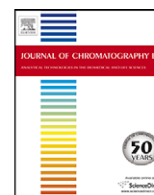
Journal of Chromatography B, 866 (2008) 154–166

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb



Review

Capillary electrophoresis analysis of biofluids with a focus on less commonly analyzed matrices☆

David K. Lloyd*

Analytical R&D, Bristol-Myers Squibb, One Squibb Drive, New Brunswick, NJ 08903, USA

ARTICLE INFO

Article history:
Received 1 November 2007
Accepted 24 January 2008
Available online 7 March 2008

Keywords:
Airway surface fluid
Sputum
Synovial fluid
Amniotic fluid
Saliva
Cerebrospinal fluid
Aqueous humor
Vitreous humor
Sweat
Capillary electrophoresis
Micellar electrokinetic capillary chromatography
Direct sample injection

ABSTRACT

The analysis by capillary electrophoresis of less commonly analyzed biofluids is reviewed. The sample matrices considered include airway surface fluid, sputum, synovial fluid, amniotic fluid, saliva, cerebrospinal fluid, aqueous humor, vitreous humor, and sweat. Many of the techniques used in the analysis of abundant and commonly tested biofluids such as plasma or urine can be applied to these other matrices, e.g. sample extraction prior to analysis. However, for some of these alternative biofluids the available sample amounts are only in the nanoliter or low microliter range, which places constraints on the sample preparation options which are available. For such samples, direct sample injection may be necessary, possibly coupled with on-capillary concentration or derivatization approaches. Particular attention is paid in this review to analyses where the sample is directly injected onto the separation capillary or where minimal sample preparation is performed.

© 2008 Elsevier B.V. All rights reserved.

Contents

1. Introduction 155
2. Sample preparation and effects of matrix composition 155
3. Analysis of airway surface fluid and sputum 156
4. Synovial fluid 157
5. Amniotic fluid 158
6. Saliva 158
7. Cerebrospinal fluid 160
8. Aqueous and vitreous humor 162
9. Sweat 163
10. Other biofluids 163
11. Conclusions 164
References 164

Abbreviations: APOC, 1-(9-anthryl)-2-propyl chloroformate; AF, amniotic fluid; ASF, airway surface fluid; BAL, bronchoalveolar lavage; BGE, background electrolyte; CBQCA, 3-(4-carboxybenzoyl)-2-quinolinecarboxaldehyde; CE, capillary electrophoresis; CE–MS, capillary electrophoresis-mass spectrometry; CD, cyclodextrin; CFSE -5-carboxyfluorescein succinimidyl ester; CIEF, capillary isoelectric focusing; CITP, capillary isotachophoresis; CNS, central nervous system; CSF, cerebrospinal fluid; CZE, capillary zone electrophoresis; EOF, electroosmotic flow; FITC, fluorescein isothiocyanate; LIF, laser induced fluorescence; NBD-F, 4-fluoro-7-nitrobenzo-2,1,3-oxadiazol; NDA, naphthalene-2,3-dicarboxaldehyde; NEM, *N*-ethylmaleimide; SDS, sodium dodecyl sulfate; SF, synovial fluid; TTAB, tetradecyltrimethylammonium bromide.

☆ This paper is part of a Special Issue dedicated to the 50th anniversary of Journal of Chromatography.

* Tel.: +1 732 2277620.
E-mail address: david.lloyd@bms.com.

1570-0232/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2008.01.058

1. Introduction

At the time of writing, a search in Scopus for the terms "capillary electrophoresis" (CE) "capillary zone electrophoresis" (CZE) or "micellar electrokinetic capillary chromatography" (MEKC) results in a listing of over 14,000 papers published since 1997. Certainly this is far less than the nearly 100,000 papers found using the search terms "liquid chromatography" or "HPLC" over the same time period, but nevertheless it represents a substantial portion of the separation sciences literature over the last decade. A very large fraction of the CE literature is devoted to analysis of biological samples, thus it is appropriate to review the use of CE in bioanalysis as part of this special issue commemorating the 50th Anniversary of the Journal of Chromatography.

Biannual reviews of bioanalytical applications of CE have appeared in Analytical Chemistry [1,2], in large part organized by type of analyte. This article is instead organized primarily on the basis of the sample matrix. There are many publications describing the use of CE to quantitate xenobiotics and endogenous compounds in blood (typically plasma or serum) and urine (e.g. see reviews [3,4]). In this paper, the focus is on CE analyses of less commonly analyzed biofluids such as sweat, cerebrospinal fluid (CSF), airway surface fluid (ASF), and a variety of other matrices. Some of these are relatively abundant, while others can only be harvested in sub-microliter volumes. Particular attention is paid to analyses where the sample is directly injected onto the separation capillary or where minimal sample preparation is performed. An important application area of CE which will not be covered in this article is single-cell analysis. This topic is reviewed in this 50th Anniversary volume by Cheng et al. [5], and has also recently been reviewed elsewhere [6].

2. Sample preparation and effects of matrix composition

Sample preparation prior to CE analysis and approaches for directly injecting biological samples have been topics of extensive research activity which has been reviewed elsewhere [7–9]. Many biological sample matrices contain high (sometimes variable) concentrations of salts. In addition, they may contain high concentrations of proteins. Both of these characteristics can cause problems in a CE analysis, thus, the composition of any biological sample plays a significant role in determining the choice of which CE analytical approach to take. A comparison of the composition of a variety of biofluids is presented in Table 1 [10–12].

Most extracellular biofluids have high concentrations of sodium and chloride, of the order of hundreds of mM. However, some, such as saliva, sweat or airway surface fluid (ASF) have lower levels. High concentrations of electrolytes in the sample can lead to peak broadening or distortion in a CE separation. Sample preparation by solid-phase or liquid–liquid extraction can effectively desalt biological samples [7–9]. When directly injecting unextracted biofluids in CE, a simple but often effective solution to peak broadening is to employ a high ionic strength background electrolyte (BGE) in which to perform the separation [13,14], such that the sample conductivity is relatively low compared to that of the BGE. In some cases, the high concentration of chloride present in many biosamples may be used to advantage for the transient isotachophoretic concentration of trace components in a suitably designed separation [15].

Blood and preparations such as plasma or serum have relatively very high protein concentrations (around 75 g/L for plasma), while sweat, urine and cerebrospinal fluid (CSF) have quite low protein concentrations (<1 g/L). Particularly with respect to protein concentration, the differences in biofluid composition illustrated in Table 1 can impact the CE approach and conditions chosen. In most modes of CE the separation occurs in solution in an open

Table 1
Normal composition of some human biological fluids

	Dry mass (units as indicated)	Total protein (g/L)	Na (mM)	K (mM)	Cl (mM)
Aqueous humor	–	0.45 (0.2–0.9)	111 (60–155)	3.2	107 (55–130)
Amniotic fluid[a]	–	2–8	≈130	≈4	≈105
Cerebrospinal fluid	10.8 g/kg (8.5–17.0)	≈0.3 (0.18–0.41)[b]	145 (137–153)	2.96 (2.6–3.3)	125 (119–131)
Plasma	80 g/L (75–100)	75.2 (66.3–88.6)	140 (134–145)	4.03 (3.5–4.6)	104 (100–109)
Saliva[c]	6 g/L (3–8)	(1.4–6.4)	14.4 (5.2–24.4)	20.7 (14–41)	16.3 (6.5–4.9)
Sweat	(3–10) g/L	0.077 (0.060–0.115)	46.8[d]	8.6[d]	29.7[d]
Synovial fluid	34 g/kg (12–48)	12 (5–18)	136	4.0	107.4 (87–138)
Tears	≈19 g/L	8.1 (4.3–12.2)	146	16.2	128
Vitreous humor	–	1.2 [12]	144 [11] (118–154)	7.7 [11] (3.3–12)	114 [11] (89–145)

Individual numbers represent average values while ranges are provided in parentheses. All data from [10] unless otherwise indicated.
[a] Values vary according to gestational age, particularly for protein content.
[b] Lumbar CSF.
[c] Unstimulated production of saliva.
[d] Adults, 17–50 years.

capillary, and interactions between sample components and the capillary walls are undesirable. However, the proteins found in many biological samples at high concentrations can bind strongly to a fused silica capillary surface under neutral or moderately acidic or basic pH conditions. Such adsorption can be directly visualized by microscopy [16] and is manifested in the separation by changes in electroosmotic flow (EOF), broad, tailing peaks which can be difficult to quantitate and may obscure large parts of the electropherogram, baseline shifts, etc. Thus if the matrix of interest does contain large quantities of protein, the choice of analytical conditions will be constrained by the need to eliminate or control undesirable effects of proteins binding to the capillary surface. One approach to dealing with proteinaceous biofluids is sample extraction or protein precipitation to prior to analysis [7–9,17]. However, if off-line sample preparation is performed on a conventional scale, the microanalytical capabilities of CE are lost. For samples such as plasma which are generally available in abundance this is not a problem, but clearly this is not appropriate when only small sample volumes are available. Developments in miniaturization of sample preparation [18] or in/at/on line approaches [19,20] are important to extend the utility of CE where sample preparation is required. Microdialysis offers an *in situ* approach to sample preparation by removal of macromolecules and is often used when sampling from tissues [21].

Alternatively, it may be possible to inject proteinaceous biofluids directly onto the separation capillary using conditions which minimize protein adsorption. A variety of approaches are possible. Different more or less permanently coated capillaries [22,23] have been developed to avoid adsorption of proteins during protein analysis. They may also be effective at reducing protein adsorption when injecting a complex biological matrix. An alternative to a permanent coating is to use dynamically coated capillaries where a coating agent is included in the BGE [24].

Instead of modifying the capillary surface, the protein components themselves may be modified. Operation at extremes of pH [25] may reduce protein adsorption (at high pH both the protein and the fused-silica capillary surface are negatively charged, while at low pH although the protein is positively charged the capillary surface is neutral; neither condition favours protein adsorption). One way to avoid adsorption of matrix proteins which has long been used in liquid chromatography is to complex them with a surfactant such as sodium dodecyl sulfate (SDS) in the mobile phase [26]. Analogous to this, SDS can be used as a component of the CE BGE to effectively reduce protein adsorption [7,27]. Although first applied [28] and later optimized [29–31] for direct injection of plasma, this approach may be used in the analysis of a variety of biofluids and several direct injection assays using surfactant additives are discussed in the following sections dealing with specific matrices.

Capillaries which become coated with biological macromolecules during use can be cleaned up between runs. A variety of post-run washing procedures optimized for the contaminants and surfaces involved have been developed [32–34]. A general limitation of CE is the relatively high limit of detection usually obtained with UV detection. Since more sensitive detection approaches may not be possible for all analytes, on-capillary concentration offers an alternative way to improve the limit of quantitation with direct injection whether by chromatographic [20] or electrophoretic processes [15,35,36].

