchanger particles were prepared for the separation of basic peptides in CEC by Horváth and coworkers [15]. The siliceous microspheres and the capillary inner wall were treated first with a heterobifunctional silanizing agent, which then reacted with 2-acrylamidoglycolic acid in the second step. On such a stationary phase, baseline separations of basic peptides with isocratic elution were obtained with good column efficiency. A tentacular anion-exchanger for protein separations in the PC-CEC mode was also developed by the same group [16]. Basic peptides were separated on a bare-silica packed column dynamically modified with cetyltrimethylammonium bromide, which was added into the mobile phase [17].

Monolithic column based capillary electrochromatography of peptides and proteins

CEC separations on monoliths are an attractive technology, mainly because it is easy to prepare the stationary phases, and the retaining frits are no longer necessary [18,19]. Separation of proteins and peptides in monolith CEC was mostly performed in the 'counterdirectional mode'. In this CEC system, the proteins and peptides migrate electrophoretically in a direction opposite to that of the EOF. Ericson and Hjertén [20] reported the separation of proteins on a column filled with a continuous bed derivatized with C_{18} groups and ammonium groups at pH 2.0. Under these conditions, the positively charged proteins tend to migrate electrophoretically to the cathode. As the surface of the continuous bed is also positively charged, the EOF is anodic. If the EOF velocity was greater than the electrophoretic velocity of solutes, the proteins will be eluted to the anode. As both the proteins and the surface of the monolith are positively charged, electrostatic interaction between analytes and the stationary phase are largely eliminated. Gradient elution generated by an HPLC instrument was also adopted for optimizing the separation of proteins [20].

There are several papers reported by Horváth's group that deal with protein and peptide analysis in the counter-directional mode. A column filled with poly(styrene-divinylbenzene) monolith bearing tertiary amino functions and octyl chains has been used for the separation of acidic and basic polypeptides at pH 3.0 [21]. A monolithic column prepared with acrylic supports functionalized with *N*-ethylbutylamine was used for the separation of the protein mixture containing ribonuclease A, insulin, α-lactalbumin and myoglobin and the four angiotensin-type peptides [22]. More recently, a monolithic column was prepared by co-polymerization of vinylbenzyl chloride and ethylene glycol dimethylacrylate and further reaction with *N*,*N*-dimethylbutylamine [23]. The separation of synthetic peptides and proteins, as well as the

tryptic digest of cytochrome *c* have been successfully performed on this column in isocratic elution conditions with acidic eluent.

Yu and coworkers [24•] attempted to separate a mixture of peptides on a negatively charged acrylic monolith prepared by photoinitiated *in situ* polymerization. It was observed that the separation of peptides was greatly improved by the addition of sodium 1-octanesulfonate as an ion-pairing reagent in the mobile phase. A mode of CEC based on the dynamic adsorption of surfactants on the uncharged monolith has been developed for separating peptides [25]. Wu *et al.* [26] reported the separation of peptides on a sulfated monolithic column with a mixed mode of reversed-phase and strong cation-exchange CEC.

Migration of ionic compounds in CEC can also be driven by electrophoretic mobility only. Gusev *et al.* [21] and Wu *et al.* [27] have achieved separations of peptides on the uncharged monolithic columns with poly (styrene-co-divinylbenzene) and poly (lauryl methacrylate-co-ethylene dimethacrylate) rods. It was observed that peptides were separated on the basis of their difference in electrophoretic mobility and hydrophobic interaction with the stationary phase, and all of the basic peptides containing histidine and lysine showed good peak symmetry [27].

In another example, a porous organic–inorganic hybrid monolith modified with silane containing large steric groups was prepared by photopolymerization of sol-gel [28], and the cationic peptides were separated on the column because the electrostatic interaction between the cationic peptides and the anionic monolith was largely eliminated.

