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Review

New approaches in clinical chemistry: on-line analyte concentration and microreaction capillary electrophoresis for the determination of drugs, metabolic intermediates, and biopolymers in biological fluids

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Abstract

The use of capillary electrophoresis (CE) for clinically relevant assays is attractive since it often presents many advantages over contemporary methods. The small-diameter tubing that holds the separation medium has led to the development of multicapillary instruments, and simultaneous sample analysis. Furthermore, CE is compatible with a wide range of detectors, including UV–Vis, fluorescence, laser-induced fluorescence, electrochemistry, mass spectrometry, radiometric, and more recently nuclear magnetic resonance, and laser-induced circular dichroism systems. Selection of an appropriate detector can yield highly specific analyte detection with good mass sensitivity. Another attractive feature of CE is the low consumption of sample and reagents. However, it is paradoxical that this advantage also leads to severe limitation, namely poor concentration sensitivity. Often high analyte concentrations are required in order to have injection of sufficient material for detection. In this regard, a series of devices that are broadly termed 'analyte concentrators' have been developed for analyte preconcentration on-line with the CE capillary. These devices have been used primarily for non-specific analyte preconcentration using packing material of the C_{18} type. Alternatively, the use of very specific antibody-containing cartridges and enzyme-immobilized microreactors have been demonstrated. In the current report, we review the likely impact of the technology of capillary electrophoresis and the role of the CE analyte concentrator–microreactor on the analysis of biomolecules, present on complex matrices, in a clinical laboratory. Specific examples of the direct analysis of physiologically-derived fluids and microdialysates are presented, and a personal view of the future of CE in the clinical environment is given. © 1997 Elsevier Science B.V.

Keywords: Reviews; Drugs; Metabolites; Biopolymers; Enzymes; Lectin

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1. Introduction

The design of the human body is far more sophisticated than that of the most advanced computer. Billions of living cells containing a diverse number of biomolecules make up the complex machinery of the human body. Many of these cells have a special function to perform that is necessary to maintain and coordinate the steady-state balance, or homeostasis, needed for smooth operation of the whole body [1,2]. Hence, an understanding of the composition of the constituent biomolecules present in biological fluids, cells, and tissue specimens is then an important parameter in assessing the 'state of health' of an individual. A significant disturbance in the homeostasis of the body triggers a variety of responses that often produces a disease state [3–6]. Much information about specific disease states has been gained by determining a certain number of biochemical parameters that are used as indicators of disease activity, as well as providing information about the intensity of the whole pathological process. Although the molecular basis of many diseases is not clearly understood, it has been recognized that the analysis of biochemical constituents of body fluids, cells, and tissues is important in making a diagnosis [3–14]. In addition, the screening and confirmation of drugs in body fluids is important in the investigation of intoxications, the detection of potential abusers of drugs, and the control of drug addicts following withdrawal therapy [15–17].

Today, modern medicine and forensic science could not operate without the widespread use of instrumentation and the performance of highly sophisticated laboratory tests. A variety of techniques have been used throughout the years, including a bewildering array of laboratory procedures. Each of these techniques has its own special usefulness, advantages and potential drawbacks. Among the most common techniques used are electrophoretic, chromatographic, radiometric, enzymatic, and immunological assays, as well as the measurement of electrolytes by ion-selective electrodes [6–14]. The use of manual techniques is declining and it is becoming crucial to use automated instruments, especially in a large hospital setting. Automation has revolutionized the practice of clinical chemistry, making it possible to perform large numbers of analysis rapidly, efficiently, and economically. Advances in automated instrumentation have also allowed multiple specimens to be analyzed simultaneously. The use of properly controlled, automated instruments increases the precision and accuracy of the test and minimizes errors in technique and measurement [6–14].

In many instances, the availability of small amounts of a biological fluid, cell, or tissue is not enough to determine their biological constituents using traditional laboratory techniques. Frequently, many applications require analytical systems capable of handling nanoliter volumes of sample containing subnanomolar quantities of material. In the clinical environment, in which the early detection of the onset of a disease process may be essential to a patient's survival, determinations of analytes at concentrations approaching even the single molecule are highly desirable. As a result of this new awareness, microscale will eventually be defined by atto- (10^{-18} mol/l), zepto- (10^{-21} mol/l), and eventually yoctomolar (10^{-24} mol/l) concentration ranges of detection. The emerging technology of capillary electrophoresis (CE) appears to offer an optimal approach to overcome some of the limitations of conventional analytical approaches. This technique was first introduced by Hjertén [18] and Virtanen [19] and subsequently by Mikkers et al. [20] and Jorgenson and Lukacs [21] over a decade ago. It consists typically of a fused-silica capillary (20–60 cm×5–100 μm I.D.) filled with an appropriate electrolyte/buffer solution, immersed in two buffer reservoirs (shown in Fig. 1). Analyte solutions can be loaded onto the capillary either hydrodynamically with pressure or vacuum, or electrokinetically with a low voltage. An appropriate voltage (10–30 kV) is

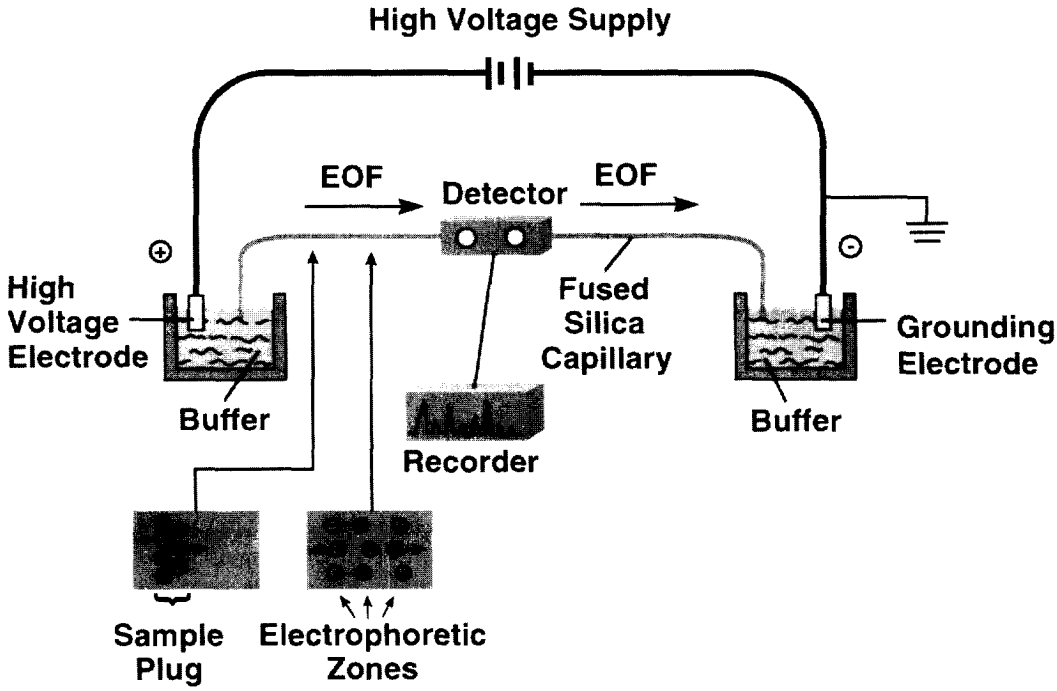

Fig. 1. Schematic diagram of a capillary electrophoresis system (not drawn to scale), showing the effect of electroosmotic flow and electrophoretic mobility during electrophoresis.

applied across the capillary and analytes migrate at variable velocities through the system, and past a detector. Analyte mobility is determined by (a) the electrophoretic mobility of individual analytes which, to a first approximation, equates to charge/mass ratio, and (b) bulk properties of the solution and its interaction with the wall of the capillary, this is referred to as the electroosmotic flow (EOF). These characteristics of CE are described in Refs. [22–36] and summarized in Fig. 1.

Modern capillary electrophoresis has emerged as a powerful tool with many advantages over traditional separation techniques. The inherent characteristics of CE, such as separation selectivity, small sample size capability, high speed of analysis, high efficiency, excellent mass sensitivity, low reagent consumption, high resolution, and high recovery (if surface adsorption is minimized) are among the many unique features of this technology. Undoubtedly, capillary electrophoresis is becoming an all-purpose technology since it has been applied to solve analytical problems related to forensic chemistry, food chemistry, clinical chemistry, biochemistry, pharmaceutical science, neuroscience, molecular biology, and environmental science [22–36]. Capillary electrophoresis continues to develop rapidly. It has been applied successfully to the separation and analysis of a variety of simple and complex molecules ranging from ions, vitamins, drugs, amino acids, and nucleotides, to peptides, oligonucleotides, and glycoproteins, including viral and subcellular particles [22–36]. More recently, CE has been used in gene diagnosis and in the Human Genome Project, and may play a crucial role in the success of that effort [37,38]. Scientists are devoting a major effort to building a high-throughput DNA sequencing system based on capillary electrophoresis [39–42]. Capillary electrophoresis has drawn upon several other technologies in its development. Applying successful aspects of other separation techniques has enabled CE to become an established technique comparable to gas chromatography (GC) and high-performance liquid chromatography (HPLC). CE possibly owes most of its technological development to gel electro-

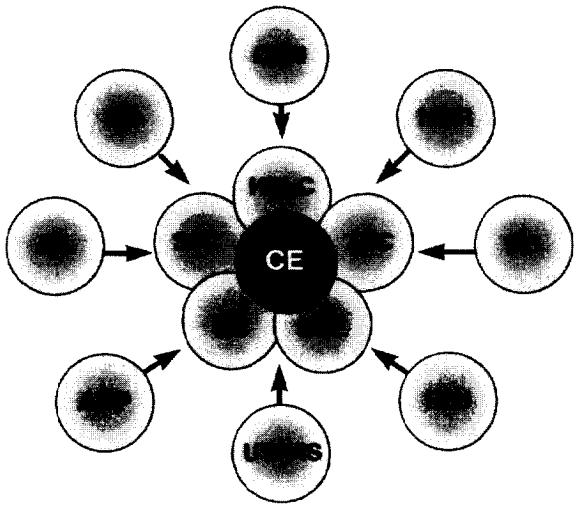

Fig. 2. Schematic diagram linking CE to other technologies. The various modes of CE complement and expand the separation capabilities of other technologies. In addition, it broadens the detection capabilities to make CE one of the most powerful techniques for characterization of substances. Abbreviations: CE, capillary electrophoresis; HPLC, high-performance liquid chromatography; SFC, supercritical fluid chromatography; GC, gas chromatography; SGE, slab-gel electrophoresis; TLC, thin-layer chromatography; CON, conductivity; CD, circular dichroism; RAD, radiometry; AMP, amperometry; UV–Vis, ultraviolet–visible; FLU, fluorometry; MS, mass spectrometry; NMR, nuclear magnetic resonance.

phoresis. In addition, CE has been coupled with several powerful detection technologies (see Fig. 2) to even further enhance the features of this separation technique for the determination and characterization of important biomolecules.

Despite the unique features of CE and the many reports of its use to analyze constituents present in biological fluids, cells, and tissue specimens, the technique has not yet been widely accepted by the community of clinical chemists. Among the few drawbacks of CE is the fact that the best performance is normally obtained in analyzing small sample volumes (typically <50 nl for a 50 μm I.D. capillary). Ultimately, this limitation leads to a relatively poor concentration limit of detection (CLOD) for CE and makes it difficult to provide sufficient sensitivity for many applications. Hence, the purpose of this manuscript is to review: (a) the major developments describing the use of capillary electrophoresis in a clinical laboratory, and (b) recent advancements in the development of methods describing the possibilities of enhancing the concentration limits of detection of CE through the use of analyte concentrator–microreactor cartridges. Currently, other devices are commercially available that increase the short light path of the existing CE capillary columns, such as the z-shaped column or

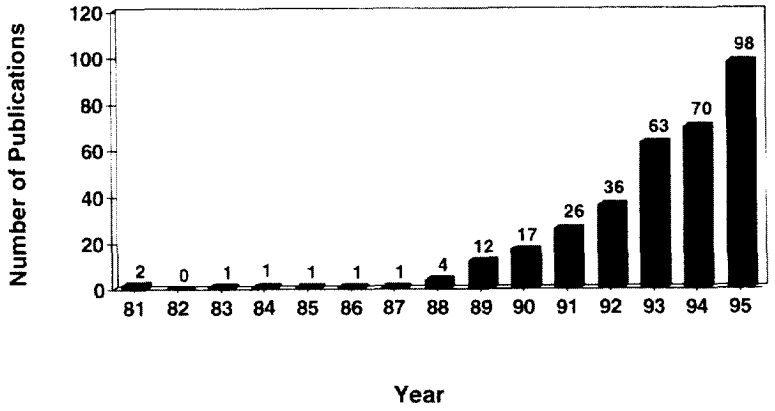

Fig. 3. Schematic diagram representing the involvement of CE in clinical chemistry. Number of publications per year using CE in the determination of drugs, metabolic intermediates and biopolymers in biological fluids, cells, and tissue specimems. This graph (not drawn to scale) also includes standard compounds of importance in the development of the CE assay that eventually will be used in the determination of that particular compound in biological fluids, cells, and tissue specimens.

high-sensitivity cell and the bubble cell. These high sensitivity cells will substantially increase the utility of CE for impurity analysis of biologicals, pharmaceuticals, and environmental compounds, among others. In this review, however, we will focus on the use of the on-line preconcentration cartridges.

2. Capillary electrophoresis in the clinical laboratory

2.1. General approach

Since the first electropherogram of the constituents of human urine reported in 1981 [21,406], the use of CE for the analysis of drugs, metabolic intermediates, and biopolymers present in biological fluids, cells, and tissue specimens has undergone an explosive growth. This is demonstrated in Fig. 3, which chronicles the number of publications in the literature pertinent to this subject. Throughout most of the early-to-mid 1980s typically only a single paper per year was published. However, with the advent of commercially available CE instruments the interest and growth has been significant.

The most common mode of CE used in such analyses has been free-solution capillary-zone electrophoresis (FSCE or CZE). However, others modes of CE, such as micellar electrokinetic-capillary chromatography (MEKC), have also been used, particular in the analysis of non-ionic species. The use of several modes of CE techniques has allowed the analysis of a wide variety of biologically derived standards and compounds present in biological fluids, cells, and tissue specimens and this is detailed in Table 1 and Refs. [43–417].

The relatively poor performance of HPLC for macromolecule analysis has made clinical chemists reluctant and skeptical of adopting CE into their environments. With the exception of a few tests still being carried out today by HPLC and GC–MS (i.e., determination of hemoglobins, amino acids, certain vitamins, hormones, and drugs), chromatography has played a relatively minor role in the routine laboratory of most hospitals. A number of drawbacks including speed, resolution, and sensitivity has accounted for the resistance to their use in larger

Table 1. Determination of drugs, metabolic intermediates, and biopolymers in biological fluids or cells using capillary electrophoresis (or microchip electrophoresis)

Analyte	Specimen	Ref.
Acetaminophen	Serum	[43]
Acetaminophen	Urine	[43]
Acetylcholine	Single cells	[44]
Adenylosuccinase	Standards	[45]
Adenylsuccinate lyase	Urine	[46]
Albumin	Plasma	[47]
Albumin	Serum	[48,49]
Alkaline phosphatase	Tissue extract	[50]
Aluminum	Serum	[51]
α-Amanitin	Urine	[52]
β-Amanitin	Urine	[52]
Amines	Single cells	[53,54]
Amino acids	Brain dialysate	[55]
Amino acids	Cerebrospinal fluid	[56–58]
Amino acids	Saliva	[59]
Amino acids	Single cells	[60,61]
Amino acids	Serum	[59,62]
Amino acids	Standards	[53,63–71]
Amino acids	Urine	[59]
Aminopyrine	Urine	[72]
Amiodarone	Standards	[73]
Amitriptyline	Standards	[74]
Amphetamine	Standards	[66]
Amphetamine	Urine	[75,76]
Amyloid P peptides	Serum	[77,78]
Analgesics	Urine	[79]
Anions	Urine	[80]
Anthracyclines	Plasma	[81]
Anthrylethanol	Standards	[82]
Antibodies	Cell extract	[83]
Antibodies	Standards	[84,85]
Antibodies	Cell media	[86]
Antibodies	Standards	[87–90]
Antidepressants	Plasma	[91]
Anti-epileptic drugs	Plasma	[92]
Anti-epileptic drugs	Saliva	[93]
Anti-epileptic drugs	Serum	[43,62,93,94]
Anti-epileptic drugs	Urine	[43]
Antigen–antibody complex	Standards	[95]
Antiinflammatory drugs	Serum	[94]
Antipyrine	Saliva	[96]
Antisense oligonucleotides	Standards	[97]
Apolipoprotein DNA	Blood	[98]
Apolipoproteins	Plasma	[99,100]
Apolipoproteins	Serum	[101,102]
Aromatic hydrocarbons	Standards	[103]
Arylsulfonamides	Standards	[104]
Aspoxicillin	Plasma	[105]
Atrazine	Standards	[106]
Banbuterol	Plasma	[107]
Barbital	Urine	[108]

Table 1. Continued

Analyte	Specimen	Ref.
Barbiturates	Serum	[109]
Barbiturates	Urine	[75,109,110]
Basic drugs	Plasma	[111]
Basic drugs	Urine	[111]
Benzodiazepines	Urine	[75,112]
Benzoylecgonine	Urine	[76]
Beta blockers	Serum	[94]
Beta blockers	Standards	[113,114]
Beta blockers	Urine	[79,114]
Bile salts	Standards	[115]
Bilirubin	Serum	[116]
Bradykinin	Single cells	[44]
Brompheniramine	Plasma	[107]
Bupivacaine	Drain fluid	[117]
Bupivacaine	Serum	[118]
Caffeine	Saliva	[119]
Caffeine	Serum	[120]
Caffeine	Standards	[73]
Caffeine	Urine	[121]
Caffeine metabolites	Urine	[121,122]
Calcium	Serum	[123]
Calcium	Urine	[124]
Carbohydrates	Single cells	[54,125]
Carbohydrates	Standards	[126,127]
Carbonic anhydrase	Standards	[128]
S-Carboxymethyl-L-cysteine	Urine	[129]
Cardiovascular drugs	Standards	[130,131]
β-Casein	Standards	[132]
Catecholamines	Lymphocytes	[133]
Catecholamines	Single cells	[134]
Cathechols	Single cells	[54]
Cefixime	Urine	[135]
Cefpiramide	Plasma	[136]
Chloramphenicol acetyl transferase	Standards	[137]
Chorionic gonadotropin	Standards	[138]
Cicletanine	Plasma	[139,140]
Cimetidine	Serum	[141]
Cimetidine	Urine	[142]
Citrate	Urine	[143]
Clenbuterol	Urine	[144]
Cocaine	Brain dialysate	[145]
Cocaine	Hair	[146]
Cocaine	Standards	[147]
Cocaine	Urine	[75,76]
Codeine	Standards	[148]
Codeine	Urine	[76]
Corticosteroids	Standards	[149]
Cortisol	Serum	[150–152]
Creatinine	Plasma	[153,154]
Creatinine	Serum	[62]
Creatinine	Urine	[124,153,155]
Cyclic nucleotides	Standards	[87,156]
Cysteine	Standards	[157]