3. Analysis of airway surface fluid and sputum

Covering the surface of the airway epithelia is a layer of liquid a few tens of microns in thickness, the airway surface fluid (ASF). A more fluid sub-layer may be covered by a complete or discontinuous mucus film. ASF has various roles in protection of the lung. Mucus can physically entrap inhaled particles and microbes, and is cleared from the airways by beating of the epithelial cilia. Furthermore, a variety of proteins and peptides with antimicrobial functions are present in ASF [37]. Mucocilliary clearance is impaired in cystic fibrosis because of changes in the inorganic ion composition of ASF [38]. Thus there is considerable interest in determination of both the small and large-molecule components of ASF. Nevertheless, analysis of ASF is challenging both because of the relative inaccessibility of the airway epithelia, as well as of the low volumes of ASF available (≈1–3 μL/cm^2 of airway epithelium).

To overcome the difficulties of sampling ASF *in vivo*, much research has been performed using cultures of bronchial epithelial cells. This approach can yield ASF proteins in hundreds of microgram quantities, e.g. for proteomic studies [39]. However, there are limitations to *in vitro* studies and so it is also necessary to sample ASF *in vivo*. One technique used for this purpose is bronchoalveolar lavage (BAL), wherein saline solution is instilled into the airways and then aspirated out again after having dissolved some ASF. A great deal of information has been obtained on ASF using BAL, for example a database on BAL proteins was recently published in this journal [40]. However, some limitations are clear: it is not possible (especially in small animals) to get very localized sampling, and the variable degree of dilution makes accurate quantitation impossible, although this can be compensated for to a certain degree through the use of urea as a probe of dilution (urea is considered to be freely permeable through the airway epithelium and thus present in ASF and plasma at the same concentrations, hence it can be used to calculate the approximate dilution of ASF in BAL samples).

Given the limitations of BAL, other approaches to sampling of ASF have been investigated. The airways are accessible via bronchoscopy in humans or intubation in smaller animals, allowing development of a variety of direct-sampling approaches. One alternative has been to apply a piece of filter paper to the airway surface, which soaks up a few microliters of ASF [41]. My group developed an approach whereby a polyethylene capillary was introduced into the airway and touched against the epithelium, allowing collection of submicroliter quantities of ASF [42]. The separation capillary is then inserted into the end of the collection capillary to directly inject a nanoliter aliquot of the collected sample onto the CE system. This capillary sampling technique is particularly suited to smaller animals such as rats, while filter paper collection has been used in larger species. A comparison of the two approaches suggests that both may potentially be subject to bias, either due to the strong force generated upon the epithelial surface due to liquid uptake into the filter paper, or the possibility of trauma to the epithelium as a sampling capillary touches the surface [43]. These sampling techniques were used to harvest ASF for analysis of the inorganic ion content. CE measurements were made of rat ASF collected into sampling capillaries, with the separation capillary being inserted into the sub-microliter volume of ASF in the collection capillary in order to inject sample. CE with both indirect UV [42] and conductivity detection [44] was used for determination of inorganic ions in ASF. These measurements as well as the analysis of proteins in ASF [45] have previously been reviewed in some detail [46]. Initially it appeared that there may be some systematic bias between the filter-paper and capillary sampling techniques [43], since generally isotonic levels of inorganic ions were determined using filter paper sampling in dogs, while those determined in rats by capillary sampling were hypotonic. Recent results using ion exchange beads to harvest ASF and X-ray microanalysis to determine inorganic ions in the collected sample [47,48] are generally in agreement with data collected in large animals by the filter paper technique and in rodents using capillary sampling and CE, leading to the conclusion that both approaches are producing essentially reliable results and that the differences observed truly reflect inter-species differences.

The capillary sampling/CE analysis approach has also been applied to the determination of ASF composition in mice [49]. Sampling of ASF from mice is more challenging than from rats due to their smaller size, and a longer intubation period was necessary (30 min vs. 3 min) to collect adequate ASF (100–300 nL) for analysis.

With an effective method for collecting ASF samples, many ASF components can be investigated by CE. Analysis of principal ASF proteins has already been mentioned [45]. NO metabolites nitrate and nitrite may also be measured in ASF by CE [50]. The physiological role of NO is a topic of intense research and various effects have been proposed in the airways, and CE has been widely used in measurement of compounds related to the l-arginine/nitric oxide pathway [51]. Nitrate and nitrite can be detected in ASF by CE with conductivity detection [44], but direct UV detection at 214 nm proved advantageous because it is more selective than conductivity detection in this analysis [50]. In particular, there is no interference from chloride, allowing the development of a faster and more specific method. This is illustrated in Fig. 1 which shows a comparison of nitrate and nitrite measurements in rat ASF using conductivity detection and direct UV absorbance detection. The capillary sampling/CE analysis approach provided results which were similar to those previously determined in BAL or sputum samples, but has the advantage of being able to sample a particular location within the airways.

Glutathione has been measured in rat ASF by CE, with a modified capillary sampling procedure [52]. After collection of approximately 1 ml of ASF from the combination of three to five capillary samplings, the ASF was acidified and derivatized with *N*-ethylmaleimide (NEM). Alkylation of the free thiol group in reduced glutathione by NEM was performed to avoid on-capillary conversion between reduced and oxidized glutathione, and this procedure also resulted in a better resolution of the two species. Limits of detection were around 10 μM with UV detection, and levels of several hundred μM were determined in rat ASF.

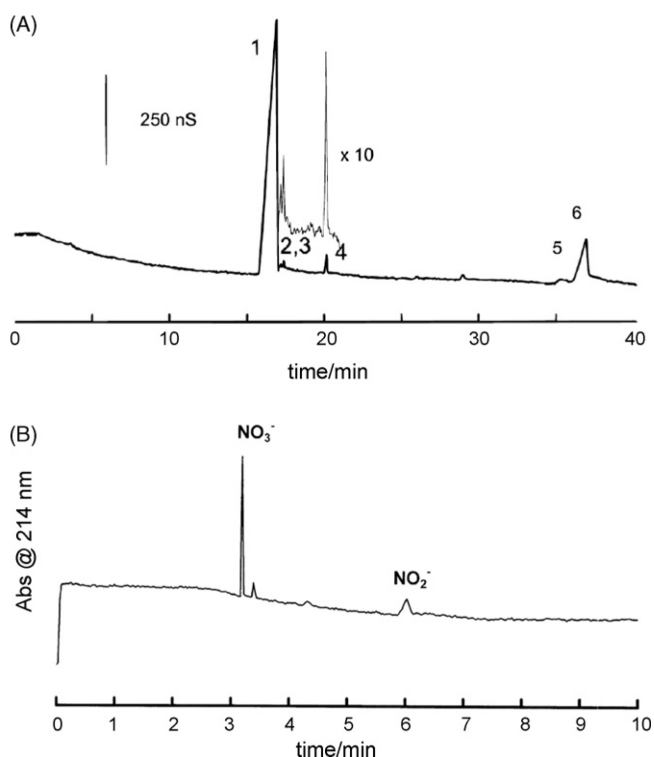


Fig. 1. (A) Analysis of nitrate, nitrite and other anions in rat airway surface fluid using CE with conductivity detection. BGE, 100 mM CHES, 40 mM LiOH/2-propanol (98:2 v/v), pH 9.3, with 80 μM spermine as EOF modifier. Peak identification: (1) chloride; (2) nitrite; (3) nitrate; (4) sulfate; (5) phosphate; (6) bicarbonate. Reproduced with permission from [34], copyright, the American Chemical Society, 1997. (B) Analysis of nitrate and nitrite in rat airway surface fluid using direct UV detection. BGE. 50 mM phophate, pH 3.0, with 0.5 mM spermine as EOF modifier. Reprinted from [50] (2001) with permission from Elsevier.

In many of the anion separations described here, spermine was used as an EOF modifier. It has a low UV absorption coefficient and fairly low conductivity, making it a useful modifier for use with UV or conductivity detection, and unlike many cationic surfactants does not cause precipitation of proteins [53] making it particularly suitable for use with directly injected biofluids. Vigorous between-run washing with 0.5 M NaOH (followed by adequate re-equilibration with BGE) also helped avoid significant problems with protein adsorption.

Various infections result in the production of significant quantities of sputum, which is comprised of a mixture of secretions coughed up from throughout the respiratory tract. Determination of the concentration of antibiotics in the sputum is of interest in developing an understanding of their penetration into lung secretions. Cephalosporins have been determined in sputum by MEKC [54,55], using a pH 9.1 borate buffer containing 50 mM SDS, the surfactant helping to avoid adsorption of sample components to the capillary surface. Vigorous between-run washing with 0.5–1 M NaOH and 100–300 mM SDS solutions was also employed. Samples were obtained by aspiration from the trachea, lyophilized, and re-dissolved in a methanol:water mixture before CE analysis (direct injection of sputum samples resulted in poor analytical reproducibility).

4. Synovial fluid

Synovial fluid (SF) bathes and lubricates the cartilage joint surfaces. In addition, it acts as a carrier for nutrients and oxygen since the joint cartilage has no blood supply. SF originates from the plasma, with small molecules and smaller proteins freely diffusing from plasma into the SF, although larger MW proteins are excluded. Total protein concentration is around 10–30 g/L (somewhat lower than plasma but higher than many other biofluids discussed here) and there is a high concentration (3–4 g/L) of hyaluronan, a major lubricating component [56]. A human knee may contain 0.5–2.0 mL of SF, a sample of which can be collected by joint aspiration. On the other hand, it may be difficult to obtain more than a few microliters from small animals, e.g. multiple aspirations from rabbit joints being required to pool a few tens of microliters of sample [57].

CE has been used for the analysis of a variety of SF components, including small inorganic ions as well as the macromolecular species. Hyaluronan is an important extracellular component in many tissues and extracellular fluids, with literature reports on its determination by electrophoresis and chromatography [58]. Surfactant-containing BGEs have been used to analyze proteins [59], however CE analysis of intact hyaluronan in SF was reported to be problematic [60] even under MEKC conditions to minimize protein adsorption. Grimshaw and co-workers [61] did successfully use similar CE conditions to directly inject SF and determine the presence and quantity of α_1-acid glycoprotein in these samples. α_1-acid glycoprotein formed a sharp peak well-resolved from other major SF proteins in the analysis.