Chip-based capillary electrochromatography of peptides and proteins

Research has now been carried out on the separation of biomolecules by CEC on the microchip scale. Regnier and coworkers [29,30] reported CEC analysis of tryptic peptides using microfabricated columns based on an array of collocate monolith support structure (COMOSS) modified by C_{18} silane. This work indicated that CEC of peptides in microfabricated columns is comparable to HPLC. Recently, the COMOSS column was introduced into a polydimethyl siloxane (PDMS) device instead of in quartz [31•], and alkyl silanes with C8-, C18-alkyl groups or polystyrene sulfonic acid were grafted to the column surfaces. Peptides from a tryptic digest of fluorescein isothiolyanate labeled bovine serum albumin were separated on the different columns, with the maximum column efficiency of 4×10^5 plates/m. Recently, 2-acrylamino-2-methylpropane sulfonic acid and methoxydimethyl octadecylsilane were covalently bonded to the surface of PDMS by the cerium(IV)-catalyzed polymerization [32]. Good reproducibility and stability with relative standard deviation for retention times below 2.6% were achieved, and separation of the complex digestion product of bovine serum albumin showed the efficiency to be about 62 000 plates/m.

Capillary electrochromatography of carbohydrates

Carbohydrates usually require derivatization prior to separation because of their lack of UV sensitivity. Gucek and Pilar [33] analyzed 1-phenyl-3-methyl-5-pyrazoloe (PMP) derivatives of several mono- and disaccharides using columns packed with octadecyl silica (ODS) particles and on-line UV detection. Suzuki *et al.* [34] reported the separation of the monosaccharides commonly found in glycoprotein as their PMP derivatives, performed on a column prepared by in-column derivatization of bare silica with a silane agent bearing both alkyl group and quaternary ammonium groups. PMP derivatives of aldopentose isomers were also excellently separated on a column prepared by *in situ* aminopropylation with 3-aminopropyltrimethoxsilane of a capillary packed with silica particles. Recently, quantification of the cellular carbohydrates chitin and glucan in peanut fungal pathogens and baker's yeast was performed on CEC with ODS packing [35]. Separation of sucrose and saccharin on CEC was reported with highly sensitive detection using condensation nucleation light scattering detection [36].

Capillary electrochromatography of nucleosides and nucleotides

CEC presents an alternative to HPLC for the separation of nucleosides and nucleotides that is more efficient and can avoid the use of gradients or ion-pair reagents. Helboe and Hansen [37] completely separated six nucleosides in less than 13 min through the optimization of buffer concentration, pH, amount of acetonitrile, temperature and voltage. A mixture of five nucleotides was also analyzed on a phenyl-bonded silica-packed column within 3 min by the short-end injection method [38]. Zhang *et al.* [39] introduced a CEC column packed with a novel stationary phase of octadecyl-sulfonated silica (ODSS), and achieved the separation of a mixture of twelve mono-, di-, and triphosphate nucleotides and four large tRNAs. It was found that the retention and selectivity of charged and relatively polar nucleosides and bases on the ODSS stationary phase are mainly based on the electrostatic interaction, hydrophilic interaction and reversed-phase mechanisms [40].

Levels of nucleosides and modified nucleosides in urine, serum or plasma have been proposed as the diagnostic markers of cancers and human immunodeficiency virus (HIV) [41,42]. Mesplet *et al.* [43,44] separated and quantified the nucleoside HIV reverse transcriptase inhibitors by short-end injection capillary electrochromatography on a β-cyclodextrin-bonded silica stationary phase, proving that CEC would be a suitable tool for both the quantitative determination and the assay of a mixture of nucleosides.

Conclusions and future prospects

The unique properties of CEC make it an attractive method for analyzing biomolecules. However, strong electrostatic interactions between the analytes and the packing surface present a challenge for the analysis of biomolecules, especially for proteins and peptides, when conventional silica-based packing materials are used. Tailor-made stationary phases for separating biomolecules therefore still have to be developed. The use of CEC is also hampered by its low detection sensitivity, which is inherent in the short optical path length, when on-line absorption detectors are used. Some methods such as sample stacking [28], on-line enrichment [45,46] and larger detection cell [2] have been adopted to increase the detection sensitivity. However, high-sensitivity detectors coupled to CEC are needed to fulfill the demand of biological analysis. Combination techniques such as CEC–MS [47,48], CEC–laser induced fluorescence detection [49] and CEC–conductive detection [50] have been reported recently, whereas detection devices, which allowed simultaneous analysis of multiple samples in order to increase sample throughput, are expected.