Table 1. Continued

Analyte	Specimen	Ref.
Cystine	Standards	[157]
Cytokines	Tissue extract	[158,159]
Cytosine-β-D-arabinoside	Plasma	[160]
Dexamethasone	Urine	[161]
Dexepin	Urine	[162]
Dextromethorphan	Standards	[73]
Dextromethorphan	Urine	[121,163]
Dextrorphan	Urine	[163]
Difenzoquat	Standards	[164]
Digoxin	Serum	[165]
Dihydrocodeine	Urine	[166]
Diltiazem	Urine	[143]
Dinaphthyl	Standards	[82]
Diquat	Serum	[167]
Diquat	Standards	[164]
Diuretics	Serum	[168]
Diuretics	Urine	[79,168]
DNA	Bone marrow	[169]
DNA	Cell extract	[170]
DNA	Lymphocytes	[171]
DNA or RNA	Microorganisms	[172–174]
DNA	Serum	[175–177]
DNA	Standards	[178–184]
DNA	White blood cells	[178]
DNA fragments	Standards	[185–189]
DNA restriction fragments	Plasma	[190]
Dopamine	Brain dialysate	[191]
Dopamine	Single cells	[192]
Drugs	Serum	[193]
Drugs	Urine	[193]
Endorphins	Tissue extract	[194]
Enkephalins	Tissue extract	[194]
Enkephalin (leucine)	Cerebrospinal fluid	[195]
Enkephalin (methionine)	Cerebrospinal fluid	[195]
Enzyme inhibitors	Standards	[196]
Ephedrine	Plasma	[197]
Ephedrine	Standards	[198]
Ephedrine	Urine	[199]
Estrogens	Urine	[200]
Felbamate	Serum	[201]
Fluconazole	Plasma	[202]
Flucytosine	Serum	[203]
Flumethasone	Urine	[161]
Fluorescein	Standards	[204]
Gabapentin	Serum	[205]
Glipizide	Urine	[206]
Globins	Blood	[207]
Globin chains	Serum	[208]
Globin chains	Blood	[209]
Glucose	Tears	[210]
Glucose-6-phosphate dehydrogenase	Standards	[211]
Glutamate	Brain dialysate	[212–214]
Glutathione	Standards	[157]
Glutathione	Tissue extract	[215]

Table 1. Continued

Analyte	Specimen	Ref.
Glutathione (reduced)	Red blood cells	[216]
Glyburide	Urine	[206]
Glycine	Standards	[53]
Glycoproteins	Cell extract	[217]
Glycoproteins	Standards	[218]
Glyphosate aminomethyl-phosphonic acid	Serum	[219]
Growth hormone	Standards	[220]
Haloperidol	Cell extract	[221]
Haloperidol	Standards	[222–225]
Haloperidol	Tissue extract	[226–230]
Haloperidol	Urine	[231–233]
Hapten–antibody complex	Standards	[234]
Hemoglobin A,S,C	Cerebrospinal fluid	[235]
Hemoglobin A,S,C	Serum	[235]
Hemoglobin A,S,C	Urine	[235]
Hemoglobins	Cerebrospinal fluid	[236]
Hemoglobins	Serum	[208,236–242]
Hemoglobins	Standards	[243,244]
Hemoglobins	Umbilical cord blood	[245,246]
Hemoglobins	Urine	[236]
Hemoglobin peptides	Serum	[247]
Herbicides	Standards	[248,249]
Heroin	Standards	[147,250,251]
Heterocyclic compounds	Standards	[252]
Hippuric acid	Serum	[253]
Hippuric acid	Urine	[254]
Homovanillic acid	Urine	[255]
Hyaluronan	Synovial tissue	[256]
Hydrocannabinol (11-nor-δ-9-tetra-hydrocannabinol-9-carboxylic acid)	Urine	[257]
7-Hydroxycoumarin	Tissue extract	[258]
7-Hydroxymethotrexate	Serum	[259]
Hydroxyproline	Serum	[260]
Hydroxyproline	Standards	[87,261]
Hypnotics	Urine	[75]
Hypoglycemic drugs	Urine	[262]
Imipramine	Standards	[74]
Immunoglobulin E (IgE)	Serum	[232,263,264]
Immunoglobulin (IgG)	Serum	[48,49]
Inorganic cations	Urine	[124]
Insulin	Standards	[265]
Iodide	Plasma	[266]
Iohexol	Serum	[267]
Ions	Saliva	[59]
Ions	Serum	[59,268–270]
Ions	Single cells	[271]
Ions	Standards	[272]
Ions	Urine	[59]
Iron	Serum	[273]
Itraconazole	Standards	[73]
Kallidin	Single cells	[44]

Table 1. Continued

Analyte	Specimen	Ref.
Ketoconazole	Standards	[73]
Lactate dehydrogenase	Single cell	[274]
Lactobionic acid	Standards	[275]
Leucine amino peptidase	Serum	[276]
Leucine amino peptidase	Urine	[276]
Lidocaine	Brain dialysate	[145]
Lipoproteins	Plasma	[47,277]
Lipoproteins	Serum	[278–284]
Lithium	Serum	[285]
Litracene	Standards	[74]
Maprotiline	Standards	[74]
Meclofenamic acid	Urine	[286]
Mephenytoin	Urine	[121]
6-Mercaptopurine	Standards	[287]
Metalloprotein	Serum	[288]
Metallothionein	Serum	[289]
Metallothionein	Tissue extract	[289–291]
Metallothionein	Urine	[289]
Methadone	Standards	[73]
Methadone	Urine	[76,292]
Methamphetamine	Urine	[293]
Methotrexate	Serum	[259]
5-Methylcytosine	Cell extract	[294]
3-Methylflavonin-8-carboxylic acid	Urine	[295]
Methylhippuric acid	Urine	[254]
Methylmalonic acid	Serum	[296,297]
Methylmalonic acid	Urine	[298]
Methyluric acid	Saliva	[119]
Methylxanthine	Saliva	[119]
Monosaccharides	Standards	[299]
Morphine	Hair	[146]
Morphine	Standards	[300]
Morphine	Urine	[76,108,286]
Morphine-3-glucuronide	Urine	[286,301]
Morphine-6-glucuronide	Urine	[286]
Muscapine	Standards	[148]
Myoglobin	Standards	[243]
Myoglobin	Urine	[302]
Naproxen	Standards	[73]
Narcotics	Urine	[79]
Neuropeptides	Brain perfusate	[303–307]
Neuropeptides	Standards	[87,264,308]
Neurotransmitters	Single cells	[60]
Nitrate	Plasma	[266,309]
Nitrate	Urine	[310]
Nitrite	Plasma	[266,309]
Nitrite	Urine	[310]
p-Nitroanilide (leucine)	Standards	[311]
Nitrophenyl-β-galactoside	Standards	[312]
Noradrenaline	Brain dialysate	[191]
Normorphine	Urine	[286]
Noscapine	Standards	[148]
Nucleic acids	Single cells	[125]
Nucleic acids	Standards	[68]

Table 1. Continued

Analyte	Specimen	Ref.
Nucleosides	Cord plasma	[313]
Nucleotides	Plasma	[314]
Nucleotides	Tissue extract	[314,315]
Organic acids	Cerebrospinal fluid	[316]
Organic acids	Saliva	[59]
Organic acids	Serum	[59,317]
Organic acids	Urine	[59,318]
Organic anions	Serum	[253,319]
Opiates	Urine	[320]
Opioids	Urine	[75]
Oxalate	Urine	[143]
Oxprenolol	Urine	[321]
Paclitaxel	Plasma	[322]
Paclitaxel	Urine	[322]
Papaverine	Standards	[148]
Paraproteins	Single cells	[125]
Paraquat	Serum	[167]
Paraquat	Standards	[164]
Paraxanthine	Saliva	[119]
PCR reaction products	Blood	[323,324]
PCR reaction products	Plasma	[190]
PCR reaction products	Serum	[325,326]
PCR reaction products	Standards	[181,327,328]
PCR reaction products	Virus	[329]
Pentobarbital	Serum	[330]
Pentobarbital	Standards	[82]
Peptides (MHC class I)	Cell extract	[331,332]
Peptides	Standards	[68,180,333–344]
Peptide mapping	Standards	[53,232,345–354]
Pharmaceuticals	Standards	[355]
Phenols	Single cells	[54]
Phenols	Standards	[356]
Phenoxyacid	Standards	[249]
Phenylalanine	Serum	[281,357]
Phenylketones	Urine	[358]
Phenylpyruvate	Urine	[359]
β-Phycoerythrin	Standards	[360]
Porphyrins	Standards	[361,362]
Porphyrins	Urine	[363]
Prazosin	Urine	[142]
Procainamide	Standards	[364]
Procaine	Brain dialysate	[145]
Proline	Standards	[87,261]
Prolyl 4-hydroxylase	Tissue extract	[365,366]
Propanolol	Serum	[367]
Propanolol	Urine	[162]
Proteins	Cerebrospinal fluid	[56,236,281,368]
Proteins	Lung fluid	[369]
Proteins	Plasma	[370]
Proteins	Saliva	[370]
Proteins	Seminal plasma	[371]
Proteins	Serum	[102,236,262] [281,372,373] [374–376] [377–379]

Table 1. Continued

Analyte	Specimen	Ref.
		[380–382]
		[383–385]
		[386,387]
Proteins	Single cells	[125,388]
Proteins	Standards	[65,68,71] [179,180,333] [334,335,338] [339,389,390] [391,392]
Proteins	Urine	[236,281,393]
Protriptyline	Standards	[74]
Purine (substitutes)	Serum	[119]
Purine (substitutes)	Urine	[119]
Purine (succinyl)	Urine	[216]
C-Reactive protein	Lung fluid	[394]
Retinol (vitamin A)	Serum	[395]
RNA	Microorganisms	[172]
Ropivacaine	Standards	[396]
Salicylate	Serum	[43]
Salicylate	Urine	[43]
Steroids	Serum	[397,398]
Sulfonylurea	Standards	[249]
Suramin	Serum	[399]
Taurine	Tissue biopsy	[216]
Terbutaline	Plasma	[107]
Terbutaline	Standards	[198]
Thalidomide	Urine	[400]
Theobromine	Saliva	[119]
Theophylline	Plasma	[401]
Theophylline	Saliva	[119]
Theophilline	Serum	[120,402]
Theophylline	Standards	[73]
Thiocyanate	Plasma	[266]
Thiol-containing compounds	Standards	[216]
Thiopental	Plasma	[403]
Thiopental	Serum	[403]
Thiopental	Standards	[82]
Transferrin isoforms	Standards	[404]
Triamterine	Urine	[359]
TRH	Standards	[225]
Unidentified (several)	Cerebrospinal fluid	[232,405]
Unidentified (several)	Urine	[405]
Unidentified (several)	Saliva	[63,405]
Unidentified (several)	Serum	[405]
Unidentified (several)	Tears	[63,87,405–409]
Urea	Urine	[155]
Uric acid	Plasma	[153,154]
Uric acid	Synovial fluid	[410]
Uric acid	Urine	[153,293]
Vanillylmandelic acid	Urine	[255]
Vitamins	Standards	[411,412]
Vitamin A	Blood	[413]
Vitamin C	Plasma	[414]
Vitamin C	Urine	[414]
Xanthine	Urine	[415]

clinical laboratories performing mostly routine automated analysis. Nevertheless, chromatographic techniques still play major roles in many specialized laboratories. It is the purpose of this review to address some of the questions raised by clinical chemists and to place the role of CE in medical diagnosis into the proper perspective.

One major reason driving the development of capillary electrophoresis is to facilitate the separation of macromolecules in solution. Larger biopolymers are resistant to mass transfer and do not separate as efficiently by partition methods, such as chromatography. Non-partitioning methods like electrophoresis are more appropriate for this application. Another reason is that in pressure-driven systems, such as HPLC, the frictional forces of the mobile phase interacting at the walls of the tubing result in a radial gradient (laminar flow) throughout the tube. These frictional forces, together with the chromatographic packing, result in a substantial pressure drop across the column. As a consequence, there is a relatively low efficiency of separation, resulting in a low number of theoretical plates for a given separation. Furthermore, limitation in sensitivity has made HPLC a difficult tool to use, in many instances, for the detection of the widely diverse constituents present in biological fluids. These characteristics, among others, have contributed to the lack of use of HPLC in clinical laboratory settings. In contrast, an electrically driven system, such as CE, experiences no pressure drop, and the radial flow profile is uniform across the capillary. This system lends itself to high separation efficiency, resulting in a much greater number of theoretical plates/meter than other contemporary chromatographic methods. This characteristic, in conjuction with many other advantageous features described previously, make CE a very attractive technology for the routine characterization and quantification of substances of clinical chemistry interest.

Currently, identification and accurate quantification of endogenous and exogenous compounds present in biological fluids, cells and tissue extracts are the center of modern biomedical research. Undoubtedly, CE is well suited to the determination of low-molecular-mass substances. Furthermore, CE represents a fully instrumental format of electrophoresis, thus providing new possibilities for the determination of proteins and oligonucleotides. Several review articles on the subject of clinical and forensic capillary electrophoresis have appeared in the literature [47, 68, 102, 216, 229, 232, 235, 236, 249, 262, 264, 281, 300, 359, 374–376, 379, 386, 405, 409]. Most of these articles described the use of various modes of CE for the determination of primarily small-molecular-mass substances present in biological tissues, and cells. Only a few papers have described the determination of proteins and peptides. Nevertheless, proteins and peptides are substances of great interest to be analyzed by CE, because of the significant roles they play in cellular processes, providing essential information in physiological and pathophysiological conditions. Biological functions of proteins and peptides are numerous, varied, and important. Presently, there are more than 300 different soluble proteins that have been determined in plasma, but most of these are below the detection limit of electrophoretic methods. Some proteins are soluble in fluids within or outside the cells, others are insoluble and structural in nature. However, nearly all proteins of interest in clinical chemistry are soluble proteins such as hemoglobin, enzymes, and plasma proteins (with the exception of fibrinogen).

Plasma proteins and peptides move between blood and other extracellular fluids by active transport as well as by passive diffusion. When cells are damaged, soluble proteins and peptides normally in cells or on their surfaces may also be present in extracellular fluid. Most of the proteins and peptides of urine and spinal fluid are derived from plasma. A few peptides are generated as degradation products of structural proteins. Ultimately, a significant amount of peptides, and/or their bioproducts, can be present in urine or other biological fluids, such as saliva, sweat, and tears. Proteins and peptides are also present in amniotic fluid, synovial fluid, seminal fluid, bile, or in pathological accumulations of fluid in the peritoneal and pleural cavities, or other important secretions [3–14]. Unfortunately, in many instances, peptides are present in these biological fluids in minute amounts, making their detection and characterization difficult by conventional methods. Currently, for routine diagnosis the detection of proteins in biological fluids can be easily and automatically performed by immunoassays. Thus, an alternative way to observe these molecules is to use

an analyte preconcentration step or derivatization with a highly absorbing or fluorescence chromophore.

Clinical research and diagnosis are becoming ever more focused on measurements of various peptides in blood [3–14]. From such measurements conclusions are drawn concerning production and elimination rates, kinetics and dose–effect relationships in organs. However, measurements in the blood are clearly hampered by several problems which render the validity of such extrapolations questionable. Several disadvantages must be considered when attempting the analysis of peptides in blood. For example, they are almost always present with large concentrations of albumin and globulins. A significant amount of the peptides of interest tends to coprecipitate with larger molecular proteins when using the harsh deproteinization methods necessary to remove interfering compounds and/or to enrich the analytes.

There has long been a need for a rapid and reliable method to measure concentrations of multiple peptides in clinical samples. Sample preparation is generally recognized as a bottleneck within the total analytical procedure because of its laborious nature. Currently, enzyme immunoassays and radioimmunoassays are being used. However, in most cases, a specific antibody is needed for every peptide to be assayed. Although specific antibodies are available for many biological serum components, and commercially available kits allow automatic, multiple and rapid routine analysis, the method could be inevitably expensive. Capillary electrophoresis has now progressed to the point where it offers a true alternative to classical electrophoresis of serum proteins and peptides in a clinical laboratory. For the routine analysis of hemoglobin variants and lipoprotein analysis, CE has much to offer.

Commercial instruments dedicated to clinical chemistry applications are now beginning to appear. Recently, Beckman Instruments introduced a capillary electrophoresis instrument dedicated to the analysis of serum proteins. The Paragon CZE™ 2000 CE instrument can use several capillaries simultaneously for the analysis of at least six serum samples [382,383]. Preliminary tests have shown that it can provide the equivalent of one separation per minute with a total run time of 6 min/separation. This multicapillary system has been designed primarily for the routine and automated performance of two tests: serum protein (SPE) and immunofixation electrophoresis (IFE). Serum proteins are separated in fixed capillaries. The automation of IFE is achieved by immunosubstraction followed by capillary electrophoresis.

Immunosubstraction is used to sequentially remove specific immunoglobulin classes from the serum. The sample is exposed to five different sepharose supports, each containing an immunoglobulin-specific binder. Three of them are specific for the heavy chains of IgG, IgA, and IgM. Two are specific for the light chains κ or λ. After incubation and sedimentation, the treated samples and an untreated control sample are separated by capillary electrophoresis. Six electropherograms are generated per sample. The class and type of monoclonal component can be determined by overlaying electropherograms from before and after immunosubstraction [382,383]. The performance of the Paragon CZE™ 2000 CE instrument is comparable to the separation obtained by agarose electrophoresis. Traditional electrophoresis carried out in agarose or cellulose acetate membranes normally separates five classical zones termed albumin, α_1, α_2, β, and γ. Similarly, capillary electrophoresis separates albumin, α_1, α_2, β_1, β_2, and γ (see Fig. 4). It is expected that further improvements in clinical capillary electrophoresis instruments will capture the market and CE may become a useful tool for routine automated analysis of clinical samples.