Since SF samples on the orders of hundreds of microliters can be obtained by joint aspiration, off-line sample preparation is an alternative to direct injection of SF [62]. Thus, around 200 μL SF could be diluted with buffer and a hyaluronase to digest the polysaccharide resulting in a mixture containing primarily a tetrasaccharide and a smaller hexasaccharide component (the authors noted that 25 μL sample would suffice if necessary). The digested mixture could be separated by CE with a phosphate/borate BGE, pH 9, and SDS, the presence of the surfactant being important to avoid interference of

the major SF proteins with the analytes in the separation, and also no doubt to reduce their interaction with the uncoated capillary surface.

The glycosaminoglycan composition of SF may provide a marker of cartilage formation and decomposition. Chondroitin 4-sulfate and chondroitin 6-sulfate disaccharides have been determined by CE at low pH after enzymatic degradation of chondroitin sulfate in SF (and in related tissues such as synovium) [57,63,64]. These methods and others described in CE publications related to these disaccharides [65] rely on low-wavelength UV detection without derivatization, providing adequate sensitivity for determination of these species in SF digests; there exist many other approaches, e.g. by HPLC [66] or polyacrylamide gel electrophoresis [67] where derivatization is required for detection.

As in many other biological matrices [51], there is interest in analysis of NO metabolites nitrate and nitrite in SF. Davies et al. [68] have reported their determination in a variety of biological matrices including SF by CE with direct UV detection. Good resolution from small anions other and SF components was achieved through the use of an EOF reversing agent (tetradecyltriethylammonium bromide) in a reversed-polarity separation. Biological samples were pretreated by ultrafiltration to remove proteins which improved migration-time reproducibility, a preparation procedure which probably also avoided protein precipitation with the cationic surfactant.

5. Amniotic fluid

Amniotic fluid (AF) offers physical protection by cushioning and helping maintain a constant temperature around the fetus. Chemically, its composition changes significantly throughout pregnancy (Table 1). The protein content is very low in early stages of pregnancy, but increases later on, with α-fetoprotein present at very high levels relative to the maternal blood [69]. AF contains many compounds related to fetal development and abnormalities [69], and proteomic studies are revealing more potential biomarkers of disease [70]. Diagnosis of several disorders is performed by DNA analysis for which various analytical techniques may be suitable including CE [71].

Compared to some of the other biofluids discussed in this review, there are relatively few reports of the use of CE to analyze AF, indeed, the first CE analysis of AF was reported as recently as 2001 [72]. In that work, several principal UV-absorbing components of AF were separated, seven of which were identified as proteins while xanthine was also a significant feature in the electropherograms. The authors investigated a variety of separation conditions which have been reported to be useful for serum protein analysis in uncoated capillaries, choosing a borate buffer at pH 9.25 as most suitable for AF analysis. EDTA was used as an additive which improved peak shape, and may well have a positive effect on long-term reproducibility by helping keep the capillary surface clean from contamination with metal ions [73]. The authors also studied various sample preparation options including dialysis to remove salts, and concluded that a simple 1:1 dilution with water provided best performance (although direct injection without prior dilution was not listed as a condition used).

Abnormal amino acid levels in blood are indicative of a variety of pathologies, and may also be useful biomarkers in AF. Acetonitrile precipitation of proteins has been used for AF sample preparation prior to CE analysis of amino acids [74]. The use of conductivity detection allowed analysis of all proteinogenic amino acids and a variety of other biogenic compounds (Fig. 2). For this group of analytes, detection limits were in the range 1.5–9 μM. Compared to simple dilution or use of sulfosalicylic acid as a precipitation agent, improved peak stacking was observed with acetonitrile precipitation similar to observations in other biofluids [17], resulting in an approximately four-fold reduction of detection limits compared to an analysis without stacking.

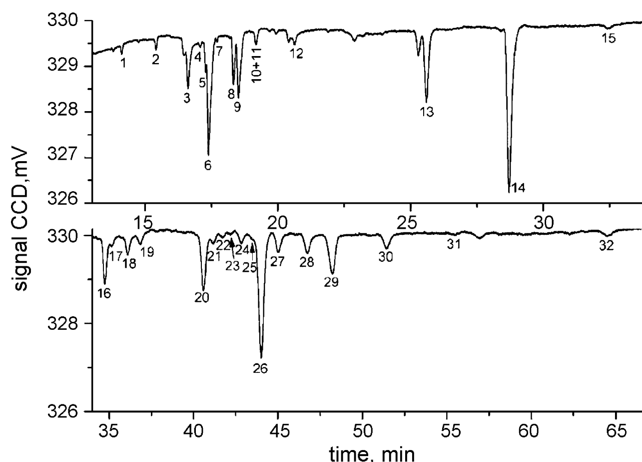


Fig. 2. Analysis of amino acids and other biogenic substances in amniotic fluid after deproteinization with acetonitrile 1:1 (v/v). BGE was 1.7 M acetic acid with 0.1% hydroxyethylcellulose (pH 2.15); detection by contactless conductivity. Peak identification: (1) ethanolamine, (2) choline, (3) creatinine, (4) β-Ala, (5) ornithine, (6) Lys, (7) GABA, (8) Arg, (9) His, (10) 1-methylhistidine, (11) 3-methylhistidine, (12) carnitine, (13) Gly, (14) Ala, (15) 2-aminobutyric acid, (16) Val, (17) Ile, (18) Leu, (19) Ser, (20) Thr, (21) Asn, (22) Met, (23) Trp, (24) Gln, (25) citrulline, (26) Glu, (27) Phe, (28) Tyr, (29) Pro, (30) cystine, (31) Asp and (32) 4-hydroxyproline. Reprinted from [74] (2006) with permission from Elsevier.

A high sensitivity analysis of estriol conjugates in AF has been reported using CE–MS/MS [75]. Detection limits of the order of 1–3 nM were achieved without any sample preconcentration (AF was passed through a 3000 MW cutoff membrane, and the filtrate injected directly). The method was validated for concentrations up to 500 nM.

As well as determination of endogenous components of AF, zidovudine and zidovudine monophosphate have been analyzed in rat AF, plasma and placental tissue by MEKC with UV detection [76]. Amniotic fluid samples underwent protein precipitation with acetonitrile, followed by evaporation of the supernatant and reconstitution in phosphate buffer. Validation data over multiple days demonstrated that the performance of the assay was suitable for drug distribution studies.

6. Saliva

Saliva is a readily available biofluid produced in liter quantities daily by humans. Analysis of saliva components is useful diagnostically for both local and systemic diseases, and measurements of pharmaceuticals or drugs of abuse may also be made in this matrix. Chiappin et al. recently published a detailed review of the opportunities presented by saliva analysis, in which they also provided a good overview of saliva production and sampling [77].

Salivary peptides and proteins have been analyzed using a variety of CE approaches. An early report described the analysis of histatins (approximately 20–40 mer histidine-rich peptides) which form the major basic peptide components of saliva. After acidification and boiling of saliva (a fairly common pretreatment for saliva, to precipitate some proteins and to inhibit proteases), CZE at pH 2.5 resulted in high-efficiency separations of the histatins in parotid saliva, with detection by UV absorbance [78]. Saliva has been used as a model biological matrix to demonstrate the utility of other approaches to separation of basic proteins, e.g. poly(diallyldimethylammonium chloride) as a capillary coating agent [79] or didodecyldimethylammonium bromide-coated nanoparticles as a capillary packing [80]. Fluorescence-based

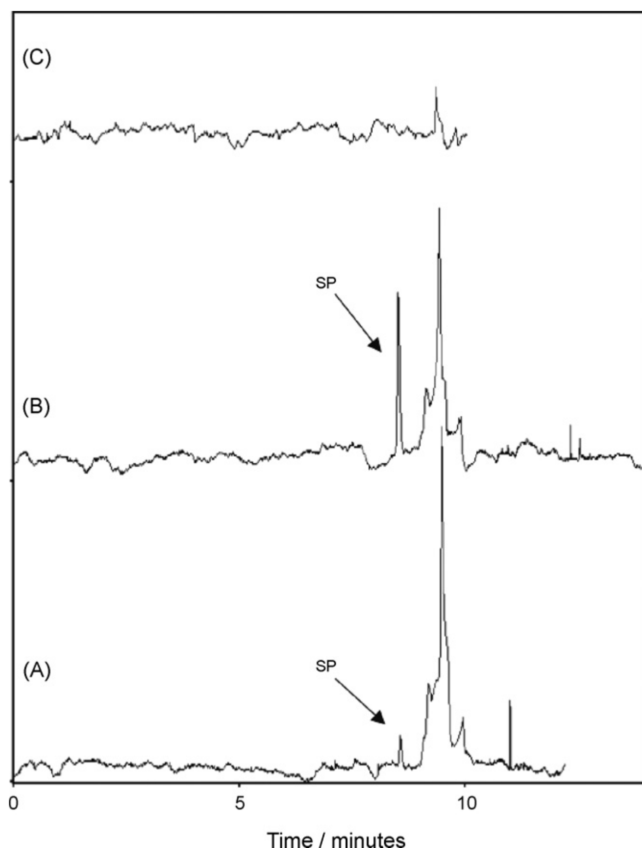


Fig. 3. Determination of NDA-derivatized substance P in saliva extract. (A) Unspiked saliva sample showing endogenous substance P at approximately 3 nM. (B) Saliva spiked with substance P. (C) Blank. BGE, 0.45 mg/mL hydroxypropylmethylcellulose in 10% acetonitrile/90% 18.8 mM borate, pH 9.3. Reprinted from [82] (2004) with permission from Elsevier.

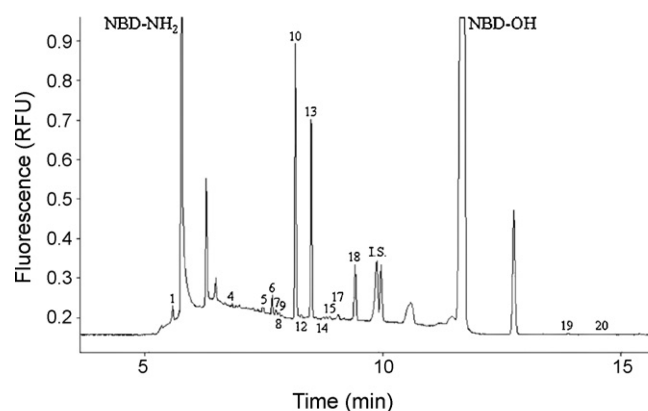


Fig. 4. Analysis of amine compounds in saliva after derivatization with NBD-F. BGE was a 20 mM borate buffer, pH 9.3 containing 7% methanol, 50 mM sodium cholate, 5 mM β-CD and 20 mM Brij 35. Peak identification: (1) arginine; (4) tyrosine; (5) lysine; (6) ornithine; (7) phenylalanine; (8) citrulline; (9) leucine/isoleucine; (10) histidine/valine; (12) glutamine; (13) proline; (14) threonine; (15) alanine; (17) serine; (18) glycine; (19) glutamic acid; (20) aspartic acid. IS is gly–gly. Reproduced from [89], with kind permission from Springer Science and Business Media.

detection has been used with fluorescence-labeled antibodies to analyze secretory immunoglubulin A in saliva by CE over the range 1-8 μg/mL (typical values found in saliva)[81]. Colón's group published a CE method for analysis of substance P in salvia, which was carefully optimized to achieve an LOD of 100 pM [82]. Saliva was passed through a C_{18} solid-phase extraction cartridge which was washed to remove more polar components before substance P was eluted with pure acetonitrile. The analyte was then derivatized with NDA. This sample preparation and the resulting low-conductivity matrix was designed to provide considerable sample concentration on-capillary by electrokinetic injection ("field amplified sample injection"). The combination of LIF detection and stacking injections led to the high sensitivity obtained. Fig. 3 shows analysis of substance P in saliva at low nanomolar levels using this method.