CEC may play a more important role than CE in peptide mapping, because a range of stationary phases and thus separating modes are available. This will be particularly relevant to the rapid, high-throughout screening of peptides derived from combinatorial libraries or from proteome investigations.

The most significant breakthrough will be from the miniaturized separation system in CEC mode with monolithic material. Although CE remains the most popular mode for chip-based separation up to now, chip-based CEC separations can be expected to overcome some inevitable shortcomings of CE such as low loading capacity and difficult compatibility with MS detection. Some pioneering work has already been done in this field [24•,50].

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Krull I, Sebag A, Stevenson R: **Specific applications of capillary electrochromatography to biopolymers, including proteins, nucleic acids, peptide mapping, antibodies, and so forth**. *J Chromatogr A* 2000, **887**:137-163.
2. Matyska M, Pesek J, Boysen R, Hearn M: **Characterization of open tubular capillary electrochromatography columns for the analysis of synthetic peptides using isocratic conditions**. *Anal Chem* 2001, **73**:5116-5125.
3. Matyska M, Pesek J, Boysen R, Hearn M: **Electrochromatographic characterization of etched chemically-modified capillaries with small synthetic peptides**. *J Chromatogr A* 2001, **924**:211-221.
4. Charvatova J, Kral V, Deyl Z: **Capillary electrochromatographic separation of aromatic amino acids possessing peptides using porphyrin derivatives as the inner wall modifiers**. *J Chromatogr B* 2002, **770**:155-163.
5. Charvatova J, Kral V, Deyl Z: **Capillary electrochromatographic study of the interactions of porphyrin derivatives with amino acids and oligopeptides**. *J Chromatogr B* 2002, **770**:165-175.
6. Rehder M, McGown L: **Open-tubular capillary electrochromatography of bovine β-lactoglobulin variants A and B using an aptamer stationary phase**. *Electrophoresis* 2001, **22**:3759-3764.
7. Huang X, Zhang J, Horváth C: **Capillary electrochromatography of proteins and peptides with porous-layer open-tubular columns**. *J Chromatogr A* 1999, **858**:91-101.
8. Xu W, Regnier F: **Electrokinetically-driven cation-exchange chromatography of proteins and its comparison with pressure-driven high-performance liquid chromatography**. *J Chromatogr A* 1999, **853**:243-256.
9. Zou H, Ye M: **Capillary electrochromatography with physically and dynamically absorbed stationary phase**. *Electrophoresis* 2000, **21**:4073-4095.
10. Pesek J, Matyska M, Sentellas S, Galceran M, Chiari M, Pirri G: **Multimodal open-tubular capillary electrochromatography analysis of amines and peptides**. *Electrophoresis* 2002, **23**:2982-2989.
11. Walhagen K, Unger K, Hearn M: **Capillary electrochromatography analysis of hormonal cyclic and linear peptides**. *Anal Chem* 2001, **73**:4924-4936.
12. Walhagen K, Unger K, Keah H, Hearn M: **Application of CEC procedures for the analysis of synthetic peptides: characterization of linear immunogenic peptides that mimic a HIV-1 gp120 epitope**. *J Pept Res* 2002, **59**:159-173.
13. Walhagen K, Unger K, Hearn M: **Influence of temperature on the behavior of small linear peptides in capillary electrochromatography**. *J Chromatogr A* 2000, **893**:401-409.
14. Ye M, Zou H, Liu Z, Ni J: **Separation of peptides by strong cation-exchange capillary electrochromatography**. *J Chromatogr A* 2000, **869**:385-394.
15. Zhang J, Zhang S, Horváth C: **Capillary electrochromatography of peptides on a column packed with tentacular weak cation-exchange particles**. *J Chromatogr A* 2002, **953**:239-249.
16. Zhang J, Huang X, Zhang S, Horváth C: **Capillary electrochromatography of proteins on an anion-exchanger column**. *Anal Chem* 2000, **72**:3022-3029.
17. Ye M, Zou H, Liu Z, Li J, Zhang Y: **Capillary electrochromatography with a silica column dynamically modified cationic surfactant**. *J Chromatogr A* 1999, **855**:137-145.
18. Zou H, Huang X, Ye M, Luo Q: **Monolithic stationary phase for liquid chromatography and capillary electrochromatography**. *J Chromatogr A* 2002, **954**:5-32.
19. Sevec F, Peters E, Sýkora D, Yu C, Fréchet J: **Monolithic stationary phase for capillary electrochromatography based on synthetic polymers: designs and applications**. *J High Resol Chromatogr* 2000, **23**:3-18.
20. Ericson C, Hjertén S: **Reversed-phase electrochromatography of proteins on modified continuous beds using normal-flow and counterflow gradients. Theoretical and practical considerations**. *Anal Chem* 1999, **71**:1621-1627.
21. Gusev I, Huang X, Horváth C: **Capillary columns with in situ formed porous monolithic packing for micro high-performance liquid chromatography and capillary electrochromatography**. *J Chromatogr A* 1999, **855**:273-290.
22. Zhang S, Huang X, Zhang J, Horváth C: **Capillary electrochromatography of proteins and peptides with a cationic acrylic monolith**. *J Chromatogr A* 2000, **887**:465-477.