A major disadvantage of traditional electrophoresis, carried out in agarose or cellulose acetate membranes, is the semi-quantitative nature of acquired data. Many errors are propagated during staining procedures due to the time of staining, temperature of the reaction, stain concentration, age of the reagent, solution used for destaining, time of destaining, and factors that contribute to create major differences in the stainability of proteins and peptides (i.e., the presence of specific 'stainable' groups in each protein or peptide). Conversely, a major advantage of CE over conventional electrophoresis for the determination of proteins and peptides is the generation of direct quantitative data. Proteins and peptides are normally monitored by CE at 230 nm or other low wavelengths utilizing the property of

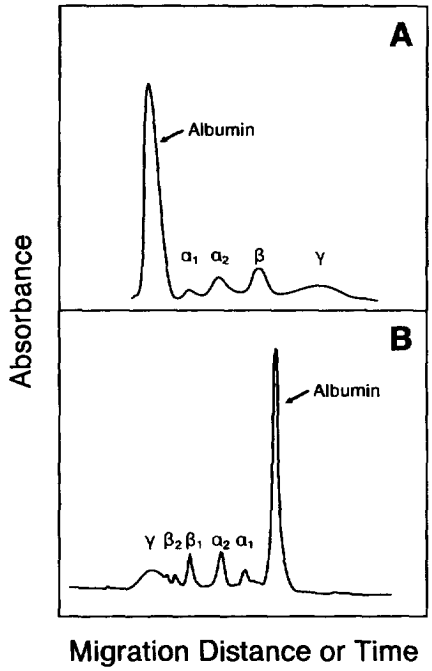

Fig. 4. Typical profile of a normal serum sample performed by (A) electrophoresis carried on cellulose acetate, stained with a dye, destained and then scanned for quantification of the resulting bands; or (B) capillary electrophoresis with direct quantification of major protein constituents of serum. Normally the profile resulting from cellulose acetate is expressed as migration distance in centimeters, and the profile resulting from capillary electrophoresis as migration time in minutes. (Figure modified from Ref. [385], reproduced with permission.)

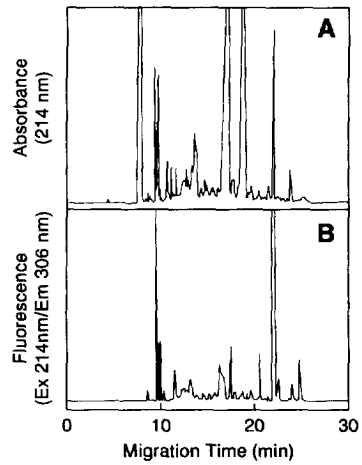

Fig. 5. Typical capillary electrophoresis profile of a normal urine sample monitored simultaneously at UV absorbance and fluorescence detection. (A) Electropherogram obtained at 214 nm; and (B) electropherogram obtained at excitation 214 nm/emission 306 nm.

intrinsic absorbance of the protein peptide bonds. An interesting improvement in the CE instrumentation is to have an instrument with two or more detectors to generate additional information [193,363]. For example, we have monitored the urinary constituents of a healthy person using simultaneously a UV–fluorescence detector (see Fig. 5 and Ref. [427]). The presence of intrinsic fluorescence for proteins and peptides that contain aromatic amino acids may prove to be of immeasurable value for the diagnosis and monitoring of a disease. Furthermore, other fluorescent analytes may also be revealed by the dual detector approach to aid the diagnosis of many disease states.

It is paradoxical that one of the noted advantages of capillary electrophoresis, namely the small volume of a conventional fused-silica capillary, also leads to a significant drawback of the technique. The total volume of the capillary is typically only 1–4 μl, and this results in a very limited loading capacity of analyte solutions. Optimal analyte resolution and separation efficiency are usually obtained when the sample injection is <2% of the total capillary volume. Ultimately, this results in poor concentration limits of detection and leads to a major problem when attempting to analyze relatively dilute analyte mixtures, particularly those derived from biological or environmental sources. In attempts to overcome the poor concentration limits of detection of capillary electrophoresis, we have developed a method for on-line sample pretreatment and analyte preconcentration. This method is currently being used by many investigators and several improvements have been reported (for reviews see Refs. [232,264]).

2.2. The use of on-line preconcentration techniques with non-specific chemical interactions

The finite volume of the CE capillary limits the focusing of large sample volumes. The volume injected into the capillary normally ranges between 1 and 30 nl. Nevertheless, in many instances, analysis of several microliters of sample solution is often

required to enable detection of analytes of interest. Therefore, it is becoming increasingly important to develop methods capable of analyzing compounds that are present in extremely low concentrations in certain diluted solutions or biological fluids. Two approaches have been used to extend the applicability of CE for analyzing dilute analyte solutions: (a) preconcentration of the analytes and (b) derivatization of the analytes. Some of the concentration-enhancement techniques include isotachophoresis, transient isotachophoresis, analyte stacking, and field amplification. Derivatization techniques include the use of a variety of different chromophores with laser-induced fluorescence detectors. In spite of all of the advantages of using these methods, there are still some disadvantages which prompted investigators to develop the technique of on-line preconcentration and on-line chemical reactions. Recently, we [232,264] reviewed general aspects of on-line preconcentration using the analyte concentrator–reactor chamber technique. It included the development of the concept and an analysis of the usefulness of the technique for the determination and characterization of substances for CE and CE coupled to mass spectrometry. In general, the analyte concentrator–microreactor chamber is a multipurpose device [53, 148, 162,187, 223–225, 230, 231, 233, 248, 263–265, 289, 293, 337, 339, 341, 343–350, 352, 354, 390, 416, 418–427] that has many unique features, such as: (a) minimizing sampling handling, (b) concentrating samples present in dilute solutions or present at low concentrations in biological fluids, (c) serving as a desalting system and as a cleanup procedure, (d) enabling the derivatization of samples to enhance detection sensitivity, (e) acting as an affinity or chromatographic column, (f) supplying a surface as a microreactor chamber to perform a variety of macromolecule interactions including peptide mapping, (g) presenting a practical approach to increasing the concentration limits of detection using UV absorbance detectors, and (h) obtaining additional information on the analyte when connected on-line with CE–mass spectrometry (CE–MS), or other detectors such as nuclear magnetic resonance (CE–NMR) or circular dichroism (CE–CD).

In the following section we outline a major advance in the detection of biomolecules present at ultra-low concentrations (e.g., fg/ml) in biological fluids using the CE on-line preconcentration technique through non-specific chemical interactions. The first published application of on-line preconcentration using an analyte concentrator containing a non-specific adsorptive phase was reported in 1993 [53,162,421]. The report described the use of a chamber containing reversed-phase HPLC packing material (uniformly sized solid microspheres coated with an adsorptive phase of the C_{18} type of chemistry) located at the capillary inlet for the concentration of, typically, amino acids, peptides to at least 6000 in molecular mass, or other small-molecular-mass compounds. The microsphere beads are held in place by two frits located at both ends of the cartridge (Fig. 6). Once the analytes are bound to the surface of the chemically bonded beads, they are freed of matrix components or chemical reagents. An appropriate buffer is placed in the CE capillary and the bound solids are then eluted from the beads. Substances are eluted in a small volume, usually 50–100 nl, of an organic solvent (or a mixture of them).

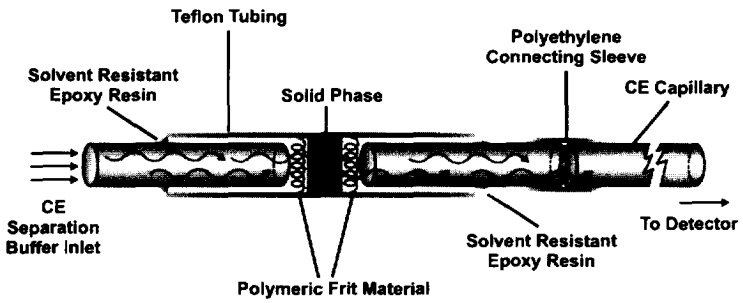

Fig. 6. Scheme of a solid-phase preconcentration-CE (spPC-CE) cartridge (not drawn to scale). (Figure modified from Ref. [232], reproduced with permission.)

The construction of the microcartridge presented some difficulties and thus some compromise of CE performance has been observed. This has been, in part, attributed to an increased back pressure that reduces hydrodynamic flow within the CE capillary [224,230,232,341,342]. In addition, as ion flow is impaired by the solid phase, the electroosmotic flow can be reduced leading to variable analyte migration times. The precise nature of these problems is not very well understood, but it is believed that the microbeads and/or the frits may be retaining material after every injection. The accumulation of the material ultimately leads to reduction of the electroosmotic flow and then to blockage of the system.

Some modifications to this original concept have been made in order to design and construct a new preconcentration-CE approach, that would decrease or remove all of the potential problems associated with the original design. The new concept is based on the development of an adsorptive phase made of an appropriate coated/impregnated membrane, installed for convenience in a Teflon cartridge system, thus replacing the beads and the frits of the original concept (Fig. 7). A major advantage of this new concept of on-line analyte concentrator, is the reduction in the volume of elution solvent required for the efficient removal of analytes from the membrane (for reviews see Refs. [232,264]). The technology that utilizes a coated/impregnated membrane as a preconcentration-CE cartridge has been termed mPC-CE [224,230,232,341,342]. In addition, when mPC-CE is used in conjuction with mass spectrometry it is termed mPC-CE–MS [224, 230, 232, 341, 342].

The analysis of clinical samples by capillary electrophoresis has benefited significantly from the use of the analyte concentrator on-line with a CE capillary or with a CE capillary coupled to mass spectrometry. The analyte concentrator enhances the sample detectability and desalts the usually high-salted biological fluid. CE–MS adds structural information to the analysis of clinical samples [224,232,264].

A major impact of CE–MS will be in the investigation of the metabolism of drugs. As shown in Fig. 8, mPC-CE–MS has been used for the analysis of drug metabolite from a patient who was receiving 0.5 mg/day of the neuroleptic drug haloperidol [224]. The current analysis by CE–MS of in vivo produced metabolites often results in incomplete information with regard to the metabolic transformation of the parent drug [221,232]. This is predominantly due to the dilute concentration of metabolites present in biological fluids, as well as to the limited loading capacity of the CE capillary. The mPC-CE–MS analysis of the urine sample, employing full-scan (125–450 Da) mass detection, revealed ions at m/z 376.4, 380.3, 354.3, and 356.3, all of which exhibited a ^{37}Cl isotope contribution. These responses could tentatively be assigned as protonated molecular ions corresponding to unmetabolized parent drug HAL, the known RHAL, and two pyridinium species, HP^+ and RHP^+, respectively. The detection of two pyridinium metabolites indicates that HAL undergoes metabolic conversion via active intermediate that is similar in nature to MPTP metabolism. MPTP is a known and powerful inducer of

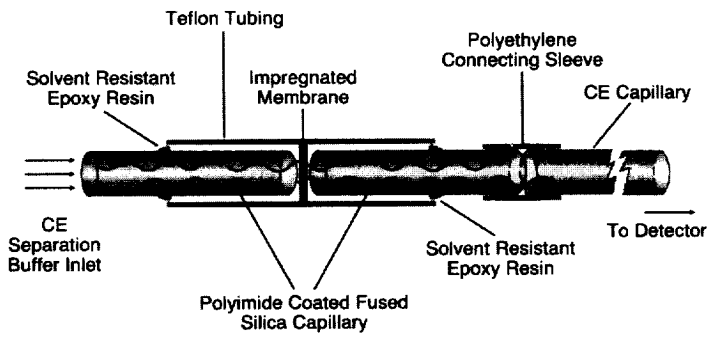

Fig. 7. Scheme of a membrane preconcentration-CE (mPC-CE) cartridge (not drawn to scale). (Figure modified from Ref. [232], reproduced with permission.)

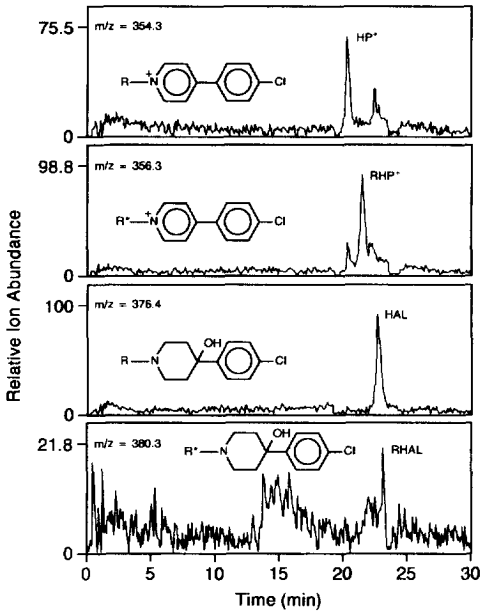

Fig. 8. mPC-CE–MS analysis of 10 μl of the supernatant solution of patient urine sample after protein removal by $ZnSO_4$ precipitation and centrifugation. Patient dose, 0.5 mg HAL/day. mPC-CE capillary, 80 cm×50 μm with a C_{18}-impregnated membrane installed in a metal cartridge at the capillary inlet. Sample clean-up was effected on-line by washing the mPC-CE capillary with CE separation buffer (10 μl). Analyte elution was carried out with methanol (80 nl) followed by separation buffer (50 m*M* NH_4OAC, 10% methanol, 1% acetic acid 100 nl). Separation voltage, 25 kV. A sheath liquid containing IPA–water–acetic acid (60:40:1, v/v/v) was delivered at 2 μl/min; ESI voltage, −3.4 kV; instrument resolution, ~1000; scan range, 125–450 Da at 2 s/decade. Detection by a PATRIC focal plane detector, 8% *m/z* window; RHAL detection of the ^{37}Cl isotope contribution of MH^+=378. (Figure modified from Ref. [232], reproduced with permission.)

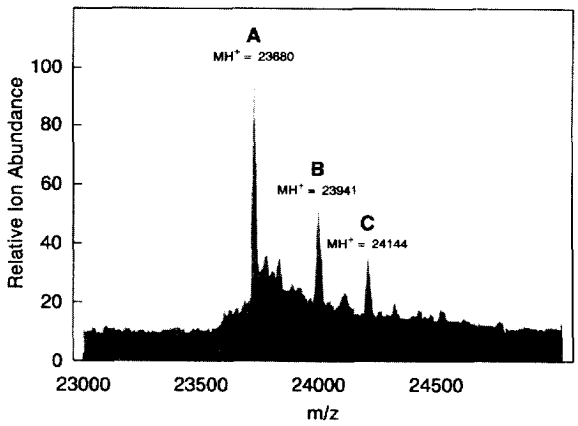

Fig. 9. Deconvoluted ESI-MS mass spectral data showing proteins that are suspected to be Bence–Jones proteins obtained from the mPC-CE–MS analysis of patient urine. One μl of patient urine was applied to an mPC-CE capillary by hydrodynamic pressure. mPC-CE capillary, 80 cm×50 μm, coated with Polybrene. An mPC-CE cartridge containing an SDB membrane was installed at the capillary inlet. Sample clean-up was effected by washing the mPC-CE capillary with CE separation buffer (1% formic acid), and analyte elution was with ~80 nl of a solution of methanol–water–acetic acid (80:20:1, v/v/v) followed by a wash with CE separation buffer (~100 μl); separation voltage, 30 kV with simultaneous application of low pressure (0.5 p.s.i.). Sheath liquid, methanol–water–acetic acid (25:75:1 v/v/v) delivered at a flow-rate of 2 μl/min. Voltage applied to an ESI interface (Finnigan MAT), 3.5 kV. Instrument resolution, 1000; scan range 700–2000 Da at 2 s/decade. Analyte detection was with a PATRIC focal plane detector (Finnigan, MAT), 8% *m/z* window. (Figure modified from Refs. [224,232], reproduced with permission.)

Parkinsonian-like symptoms in both animals and humans after metabolism.

Similarly, in Fig. 9, we describe the use of mPC-CE–MS in the analysis of Bence–Jones protein in urine samples from a patient suffering from multiple myeloma [224,232]. Direct analysis of a Bence–Jones patient urine as a confirmatory test, would be beneficial in terms of reduced time and cost as compared to existing assays. The deconvoluted spectrum showed a family of proteins, similar in mass to Bence–Jones protein. Furthermore, the mass difference between ions B and C is ~203 a.m.u. and indicates the heterogeneity of a glycoprotein (a mass difference of ~203 representing the possible presence of a glucosamine residue). This example not only demonstrates the applicability of mPC-CE–MS to clinical assay but shows the high level of information that can be achieved by this technology.

2.3. The use of on-line preconcentration techniques with high-affinity chemical interactions

Another approach to concentrate samples is using solid supports containing immobilized ligands that have specific interactions for their corresponding counterpart biomolecules. Such substances are best represented by antigen–antibodies and lectin–carbohydrates which have high affinity for each other. The use of these immobilized affinity reagents is of great

interest for the characterization of substances present in complex matrices, such as biological fluids.

Immobilized antibodies and antigens have long been used as affinity reagents. The method suffers from low binding capacity and specificity limited by the quality of the antibodies. Harsh elutions are often necessary, causing irreversible changes in the antigen or antibody. As a result, for some time antibody immunoadsorbents never achieved the widespread use originally expected. The introduction of monoclonal antibodies, however, began a new era – allowing columns with any desired specificity and high binding capacity, requiring only mild elution conditions. The nearly infinite supply of reagents made commercial applications feasible. Besides, many of the difficulties associated with generating monoclonal antibodies by B-cell immortalization can be overcome by engineering and expressing antibody fragments in *E. coli*, using phage display [428]. Furthermore, recombinant Protein A is now commercially available. This protein has been modified to favor an optimal orientation of the ligand when coupled to a solid support. The resulting Protein A has an increased binding capacity suitable for an optimal binding to monoclonal antibodies [429].

Today, affinity columns have found their greatest use in purifying (and characterizing) high-priced biologicals isolated from tissue and recombinant cell culture. On-line immunoaffinity isotachophoresis [430] and on-line immunoaffinity chromatography [431] have previously been described. Furthermore, a technique termed affinophoresis [439] is gaining popularity for the separation of various substances having affinity for each other.