Some recent publications have described CE-based approaches to analysis of the saliva proteome. Capillary isoelectric focusing (CIEF) has been used in combination with nano-reversed phase HPLC and mass spectrometry to profile and identify over a thousand salivary proteins [83]. Most were of human origin, but 31 were of bacterial origin, unsurprising given the nature of a saliva sample (the possibility of using CZE–MS to identify specific bacterial peptides had previously been reported for bacteria cultured from microbially spiked saliva [84]). Capillary isotachophoresis (CITP) was later used in place of CIEF to give selective enhancement of trace proteins [85] resulting in an even higher number of identified salivary components. In both these approaches, fractionated material from the initial CE step was loaded onto reversed-phase trapping columns before nano-LC. Although not capillary in format, the use of free-flow electrophoresis for pre-fractionation of salivary tryptic peptides before micro-LC–MS has also been reported [86].

The reports of amino acid analysis in saliva by CE have mostly described the use of various derivatization approaches with fluorescence detection. An acid plug injected after the sample was used to stack 9-fluorenylmethyl chloroformate-derivatized amino acids in saliva and other biological matrices [87]. Major ions such as chloride are unaffected by the acid plug and migrate through it when the electric field is applied, but the weak acid components are neutralized and stack at the boundary of the acid plug until its pH is modified by the BGE, after which the weak acids re-ionize and start to migrate. Saliva and blood samples were deproteinized by acetonitrile precipitation, then the supernatant was dried and reconstituted in water before analysis. Fluorescein-labeled amines were separated and detected in saliva using a microchip CE apparatus [88], but individual components were not identified in this analysis used for profiling pre- and post-exercise effects on saliva. Fluorescein isothiocyanate (FITC) derivatized amino acids were analyzed in saliva by CZE with a pH 9.5 borate BGE. The derivatization involved a 1:20 dilution of saliva in buffer, and no further sample preparation was required, however only data on proline and glycine in saliva were shown. Recently, an MEKC method was described for the analysis of 4-fluoro-7-nitrobenzo-2,1,3-oxadiazol (NBD-F) labeled amine metabolites in saliva, plasma and urine [89]. One sample preparation approach involved preparation of 5 μL of each biofluid by acetonitrile precipitation followed by derivatization and Fig. 4 illustrates the analysis of saliva using this method. For plasma an alternative approach was also demonstrated where only 100 nL of sample was directly reacted without protein precipitation and then injected onto the separation capillary; a similar approach would likely be feasible with other biofluids such as saliva.

Sixteen amine metabolites in saliva were identified by spiking, with detection limits typically in the low tens of nanomolar range. To avoid derivatization, contactless conductivity detection has been employed for amino acid analysis with direct injection of saliva [90]. Hydroxyethylcellulose was used as an additive to the 2.3 M acetic acid BGE to avoid contamination of the capillary surface. Detection limits were in the tens of micromolar range, allowing analysis of proline and glycine in saliva. Small organic acids may be directly determined by CE with amperometric detection; lactate has been measured in this way in diluted saliva, with a run time of less than 3 min [91].

Several reports deal with the analysis of inorganic ions in saliva by CE. UV absorbing anions nitrate, nitrite and thiocyanate

were determined in saliva by MEKC employing *N*-tetradecyl-*N*,*N*-dimethyl-3-ammonio-1-propanesulfonate (Zwittergent-3-14) as a surfactant additive to the BGE [92]. Saliva was injected without deproteinization with the surfactant effectively protecting the capillary surface from modification (although the cationic surfactant cetyltrimethylammonium chloride which was also investigated did not eliminate modification of the capillary due to matrix components). Alternatively, CZE with direct UV detection was described for thiocyanate analysis, using a 0.1 M β-alanine-HCl BGE, with the saliva preparation step being a 20-fold dilution in water [93]. A similar sample preparation was employed before CITP to determine nitrate, nitrite, iodide and thiocyanate in saliva from smokers and non-smokers [94]. Polyvinylpyrrolidine was used as an additive in the HCl leading electrolyte, since it was found to modify the mobilities of the analytes leading to fully separated rather than mixed zones. The LODs achieved were in the low μM range, similar to CZE with UV detection [93]. Tanaka et al. [95] used a triple-coating of the capillary surface with polybrene-dextran sulfate-polybrene to achieve flow reversal and limit protein adhesion in the CZE analysis of nitrate and nitrite. However, to achieve good reproducibility preconditioning of the capillary with polybrene was required prior to each sample injection; since saliva was diluted with an approximately equal volume of acetonitrile, it was considered that the acetonitrile may cause stripping of the coating. Unlike some other reports where saliva was used purely as a model matrix, this work did describe the use of the method in support of an extended study of the diurnal variation in salivary anions. In another report, the same group described the development of a chip-based CE analysis for nitrate and nitrite, with a run time of 15 s [96]. Metal ion separations in saliva have been reported using indirect UV detection, with either a copper (II) acetate-ethylenediamine BGE [97] or an imidazole-containing BGE [98]. In the latter report on analysis of lead in saliva, a selective extraction procedure was employed prior to CE, using the surfactant polyethylene-glycolmono-*p*-nonylphenylether which preferentially forms a complex with $[Pb(OH)]^+$ but not with other metals [98]. Most of the above papers concerned the analysis of human saliva, but one article describes the analysis of inorganic ions in cockroach saliva [99]. Salivary glands were dissected from the insect and suspended in cockroach physiological saline. After stimulation with dopamine or serotonin, saliva secreted at a rate of around 300 nL/min was collected from the salivary duct and diluted in 100 μL water before CE analysis.

A number of authors have reported the analysis of drugs in saliva. Thormann et al. used saliva as one of several matrices in investigations of suitable MEKC conditions for the direct injections of biofluids [100,101]. Antipyrene was measured in saliva using MEKC with SDS as micellar additive which minimized the effects of the sample matrix and helped ensure repeatability of the assay [102]. Enantiomers of albendazole sulfoxide were concentrated from saliva by liquid–liquid extraction (LLE) before separation using CE with sulfated-β-cyclodextrin (CD) as an additive [103]. With UV absorbance detection, LODs of around 125 ng/mL were possible.

7. Cerebrospinal fluid

CSF is a clear liquid which surrounds the central nervous system (CNS) structures. A total volume of 125–150 mL CSF in an adult human is contained primarily in the spinal canal, the ventricles of the brain and in the subarachnoid space. CSF is separated from the blood by the blood–brain barrier which allows free diffusion of small polar species such as dissolved O_2 and CO_2, but which does not allow passive transport of macromolecules (although active transport of many proteins does occur). CSF is secreted by the choroid plexus and ventricular membrane. Isolated from the plasma, it contains a variety of chemical species which can be specifically related to the CNS. Analysis of the composition of CSF is well established in the diagnosis of neurological diseases such as multiple sclerosis, acute bacterial meningitis, viral neuro-infections, or autoimmune diseases [104], and with the application of increasingly sophisticated analytical approaches the CSF proteome is yielding new biomarkers of neurological disorders [105,106].

With a low protein concentration compared to other biofluids considered thus far, CSF may be considered a more analysis-friendly matrix than plasma. However, where sample is directly injected, most of the CE analyses of CSF still employ approaches which limit modification of the capillary surface.

Since the protein composition of CSF is of considerable diagnostic value [104,107] it is not surprising to find several publications related to CE of CSF proteins. An early paper described CZE using a hydrophilic-coated capillary to achieve high efficiency separations of 20–25 components in undiluted CSF, many of them proteins [108]. CE was compared to high resolution planar agarose gel electrophoresis for determination of elevated γ-globulin as a diagnostic indicator of multiple sclerosis [109]. A high-pH buffer was used modified with polyethylene glycol and a zwitterionic additive to improve resolution of the γ-globulin region of the separation. CSF samples were concentrated approximately 30-fold by ultrafiltration prior to analysis by CE and planar agarose gel electrophoresis, and although some preliminary results were shown on unconcentrated CSF, improvements in detection were considered necessary before this approach could really be feasible. A similar separation with high pH borate BGE without additives was shown by Ivanova et al. [110]; a fairly lengthy washing procedure (5 min each 1 M NaOH, 0.1M NaOH, water and buffer) was required between runs to ensure adequate reproducibility. It was suggested that determination of abnormal CSF γ-globulin patterns was possible without preconcentration although no quantitative data were shown. Surfactant-containing BGEs may also be useful for determination of CSF γ-globulins. A separation using borate buffer at pH 10 with 25 mM SDS was shown to resolve components in directly injected CSF which correlated well with results from gel isoelectric focusing [111]. Again, the importance of appropriate capillary washing procedures was highlighted, with the use of both 0.1 M NaOH and SDS-containing solutions.

Hiraoka et al. [112,113] used miniconcentrators to prepare CSF fractions containing components in the 10,000–50,000 MW range, and separated these using a variety of CE techniques – CZE, capillary gel electrophoresis (CGE) and CIEF – in the analysis of β_2-microglobulin, β-trace protein and other low MW proteins. High resolution CIEF separations were shown by Manabe et al. [114], who constructed an apparatus to dialyse 20–30 μL volumes of CSF to desalt the sample. About 70 fully or partially resolved peaks were obtained, many of which appeared to correspond to plasma proteins and some of which were unique to CSF.

Quite a different approach was applied to CSF protein analysis by Tseng et al. [115] who used pH 9 or 10 Tris buffer, containing up to 2% polyethylene oxide which provided sieving and capillary wall-coating functions in this separation. Moreover, a plug of SDS was introduced just before the protein-containing sample, and binding of the SDS to the analytes improved efficiency. Native fluorescence detection using laser excitation at 266 nm was employed for detection. β-trace protein and human serum albumin in untreated CSF were separated in this way. The performance of this separation with more conventional UV detection was not described.