23. Zhang S, Zhang J, Horváth C: **Rapid separation of peptides and proteins by isocratic capillary electrochromatography at elevated temperature**. *J Chromatogr A* 2001, **914**:189-200.

24. • Yu C, Svec F, Fréchet J: **Towards stationary phases for chromatography on a microchip: molded porous polymer monoliths prepared in capillaries by photoinitiated in situ polymerization as separation media for electrochromatography**. *Electrophoresis* 2000, **21**:120-127.
The authors carried out their study using the capillary format and CEC separation as a model for a microchip containing a bed of monolithic separation medium prepared by *in situ* UV-initiated polymerization.

25. Wu R, Zou H, Ye M, Lei Z, Ni J: **Separation of basic, acidic and neutral compounds by capillary electrochromatography using uncharged monolithic capillary columns modified with anionic and cationic surfactants**. *Electrophoresis* 2001, **22**:544-551.

26. Wu R, Zou H, Fu H, Jin W, Ye M: **Separation of peptides on mixed mode of reversed-phase and ion-exchange capillary electrochromatography with a monolithic column**. *Electrophoresis* 2002, **23**:1239-1245.

27. Wu R, Zou H, Ye M, Lei Z, Ni J: **Capillary electrochromatography for separation of peptides driven with electrophoretic mobility on monolithic column**. *Anal Chem* 2001, **73**:4918-4923.

28. Dulay M, Quirino J, Bennett B, Zare R: **Bonded-phase photopolymerized sol-gel monoliths for reversed phase capillary electrochromatography**. *J Sep Sci* 2002, **25**:3-9.

29. He B, Tait N, Regnier F: **Fabrication of nanocolumns for liquid chromatography**. *Anal Chem* 1998, **70**:3790-3797.

30. He B, Ji J, Regnier F: **Capillary electrochromatography of peptides in a microfabricated system**. *J Chromatogr A* 1999, **853**:257-262.

31. • Slentz B, Penner N, Lugowska E, Regnier F: **Nanoliter capillary electrochromatography columns based on collocated monolithic support structures molded in poly(dimethylsiloxane)**. *Electrophoresis* 2001, **22**:3736-3743.
Instead of using quartz, the authors introduced the COMOSS columns into a PDMS device, which is cheaper and easier to chemically modify than quartz.

32. Slentz B, Penner N, Regnier F: **Capillary electrochromatography of peptides on microfabricated poly (dimethylsiloxane) chips modified by cerium (IV)-catalyzed polymerization**. *J Chromatogr A* 2002, **948**:225-233.

33. Gucek M, Pilar B: **Capillary electrochromatography of 1-phenyl-3-methyl-5-pyrazolone derivatives of some mono- and disaccharides**. *Chromatographia* 2000, **51**:S139-S142.

34. Suzuki S, Kuwahara Y, Makiura K, Honoda S: **Preparation of various silica-based columns for capillary electrochromatography by in-column derivatization**. *J Chromatogr A* 2000, **873**:247-256.