The affinity purification (or characterization) designer must choose antibodies carefully. When covalently bonded to solid supports, polyclonal antibodies often lose antigen-binding capacity. Monoclonal antibodies lack the diversity of structures found in polyclonal antisera. Thus, the antibody with the best binding properties in solution may not necessarily retain its affinity when coupled. It is known that the antigen-binding efficiency/to a solid support varied widely when determining the amount of bound antigen/mg of bound antibody. In addition, the chemistry used for the immobilization of either

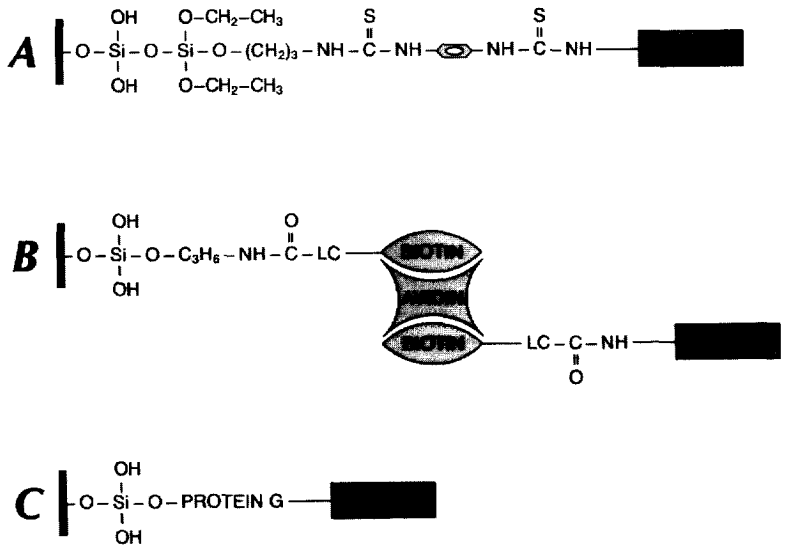

Fig. 10. Schematic representation of the the various chemical reactions available to covalently immobilize an antibody to the surface of a glass bead or column. (A) Glass beads were silylated with 3-aminopropyltriethoxysilane and then reacted with 1,4-phenylene diisothiocyanate before being conjugated to a monoclonal antibody. For details see Ref. [293]. (B) An antibody can be immobilized using the biotin–avidin complex. LC refers to a long-chain spacer chemical unit necessary to facilitate an optimal interaction between the immobilized antibody and the correspondent antigen. For details see Refs. [345,352], as used for the immobilization of trypsin to a solid support. (C) Immunoglobulin G was immobilized using covalently linked protein G. For details see Ref. [265].

the antigen or the antibody (or any other ligand) will significantly affect the efficiency of the system. Some investigators have suggested that coupling an antibody via its Fc-portion to immobilized Protein A (or Protein G) would affect binding capacity less than covalent linkage. In any case, the choice of selecting a monoclonal antibody with high-affinity properties should be based on the antibody's performance when linked to the solid support, not in solution. At present, three approaches have been used for immobilizing proteins to the CE microreactor (see Fig. 10).

For the purpose of clarification, the terminology of affinity capillary electrophoresis should be subdivided at least into two categories: (a) the interaction of an antigen with an antibody or a fraction of an antibody (or other ligands with high affinity for each other) in a test tube (off-line) and then separation of the reaction components by capillary electrophoresis [77,104,128,132,150,151,211,220,275, 276,299,340,361,364,394], and (b) the interaction of an antigen with an immobilized antibody (or other ligands of high affinity for each other) in a micro-

Table 2
Determination of drugs, metabolic intermediates, and biopolymers in biological fluids using affinity capillary electrophoresis (or affinity microchip electrophoresis)[a]

Analyte	Specimen	Ref.
Amyloid P component	Serum	[77]
Arylsulfonamides	Standards	[104]
Atrazine	Standards	[106]
Carbonic anhydrase	Standards	[128]
Cortisol	Serum	[150,151]
Cortisol	Standards	[152]
Glucose-6-phosphate dehydrogenase	Standards	[211]
Glycopeptides	Urine	[416]
Growth hormone	Standards	[220]
HSA–antiHSA	Standards	[417]
Immunoglobulin E (IgE)	Serum	[263]
Insulin	Standards	[132,265]
Lactobionic acid	Standards	[275]
Leucine amino peptidase	Standards	[74]
Leucine-*p*-nitroanilide	Standards	[311]
Methamphetamine	Urine	[293]
Monosaccharides	Standards	[299]
Nitrophenyl-β-galactoside	Standards	[312]
Nitrosoprocainamide	Standards	[364]
Nucleic acid mapping	Standards	[187]
Peptides (derivatized)	Standards	[354]
Peptide mapping	Standards	[345–352,391]
Porphyrins	Standards	[361]
Procainamide	Standards	[364]
Proteins	Standards	[390]
C-Reactive protein	Standards	[394]
Triazine herbicides	Standards	[248]

[a] Affinity capillary electrophoresis (or affinity microchip electrophoresis) refers to the interaction (and separation by CE) of an antigen/antibody, sugar/lectin, substrate/enzyme, ligand/macromolecule, or any other ligand with high affinity for another biomolecule. The specific binding can be carried out outside the capillary or channel (off-line) and the resulting components of the reaction can then be separated by capillary electrophoresis. Alternatively, the specific binding can be carried out within the capillary or channel (on-line) in which all the reagents are part of the separation buffer, and the enzyme, for example, is added to the capillary to react with the substrate to form the corresponding product and then separated by capillary electrophoresis. In addition, a more convenient way to carry an on-line affinity capillary electrophoresis is to immobilize the antibody, enzyme, receptor or the corresponding ligands in order to perform the reaction on the microreactor or channel and then separate the bound substance and/or the resulting products by capillary electrophoresis.

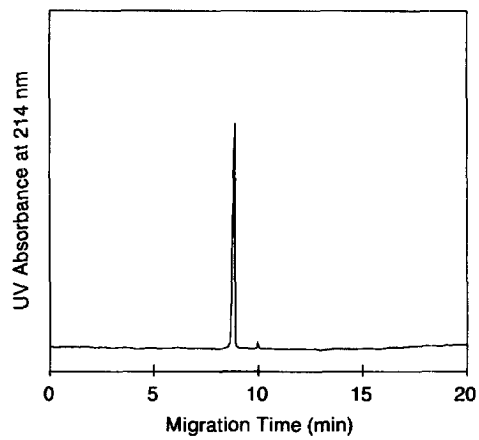

Fig. 11. Electropherogram of methamphetamine separated from a complex matrix. A spPC-CE cartridge made of controlled porous glass contained an immobilized antibody directed against methamphetamine. The cartridge was approximately 5 mm×100 μm I.D., and it was located 7 cm from the inlet of the capillary. The separation capillary column consisted of a 60 cm×75 μm fused-silica capillary. A urine sample spiked with methamphetamine was applied to the capillary by using a slow-speed controlled-vacuum pump and then washed with separation buffer. The bound methamphetamine was eluted from the cartridge with 50 nl of 50 m*M* Hepes/NaOH buffer, pH 7.2, containing 2 *M* $MgCl_2$ and 25% ethyleneglycol. The separation was carried out in 50 m*M* sodium tetraborate buffer, pH 8.3. Separation was accomplished at approximately 100 V/cm for the first 10 min and then at 300 V/cm for the rest of the run.

reactor (on-line) and then elution and separation of the antigen by capillary electrophoresis [232,248,263–265,293,312,354,390]. This section of the paper will address only the interaction of an antigen with an antibody (or other ligands) using the microreactor technique that we termed on-line immunoaffinity capillary electrophoresis (on-line IACE). A summary of the various reports using affinity capillary electrophoresis is presented in Table 2.

The first published application of on-line IACE was reported in 1991 [293]. The report described the use of an immobilized antibody for the determination of methamphetamine in urine samples. The antigen–antibody reaction was allowed to take place in a microreactor of approximately 5 mm in length and located near the injection side of the capillary. The fabrication of the microreactor was made using controlled-porous glass to which the purified monoclonal antibody was immobilized [293]. The glass beads were held in place by two porous frit structures. Once the methamphetamine was specifically bound to the immobilized antibody, fresh buffer was applied to the column to eliminate the excess of salt and other nonrelevant matrix components present in urine. The capillary column was then equilibrated with fresh buffer and the bound methamphetamine was eluted from the complex antigen–antibody using nanoliter quantities of an acidic buffer [293]. Improvements in the elution procedure have been made, and a more effective buffer is currently used [263]. Fifty nanoliters of Hepes/NaOH buffer, pH 7.2, containing 2 *M* $MgCl_2$ and 25% ethylene glycol, although a viscous solution, seems to be an optimized elution system. As shown in Fig. 11, a single peak of the eluted material was obtained under these experimental conditions.

Even though non-affinity microreactors adsorb many more substances than an affinity microreactor, the preconcentration step is carried out with low selectivity. A big advantage of an affinity microreactor is the high selectivity of analyte preconcentration. In principle, one substance or one group of closely related substances should be captured by the immobilized antibody, metal, lectin, or any appropriate affinity ligand. If the affinity constant and the number of affinity ligands per surface area of an affinity cartridge is larger than a non-affinity car-

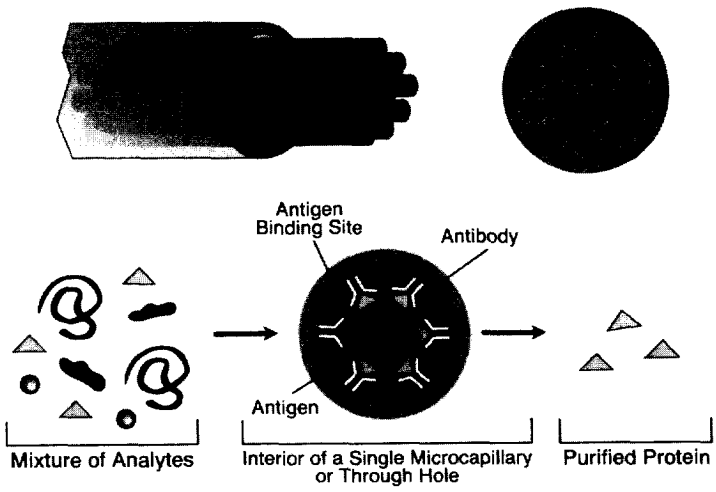

Fig. 12. Scheme of an analyte concentrator–microreactor made of multiple microcapillaries associated in bundle or from a piece of solid glass rod that has been drilled with a laser beam to produce a plurality of small-diameter rod passages or through holes. An antibody directed against immunoglobulin E was immobilized to the surface of every microcapillary or through hole. A serum sample containing high titers of IgE was applied to the capillary and then washed with separation buffer. The bound IgE was eluted from the cartridge with 100 nl of 50 m*M* Hepes/NaOH buffer, pH 7.2, containing 2 *M* $MgCl_2$ and 25% ethylene glycol. The separation was carried out in 50 m*M* sodium tetraborate buffer, pH 8.3. A major improvement of this design is the absence of frits allowing an uninterrupted uniform electroosmotic flow-rate and several re-uses of the cartridge. For details see Ref. [263]. (Figure modified from Refs. [232,263], reproduced with permission.)

tridge, analyte concentration is greater. Efficiency of the binding of ligands per surface area is more important than the length of the cartridge.

The main attractive features of the affinity microreactor conducted on a solid support on-line with CE are: (a) the accomplishment of an affinity reaction in a short period of time, (b) the consumption of a smaller amount of reagent, (c) the separation of the main compound from nonrelevant matrix components, and (d) the re-usability of the cartridge. These features prompted several investigators to explore other principles of affinity.

A second approach came from Cai and El Rassi [248,390]. They used different surface-bound functions for the affinity capture and concentration of small molecules and proteins. For the small molecules, prometon and prometryne, a surface-bound oligomeric octadecyl function was used to capture these herbicides. Acetonitrile was used as the desorbing agent. In the case of proteins, iminodiacetic acid metal with chelating properties for the selective on-line preconcentration of dilute protein samples was used. Only proteins with affinity for the chelated metal were concentrated. In this particular report, carbonic anhydrase was used as model protein to illustrate the principle of specific capture and concentration using Zn(II) as the metalic ligand. As a desorbing agent, to remove the bound protein from complex, EDTA was used since it forms a stronger complex with metals thus competing with the binding.

Unfortunately, in both examples, instead of using an analyte concentrator containing microbeads or membranes similar to the one described in Fig. 6 or Fig. 7, a piece of capillary (20 cm×50 μm I.D.) was used as the affinity capture microreactor in tandem with a 60-cm separation capillary. The efficiency of surface area was low since only a factor ranging from 10- to 35-fold enhancement for the concentration of the herbicides and 25-fold for proteins was obtained [248,390].

A third approach came from Cole and Kennedy [265] These authors applied a selective preconcentration step for CE using protein G immunoaffinity capillary chromatography. However, in this case the immunoaffinity was carried out off-line in a larger capillary. In addition, a required collection of desorbed fractions was necessary before introduction into the CE capillary.

Recent reports [232,263,264] utilize a cartridge made of multiple capillary bundles or a piece of solid glass predrilled with a laser beam to form multiple through holes (Fig. 12). There are no beads nor frits in this model allowing for a more uniform electroosmotic flow. Specific antibodies directed against immunoglobulin E (IgE) were covalently bound to the surface of every microcapillary. Serum was applied directly into the capillary. After binding, the capillary was rinsed with buffer, and IgE was eluted with an optimized buffer (see Fig. 13).

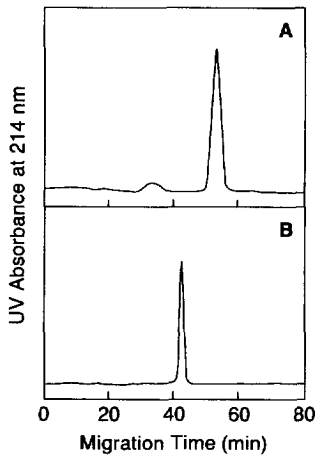

Fig. 13. Immunoaffinity microreactor–electropherograms of serum IgE. The cartridge was approximately 5 mm×500 μm I.D. when fabricated as a bundle, or 150 μm I.D. when fabricated from a solid rod. The location was about 7 cm from the inlet of the capillary. The separation capillary column consisted of a 65 cm×75 μm fused-silica capillary. Approximately 20 μl of a serum sample containing high titers of IgE were applied to the capillary (containing the cartridge with immobilized antibody against IgE) followed by a clean-up procedure consisting of separation buffer to remove salts and other serum constituents. The bound IgE was eluted from the cartridge with 100 nl of 50 m*M* Hepes/NaOH buffer, pH 7.2, containing 2 *M* $MgCl_2$ and 25% ethylene glycol. The separation was carried out in 50 m*M* sodium tetraborate buffer, pH 8.3, at approximately 300 V/cm. The separated components were monitored at 214 nm. A typical electropherogram was obtained using the cartridge fabricated of multiple capillaries associated in bundles (panel A), or using the cartridge fabricated from a solid glass rod having a plurality of small-diameter rod passages or through holes (panel B). For details see Ref. [263]. (Figure modified from Refs. [232,263], reproduced with permission.)

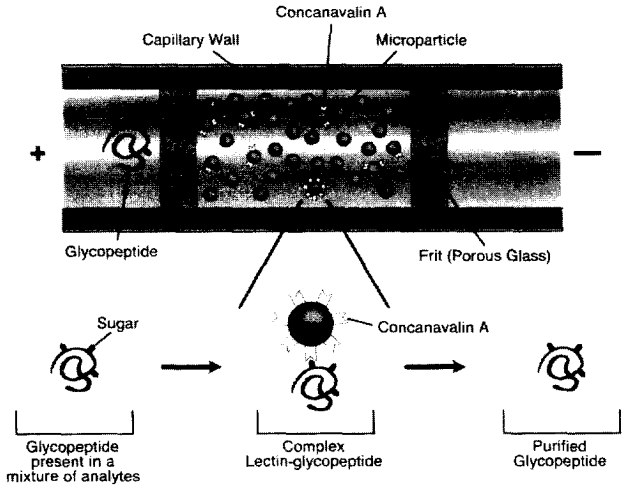

Fig. 14. Schematic of a microreactor containing an immobilized lectin. Controlled-porous glass beads were activated, conjugated with concanavalin A and installed inside a portion of a fused-silica capillary. For details see Ref. [416]. (Figure modified from Ref. [416], reproduced with permission.)

Another approach using the concept of on-line affinity capillary electrophoresis is the use of immobilized lectins and enzymes on the surface of microbeads or directly on the inner surface of the capillary. Lectins are carbohydrate-binding proteins of non-immunoglobulin nature. The use of lectins from plant and animal origin for studying animal cell glycoconjugates has a long and productive history [416,432–436]. By definition, most lectins are multivalent proteins having multiple subunits, and the interaction of a conjugate with a lectin is governed by the binding specificity and affinity of each subunit for a glycoconjugate [416,432–436]. Today, many different lectins are commercially available and used for purification of glycoproteins, glycopeptides, and glycolipids [416,432–436] by lectin affinity chromatography.

Many lectins with well-characterized sugar-binding activities could be immobilized in any of the various models of the analyte concentrator–microreactor cartridge and employed for affinity interaction. In fact, we have immobilized concanavalin A into controlled-porous glass beads and fabricated an analyte concentrator cartridge (see Fig. 14 and Ref. [416]). In order to differentiate this affinity method from on-line IACE we have termed it on-line lectin affinity capillary electrophoresis (on-line LACE). As shown in Fig. 15, we describe the use of on-line LACE in the analysis of suspected glycopeptides present in the urine obtained from a pool of six normal individuals and from a patient suffering of pancreatic cancer and complications to the liver and other organs.

Enzymes immobilized on the surface of microbeads or on the inner surface of the capillary, to perform on-line enzyme affinity capillary electrophoresis (or on-line EACE), have also been used. The interaction of the immobilized enzyme with the substrate enables to quantitate chemical conversion of substrate into product, as well as to evaluate the binding and kinetic constants of an enzymatic reaction [312].

On-line IACE, LACE, and EACE show great promise for rapid processing of clinical samples. Furthermore, these techniques enable to detect minute quantities of analytes present in complex biological matrices with great selectivity.