As noted earlier, there is significant interest in the CSF proteome [105,106]. CE–MS was used by Wittke et al. [106] to determine intact low MW proteins in CSF. Sample pretreatment was by ultrafiltration followed by chromatographic purification on a C2 column, lyophilization and then reconstitution in water. These samples were subsequently analyzed by CE with

a formic acid, 20% acetonitrile BGE, with detection by time-of-flight (TOF)-MS. Using this approach, around 450 individual proteins and polypeptides were resolved by mass and/or migration time. Several potential biomarkers for Alzheimer's disease and schizophrenia were reported. Meanwhile, Wetterhall et al. [116] have described the CE–MS analysis of tryptic peptides from CSF. CE–electrospray injection–Fourier transform ion cyclotron resonance–mass spectrometry (CE–ESI–FTICR–MS) was compared with infusion ESI–FTICR–MS, the inclusion of the separation step allowing greater overall resolution with the use of smaller amounts of sample.

Moving down in molecular weight to a different class of analytes, many smaller peptides are of significant interest in CSF. Several authors have analyzed enkephalins spiked into CSF as a model system. An early bioanalytical CE paper described a CE–MS approach to analysis of leu- and met-enkephalin spiked in CSF [117], which were analyzed after protein precipitation or solid-phase extraction, with a stable-label internal standard, resulting in an approximately 2 μg/mL limit of detection. A recent paper describes sample preparation of CSF, using an elegant on-line multidimensional system with size-exclusion for removal of major proteins and a reversed-phase trapping system for chromatographic concentration, after which on-capillary stacking may also be performed [118]. Using this approach, a limit of quantitation of around 2.5 μg/mL for enkephalins spiked into CSF was possible with UV detection. Off-line preparation can also be used to achieve fairly similar results [119]. However, all of these approaches were demonstrated on spiked CSF, with concentrations of enkephalins some three orders of magnitude higher than endogenous levels. Refinement of the on-line sample preparation reduced detection limits for spiked enkephalins to around 100 ng/mL from 20 μL CSF samples [120] with UV detection, a very significant improvement, but still not quite adequate for determination of endogenous enkephalins.

In order to achieve lower detection limits more suitable to the analysis of endogenous levels of peptides in CSF, laser-induced fluorescence (LIF) detection has been used coupled with CE. MEKC with LIF detection was used for analysis of the pentapeptide enterostatin [121]. Offline sample preparation was performed using protein precipitation with trichloroacetic acid followed by derivatization with naphthalene-2,3-dicarboxaldehyde (NDA). With excitation from an argon ion laser, an LOD of around 2 ng/mL was achieved. Good assay recovery was demonstrated and endogenous levels of around 18 ng/mL enterostatin were measured. For the ultimate selectivity in sample preparation, immunoaffinity methods have been used [122,123] in the analysis of a variety of cytokines in CSF, with the antibody immobilized at the head of the capillary or separation channel. Using an immobilized FAb fragment for selective enrichment of the analyte, followed by on-capillary derivatization and diode-laser based fluorescence detection, LODs in the low ng/mL range were achieved in a capillary-format instrument [122], while in a more recent chip-format experiment LODs in the pg/mL range were demonstrated [123]. In both these reports, fairly extensive validation studies were performed including comparison with standard immunoassays. In Fig. 5, the cytokine profiles of CSF from subjects with different head injuries are shown, using a chip-based instrument. The selective sample preparation and detection scheme results in a fairly limited resolution requirement for the CE analysis, and the chip-based separation takes <2 min.

Quite a number of papers have been published on amino acid analysis in CSF, as well as publications on other small organic acids and amines. In almost all of the literature related to amino acids in CSF, detection is performed by LIF, giving more than adequate concentration sensitivity (many amino acids are present at low μM concentrations in CSF). The first report of quantitative amino acid data from CSF using MEKC was from Bergquist et al. [124], with 3-(4-carboxybenzoyl)-2-quinolinecarboxaldehyde

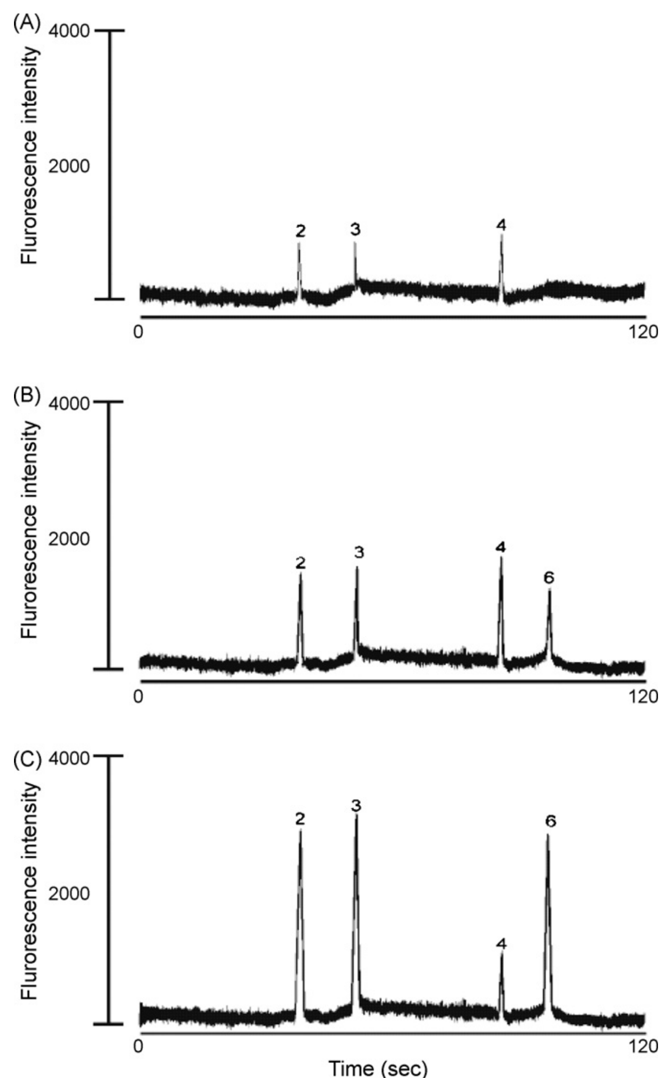


Fig. 5. Separation of four inflammatory cytokines in CSF using chip-based CE with immunoaffinity sample preparation. A, B and C are samples from patients following head trauma, and illustrate different cytokine levels which may be correlated with the clinical outcome. Analytes were selectively removed from 500 nL CSF samples by FAb fragments immobilized in the separation channel after which they were labeled *in situ* followed by electrophoresis in a 75 mm long separation channel. Peak identification: (2) TNF-α; (3) IL-1β; (4) IL-6; IL-8. Reproduced with permission from [123] (2004), copyright Wiley-VCH Verlag GmbH & Co. KGaA.

(CBQCA) as a derivatizing agent with argon ion laser excitation at 488 nm for detection. Derivatization was performed in just 10 μL of CSF, and detection limits ranged from 0.2 to 100 μM for the ten amino acids identified. Since then a variety of other fluorescent labels have been used, e.g. fluorescein isothiocyanate (FITC) [125-127], 1-(9-anthryl)-2-propyl chloroformate (APOC) [128], 5-carboxyfluorescein succinimidyl ester (CFSE) [129] or NDA [130–132] in combination with lasers operating in the visible region. Alternatively, UV lasers can be used to excite native fluorescence from tryptophan, tyrosine, and small organic acids which may be of interest in CSF [133,134]. For derivatized samples, low nanomolar detection limits have been reported in recent papers using NDA [130–132], with on-capillary stacking contributing to obtaining a higher sensitivity [132]. For chiral analysis of CSF amino acids, reaction with either (+)- or (−)-APOC was performed for the dual purpose of providing a fluorophore for detection and as a chiral derivatizing agent to form diasteroisomers to achieve chiral resolution [128]. 351 nm light from an argon ion laser was used to excite fluorescence (resulting in low-nM LODs), and MEKC separation was

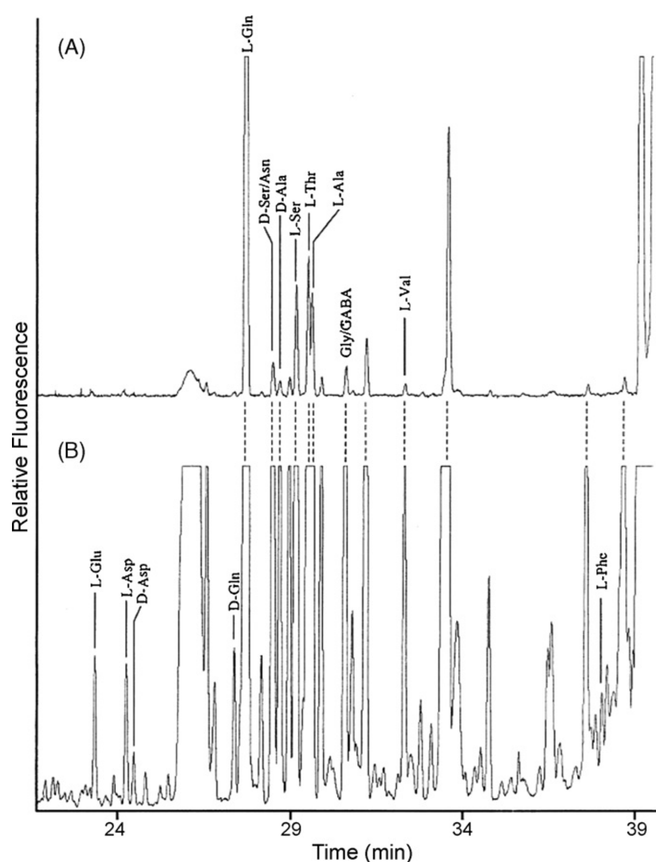


Fig. 6. CSF amino acids separated by MEKC with LIF detection, after derivatization with (+)-APOC. Low and high sample loadings are shown: (A) 1.2 nL injection of 100-fold diluted sample. (B) 5 nL injection of 10-fold diluted sample. BGE, 20 mM borax buffer, pH 9.8 with 20 mM SDS and 7.5 mM SDC. Reprinted from [128] (2000) with permission from Elsevier.

performed using a BGE containing a mixture of SDS and sodium deoxycholate (Fig. 6). Samples were derivatized with both (+)- and (−)-APOC, which helped demonstrate which of the peaks resolved were in fact due to amino acid enantiomeric pairs.