35. Zhang M, Melouk H, Chenault K, El Rassi Z: **Determination of cellular carbohydrates in peanut fungal pathogens and baker's yeast by capillary electrophoresis and electrochromatography**. *J Agric Food Chem* 2001, **49**:5265-5269.

36. Guo W, Koropchak J, Yan C: **Sensitive, universal detection for capillary electrochromatography using condensation nucleation light scattering detection**. *J Chromatogr A* 1999, **849**:587-597.

37. Helboe T, Hansen S: **Separation of nucleosides using capillary electrochromatography**. *J Chromatogr A* 1999, **836**:315-324.

38. Calhours X, Morin P, Agrofoglio L, Dreux M: **Fast separation of nucleosides by capillary electrochromatography on non-endcapped phenyl-bonded silica phase using short-end injection method**. *J High Resol Chromatogr* 2000, **23**:138-142.

39. Zhang M, Yang C, El Rassi Z: **Capillary electrochromatography with novel stationary phases. 3. Retention behavior of small and large nucleic acids on octadecyl-sulfonated-silica**. *Anal Chem* 1999, **71**:3277-3282.

40. Zhang M, El Rassi Z: **Capillary electrochromatography with novel stationary phases. II. Studies of the retention behavior of nucleosides and bases on capillaries packed with octadecyl-sulfonated-silica microparticles**. *Electrophoresis* 1999, **20**:31-36.

41. Liebich H, Stefano C, Wixforth A, Schmid H: **Quantitation of urinary nucleosides by high-performance liquid chromatography**. *J Chromatogr A* 1997, **763**:193-197.

42. Pane F, Intrieri M, Calcagno G, Izzo E, Liberti A, Salvatore F, Sacchetti L: **Diagnostic and discriminatory efficiency of eight serum modified nucleosides in HIV infection and in at-risk subjects**. *J Liq Chromatogr* 1993, **16**:1229-1248.

43. Mesplet N, Morin P, Francois C, Agrofoglio L: **Simultaneous quantitation of nucleoside HIV-1 reverse transcriptase inhibitors by short-end injection capillary electrochromatography on a β-cyclodextrin-bonded silica stationary phase**. *J Chromatogr A* 2001, **927**:161-168.

44. Mesplet N, Morin P, Francois C, Agrofoglio L: **Concurrent analysis of nucleoside reverse transcriptase inhibitors in a pool of endogenous nucleosides by short-end injection-capillary electrochromatography on a β-cyclodextrin-bonded stationary phase**. *Electrophoresis* 2002, **23**:1263-1271.

45. Quirino J, Dulay M, Zare R: **On-line preconcentration in capillary electrochromatography using a porous monolith together with solvent gradient and sample stacking**. *Anal Chem* 2001, **73**:5557-5563.

46. Thomas D, Rakestraw D, Schoeniger J, Lopez-Avila V, Van Emon J: **Selective trace enrichment by immunoaffinity capillary electrochromatography on-line with capillary zone electrophoresis-laser-induced fluorescence**. *Electrophoresis* 1999, **20**:57-66.

47. Gaspari M, Guček M, Walhagen K, Vreeken R, Verheij E, Tjaden U, Van der Greef J: **Ion trap mass spectrometry as detector for capillary electrochromatography of peptides: possibilities and limitations**. *J Microcol Sep* 2001, **13**:243-249.

48. Guček M, Gaspari M, Walhagen K, Vreeken R, Verheij E, Van der Greef J: **Capillary electrochromatography/nanoelectrospray mass spectrometry for attomole characterization of peptides**. *Rapid Commun Mass Spectrom* 2000, **14**:1448-1454.

49. Nagaraj S, Karnes H: **Visible diode laser induced fluorescence detection of doxorubicin in plasma using pressurized capillary electrochromatography**. *Biomed Chromatogr* 2000, **14**:234-242.

50. Galloway M, Stryjewski W, Henry A, Ford S, Llopis S, McCarley R, Soper S: **Contact conductivity detection in poly(methyl methacrylate)-based microfluidic devices for analysis of mono- and polyanionic molecules**. *Anal Chem* 2002, **74**:2407-2415.