3. Future directions of CE in the clinical laboratory

A clear trend in current analytical practice is the handling of ever smaller amounts of sample. For

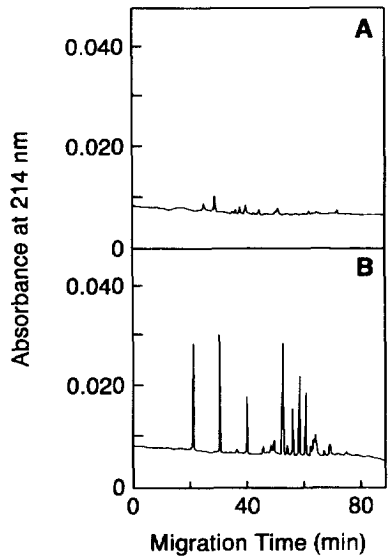

Fig. 15. Immunoaffinity microreactor-electropherograms of urine glycopeptides. Approximately 50 μl of a urine sample containing high levels of glycopeptides were applied to the capillary (containing the cartridge with immobilized concanavalin A) followed by a clean-up procedure consisting of separation buffer to remove salts and other urine constituents. The bound glycopeptides were eluted from the cartridge with 100 nl of 50 m*M* Tris–HCl buffer, pH 7.4, containing 0.3 *M* α-D-mannopyranoside and 25% ethylene glycol. The separation was carried out in 50 m*M* sodium tetraborate buffer, pH 8.3. A typical electropherogram was obtained from a freshly collected clean-catch urine specimen obtained from a pool of six normal individuals (panel A), or from a urine specimen obtained from a patient suffering of pancreatic cancer and complications to the liver and other organs (panel B). For details see Ref. [416]. (Figure modified from Ref. [416], reproduced with permission.)

example, the determination of components in single cells is an active area under development [54,60,61,134,274,360,388].

Another exciting technology currently under development is the operation of capillary electrophoresis on microchips [437,438,449]. Excellent separation has already been achieved. Critical to the success of chip technology is the utilization of electroosmotic rather than pressurized bulk flow, the former being easy to control and manipulate. While much more needs to be done on the development of CE in chips, it is clear that such systems could be inexpensive to manufacture, would allow analysis in difficult places, and would achieve high-efficiency, high-speed separations.

One additional area is presently being investigated to make capillary electrophoresis applicable to a completely automated high-throughput technology. Conventional high-throughput assay systems (for example, immunoassays, enzyme assays, DNA reverse dot blots) usually involve highly repetitive binding measurements in a microtiter format. This requires preparation of large quantities of reagents, followed by deposition into individual microtiter wells. Because of the effort required to prepare these assays, the number of tests is usually limited, and customarily performed in the 96-well format. Both academic and commercial laboratories have put substantial effort into automating, miniaturizing and expanding the multiple assay format.

The possibility of using capillary electrophoresis as a high-throughput technology may have several advantages over currently used technology: (a) utilization of small amounts of reagents and solvents; (b) separation of components, permitting the identification of substances of interest; (c) generation of structural information if coupled to a mass spectrometer; and (d) the possibility of using capillary array electrophoresis, in which many capillaries are run and analyzed simultaneously [40,42]. These

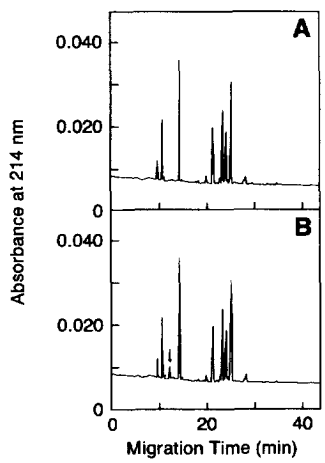

Fig. 16. Typical electropherogram obtained from rat blood microdialysate. (A) Electropherogram of the components present in the microdialysate under baseline conditions. (B) Electropherogram of the components present in the microdialysate after a single injection of amphetamine. The presence of amphetamine is indicated by an arrow. (Figure modified from Ref. [450], reproduced with permission.)

improvements are expected to provide shorter analysis times, higher sample throughput, increased automation, and improved analysis software.

Another potential use of capillary electrophoresis to aid diagnosis is in conjuction with perfusion techniques, such as microdialysis. In the last decade, the use of semipermeable membranes in sample-preparation procedures has gained popularity, because of the possibility of removing macromolecular sample components in an automated way [440–444]. In vivo microdialysis has been used to collect samples containing various putative small-molecular-mass substances from the interstitial fluid of organs, or from the blood, thus allowing their analysis and characterization [440–444].

An important yet often neglected parameter in dialysis is the membrane pore size, expressed as the molecular mass cut-off value, which is defined as the molecular mass of the smallest solute that is more than 90% retained. In order to obtain an optimum separation between macromolecular matrix-compo-

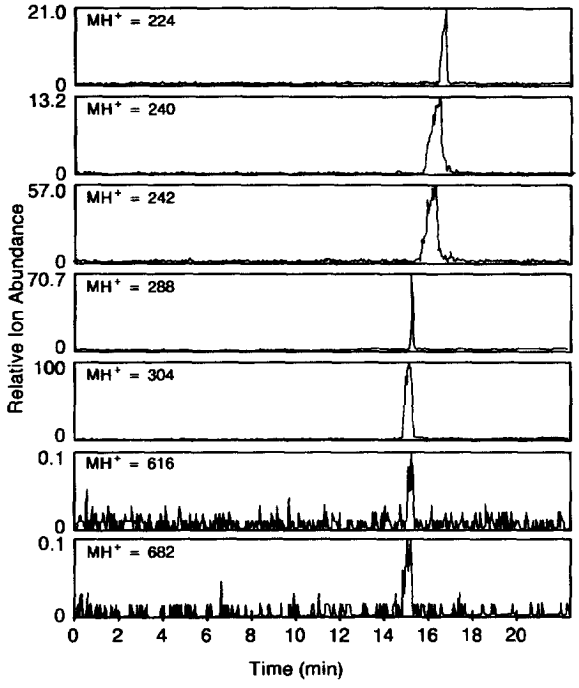

Fig. 17. mPC-CE–MS analysis of rat blood microdialysate sample. These data were acquired using a styrene–divinyl benzene (SDB) membrane cartridge, preconditioned with methanol and CE separation buffer (0.1% formic acid). The CE capillary was 75 cm×50 μm I.D., polybrene coated. Separation voltage, −10 kV (2 μA). Ten μl of sample were loaded onto the cartridge. The mPC-CE was washed with ~7 μl of CE separation buffer. Analytes were then eluted from the membrane with 80% MeOH in water (~140 nl) and followed by ~140 nl of CE separation buffer. The mass spectrometer was a Finnigan MAT 900 with a focal plane detector, which was used with a 4% mass window. The electrospray source was a Finnigan design with a heated capillary (at 250°C) as the isolation between atmosphere and the first stage of vacuum. The scan range was 200–2000 at 2 s/decade. Resolution was ~1000, electrospray voltage was 3.6 kV and accelerating voltage was 4.7 kV. A sheath liquid of IPA–water–acetic acid (60:40:1) was delivered coaxially to the CE capillary at 3 μl/min. Further studies are in progress to identify these compounds.

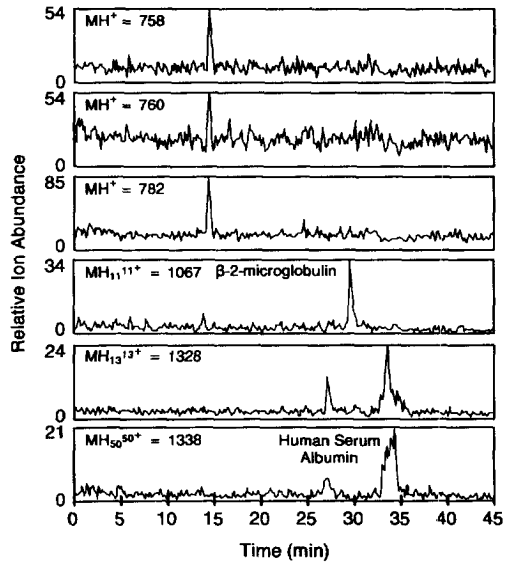

Fig. 18. mPC-CE–MS analysis of human aqueous humor sample. These data were acquired using a C_8 silica-based membrane cartridge, preconditioned with methanol and CE separation buffer (0.1% formic acid). The CE capillary was 70 cm×50 μm I.D, polybrene coated. Separation voltage, −10 kV (2 μA). Seven μl of sample were loaded onto the cartridge. The mPC-CE was washed with ~7 μl of CE separation buffer. Analytes were then eluted from the membrane with 80% MeOH in water (~140 nl) and followed by ~140 nl of CE separation buffer. The mass spectrometer was a Finnigan MAT 900 with a focal plane detector, which was used with a 4% mass window. The electrospray source was a Finnigan design with a heated capillary (at 250°C) as the isolation between atmosphere and the first stage of vacuum. The scan range was 700–2500 at 2 s/decade. Resolution was ~1500, electrospray voltage was 3.6 kV and accelerating voltage was 4.7 kV. A sheath liquid of IPA–water–acetic acid (60:40:1) was delivered coaxially to the CE capillary at 3 μl/min. Further studies are in progress to identify these compounds.

nents and the analytes of interest, the pore size has to be carefully optimized to find an acceptable compromise between a high-membrane flux of the analytes and sufficient removal of the interfering compounds. The membrane material is not supposed to be involved in the separation process, but electrostatic as well as hydrophobic interactions with analytes have been reported [444]. The most frequently used materials are reconstituted cellulose and cellulose acetate, and the nature of the interactions between these polymers and small solutes, in particular peptides, is not very well understood.

Although microdialysis techniques have been used since 1972, after being introduced by Delgado et al. [445], more recently microdialysis has been coupled to capillary electrophoresis to determine analytes present in microdialysates of brain tissue, blood vessels, and others (see Refs. [54,55,145,212–214,304,446–448]). We have just embarked upon analyzing and characterizing peptides and other small-molecular-mass compounds present in microdialysis samples, by CE and CE–MS. Fig. 16 shows a typical CE profile of substances present in a microdialysate. Fig. 17 shows a CE–MS profile of a microdialysate. It is quite feasible that the combination of all three techniques (microdialysis, capillary electrophoresis, and mass spectrometry), would provide useful information to the medical team involved in the diagnosis and treatment of disease or intoxication. Similarly, we have used CE–MS for the characterization of substances present in aqueous humor (Fig. 18).

4. Conclusions

Currently, the techniques of on-line preconcentration for CE continue to be refined; nevertheless, several points of special interest have emerged. (a) The reproducibility of the separation profile and quantitative determination of an analyte depend of the fabrication of the cartridge, including the quality of the frits. Under optimal conditions, analyte migration time and peak area are under 2% R.S.D. (b) The time of regeneration of the cartridge depends of the length of the packaging material (e.g., 1–5 mm) and the length and I.D. of the separation column. Using pressure or vacuum, the regeneration time typically varies from 2 to 5 min. (c) A good column containing beads can be used at least 10 times; a good column containing membranes can be used at least 20 times. If there is enough porosity in the membranes or frits to allow normal electroosmotic flow, and if the packing of the beads is carried out by vibration rather than by high pressure, one can obtain several runs per cartridge. In some instances, the use of lipemic serum or other complex biological matrices may limit the use of the cartridge. Future commercialization of the analyte concentrator–microreactor cartridges may solve many of the problems faced today.

In summary, capillary electrophoresis is slowly gaining popularity as a tool with great potential for routine automated performance of samples in a clinical laboratory. In particular, the CE determination of minute amounts of analytes present in tissue biopsies [159] and single cells [54,60,61,134,274,360,388] offers new and interesting diagnostic possibilities.

5. List of abbreviations

AMP	amperometry
CD	circular dichroism
CE	capillary electrophoresis
CON	conductivity
ESI	electrospray ionization
FITC	fluorescein isothiocyanate
FLU	fluorometry
HAL	haloperidol
Hepes	(*N*-[2-hydroxyethylpiperazine-*N*'-[2-ethane-sulfonic acid])
HP^+	4-(4-chlorophenyl)-1-[4-(4-fluorophenyl)-4-oxobutyl]-pyridinium
HPLC	high-performance liquid chromatography
IPA	isopropanol
LOD	limits of detection
LC	long-chain spacer
MH^+	pseudo-molecular ion
MPTP	*N*-methyl-4-phenyl-1,2,3,6-tetrahydro-pyridine
MS	mass spectrometry
NH_4OAc	ammonium acetate
NMR	nuclear magnetic resonance

RA	radiometric
RHAL	4-(4-chorophenyl)-1-[4-(4-fluoro-phenyl)-4-hydroxybutyl]-4-piperidinol
RHP	reduced haloperidol
SBD	styrene–divinyl benzene copolymer
SFC	supercritical fluid chromatography
SGE	slab-gel electrophoresis
TLC	thin-layer chromatography
UV–Vis	ultraviolet–visible

References

[1] P. Schulz, Med. Hypotheses 42 (1994) 57–62.
[2] P. Schulz, R. Knabe, Med. Hypotheses 42 (1994) 63–68.
[3] R. Gräsbeck, T. Alstron (Eds.), Reference Values in Laboratory Medicine. The Current State of the Art, John Wiley & Sons. New York, 1981.
[4] B.F. Trump, A. Laufer, R.T. Jones (Eds.), Cellular Pathobiology of Human Disease, Gustav Fisher, New York, 1983.
[5] D.S. Latchman, New Engl. J. Med. 334 (1996) 28–33.
[6] N.W. Tietz (Ed.), Fundamentals of Clinical Chemistry, 3rd ed., W.B. Saunders Company, Philadelphia, PA, 1987.
[7] A.H. Gowenlock, J.R. McMurray, D.M. McLauchlan (Eds.), Varley's Practical Clinical Biochemistry, CRC Press, Boca Raton, FL, 1988.
[8] J.B. Henry, Clinical Diagnosis Management By Laboratory Methods, W.B. Saunders Company, Philadelphia, PA, 1991.
[9] W.J. Marshall, Clinical Chemistry, 2nd ed., Gower Medical Publishing, London, UK, 1992.
[10] M.L. Bishop, J.L. Duben-Engelkirk, E.P. Fody (Eds.), Clinical Chemistry. Principles, Procedures, Correlations, 3rd ed., Lippincott-Raven Publisher, Philadelphia, PA, 1996.
[11] J.P. Felber, Adv. Clin. Chem. 20 (1978) 129–179.
[12] R.A. Hartwick, P.R. Brown, Adv. Clin. Chem. 21 (1980) 25–99.
[13] P.M.S. Clark, L.J. Kricka, Adv. Clin. Chem. 22 (1981) 247–296.
[14] I.N. Papadoyannis, HPLC in Clinical Chemistry, Chromatographic Science Series, vol. 54, Marcel Dekker, New York, 1990.
[15] J. Chamberlain, Analysis of Drugs in Biological Fluids, CRC Press, Boca Raton, FL, 1985.
[16] D.G. Deutsch (Ed.), Analytical Aspects of Drug Testing, John Wiley & Sons, New York, 1989.
[17] J.H. Mendelson, N.K. Mello, New Engl. J. Med. 334 (1996) 965–972.
[18] S. Hjertén, Chromatogr. Rev. 9 (1967) 122–219.
[19] R. Virtanen, Acta Polytech. Scand. Ch. 123 (1974) 1–67.
[20] F.E.P. Mikkers, F.M. Everaerts, T.P.E.M. Verheggen, J. Chromatogr. 169 (1979) 11–20.
[21] J.W. Jorgenson, K.D. Lukacs, Anal. Chem. 53 (1981) 1298–1302.
[22] P.D. Grossman, J.C. Colburn (Eds.), Capillary Electrophoresis: Theory and Practice, Academic Press, San Diego, CA, 1992.
[23] S.F.Y. Li, Capillary Electrophoresis: Principles, Practice and Applications, Elsevier, Amsterdam, 1992.
[24] J. Vindevogel, P. Sandra, Introduction to Micellar Electrokinetic Chromatography, Hüthig Buch Verlag, Heidelberg, 1992.
[25] J.E. Wiktorowicz (Ed.), Capillary Electrophoresis, Academic Press, New York, 1992.
[26] F. Foret, L. Krivánková, P. Bocek, Capillary Zone Electrophoresis, VCH, Weinheim, 1993.
[27] N.A. Guzman (Ed.), Capillary Electrophoresis Technology, Marcel Dekker, New York, 1993.
[28] P. Jandik, G. Bonn, Capillary Electrophoresis of Small Molecules and Ions, VCH Publishers, Cambridge, 1993.
[29] R. Weinberger, Practical Capillary Electrophoresis, Academic Press, San Diego, CA, 1993.
[30] P. Camilleri (Ed.), Capillary Electrophoresis: Theory and Practice, CRC Press, Boca Raton, FL, 1993.
[31] R. Kuhn, Hoffstetter-Kuhn, Capillary Electrophoresis: Principles and Practice, Springer-Verlag, Berlin, 1993.
[32] J.P. Landers (Ed.), Handbook of Capillary Electrophoresis, CRC Press, Boca Raton, FL, 1994.
[33] H. Engelhard, W. Beck, T. Schmitt, Kapillarelektrophorese. Methoden und Möglichkeiten, Vieweg Publishing, Wiesbaden, 1994.
[34] K.D. Altria, Capillary Electrophoresis Guidebook, Chapman & Hall, London, 1995.
[35] D.R. Baker, Capillary Electrophoresis John Wiley & Sons, New York, 1995.
[36] P.G. Righetti (Ed.), Capillary Electrophoresis in Analytical Biotechnology, CRC Press, Boca Raton, FL, 1996.
[37] A. Schlenck, S. Visvikis, M. O'Kane, G. Siest, Biomed. Chromatogr. 10 (1996) 48–50.
[38] S. Kim, H.J. Yoo, J.H. Hahn, Anal. Chem. 68 (1996) 936–939.
[39] L.M. Smith, Nature 349 (1991) 812–813.
[40] R.A. Mathies, X.C. Huang, Nature 359 (1992) 167–169.
[41] J.A. Luckey, L.M. Smith, Anal. Chem. 65 (1993) 2841–2850.
[42] J. Bashkin, M. Bartosiewicz, D. Roach, J. Leong, D. Barker, R. Johnston, J. Cap. Elec. 3 (1996) 61–68.
[43] J. Caslavska, S. Lienhard, W. Thormann, J. Chromatogr. 638 (1993) 335–342.
[44] H.A. Fishman, O. Orwar, R.H. Scheller, R.N. Zare, Proc. Natl. Acad. Sci. USA 92 (1995) 7877–7881.
[45] C. Salerno, C. Crifo, Anal. Biochem. 226 (1995) 377–379.
[46] M. Gross, B.S. Gathof, P. Kölle, U. Gresser, Electrophoresis 16 (1995) 1927–1929.
[47] G.L. Klein, C.R. Jolliff, in: J.P. Landers (Ed.), Handbook of Capillary Electrophoresis, Chapter 16, CRC Press, Boca Raton, FL, 1994, pp. 419–457.
[48] T. Manabe, H. Yamamoto, T. Okuyama, Electrophoresis 10 (1989) 172–177.
[49] N.A. Guzman, L. Hernandez, C.M. Berck, J.P. Advis, 'Cis '89 Tokyo', International Symposium on Chromatography. Tokyo, Japan, October 17–20, Abstract I-P-32 (1989).