CE–MS has been used to measure tryptophan and its metabolites in the kynurenic pathway in CSF [135]. The use of time-of-flight (TOF) MS detection was required to give suitably rapid scanning for detection of peaks <10 s in width, and detection limits around 20 nM were achieved. To achieve good analytical reproducibility, deactivation of the capillary surface was performed using a pre-rinse with M7C4I, a quaternary ammonium salt containing an alkyl iodine functionality [22].

Changes in the CSF levels of some small organic acids such as lactic acid may be associated with disease states, and there are a few papers devoted to their analysis by CE in CSF. Ultrafiltered CSF was analyzed by CE with low wavelength (185 nm) UV detection, using tetradecyltrimethylammonium bromide (TTAB) as a capillary coating and flow modification agent in the BGE, to determine species such as oxalate, fumarate, acetate, pyruvate, lactate, glutamate and ascorbate with detection limits in the low μg/mL range [136]. Saavedra and Barbas determined D- and L-lactic acid in CSF, considering that the D-form may be a byproduct of microbial metabolism [23]. Centrifuged and diluted (1:4 with water) CSF, plasma and urine samples were separated using hydroxypropyl-β-CD as a chiral selector in a pH 6 phosphate buffer, with a polyacrylamide coated capillary. Optimization of the separation was performed via a formal experimental design, and an extensive validation was performed for plasma samples. Direct injection of untreated CSF was investigated in the analysis of oxalic, citric, lactic, glycolic, and 2- and 3-hydroxybutyric acids [14]. A triple-coating of the capillary surface with polybrene-dextran sulfate-polybrene was used to avoid protein adsorption, and a high concentration (200 mM phosphate) BGE was employed to achieve stacking of relatively large-volume CSF samples (2% of the capillary was filled). LODs between 2 and 8 μg/mL were thus obtained.

Since the blood–brain-barrier is an effective obstacle to the transport of many drugs into CSF, it is important to be able to measure CSF levels of therapeutic agents in order to determine their penetration into this compartment. In particular, antimicrobial agents need to be administered in such a way that the minimum inhibitory concentration (often quite a high concentration) is exceeded in CSF, and several papers exist describing their determination by CE. An early paper described the analysis of fosfomycin, a low MW antibiotic which has no UV chromophore useful for detection [137]. Fosfomycin was analyzed by indirect-UV, with sodium-4-hydroxybenzoate as an absorbing anion in the high-pH BGE and cetrimide as a wall-coating flow-reversal agent, resulting in an LOD of 1 μg/mL, with simple ultrafiltration for sample preparation. Chen and co-workers have published several papers using similar MEKC conditions with an SDS-containing Tris buffer for the direct injection analysis of antiviral [138] and antibiotic [139] compounds. Klekner et al described the application of their MEKC method in the monitoring of cefazolin in CSF and wound fluid after prophylactic administration of the drug during neurosurgery [140]. Enantiomers of the anthelmintic agent albendazole have been determined in CSF after a liquid–liquid extraction procedure. The sample cleanup allowed use of a neutral pH buffer in uncoated capillaries, with sulfated β-CD to provide chiral recognition [141].

As in many other biofluids, CE measurements of nitrate and nitrite as markers of NO production have been performed in CSF. For example, ultrafiltered CSF was analyzed using commercial CE anion analysis reagents in studies of lupus or brain hemorrhage [142,143]. Directly injected CSF could be analyzed for nitrate and nitrite using a 100 mM borate buffer at pH 10. The high ionic strength and pH minimized interactions of the CSF proteins with the uncoated capillary [144].

8. Aqueous and vitreous humor

The aqueous humor fills the anterior chamber of the eye behind the cornea, while the vitreous humor fills the space between the lens and the retina. The aqueous humor is similar to CSF in composition [10], with low levels of protein. The vitreous humor composition is broadly comparable [11,12], but it is made gelatinous by structural components such as collagen and hyaluronan. Because of the difficulty of sampling these materials from healthy human subjects there is relatively little interest in their analysis for diagnostic purposes. Indeed, a lot of data on their composition comes from post-mortem samples, and one of the applications of vitreous humor analysis is the forensic estimation of the post-mortem interval via monitoring of potassium levels, since vitreous humor potassium gradually increases due to autolysis after death [145]. An indirect-UV method was reported for vitreous humor potassium measurements, using imidazole as a buffer and background absorber, and 18-crown-6-ether and hydroxyisobutyric acid as additives to achieve selectivity for potassium [146]. Aliquots of samples of vitreous humor (approximately 1.5 mL was aspirated per eye) were then diluted in an aqueous solution of barium (internal standard) before injection onto the CE. This work was extended by using smaller (50 μL) samples, which was considered to reduce artifactual release of potassium during sampling [147]. Compared to techniques such as flame photometry and ion selective electrodes often used in clinical laboratories, the CE separation gave

greater selectivity, and the ability of the CE approach to determine multiple analytes simultaneously lead to an improved approach for determination of the post-mortem interval whereby several cations were simultaneously measured and correlated with post-mortem interval [148].

Various authors have reported determination of macromolecules in vitreous and aqueous humor. Similar to their work in SF, Grimshaw et al. determined hyaluronan in vitreous humor [149]. Using an approach adapted from their work with CSF [108], Cowdrey et al were able to profile proteins in 50–150 μL samples of aqueous humor obtained from subjects undergoing surgery [150]. A borate buffer pH 9.4 with added methylcellulose revealed proteins such as albumin, transferrin and IgG, as well as organic anions such as ascorbic and lactic acid. Membrane preconcentration has been applied to aqueous humor samples in the CE analysis of proteins with UV [151] and MS [152] detection. Preconcentration allowed the identification of a number of species including apolipoprotein A1, immunoglobulin fragments, and β-2 microglobulin.

Amino acids in aqueous humor were derivatized with CBQCA, followed by separation using a novel MEKC-polyethylene oxide separation matrix [153]. Samples (approximately 100 μL) were initially diluted 1:1 with acetic acid to stop the activity of endogenous proteases, before reaction with CBQCA. The method was used to investigate the levels of amino acids associated with proliferative diabetic retinopathy. Underivatized samples from a similar patient group were analyzed for nitrate by CE with direct UV detection [154]. The 1:1 acid-diluted samples were injected directly. Interestingly, a calibration curve prepared from aqueous standards gave a significant bias compared to quantitation using standard addition, the latter procedure giving results which accorded well with results from the Greiss reaction or NO chemiluminescence. This matrix effect may be due to the viscosity of the sample affecting hydrodynamic injection, as previously noted in analysis of saliva [102]. Ascorbic and uric acid have been determined in aqueous humor by CZE after 1:20 dilution of the sample with water [155]. This preparation was adequate to avoid any matrix effects on the capillary, using a pH 8.8 tricine buffer.

A variety of drugs have been measured using CE in aqueous and vitreous fluids. The previously mentioned fosfomycin analysis in CSF was also applied to aqueous humor, with simple ultrafiltration of the sample [137]. An MEKC-UV method with low μg/mL detection limits for barbiturates was proposed for toxicological analysis of vitreous humor [156]. Brimonidine is used to regulate intra-ocular pressure, hence its analysis in aqueous humor is of interest. 100 μL samples of aqueous humor were taken after topical application of brimonidine solution, and analyzed by CZE in pH 9.3 borate buffer after ultrafiltration [157]. CZE with a borate buffer and methylcellulose additive has also been used to determine penetration of oral and topical ciprofloxacin into aqueous humor [158], with sample preparation simply consisting of addition of 5 μL of an internal standard solution to 20 μL of aqueous humor before direct injection analysis.

9. Sweat

As a matrix, sweat is low in protein, but contains fairly high, variable amounts of salt (Table 1). The variability in amount and rate of sweat production means that collection of sample may be either a trivial matter, or a considerable challenge as in the case of insensible perspiration (perspiration which evaporates before it can be noticed as sweat upon the skin). There has been significant analytical interest in analysis of drugs of abuse in sweat [159], using sweat-collecting patches. Although CE has not to date been used to test for drugs of abuse in sweat collected in this way, it has been proposed as a method to determine sodium and potassium in sweat patches as a method of determining the collected volume [160]. Since the sensitivity required was not high, indirect UV detection with imidazole as a background absorber could be used. Hirokawa et al. [161] used CE with indirect UV detection (4-methylbenzylamine as a background absorber in an acidic BGE) to analyze a variety of cations in sweat, including metal ions, small organic amines and amino acids. The BGE also included hydroxyisobutyric acid and 18-crown-6 as complexing agents to fine-tune separation selectivity. Interestingly, this work focused on the measurement of ions in insensible perspiration as opposed to moisture from active sweating. Perspiration was collected into a collection solution (ultra-pure water) held against the skin in a small vial for a period of minutes, with the collection solution being directly analyzed by CE without further preparation. In Fig. 7, application of this method is illustrated in the analysis of cationic sweat components from finger and forearm sweat. Pyruvate has been determined in sweat using CE with electrochemical (EC) detection [162]. Since sample was collected from an exercising individual, the available quantity was not a problem, and the high sensitivity of the detection system used mean that sample dilution was required. CE with EC detection was also used for separation and detection of taurine in sweat [163].

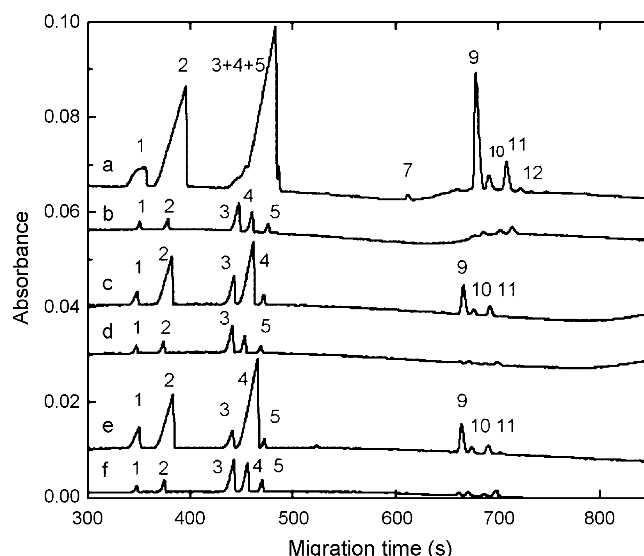


Fig. 7. Electropherograms of cationic components of finger (a, c and e) and forearm (b, d, f) sweat from three healthy volunteers. BGE: 10 mM 4-methylbenzylamine, 6.5 mM HIBA, 2 mM 18-crown-6, pH 4.25 (adjusted with 2-ethyl-*n*-butyric acid). Peaks: (a) (1) NH_4^+; (2) K^+; (3) Ca^{2+}; (4) Na^+; (5) Mg^{2+}; (7) DEA; (9) ornithine; (10) histidine; (11) lysine; (12) arginine. Reprinted from [161] (2007) with permission from Elsevier.

It is somewhat surprising that there are not more publications dealing with the CE analysis of sweat. Sweat is an analytically friendly biofluid, with very low protein concentrations. Particularly when only small volumes are available, CE should be considered to be an attractive analytical option.

10. Other biofluids

Analysis of tear fluid by CE was reviewed a few years ago [46], at which time there were a few applications related to the analysis of proteins, peptides, carbohydrates and inorganic ions. Direct sampling of tear fluid from the eye can be performed using capillary tubes or adsorbent paper strips, resulting in samples from hundreds of nL to a few μL in volume from unstimulated subjects. A commercial chip-based protein analysis system has recently been used to generate protein profiles in tear fluid giving results comparable to SDS-PAGE gels [164]. A high-resolution separation system was

described coupling CE to fractionate tear fluid onto a MALDI target before TOF-MS analysis [165]. Tear samples were directly injected onto a cationic-coated capillary, and on-target tryptic digestion demonstrated that lactoferrin was a principal component.

Low volumes of sample (low μL or less) may be obtained from the renal tubules. CE provides microanalytical capabilities to simultaneously monitor multiple cations or multiple anions to study ion transport along a renal tubule. In one reported analysis, around 1 μL of tubular fluid was collected by micropuncture, and 20–30 nL of this sample was pipetted into acid or water diluents. CE analysis was performed either by conductivity for cations [166] or indirect-UV absorbance for anions [167]. The ability to measure multiple species simultaneously in a microsample of fluid was considered the major advantage of CE for these analyses. In a related application, iothalamate has been determined by CE as a marker of single-nephron glomerular filtration rate in a microperfusion experiment [168,169]. Samples were again diluted in water, and analyzed by CZE with UV detection using a high-pH borate buffer. In the latter study, a comparison was made with a reference technique using radiolabeled inulin, demonstrating good agreement between the two approaches. Use of a non-radiolabeled marker with simple, quantitative analysis was seen as a clear advantage for the iothalamate/CE approach.

Fluid obtained from wounds or pus may be of interest for the purposes of identifying infecting microbes, or for determination of antimicrobial agents. Clearly the matrix may be very variable, and can contain large amounts of cellular debris. For microbial identification, pus and wound fluid were cultured after which bacterial cells were washed, digested with trypsin, and the resulting peptides analyzed by CE–MS [170]. In the analysis of antibiotics, fosfomycin was analyzed in pus, which was treated by the addition of methanol and centrifugation to precipitate proteins and cell debris [171]. The supernatant was injected directly onto the CE, with indirect UV detection for the non-UV-absorbing fosfomycin resulting in an LOD of 4.5 μg/mL.

11. Conclusions

This review illustrates the broad range of extracellular fluids which have been analyzed using CE techniques. Many of the approaches applied are similar to those in the more commonly analyzed biofluids such as plasma or urine, with the exact combination of sample preparation and separation techniques governed by the characteristics of the matrix. In many cases a limitation is the relatively low concentration sensitivity of CE, and various derivatization or preconcentration methods have been developed to circumvent this constraint. Some of the most exciting applications of CE remain those where its microanalytical abilities are used to the full. Some of the biofluids discussed in this review are abundantly available, hence the utility of CE is simply as an alternative analytical approach in competition to LC–MS and other analytical techniques. On the other hand, when one wants to characterize multiple species from a sub-microliter biosample, CE remains an analytical tool of choice.

References

[1] W.W.C. Quigley, N.J. Dovichi, Anal. Chem. 76 (2004) 4645.
[2] J. Kraly, M.A. Fazal, R.M. Schoenherr, R. Bonn, M.M. Harwood, E. Turner, M. Jones, N.J. Dovichi, Anal. Chem. 78 (2006) 4097.
[3] A.C. Servais, J. Crommen, M. Fillet, Electrophoresis 27 (2006) 2616.
[4] R. Jabeen, D. Payne, J. Wiktorowicz, A. Mohammad, J. Petersen, Electrophoresis 27 (2006) 2413.
[5] W.-H. Huang, F. Ai, Z.-L. Wang, J.-K. Cheng, J. Chromatogr. B 866 (2008) 104.
[6] I.G. Arcibal, M.F. Santillo, A.G. Ewing, Anal. Bioanal. Chem. 51 (2007) 387.
[7] D.K. Lloyd, J. Chromatogr. A 735 (1996) 29.
[8] J.R. Veraart, H. Lingeman, U.A.Th. Brinkman, J. Chromatogr. A 856 (1999) 483.
[9] J. Hernaĭndez-Borges, T.M. Borges-Miquel, M.A. Rodriĭguez-Delgado, A. Cifuentes, J. Chromatogr. A 1153 (2007) 214.
[10] C. Lentner, C. Lentner, A. Wink, Geigy Scientific Tables, Ciba-Geigy Ltd, Basle, 1981.
[11] P.L. Altman, D.S. Dittmer, Biology Data Book, vol. III, 2nd ed., Federation of American Societies for Experimental Biology, Bethesda, MD, 1974.
[12] A.D. Theocharis, N. Papageorgakopoulou, E. Feretis, D.A. Theocharis, Biochimie 84 (2002) 1235.
[13] S. Li, K. Fried, I.W. Wainer, D.K. Lloyd, Chromatographia 35 (1993) 216.
[14] R. Ramautar, G.W. Somsen, G.J. De Jong, Anal. Bioanal. Chem. 387 (2007) 293.
[15] L. Krivaĭnkovaĭ, P. Pantuckovaĭ, P. Gebauer, P. Bocek, J. Caslavska, W. Thormann, Electrophoresis 24 (2003) 505.
[16] H. Watzig, S. Kaupp, M. Graf, Trends Anal. Chem. 22 (2003) 588.
[17] Z.K. Shihabi, J. Capillary Electrophor. 2 (1995) 267.
[18] Y. Saito, K. Jinno, J. Chromatogr. A 1000 (2003) 53.
[19] B. Santos, M. Valcárcel, B.M. Simonet, A. Ríos, Trends Anal. Chem. 25 (2006) 968.
[20] P. Puig, F. Borrull, M. Calull, C. Aguilar, Trends Anal. Chem. 26 (2007) 664.
[21] J. Ruiz-Jiménez, M.D.L. de Castro, Trends Anal. Chem. 25 (2006) 563.
[22] P.G. Righetti, C. Gelfi, R. Sebastiano, A. Citterio, J. Chromatogr. A 1053 (2004) 15.
[23] L. Saavedra, C. Barbas, J. Chromatogr. B 766 (2002) 235.
[24] C. Lanz, M. Kuhn, F. Bortolotti, F. Tagliaro, W. Thormann, J. Chromatogr. A 979 (2002) 43.
[25] F. Bortolotti, G. De Paoli, R. Gottardo, M. Trattene, F. Tagliaro, J. Chromatogr. B 800 (2004) 239.
[26] F.J. DeLuccia, M. Arunyanart, L.J. Cline-Love, Anal. Chem. 57 (1985) 1564.
[27] A. Kunkel, H. Watzig, Electrophoresis 20 (1999) 2379.
[28] T. Nakagawa, Y. Oda, A. Shibukawa, H. Fukuda, H. Tanaka, Chem. Pharm. Bull. 37 (1989) 707.
[29] A. Schmutz, W. Thormann, Electrophoresis 15 (1994) 51.
[30] A. Schmutz, W. Thormann, Electrophoresis 15 (1994) 1295.
[31] H. Watzig, D.K. Lloyd, Electrophoresis 16 (1995) 57.
[32] D.K. Lloyd, H. Watzig, J. Chromatogr. B 663 (1995) 400.
[33] M. Graf, R. Galera Garcia, H. Wätzig, Electrophoresis 26 (2005) 2409.
[34] A. Suratman, H. Wätzig, Electrophoresis 28 (2007) 2324.
[35] M.C. Breadmore, Electrophoresis 28 (2007) 254.
[36] Z. Malá, L. Křivánková, P. Gebauer, P. Boček, Electrophoresis 28 (2007) 243.
[37] E.P. Lillehoj, K.C. Kim, Arch. Pharmacal Res. 25 (2002) 770.
[38] M.J. Welsh, M.P. Anderson, D.P. Rich, L.S. Ostedgaard, R.J. Gregory, S.H. Cheng, A. Smith, in: P.B. David (Ed.), Lung Biology in Health and Disease: Cystic Fibrosis, Dekker, New York, 1993, p. 29.
[39] G. Candiano, M. Bruschi, N. Pedemonte, L. Musante, R. Ravazzolo, S. Liberatori, L. Bini, L.J.V. Galietta, O. Zegarra-Moran, Am. J. Physiol. Lung Cell. Mol. Physiol. 292 (2007) L185.
[40] I. Noel-Georis, A. Bernard, P. Falmagne, R. Wattiez, J. Chromatogr. B 771 (2002) 221.
[41] L. Joris, I. Dab, P.M. Quinton, Am. Rev. Respir. Dis. 148 (1993) 1633.
[42] J.C. Transfiguracion, C. Dolman, D.H. Eidelman, D.K. Lloyd, Anal. Chem. 67 (1995) 2937.
[43] J.S. Landry, C. Landry, E.A. Cowley, K. Govindaraju, D.H. Eidelman, Ped. Pulmonol. 37 (2004) 149.
[44] K. Govindaraju, E.A. Cowley, D.H. Eidelman, D.K. Lloyd, Anal. Chem. 69 (1997) 2793.
[45] K. Govindaraju, E.A. Cowley, D.H. Eidelman, D.K. Lloyd, J. Chromatogr. B 705 (1998) 223.
[46] K. Govindaraju, D.K. Lloyd, J. Chromatogr. B 745 (2000) 127.
[47] V. Vanthanouvong, I. Kozlova, G.M. Roomans, Microsc. Res. Tech. 68 (2005) 6.
[48] I. Kozlova, V. Vanthanouvong, M. Johannesson, G.M. Roomans, Upsala J. Med. Sci. 111 (2006) 137.
[49] E.A. Cowley, K. Govindaraju, C. Guilbault, D. Radzioch, D.H. Eidelman, Am. J. Physiol. Lung Cell. Mol. Physiol. 278 (2000).
[50] K. Govindaraju, M. Toporsian, M.E. Ward, D.K. Lloyd, E.A. Cowley, D.H. Eidelman, J. Chromatogr. B 762 (2001) 147.
[51] D.Y. Boudko, J. Chromatogr. B 851 (2007) 186.
[52] K. Govindaraju, V. Govindaraju, D.H. Eidelman, J. Chromatogr. B 788 (2003) 369.
[53] K. Govindaraju, A. Ahmed, D.K. Lloyd, J. Chromatogr. A 768 (1997) 3.
[54] A. Klekner, K. Bagyi, L. Bognar, A. Gaspar, M. Andrasi, J. Szabo, J. Clin. Microbiol. 44 (2006) 3418.
[55] M. Andrási, A. Gáspár, Á. Klekner, J. Chromatogr. B 846 (2007) 355.
[56] N. Gerwin, C. Hops, A. Lucke, Adv. Drug Delivery Rev. 58 (2006) 226.
[57] F.M. Price, J.R. Levick, R.M. Mason, J. Physiol. 495 (1996) 803.
[58] K. Kakehi, M. Kinoshita, S.I. Yasueda, J. Chromatogr. B 797 (2003) 347.
[59] M.A. Strege, A.L. Lagu, J. Chromatogr. A 780 (1997) 285.
[60] J.M. Duffy, J. Grimshaw, A. Kane, R.A.B. Mollan, P.L. Spedding, J. Trocha-Grimshaw, Anal. Proc. 31 (1994) 257.
[61] A. Rice, J. Grimshaw, J. Trocha-Grimshaw, P. McCarron, G.B. Wisdom, J. Chromatogr. A 772 (1997) 305.
[62] J. Grimshaw, J. Trocha-Grimshaw, W. Fisher, A. Rice, S. Smith, P. Spedding, J. Duffy, R. Mollan, Electrophoresis 17 (1996) 396.
[63] M. Sharif, D.J. Osborne, K. Meadows, S.M. Woodhouse, E.M. Colvin, L. Shepstone, P.A. Dieppe, Br. J. Rheumatol. 35 (1996) 951.
[64] M.P. Brown, L.A. West, K.A. Merritt, A.H.K. Plaas, Am. J. Vet. Res. 59 (1998) 786.