[50] D.B. Craig, J.C.Y. Wong, N.J. Dovichi, Anal. Chem. 68 (1996) 697–700.
[51] A. Takatsu, S. Eyama, A. Uchiumi, Chromatographia 40 (1995) 125–128.
[52] O. Brüggemann, M. Meder, R. Freitag, J. Chromatogr. A 744 (1996) 167–176.
[53] A.M. Hoyt Jr., S.C. Beale, J.P. Larmann Jr., J.W. Jorgenson, J. Microcol. Sep. 5 (1993) 325–333.
[54] S.D. Gilman, A.G. Ewing, J. Cap. Elec. 2 (1995) 1–13.
[55] S.M. Lunte, C.E. Lunte, Adv. Chromatogr. 36 (1996) 383–432.
[56] A. Hiraoka, I. Miura, M. Hattori, I. Tominaga, S. Machida, Biol. Pharm. Bull. 16 (1993) 949–952.
[57] J. Bergquist, S.D. Gilman, A.G. Ewing, R. Ekman, Anal. Chem. 66 (1994) 3512–3518.
[58] G. Nouadje, H. Rubie, E. Chatelut, P. Canal, M. Nertz, Ph. Puig, F. Couderc, J. Chromatogr. A 717 (1995) 293–298.
[59] W. Thormann, S. Molteni, J. Caslavska, A. Schmutz, Electrophoresis 15 (1994) 3–12.
[60] R.T. Kennedy, M.D. Oates, B.R. Cooper, B. Nickerson, J.W. Jorgenson, Science 246 (1989) 57–63.
[61] J.A. Jankowski, S. Tracht, J.V. Sweedler, Trends Anal. Chem. 14 (1995) 170–176.
[62] A. Schmutz, W. Thormann, Electrophoresis 15 (1994) 51–61.
[63] J.W. Jorgenson, K.D. Lukacs, Science 222 (1983) 266–272.
[64] M.J. Gordon, X. Huang, S.L. Pentoney, R.N. Zare, Science 242 (1989) 224–228.
[65] B.L. Karger, A.S. Cohen, A. Guttman, J. Chromatogr. 492 (1989) 585–614.
[66] L. Hernandez, R. Marquina, J. Escalona, N.A. Guzman, J. Chromatogr. 502 (1990) 247–255.
[67] L. Hernandez, N. Joshi, J. Escalona, N.A. Guzman, J. Chromatogr. 559 (1991) 183–196.
[68] Z. Deyl, R. Struzinsky, J. Chromatogr. 569 (1991) 63–122.
[69] L. Hernandez, N. Joshi, P. Verdeguer, N.A. Guzman, Capillary Electrophoresis Technology, Chapter 19, in: N.A. Guzman (Ed.), Marcel Dekker, New York, 1993, pp. 605–614.
[70] K.C. Chan, G.M. Janini, G.M. Muschik, H.J. Issaq, J. Chromatogr. A 653 (1993) 93–97.
[71] S.D. Gilman, J.J. Pietron, A.G. Ewing, J. Microcol. Sep. 6 (1994) 373–384.
[72] W. Zhou, J. Liu, E. Wang, J. Chromatogr. A 715 (1995) 355–360.
[73] C.-X. Zhang, F. von Heeren, W. Thormann, Anal. Chem. 67 (1995) 2070–2077.
[74] S.H. Hansen, I. Bjørnsdottir, J. Tjørnelund, J. Pharm. Biomed. Anal. 13 (1995) 489–495.
[75] P. Wernly, W. Thormann, Anal. Chem. 63 (1991) 2878–2882.
[76] P. Wernly, W. Thorman, Anal. Chem. 64 (1992) 2155–2159.
[77] N.H.H. Heegaard, F.A. Robey, Anal. Chem. 64 (1992) 2479–2482.
[78] N.H.H. Heegaard, P. Roepstorff, J. Cap. Elec. 2 (1995) 219–223.
[79] E. Gonzalez, J.J. Laserna, Electrophoresis 15 (1994) 240–243.
[80] B.J. Wildman, P.E. Jackson, W.R. Jones, P.G. Alden, J. Chromatogr. 546 (1991) 459–466.
[81] N.J. Reinhoud, U.R. Tjaden, H. Irth, J. van der Greef, J. Chromatogr. 574 (1992) 327–334.
[82] H. Nishi, N. Fujimura, H. Yamagushi, T. Fukuyama, M. Matsuo, S. Terabe, in K. Jinno, P. Sandra (Eds.), Twelfth International Symposium on Capillary Chromatography Industrial Publishing & Consulting, Tokyo, 1990, pp. 640–645.
[83] D.J. Kroon, S. Goltra, B. Sharma, J. Cap. Elec. 2 (1995) 34–39.
[84] N.A. Guzman, M.A. Trebilcock, J.P. Advis, Anal. Chim. Acta 249 (1991) 247–255.
[85] M.A. Costello, C. Woititz, J. De Feo, D. Stremlo, L.-F.L. Wen, D.J. Palling, K. Iqbal, N.A. Guzman, J. Liq. Chromatogr. 15 (1992) 1081–1097.
[86] N. Bihoreau, C. Ramon, R. Vincentelli, J.-P. Levillain, F. Troalen, J. Cap. Elec. 2 (1995) 197–202.
[87] N.A. Guzman, B.G. Hoebel, L. Hernandez, BioPharm 2 (1989) 22–37.
[88] N.A. Guzman, L. Hernandez, In: T.E. Hugli (Ed.), Techniques in Protein Chemistry, Chapter 44, Academic Press, San Diego, 1989, pp. 456–467.
[89] S. Kundu, C. Fenters, J. Cap. Elec. 2 (1995) 273–277.
[90] T.J. Pritchett, BioPharm 8 (1995) 38–45.
[91] K.-J. Lee, J.J. Lee, D.C. Moon, J. Chromatogr. 616 (1993) 135–143.
[92] K.-J. Lee, G.S. Hoe, N.J. Kim, D.C. Moon, J. Chromatogr. 608 (1992) 243–250.
[93] W. Thormann, S. Lienhard, S. Wernly, J. Chromatogr. 636 (1993) 137–148.
[94] A. Schmutz, W. Thormann, Electrophoresis 15 (1994) 1295–1303.
[95] R.G. Nielsen, E.C. Rickard, P.F. Santa, D.A. Sharknas, X. Sittampalam, J. Chromatogr. 539 (1991) 177–185.
[96] D. Perrett, G.A. Ross, J. Chromatogr. A 700 (1995) 179–186.
[97] A.S. Cohen, D.L. Smisek, B.H. Wang, Adv. Chromatogr. 36 (1996) 127–162.
[98] R. Lehmann, M. Koch, M. Pfohl, W. Voelter, H.-U. Häring, H.M. Liebich, J. Chromatogr. A 744 (1996) 187–194.
[99] T. Tadey, W.C. Purdy, J. Chromatogr. 583 (1992) 111–115.
[100] T. Tadey, W.C. Purdy, J. Chromatogr. A 652 (1993) 131–138.
[101] H.M. Liebich, R. Lehmann, A.E. Weiler, G. Grübler, W. Voelter, J. Chromatogr. A 717 (1995) 25–31.
[102] R. Lehmann, H.M. Liebich, G. Grübler, W. Voelter, Electrophoresis 16 (1995) 998–1001.
[103] C. Yan, R. Dadoo, H. Zhao, R.N. Zare, Anal. Chem. 67 (1995) 2026–2029.
[104] L.Z. Avila, Y.-H. Chu, E.C. Blossey, G.M. Whitesides, J. Med. Chem. 36 (1993) 126–133.
[105] H. Nishi, T. Fukuyama, M. Matsuo, J. Chromatogr. 515 (1990) 245–255.
[106] K. Ensing, A. Paulus, J. Pharm. Biomed. Anal. 14 (1996) 305–315.
[107] S. Palmarsdsttir, L.-E. Edholm, J. Chromatogr. A 693 (1995) 131–143.

[108] L. Steinmann, W. Thormann, J. Cap. Elec. 2 (1995) 81–88.
[109] W. Thormann, P. Meier, C. Marcolli, F. Binder, J. Chromatogr. 545 (1991) 445–460.
[110] K.E. Ferslew, A.N. Hagardon, W.F. McCormick, J. Forensic Sci. 40 (1995) 245–249.
[111] G.L. Chee, T.S.M. Wan, J. Chromatogr. 612 (1993) 172–177.
[112] M. Schafroth, W. Thormann, D. Allemann, Electrophoresis 15 (1994) 72–78.
[113] P. Lukkari, A. Ennelin, H. Sirén, M.-L. Riekkola, J. Liq. Chromatogr. 16 (1993) 2069–2079.
[114] P. Lukkari, H. Sirén, M. Pantsar, M.-L. Riekkola, J. Chromatogr. 632 (1993) 143–148.
[115] M.A. Schwarz, R.H.H. Neubert, H.H. Rüttinger, J. Chromatogr. A 745 (1996) 135–143.
[116] N. Wu, J.V. Sweedler, M. Lin, J. Chromatogr. B 654 (1994) 185–191.
[117] H. Wolfisberg, A. Schmutz, R. Stotzer, W. Thormann, J. Chromatogr. A 652 (1993) 407–416.
[118] H. Soini, M.-L. Riekkola, M.V. Novotny, J. Chromatogr. 608 (1992) 265–274.
[119] W. Thormann, A. Minger, S. Molteni, J. Caslavska, P. Gebauer, J. Chromatogr. 593 (1992) 275–288.
[120] C.-X. Zhang, W. Thormann, J. Cap. Elec. 1 (1994) 208–217.
[121] J. Caslavska, E. Hufschmid, R. Theurillat, C. Desiderio, H. Wolfisberg, W. Thormann, J. Chromatogr. B 656 (1994) 219–231.
[122] R. Guo, W. Thormann, Electrophoresis 14 (1993) 547–553.
[123] R. Zhang, H. Shi, Y. Ma, J. Microcol. Sep. 6 (1994) 217–221.
[124] X. Xu, W.T. Kok, J.C. Kraak, H. Poppe, J. Chromatogr. B 661 (1994) 35–45.
[125] M.V. Novotny, Curr. Opin. Biotechnol. 7 (1996) 29–34.
[126] P. Oefner, C. Chiesa, G. Bonn, Cs. Horváth, J. Cap. Elec. 1 (1994) 5–24.
[127] Z. El Rassi, Y. Mechref, Electrophoresis 17 (1996) 275–301.
[128] Y.-H. Chu, L.Z. Avila, H.A. Biebuyck, G.M. Whitesides, J. Med. Chem. 35 (1992) 2915–2917.
[129] Y. Tanaka, W. Thormann, Electrophoresis 11 (1990) 760–764.
[130] A.E. Bretnall, G.S. Clarke, J. Chromatogr. A 700 (1995) 173–178.
[131] A.E. Bretnall, G.S. Clarke, J. Chromatogr. A 745 (1996) 145–154.
[132] N.M. Schultz, R.T. Kennedy, Anal. Chem. 65 (1993) 3161–3165.
[133] J. Bergquist, A. Tarkowski, R. Ekman, A. Ewing, Proc. Natl. Acad. Sci. USA 91 (1995) 2912–12916.
[134] T.M. Olefirowicz, A.G. Ewing, Chimia 45 (1991) 106–108.
[135] S. Honda, A. Taga, K. Kakehi, S. Koda, Y. Okamoto, J. Chromatogr. 590 (1992) 364–368.
[136] T. Nakagawa, Y. Oda, A. Shibukawa, H. Fukuda, H. Tanaka, Chem. Pharm. Bull. 37 (1989) 707–711.
[137] J.P. Landers, M. Schuchard, T. Sismelich, T.C. Spelsberg, J. Chromatogr. 603 (1992) 247–257.
[138] D.E. Morbeck, B.J. Madden, D.J. McCormick, J. Chromatogr. A 680 (1994) 217–227.
[139] J. Pruñonosa, R. Obach, A. Diez-Cascón, L. Gouesclou, J. Chromatogr. 574 (1992) 127–133.
[140] J. Pruñonosa, R. Obach, A. Diez-Cascón, L. Gouesclou, J. Chromatogr. 581 (1992) 219–226.
[141] H. Soini, T. Tsuda, M.V. Novotny, J. Chromatogr. 559 (1991) 547–558.
[142] H. Soini, M.L. Riekkola, M.V. Novotny, J. Chromatogr. A 680 (1994) 623–634.
[143] R.P. Holmes, Clin. Chem. 41 (1995) 1297–1301.
[144] S. Lalljie, J. Vindevogel, P. Sandra, J. Cap. Elec. 1 (1994) 241–245.
[145] L. Hernandez, N.A. Guzman, B.G. Hoebel, Psycopharmacology 105 (1991) 264–268.
[146] F. Tagliaro, C. Poiesi, R. Aiello, R. Dorizzi, S. Ghielmi, M. Marigo, J. Chromatogr. 638 (1993) 303–309.
[147] L. Hernandez, B.G. Hoebel, N.A. Guzman, The 40th Pittsburgh Conference and Exposition on Analytical Chemistry and Applied Spectroscopy. Atlanta, Georgia, March 6–10, 1989, Abstract 1423.
[148] A.J.J. Debets, M. Mazereeuw, W.H. Voogt, D.J. van Iperen, H. Lingeman, K.-P. Hupe, U.A.T. Brinkman, J. Chromatogr. 608 (1992) 151–158.
[149] J.H. Jumppanen, S.K. Wiedmer, H. Siren, M.-L. Riekkola, H. Haario, Electrophoresis 15 (1994) 1267–1272.
[150] D. Schmalzing, W. Nashabeh, X.-W. Yao, R. Mhatre, F.E. Regnier, N.B. Afeyan, M. Fuchs, Anal. Chem. 67 (1995) 606–612.
[151] D. Schmalzing, W. Nashabeh, M. Fuchs, Clin. Chem. 41 (1995) 1403–1406.
[152] L.B. Koutny, D. Schmalzing, T.A. Taylor, M. Fuchs, Anal. Chem. 68 (1996) 18–22.
[153] M. Miyake, A. Shibukawa, T. Nakagawa, In: K. Jinno, P. Sandra (Eds.), Twelfth International Symposium on Capillary Chromatography, Industrial Publishing & Consulting, Tokyo, 1990, pp. 651–661.
[154] M. Miyake, A. Shiugawa, T. Nakagawa, J. High Resolut. Chromatogr. 14 (1991) 181–185.
[155] N.A. Guzman, C.M. Berck, L. Hernandez, J.P. Advis, J. Liq. Chromatogr. 13 (1990) 3833–3848.
[156] L. Hernandez, B.G. Hoebel, N.A. Guzman, In: Cs. Horváth, J.G. Nikelly (Eds.), Analytical Biotechnology. Capillary Electrophoresis and Chromatography, Chapter 3, ACS Symposium Series 434, American Chemical Society, Washington, DC, 1990, pp. 50–59.
[157] J.-G. Chen, S.J. Woltman, S.G. Weber, Adv. Chromatogr. 36 (1996) 273–313.
[158] T.M. Phillips, LC·GC 11 (1993) 36–42.
[159] T.M. Phillips, P.L. Kimmel, J. Chromatogr. B 656 (1994) 259–266.
[160] D.K. Lloyd, A.M. Cypess, I.W. Wainer, J. Chromatogr. 568 (1991) 117–124.
[161] X. Gu, M. Meleka-Boules, C.-L. Chen, J. Cap. Elec. 3 (1996) 43–49.
[162] M.E. Swartz, M. Merion, J. Chromatogr. 632 (1993) 209–213.

[163] S. Li, K. Fried, I.W. Wainer, D.K. Lloyd, Chromatographia 35 (1993) 216–222.
[164] M.C. Carneiro, L. Puignou, M.T. Galceran, J. Chromatogr. A 669 (1994) 217–224.
[165] X. Liu, Y. Xu, P.C. Michael, Anal. Chem. 67 (1995) 3211–3218.
[166] E. Hufschmid, R. Theurillat, U. Martin, W. Thormann, J. Chromatogr. B 668 (1995) 159–170.
[167] M. Tomita, T. Okuyama, Biomed. Chromatogr. 6 (1992) 91–94.
[168] J. Jumppanen, H. Sirén, M.-L. Riekkola, J. Chromatogr. A 652 (1993) 441–450.
[169] A.W.H.M. Kuypers, P.M.W. Willems, M.J. van der Schans, P.C.M. Linssen, M.C. Wessels, C.H.M.M. De Bruijn et al., J. Chromatogr. 621 (1993) 149–156.
[170] M. Oto, T. Suehiro, Y. Akiyama, Y. Yuasa, Clin. Chem. 41 (1995) 482–483.
[171] C. Gelfi, A. Orsi, F. Leoncini, P.G. Righetti, I. Spiga, P. Carrera, M. Ferrari, BioTechiques 19 (1995) 254–263.
[172] H.E. Schwartz, K.J. Ulfelder, F.-T. Chen, S.L. Pentoney Jr., J. Cap. Elec. 1 (1996) 36–54.
[173] S. Bay, H. Starke, J.Z. Zhang, J.F. Elliot, L.D. Coulson, N.A. Dovichi, J. Cap. Elec. 1 (1994) 121–126.
[174] T.A. Felmlee, R.P. Oda, D.A. Persing, J.P. Landers, J. Chromatogr. A 717 (1995) 127–137.
[175] B.R. McCord, J.M. Jung, E.A. Holleran, J. Liq. Chromatogr. 16 (1993) 1963–1981.
[176] C. Gelfi, P.G. Righetti, C. Magnani, L. Cremonesi, M. Ferrari, Clin. Chim. Acta 229 (1994) 181–189.
[177] T.A. Felmlee, P.S. Mitchell, K.J. Ulfelder, D.H. Persing, J.P. Landers, J. Cap. Elec. 2 (1995) 125–130.
[178] C. Haber, I. Silvestri, S. Röösli, W. Simon, Chimia 45 (1991) 117–121.
[179] J.P. Landers, Trends Biochem. Sci. 18 (1993) 409–414.
[180] J.P. Landers, R.P. Oda, T.C. Spelsberg, J.A. Nolan, K.J. Ulfelder, Biotechniques 14 (1993) 98–111.
[181] B.R. McCord, D.L. McClure, J.M. Jung, J. Chromatogr. A 652 (1993) 75–82.
[182] E. Kenndler, H. Poppe, J. Cap. Elec. 1 (1994) 144–157.
[183] J. Cheng, T. Kasuga, N.D. Watson, K.R. Mitchelson, J. Cap. Elec. 2 (1995) 24–29.
[184] K.R. Mitchelson, J. Cheng, J. Cap. Elec. 2 (1995) 137–143.
[185] D.N. Heiger, A.S. Cohen, B.L. Karger, J. Chromatogr. 516 (1990) 33–48.
[186] M. Strege, A. Lagu, Anal. Chem. 63 (1991) 1233–1236.
[187] W. Nashabeh, Z. El Rassi, J. Chromatogr. 596 (1992) 251–264.
[188] K.J. Ulfelder, H.E. Schwartz, J.M. Hall, F.J. Sunzeri, Anal. Biochem. 200 (1992) 260–267.
[189] T. Izumi, M. Yamaguchi, J. Chromatogr. A 652 (1993) 41–46.
[190] H.E. Schwartz, K.J. Ulfelder, F.J. Sunzeri, M.P. Busch, R.G. Brownlee, J. Chromatogr. 559 (1991) 267–283.
[191] F. Robert, L. Bert, L. Denoroy, B. Renaud, Anal. Chem. 67 (1995) 1838–1844.
[192] T.M. Olefirowicz, A.G. Ewing, J. Neurosci. Methods 34 (1990) 11–15.
[193] J. Caslavska, E. Gassmann, W. Thormann, J. Chromatogr. A 709 (1995) 147–156.
[194] H.G. Lee, J.-L. Tseng, R.R. Becklin, D.M. Desiderio, Anal. Biochem. 229 (1995) 188–197.
[195] W.M. Mück, J.D. Henion, J. Chromatogr. 495 (1989) 41–59.
[196] J. Cao, R.F. Cross, J Chromatogr A 695 (1995) 297–308.
[197] S. Pálmarsdóttir, B. Lindegård, P. Deininger, L.-E. Edholm, L. Mathiasson, J.A. Jönsson, J. Cap. Elec. 2 (1995) 185–189.
[198] R.L. Sheppard, X. Tong, J. Cai, J.D. Henion, Anal. Chem. 67 (1995) 2054–2058.
[199] M. Chicharro, A. Zapardiel, E. Bermejo, J.A. Perez, L. Hernandez, J. Chromatogr. 622 (1993) 103–108.
[200] A.J. Ji, M.F. Nunez, D. Machacek, J.E. Ferguson, M.F. Iossi, P.C. Kao, J.P. Landers, J. Chromatogr. B 669 (1995) 15–26.
[201] Z.B. Shihabi, K.S. Oles, Clin. Chem. 40 (1994) 1904–1908.
[202] F. von Heeren, R. Tanner, R. Theurillat, W. Thormann, J. Chromatogr. A 745 (1996) 165–172.
[203] A. Schmutz, W. Thormann, Ther. Drug Monit. 16 (1994) 483–490.
[204] Z. Liang, N. Chiem, G. Ocvirk, T. Tang, K. Fluri, D.J. Harrison, Anal. Chem. 68 (1996) 1040–1046.
[205] L.L. Garcia, Z.K. Shibahi, K. Oles, J. Chromatogr. B 669 (1995) 157–162.
[206] M. Nuñez, J.E. Ferguson, D. Machacek, G. Jacob, R.P. Oda, G.M. Lawson, J.P. Landers, Anal. Chem. 67 (1995) 3668–3675.
[207] H.-Y. Law, I. Ng, J. Ong, C.-N. Ong, Biomed. Chromatogr. 8 (1994) 175–179.
[208] M. Zhu, R. Rodriguez, T. Wehr, C. Siebert, J. Chromatogr. 608 (1992) 225–237.
[209] C.-N. Ong, L.S. Liau, H.Y. Ong, J. Chromatogr. 576 (1992) 346–350.
[210] S.A. Perez, L.A. Colón, Electrophoresis 17 (1996) 352–358.
[211] J. Bao, F.E. Regnier, J. Chromatogr. 608 (1992) 217–224.
[212] L. Hernandez, J. Escalona, P. Verdeguer, N.A. Guzman, J. Liq. Chromatogr. 16 (1993) 2149–2160.
[213] L. Hernandez, S. Tucci, N.A. Guzman, X. Paez, J. Chromatogr. A 652 (1993) 393–398.
[214] L. Hernandez, N. Joshi, E. Murzi, P. Verdeguer, J.C. Mifsud, N. Guzman, J. Chromatogr. A 652 (1993) 399–405.
[215] N. Ercal, K. Le, P. Treeratphan, R. Mattews, Biomed. Chromatogr. 10 (1996) 15–18.
[216] E. Jellum, A.K. Thorsrud, E. Time, J. Chromatogr. 559 (1991) 455–465.
[217] R.P. Oda, B.J. Madden, J.P. Landers, Adv. Chromatogr. 36 (1996) 163–199.
[218] J.P. Landers, R.P. Oda, B. Madden, T.C. Spelsberg, Anal. Biochem. 205 (1992) 115–124.
[219] M. Tomita, T. Okuyama, Y. Nigo, B. Uno, S. Kawai, J. Chromatogr. Biomed. Appl. 571 (1991) 324–330.
[220] K. Shimura, B.L. Karger, Anal. Chem. 66 (1994) 9–15.
[221] A.J. Tomlinson, L.M. Benson, S. Naylor, J. Cap. Elec. 1 (1994) 127–135.

[222] A.J. Tomlinson, L.M. Benson, S. Naylor, LC·GC 12 (1994) 122–130.
[223] L.M. Benson, A.J. Tomlinson, S. Naylor, J. High Resolut. Chromatogr. 17 (1994) 671–673.
[224] A.J. Tomlinson, L.M. Benson, R.P. Oda, W.D. Braddock, B.L. Riggs, J.A. Katzmann, S. Naylor, J. Cap. Elec. 2 (1995) 97–104.
[225] A.J. Tomlinson, S. Naylor, J. High Resolut. Chromatogr. 18 (1995) 384–386.
[226] A.J. Tomlinson, L.M. Benson, J.P. Landers, G.F. Scanlan, J. Fang, J.W. Gorrod, S. Naylor, J. Chromatogr. A 652 (1993) 417–426.
[227] A.J. Tomlinson, L.M. Benson, S. Naylor, Am. Lab. 26 (1994) 29–36.
[228] S. Naylor, L.M. Benson, A.J. Tomlinson, J. Cap. Elec. 1 (1994) 181–189.
[229] S. Naylor, L.M. Benson, A.J. Tomlinson, In: J.P. Landers (Ed.), Handbook of Capillary Electrophoresis, Chapter 17, CRC Press, Boca Raton, FL, 1994, pp. 459–491.
[230] A.J. Tomlinson, L.M. Benson, W.D. Braddock, R.P. Oda, J. High Resolut. Chromatogr. 17 (1994) 729–732.
[231] A.J. Tomlinson, L.M. Benson, R.P. Oda, W.D. Braddock, M.A. Strausbauch, P.J. Wettstein, S. Naylor, J. High Resolut. Chromatogr. 17 (1994) 669–671.
[232] A.J. Tomlinson, N.A. Guzman, S. Naylor, J. Cap. Elec. 2 (1995) 247–266.
[233] A.J. Tomlinson, L.M. Benson, W.D. Braddock, R.P. Oda, S. Naylor, J. High Resolut. Chromatogr. 18 (1995) 381–383.
[234] M.H.A. Busch, H.F.M. Boelens, J.C. Kraak, X. Poppe, A.A.P. Meekel, M. Resmini, J. Chromatogr. A 744 (1996) 195–203.
[235] F.-T.A. Chen, C.-M. Liu, Y.- Z Hsieh, J.C. Sternberg, Clin. Chem. 37 (1991) 14–19.
[236] Z.K. Shihabi, Ann. Clin. Lab. Sci. 22 (1992) 398–405.
[237] N. Ishioka, N. Iyori, J. Noji, S. Kurioka, Biomed. Chromatogr. 6 (1992) 224–226.
[238] M. Zhu, T. Wehr, V. Levi, R. Rodriguez, K. Shiffer, A.C. Zhu, J. Chromatogr. A 652 (1993) 119–129.
[239] S. Molteni, H. Frischknecht, W. Thormann, Electrophoresis 15 (1994) 22–30.
[240] J.M. Hempe, R.D. Craver, Clin. Chem. 40 (1994) 2288–2295.
[241] A. Sahin, Y.R. Lalehi, R. Ortancil, J. Chromatogr. A 709 (1995) 121–125.
[242] M. Castagnola, I. Messana, L. Cassiano, R. Rabino, D.V. Rossetti, B. Giardina, Electrophoresis 16 (1995) 1492–1498.
[243] J. Wu, J. Pawliszyn, Anal. Chem. 67 (1995) 2010–2014.
[244] T.J. Pritchett, Electrophoresis 17 (1996) 1195–1201.
[245] P.G. Righetti, C. Gelfi, J. Cap. Elec. 1 (1994) 27–35.
[246] M. Conti, C. Gelfi, P.G. Righetti, Electrophoresis 16 (1995) 1485–1491.
[247] G.A. Ross, P. Lorkin, D. Perret, J. Chromatogr. 636 (1993) 69–79.
[248] J. Cai, Z. El Rassi, J. Liq. Chromatogr. 15 (1992) 1179–1192.
[249] W.C. Brumley, J. Chromatogr. Sci. 33 (1995) 670–685.
[250] R. Weinberger, I.S. Lurie, Anal. Chem. 63 (1991) 823–827.
[251] V.C. Trenerry, R.J. Wells, J. Robertson, J. Chromatogr. Sci. 32 (1994) 1–6.
[252] C. Bjergegaard, H. Simonsen, H. Sørensen, J. Chromatogr. A 680 (1994) 561–569.
[253] A.C. Schoots, T.P.E.M. Verheggen, P.M.J.M. De Vries, F.M. Everaerts, Clin. Chem. 36 (1990) 435–440.
[254] K.-L. Lee, J.J. Lee, D.C. Moon, Electrophoresis 15 (1994) 98–102.
[255] H.J. Issaq, K. Delviks, G.M. Janini, G.M. Muschik, J. Liq. Chromatogr. 15 (1996) 3193–3201.
[256] J. Grimshaw, J. Trocha-Grimshaw, W. Fisher, A. Rice, S. Smith, P. Spedding, J. Duffy, X. Mollan, Electrophoresis 17 (1996) 396–400.
[257] P. Wernly, W. Thormann, J. Chromatogr. 608 (1992) 251–256.
[258] D.P. Bogan, A.J. Killard, R. O'Kennedy, J. Cap. Elec. 2 (1995) 241–245.
[259] M.C. Roach, P. Gozel, R.N. Zare, J. Chromatogr. 426 (1988) 129–140.
[260] K.C. Chan, G.M. Janini, G.M. Muschik, H.J. Issaq, J. Chromatogr. 622 (1993) 269–273.
[261] N.A. Guzman, J. Moschera, K. Iqbal, A.W. Malick, J. Liq. Chromatogr. 15 (1992) 1163–1177.
[262] J.P. Landers, Clin. Chem. 41 (1995) 495–509.
[263] N.A. Guzman, J. Liq. Chromatogr. 18 (1995) 3751–3768.
[264] A.J. Tomlinson, L.M. Benson, N.A. Guzman, S. Naylor, J. Chromatogr. A 744 (1996) 3–15.
[265] L.J. Cole, R.T. Kennedy, Electrophoresis 16 (1995) 549–556.
[266] C. Bjergegaard, P. Moller, H. Sørensen, J. Chromatogr. A 717 (1995) 409–414.
[267] Z.K. Shihabi, M.S. Constaninescu, Clin. Chem. 38 (1992) 2117–2120.
[268] X. Huang, T.-K.J. Pang, M.J. Gordon, R.N. Zare, Anal. Chem. 59 (1987) 2747–2749.
[269] X. Huang, M.J. Gordon, R.N. Zare, J. Chromatogr. 425 (1988) 385–390.
[270] W. Buchberger, K. Winna, M. Turner, J. Chromatogr. A 671 (1994) 375–382.
[271] Q. Li, E.S. Yeung, J. Cap. Elec. 1 (1994) 55–61.
[272] C. Haber, W.R. Jones, J. Soglia, M. McGlynn, A. Caplan, J.R. Reineck, C. Krstanovic, J. Cap. Elec. 3 (1996) 1–11.
[273] P. Che, J. Xu, H. Shi, Y. Ma, J. Chromatogr. B 669 (1995) 45–51.
[274] Q. Xue, E.S. Yeung, Anal. Chem. 66 (1994) 1175–1178.
[275] S. Honda, A. Taga, S. Suzuki, K. Kahehi, J. Chromatogr. 597 (1992) 377–382.
[276] K.J. Miller, I. Leesong, J. Bao, F.E. Regnier, F.E. Lyte, Anal. Chem. 65 (1993) 3267–3270.
[277] I.D. Cruzado, A.Z. Hu, R.D. Macfarlane, J. Cap. Elec. 3 (1996) 25–29.
[278] G. Schmitz, U. Borgmann, G. Assmann, J. Chromatogr. 320 (1985) 253–262.
[279] O. Josic, A. Böttcher, G. Schmitz, Chromatographia 30 (1990) 703–706.
[280] G. Nowicka, G. Schmitz, Klin. Wochens. 68 (1990) 119–120.

[281] E. Jellum, J. Cap. Elec. 1 (1994) 97–105.
[282] G. Schmitz, C. Möllers, Electrophoresis 15 (1994) 31–39.
[283] A. Goux, A. Athias, L. Persegol, L. Lagrost, P. Gambert, Anal. Biochem. 218 (1994) 320–324.
[284] A.Z. Hu, I.D. Cruzado, J.W. Hill, C.J. McNeal, R.D. Macfarlane, J. Chromatogr. A 717 (1995) 33–39.
[285] I. Valásková, J. Balázová, E. Havránek, J. Chromatogr. B 674 (1995) 310–313.
[286] M.R. Taylor, S.A. Westwood, D. Perret, J. Chromatogr. A 745 (1996) 155–163.
[287] S.R. Rabel, C.M. Riley, R. Trueworthy, J.F. Stobaugh, J. Cap. Elec. 2 (1995) 57–61.
[288] M.P. Richards, J.H. Beattie, J. Cap. Elec. 1 (1994) 196–207.
[289] J.H. Beattie, R. Self, M.P. Richards, Electrophoresis 16 (1995) 322–328.
[290] M.P. Richards, P.J. Aagaard, J. Cap. Elec. 1 (1994) 90–95.
[291] M.P. Richards, J.H. Beattie, J. Chromatogr. B 669 (1995) 27–37.
[292] S. Molteni, J. Caslavska, D. Alleman, W. Thormann, J. Chromatogr. B 658 (1994) 355–367.
[293] N.A. Guzman, M.A. Trebilcock, J.P. Advis, J. Liq. Chromatogr. 14 (1991) 997–1015.
[294] M.L. Cunningham, D.L. Spencer, W.J. Caspary, H. Stopper, A. Phillips, J. Cap. Elec. 2 (1995) 30–33.
[295] C.-X. Zhang, Z.-P. Sun, D.-Y. Ling, J.-S. Zheng, J. Guo, X.-Y. Li, J. Chromatogr. 612 (1993) 287–294.
[296] J. Schneede, P.M. Ueland, Anal. Chem. 67 (1995) 812–819.
[297] D.R. Franke, D.B. Marsh, K.L. Nuttall, J. Cap. Elec. 3 (1996) 125–129.
[298] D.B. Marsh, K.L. Nuttall, J. Cap. Elec. 2 (1995) 63–67.
[299] K. Shimura, K.-i. Kasai, Anal. Biochem. 227 (1995) 186–194.
[300] D.M. Northrop, B.R. McCord, J.M. Butler, J. Cap. Elec. 1 (1994) 158–168.
[301] P. Wernly, W. Thormann, D. Bourquin, R. Brenneisen, J. Chromatogr. 616 (1993) 305–310.
[302] Z.K. Shihabi, J. Chromatogr. B 669 (1995) 53–58.
[303] J.P. Advis, L. Hernandez, N.A. Guzman, Peptide Res. 2 (1989) 389–394.
[304] N.A. Guzman, J.P. Advis, L. Hernandez, In: J.J. Villafranca (Ed.), Current Research in Protein Chemistry: Techniques, Structure, and Function, Chapter 19, Academic Press, San Diego, CA, 1990, pp. 203–216.
[305] N.A. Guzman, M.A. Trebilcock, J.P. Advis, In: J.J. Villafranca (Ed.), Techniques in Protein Chemistry II, Chapter 4, Academic Press, San Diego, CA, 1991, pp. 37–54.
[306] J.P. Advis, K. Iqbal, A.W. Malick, N.A. Guzman, In: F. de Pablo, C.G. Scanes, B.D. Weintraub (Eds,), Handbook of Hormonal Assay Techniques, Chapter 7, Academic Press, San Diego, CA, 1993, pp. 127–144.
[307] J.P. Advis, N.A. Guzman, J. Liq. Chromatogr. 16 (1993) 2129–2148.
[308] D. Perrett, A. Birch, G. Ross, Tech. Stud. Neuropept. 22 (1994) 127–130.
[309] T. Ueda, T. Maekawa, D. Sadamitsu, S. Oshita, K. Ogino, K. Nakamura, Electrophoresis 16 (1995) 1002–1004.
[310] G.M. Janini, K.C. Chan, G.M. Muschik, H.J. Isaaq, J. Chromatogr. B 657 (1994) 419–423.
[311] B.J. Harmon, I. Leesong, F.E. Regnier, J. Chromatogr. A 726 (1996) 193–204.
[312] Y. Yoshimoto, A. Shibukawa, H. Sasagawa, S. Nitta, T. Nakagawa, J. Pharm. Biomed. Anal. 13 (1995) 483–488.
[313] T. Grune, G.A. Ross, H. Schmidt, W. Siems, D. Perret, J. Chromatogr. 636 (1993) 105–111.
[314] A.-L. Nguyen, J.H.T. Luong, C. Masson, Anal. Chem. 62 (1990) 2490–2493.
[315] T. Tsuda, K. Takagi, T. Watanabe, T. Satake, J. High Resolut. Chromatogr. Chromatogr. Commun. 11 (1988) 721–723.
[316] A. Hiraoka, J. Akai, I. Tominaga, M. Hattori, H. Sasaki, T. Arato, J. Chromatogr. A 680 (1994) 243–246.
[317] V. Dolník, J. Dolníková, J. Chromatogr. A 760 (1995) 269–277.
[318] M. Shirao, R. Furuta, S. Suzuki, H. Nakazawa, S. Fujita, T. Maruyama, J. Chromatogr. A 680 (1994) 247–251.
[319] C.J. Petucci, H.L. Kantes, T.G. Strein, H. Veening, J. Chromatogr. B 668 (1995) 241–251.
[320] R.B. Taylor, A.S. Low, R.G. Reid, J. Chromatogr. B 675 (1996) 213–223.
[321] F. Li, S.F. Cooper, S.R. Mikkelsen, J. Chromatogr. B 674 (1995) 277–285.
[322] G. Hempel, D. Lehmkuhl, S. Krümpelmann, G. Blaschke, J. Boos, J. Chromatogr. A 745 (1996) 173–179.
[323] D. Del Principe, M.P. Iampieri, D. Germani, A. Menichelli, G. Novelli, B. Dallapiccola, J. Chromatogr. 638 (1993) 277–281.
[324] J.M. Kolesar, J.D. Rizzo, J.D. Kuhn, J. Cap. Elec. 2 (1995) 287–290.
[325] C. Gelfi, A. Orsi, P.C. Righetti, M. Zanussi, P. Carrera, M. Ferrari, J. Chromatogr. B 657 (1994) 201–205.
[326] M. Nesi, P.G. Righetti, M.C. Patrosso, A. Ferlini, M. Chiari, Electrophoresis 15 (1994) 644–646.
[327] H. Arakawa, K. Uetanaka, M. Maeda, A. Tsuji, Y. Matsubara, K. Narisawa, J. Chromatogr. A 680 (1994) 517–523.
[328] C. Gelfi, A. Orsi, P.G. Righetti, V. Brancolini, L. Cremonesi, M. Ferrari, Electrophoresis 15 (1994) 640–643.
[329] E.F. Rossomando, L. White, K.J. Ulfelder, J. Chromatogr. B 656 (1994) 159–168.
[330] Z.K. Shihabi, J. Liq. Chromatogr. 16 (1993) 2059–2068.
[331] A.J. Tomlinson, L.M. Benson, S. Jameson, S. Naylor, Electrophoresis 17 (1996) 1801–1807.
[332] A.J. Tomlinson, S. Jameson, S. Naylor, J. Chromatogr. A 744 (1994) 273–278.
[333] R.D. Smith, H.R. Udseth, C.J. Barinaga, C.G. Edmonds, J. Chromatogr. 559 (1991) 197–208.
[334] J.P. Larmann Jr., A.V. Lemmo, A.W. Moore Jr., J.W. Jorgenson, Electrophoresis 14 (1993) 547–553.
[335] K.C. Chan, G.M. Janini, G.M. Muschik, H.J. Issaq, J. Liq. Chromatogr. 16 (1993) 1877–1890.
[336] R.P. Oda, B.J. Madden, J.C. Morris, T.C. Spelsberg, J.P. Landers, J. Chromatogr. A 680 (1994) 341–351.
[337] M.A. Strausbauch, B.J. Madden, P.J. Wettstein, J.P. Landers, Electrophoresis 16 (1995) 541–548.
[338] C. Schöneich, A.F.R. Hühmer, S.R. Rabel, J.F. Stobaugh, S.D.S. Jois, C.K. Larive, T.J. Siahaan, T.C. Squier, D.J. Bigelow, T.D. Williams, Anal. Chem. 67 (1995) 155R–181R.

[339] L. Licklider, W.G. Kuhr, M.P. Lacey, T. Keough, M.P. Purdon, R. Takigiku, Anal. Chem. 67 (1995) 4170–4177.
[340] T.J. Pritchett, R.A. Evangelista, F.-T.A. Chen, J. Cap. Elec. 2 (1995) 145–149.
[341] A.J. Tomlinson, S. Naylor, J. Cap. Elec. 2 (1995) 225–233.
[342] A.J. Tomlinson, S. Naylor, J. Liq. Chromatogr. 18 (1995) 3591–3615.
[343] A.J. Tomlinson, W.D. Braddock, L.M. Benson, R.P. Oda, S. Naylor, J. Chromatogr. B 669 (1995) 67–73.
[344] M.A. Strausbauch, J.P. Landers, P.J. Wettstein, Anal. Chem. 68 (1996) 306–314.
[345] L.N. Amankwa, W.G. Kuhr, Anal. Chem. 64 (1992) 1610–1613.
[346] N.A. Guzman, Sixth Annual Symposium of the Protein Society, San Diego, California, July 25–29, 1992, T-76.
[347] N.A. Guzman, L. Hernandez, J.P. Advis, The Third Annual Frederick Conference on Capillary Electrophoresis, Frederick, Maryland, October 20–21, 1992, p. 32.
[348] N.A. Guzman, C.L. Gonzalez, Seventh Annual Symposium of the Protein Society, San Diego, California, July 24–28, 1993, T-315.
[349] W.G. Kuhr, L. Licklider, L. Amankwa, Anal. Chem. 65 (1993) 277–282.
[350] L.N. Amankwa, W.G. Kuhr, Anal. Chem. 65 (1993) 2693–2697.
[351] H.-T. Chang, E.S. Yeung, Anal. Chem. 65 (1993) 2947–2951.
[352] L. Licklider, W.G. Kuhr, Anal. Chem. 66 (1994) 4400–4407.
[353] T. Kornfelt, A. Vinther, G.N. Okafo, P. Camilleri, J. Chromatogr. A 726 (1996) 223–228.
[354] N.A. Guzman, In: P.G. Righetti (Ed.), Capillary Electrophoresis: An Analytical Tool in Biotechnology, Chapter 4, CRC Press, Boca Raton, FL, 1996, pp. 101–121.
[355] S. Fazio, R. Vivilecchia, L. Lesueur, J. Sheridan, Am. Biotechnol. Lab. 8 (1990) 10–17.
[356] S. Terabe, K. Otsuka, K. Ishikawa, A. Tsuchiya, T. Ando, Anal. Chem. 56 (1984) 111–113.
[357] F. Tagliaro, S. Moretto, R. Valentini, G. Gambaro, C. Antonioli, M. Moffa, L. Tato, Electrophoresis 15 (1994) 94–97.
[358] V. Dolník, J. Microcol. Sep. 6 (1994) 63–67.
[359] Y. Xu, Anal. Chem. 67 (1995) 463R–473R.
[360] D. Chen, N.J. Dovichi, Anal. Chem. 68 (1996) 690–696.
[361] G.E. Barker, W.J. Horvath, C.W. Huie, R.A. Hartwick, J. Liq. Chromatogr. 16 (1993) 2089–2101.
[362] N. Wu, B. Li, J.V. Sweedler, J. Liq. Chromatogr. 17 (1994) 1917–1927.
[363] R. Weinberger, E. Sapp, S. Moring, J. Chromatogr. 516 (1990) 271–285.
[364] C.V. Thomas, A.C. Cater, J.J. Wheeler, J. Liq. Chromatogr. 16 (1993) 1903–1921.
[365] N.A. Guzman, C.L. Gonzalez, M.A. Trebilcock, 6th International Symposium on HPLC of Proteins, Peptides and Polynucleotides, Baden-Baden, Germany, October 20–22, 1986, Abstract 48 (1009).
[366] N.A. Guzman, W.Q. Ascari, K.R. Cutroneo, R.J. Desnick, J. Cell. Biochem. 48 (1992) 172–189.
[367] I. Morita, J. Sawada, J. Chromatogr. 641 (1993) 375–381.
[368] G. Cowdrey, M. Firth, G. Firth, Electrophoresis 16 (1995) 1922–1926.
[369] L.R. Gurley, J.S. Buchanan, J.E. London, D.M. Stavert, B.E. Lehnert, J. Chromatogr. 559 (1991) 411–429.
[370] A. Guttman, Electrophoresis 17 (1996) 1333–1341.
[371] J. Tie, S. Tsukamoto, S. Oshida, Jpn. J. Legal Med. 47 (1993) 295–301.
[372] F.-T.A. Chen, J. Chromatogr. 559 (1991) 445–453.
[373] E. Jellum, Fourth International Symposium on High Performance Capillary Electrophoresis, Amsterdam, The Netherlands, February 9–13, 1992, p. 28.
[374] F.-T.A. Chen, Clin. Chem. 38 (1992) 1651–1653.
[375] J.W. Kim, J.H. Park, J.W. Park, H.J. Doh, G.S. Heo, K.-J. Lee, Clin. Chem. 39 (1993) 689–692.
[376] F.-T.A. Chen, J.C. Sternberg, Electrophoresis 15 (1994) 13–21.
[377] O.W. Reif, R. Lausch, R. Freitag, Int. Lab. 9 (1994) 10–14.
[378] M.A. Jenkins, E. Kulinskaya, H.D. Martin, M.D. Guerin, J. Chromatogr. B 672 (1995) 241–251.
[379] V. Dolník, J. Chromatogr. A 709 (1995) 99–110.
[380] R. Lehmann, G. Grübler, W. Voelter, H. Liebich, Wiener Klin. Wochenschr. 107 (1995) 8.
[381] M.A. Jenkins, M.D. Guerin, Ann. Clin. Biochem. 32 (1995) 493–497.
[382] H. Bernon, F. Arpin, J. Bienvenu, M. Somenzini, F. Aguzzi, The International Congress, Electrophoresis '95, Paris, France, August 28–29, 1995, Abstract 32.
[383] C. Blessum, R. Falcou, The International Congress, Electrophoresis '95, Paris, France, August 28–29, 1995, Workshop-Room 2.
[384] M. Huang, D. Mitchell, M. Bigelow, J. Chromatogr. B 677 (1996) 77–84.
[385] R. Lehmann, H.M. Liebich, W. Voelter, J. Cap. Elec. 3 (1996) 89–110.
[386] M.A. Jenkins, M.D. Guerin, J. Chromatogr. B 682 (1996) 23–34.
[387] R. Clark, J.A. Katzmann, E. Wiegert, C. Namyst-Goldberg, L. Sanders, R.P. Oda, R.A. Kyle, J.P. Landers, J. Chromatogr. A 744 (1996) 205–213.
[388] T.T. Lee, E.S. Yeung, Anal. Chem. 64 (1992) 3045–3051.
[389] N.A. Guzman, L. Hernandez, S. Terabe, In: Cs. Horváth, J.G. Nikelly (Eds.), Analytical Biotechnology. Capillary Electrophoresis and Chromatography, Chapter 1, ACS Symposium Series 434, American Chemical Society, Washington, DC, 1990, pp. 1–35.
[390] J. Cai, Z. El Rassi, J. Liq. Chromatogr. 16 (1993) 2007–2024.
[391] N.A. Guzman, L. Hernandez, J.P. Advis, Fifth International Symposium on High Performance Capillary Electrophoresis, Orlando, FL, January 25–28, 1993, W-58.
[392] N. Cohen, E. Grushka, J. Cap. Elec. 1 (1994) 112–115.
[393] M.A. Jenkins, T.D. O'Leary, M.D. Guerin, J. Chromatogr. B 662 (1994) 108–112.
[394] N.H.H. Heegaard, F.A. Robey, J. Immunol. Methods 166 (1993) 103–110.
[395] Y. Ma, Z. Wu, H.C. Furr, C. Lammi-Keefe, N.E. Craft, J. Chromatogr. 616 (1993) 31–37.
[396] C.E. Sänger-van de Griend, K. Gröningsson, J. Pharm. Biomed. Anal. 14 (1996) 295–304.

[397] M.A. Abubaker, M.G. Bissell, J.R. Petersen, Clin. Chem. 41 (1995) 1369–1370.
[398] M.A. Abubaker, M.G. Bissell, J.R. Petersen, J. Cap. Elec. 2 (1995) 105–110.
[399] L.L. Garcia, Z.K. Shibahi, J. Liq. Chromatogr. 16 (1993) 2049–2057.
[400] C. Weinz, G. Blaschke, J. Chromatogr. B 674 (1995) 287–292.
[401] I.M. Johannsson, M.-B. Grön-Rydberg, B. Schmekel, J. Chromatogr. A 652 (1993) 487–493.
[402] L. Steinmann, J. Caslavska, W. Thormann, Electrophoresis 16 (1995) 1912–1916.
[403] P. Meier, W. Thormann, J. Chromatogr. 559 (1991) 505–513.
[404] R.P. Oda, J.P. Landers, Electrophoresis 17 (1996) 431–437.
[405] N.A. Guzman, C.L. Gonzalez, M.A. Trebilcock, L. Hernandez, C.M. Berck, J.P. Advis, In: N.A. Guzman (Ed.), Capillary Electrophoresis Technology, Chapter 22, Marcel Dekker, New York, 1993, pp. 643–672.
[406] J.W. Jorgenson, K.D. Lukacs, Clin. Chem. 27 (1981) 1551–1553.
[407] N.A. Guzman, L. Hernandez, J.P. Advis, J. Liquid Chromatogr. 12 (1989) 2563–2574.
[408] N.A. Guzman, C.M. Berck, International Meeting of the Electrophoresis Society, Washington DC, March 9–21, 1991, Abstract 105.
[409] D. Perrett. In: P. Camilleri (Ed.), Capillary electrophoresis. Theory and Practice, Chapter 9, Boca Raton, CRC Press Inc., Boca Raton, FL, 1993, pp. 371–407.
[410] J. Grimshaw, J. Trocha-Grimshaw, W. Fisher, A. Kane, P. McCarron, A. Rice, P.L. Spedding, J. Duffy, R.A.B. Mollan, J. Cap. Elec. 2 (1995) 40–45.
[411] S. Fujiwara, J. Chromatogr. 447 (1988) 133–140.
[412] R.L. Boso, M.S. Bellini, I. Miksík, Z. Deyl, J. Chromatogr. 709 (1995) 11–19.
[413] H. Shi, Y. Ma, J.H. Humphrey, N.E. Craft, J. Chromatogr. B 665 (1995) 89–96.
[414] E.V. Koh, M.G. Bissell, R.K. Ito, J. Chromatogr. 633 (1993) 245–250.
[415] Z.K. Shihabi, M.E. Hinsdale, A.J. Bleyer, J. Chromatogr. B 669 (1995) 163–169.
[416] N.A. Guzman, J. Liq. Chromatogr. (1998) in press.
[417] Y.-H. Chu, W.J. Lees, A. Stassinopoulos, C.T. Walsh, Biochemistry 33 (1994) 10616–10621.
[418] N.A. Guzman, US Patent 5,045,172, September 3, 1991.
[419] N.A. Guzman, World Patent WO93/05390, March 18, 1993.
[420] N.A. Guzman, US Patent 5,202,010, April 13, 1993.
[421] M. Fuchs, M. Merion, US Patent 5,246,577, September 21, 1993.
[422] H.A. Fishman, J.B. Shear, L.A. Colon, R.N. Zare, J.V. Sweedler, US Patent 5,318,680, June 7, 1994.
[423] N. Brenner, R.H. Palmieri, US Patent 5,340,452, August 23, 1994.
[424] M. Fuchs, M. Merion, European Patent EP0 459,241B1, October 26, 1994.
[425] J. Wiktorowicz, US Patent 5,423,966, June 13, 1995.
[426] S. Naylor, A.J. Tomlinson, L.M. Benson, W.D. Braddock, R.P. Oda, US Patent Application 8/423,220: 1995.
[427] N.A. Guzman, S.S. Park, Tenth Symposium of the Protein Society. San Jose, California, August 3–7, 1996, Abstract T-205.
[428] T.J. Vaughan, A.J. Williams, K. Pritchard, J.K. Osbourn, A.R. Pope, J.C. Earnshaw, J. McCafferty, R.A. Hodits, J. Wilton, K.S. Johnson, Nature Biotechnol. 14 (1996) 309–314.
[429] C.M. Johansson, H.J. Johansson, IBC Conferences: Monoclonal Antibody Purification, La Jolla, California, May 16–17, 1996, Abstract M-112.
[430] V. Kasicka, Z. Prusnik, J. Chromatogr. 273 (1983) 117–128.
[431] R. Bagnatti, V. Ramazza, M. Zucchi, A. Simonella, F. Leone, A. Bellini, R. Fanelli, Anal. Chem. 235 (1996) 119–126.
[432] N.A. Guzman, R.A. Berg, D.J. Prockop, Biochem Biophys. Res. Commun. 73 (1976) 279–285.
[433] F. Lampreave, M.A. Alava, A. Piñeiro, Trends Anal. Chem. 15 (1996) 122–129.
[434] N. Sharon, H. Lis, Science 246 (1989) 227–234.
[435] N. Sharon, Trends Biochem. Sci. 18 (1993) 221–226.
[436] R.C. Cummnings, Methods Enzymol. 230 (1994) 66–86.
[437] S.C. Jacobson, J.M. Ramsey, Electrophoresis 16 (1995) 481–486.
[438] A.W. Moore Jr., S.C. Jacobson, J.M. Ramsey, Anal. Chem. 67 (1995) 4184–4189.
[439] K. Shimura, J. Chromatogr. 510 (1990) 251–270.
[440] P. Lönnroth, U. Smith, J. Intern. Med. 227 (1990) 295–300.
[441] P. Arner, J. Bülow, Clin. Sci. 85 (1993) 247–256.
[442] U. Ungerstedt, J. Intern. Med. 230 (1991) 365–373.
[443] C.E. Lunte, D.O. Scott, P.T. Kissinger, Anal. Chem. 63 (1991) 773A–780A.
[444] K.M. Kendrick, Methods Enzymol. 168 (1990) 182–205.
[445] J.M.R. Delgado, F.V. Feudis, R.H. Roth, D.K. Ryugo, B.M. Mitruka, Arch. Int. Pharmacodyn. Ther. 198 (1972) 9–21.
[446] L.A. Dawson, J.M. Stow, C.T. Dourish, C. Routledge, J. Chromatogr. A 700 (1995) 81–87.
[447] S.Y. Zhou, H. Zuo, J.F. Stobaugh, C.E. Lunte, S.M. Lunte, Anal. Chem. 67 (1995) 594–599.
[448] F. Robert, L. Bert, L. Denoroy, B. Renaud, Anal. Chem. 67 (1995) 1838–1844.
[449] F. von Heeren, E. Verpoorte, A. Manz, W. Thormann, J. Microcol. Sep. 8 (1996) 373–381.
[450] P. Rada, L. Hernandez, S.S. Park, N.A. Guzman, J. Cap. Electr., (1998) in press.