[65] N.K. Karamanos, S. Axelsson, P. Vanky, G.N. Tzanakakis, A. Hjerpe, J. Chromatogr. A 696 (1995) 295.
[66] N. Volpi, Anal. Biochem. 277 (2000) 19.
[67] E.G. Karousou, M. Viola, A. Genasetti, D. Vigetti, D. De Luca, N.K. Karamanos, A. Passi, Biomed. Chromatogr. 19 (2005) 761.
[68] C.A. Davies, D. Perrett, Z. Zhang, B.R. Nielsen, D.R. Blake, P.G. Winyard, Electrophoresis 20 (1999) 2111.
[69] B. Gulbis, C. Gervy, E. Jauniaux, Early Hum. Dev. 52 (1998) 211.
[70] P.A. Queloz, D. Crettaz, L. Thadikkaran, V. Sapin, D. Gallot, J. Jani, J. Deprest, D. Leĩmery, S. Barelli, J.D. Tissot, J. Chromatogr. B 850 (2007) 336.
[71] C. Gelfi, G. Cossu, P. Carta, M. Serra, P.G. Righetti, J. Chromatogr. A 718 (1995) 405.
[72] C.J. Stewart, R.K. Iles, D. Perrett, Electrophoresis 22 (2001) 1136.
[73] M.E.P. Hows, L.N. Alfazema, D. Perrett, LC–GC 15 (1997) 1034.
[74] P. Tuma, E. Samcova, K. Andelova, J. Chromatogr. B 839 (2006) 12.
[75] S.H. Cho, H.J. Byung, W.Y. Lee, C.C. Bong, Rapid Commun. Mass Spectrom. 20 (2006) 2995.
[76] Y. Alnouti, C.A. White, M.G. Bartlett, Biomed. Chromatogr. 18 (2004) 523.
[77] S. Chiappin, G. Antonelli, R. Gatti, E.F. De Palo, Clin. Chim. Acta 383 (2007) 30.
[78] K. Lal, L. Xu, J. Colburn, A.L. Hong, J.J. Pollock, Arch. Oral Biol. 37 (1992) 7.
[79] C.J. Yu, W.L. Tseng, Electrophoresis 27 (2006) 3569.
[80] C.J. Yu, C.L. Su, W.L. Tseng, Anal. Chem. 78 (2006) 8004.
[81] C.M. Liu, K.H. Tung, T.H. Chang, C.C. Chien, M.H. Yen, J. Chromatogr. B 791 (2003) 315.
[82] V.A. Frerichs, J.K. Herrmann, A. Aguirre, L.A. Colón, Microchem. J. 78 (2004) 135.
[83] T. Guo, P.A. Rudnick, W. Wang, C.S. Lee, D.L. Devoe, B.M. Balgley, J. Proteome. Res. 5 (2006) 1469.
[84] A. Hu, P.J. Tsai, Y.P. Ho, Anal. Chem. 77 (2005) 1488.
[85] X. Fang, L. Yang, W. Wang, T. Song, C.S. Lee, D.L. DeVoe, B.M. Balgley, Anal. Chem. 79 (2007) 5785.
[86] H. Xie, N.L. Rhodus, R.J. Griffin, J.V. Carlis, T.J. Griffin, Mol. Cell. Proteom. 4 (2005) 1826.
[87] Y. Han, M. Zuo, L. Qi, K. Liu, L. Mao, Y. Chen, Electrophoresis 27 (2006) 4240.
[88] Y. Tanaka, N. Naruishi, Y. Nakayama, T. Higashi, S.I. Wakida, J. Chromatogr. A 1109 (2006) 132.
[89] H.M. Tseng, Y. Li, D.A. Barrett, Anal. Bioanal. Chem. 388 (2007) 433.
[90] P. Coufal, J. Zuska, T. van de Goor, V. Smith, B. Gas, Electrophoresis 24 (2003) 671.
[91] Q. Dong, R. Dong, M. Jin, W. Jin, J. Chromatogr. B 774 (2002) 121.
[92] M. Mori, W. Hu, J.S. Fritz, H. Tsue, T. Kaneta, S. Tanaka, Anal. Bioanal. Chem. 370 (2001) 429.
[93] Z. Glatz, S. Nováková, H. Štěrbová, J. Chromatogr. A 916 (2001) 273.
[94] J. Sádecká, J. Polonsky, Talanta 59 (2003) 643.
[95] Y. Tanaka, N. Naruishi, H. Fukuya, J. Sakata, K. Saito, S.I. Wakida, J. Chromatogr. A 1051 (2004) 193.
[96] T. Miyado, Y. Tanaka, H. Nagai, S. Takeda, K. Saito, K. Fukushi, Y. Yoshida, S.I. Wakida, E. Niki, J. Chromatogr. A 1051 (2004) 185.
[97] M. Mori, W. Hu, K. Hasebe, S. Tanaka, K. Tanaka, Anal. Bioanal. Chem. 374 (2002) 75.
[98] M.O. Luconi, R.A. Olsina, L.P. Fernández, M.F. Silva, J. Hazard. Mater. 128 (2006) 240.
[99] K. Rietdorf, I. Lang, B. Walz, J. Insect Physiol. 49 (2003) 205.
[100] W. Thormann, A. Minger, S. Molteni, J. Caslavska, P. Gebauer, J. Chromatogr. 593 (1992) 275.
[101] W. Thormann, S. Lienhard, P. Wernly, J. Chromatogr. 636 (1993) 137.
[102] D. Perrett, G.A. Ross, J. Chromatogr. A 700 (1995) 179.
[103] F. Prost, J. Caslavska, W. Thormann, J. Sep. Sci. 25 (2002) 1043.
[104] F. Deisenhammer, A. Bartos, R. Egg, N.E. Gilhus, G. Giovannoni, S. Rauer, F. Sellebjerg, Eur. J. Neurol. 13 (2006) 913.
[105] M.J. Romeo, V. Espina, M. Lowenthal, B.H. Espina, E.F. Petricoin Iii, L.A. Liotta, Exp. Rev. Proteom. 2 (2005) 57.
[106] S. Wittke, H. Mischak, M. Walden, W. Kolch, T. Rädler, K. Wiedemann, Electrophoresis 26 (2005) 1476.
[107] S. D'Aguanno, P. Del Boccio, S. Bernardini, E. Ballone, C. Di Ilio, G. Federici, A. Urbani, Clin. Chem. Lab. Med. 45 (2007) 437.
[108] G. Cowdrey, M. Firth, G. Firth, Electrophoresis 16 (1995) 1922.
[109] E. Sanders, J.A. Katzmann, R. Clark, R.P. Oda, Z. Shihabi, J.P. Landers, Clin. Chem. Lab. Med. 37 (1999) 37.
[110] M. Ivanova, E. Tzvetanova, V. Jetcheva, F. Kilár, J. Biochem. Biophys. Methods 53 (2002) 141.
[111] I. Kanakis, C. Stavropoulou, F.N. Lamari, Y. Heliopoulos, H. Iordanidou, H. Piperidou, D. Monos, N.K. Karamanos, Chromatographia 58 (2003) 513.
[112] A. Hiraoka, T. Arato, I. Tominaga, A. Anjyo, J. Pharm. Biomed. Anal. 15 (1997) 1257.
[113] A. Hiraoka, T. Arato, I. Tominaga, N. Eguchi, H. Oda, Y. Urade, J. Chromatogr. B 697 (1997) 141.
[114] T. Manabe, H. Miyamoto, K. Inoue, M. Nakatsu, M. Arai, Electrophoresis 20 (1999) 3677.
[115] W.L. Tseng, Y.W. Lin, H.T. Chang, Anal. Chem. 74 (2002) 4828.
[116] M. Wetterhall, M. Palmblad, P. Håkansson, K.E. Markides, J. Bergquist, J. Proteome Res. 1 (2002) 361.
[117] W.M. Muck, J.D. Henion, J. Chromatogr. 495 (1989) 41.
[118] T. Stroink, P. Schravendijk, G. Wiese, J. Teeuwsen, H. Lingeman, J.C.M. Waterval, A. Bult, G.J. de Jong, W.J.M. Underberg, Electrophoresis 24 (2003) 1126.
[119] Y. Huang, J. Duan, X. Jiang, H. Chen, G. Chen, J. Sep. Sci. 28 (2005) 2534.
[120] F.W.A. Tempels, G. Wiese, W.J.M. Underberg, G.W. Somsen, G.J. de Jong, J. Chromatogr. B 839 (2006) 30.
[121] S. Zhao, C. Prasad, H.J.F. Robertson, Y.M. Liu, Anal. Bioanal. Chem. 369 (2001) 220.
[122] T.M. Phillips, B.F. Dickens, Electrophoresis 19 (1998) 2991.
[123] T.M. Phillips, Electrophoresis 25 (2004) 1652.
[124] J. Bergquist, S. Douglass Gllman, A.G. Ewing, R. Ekman, Anal. Chem. 66 (1994) 3512.
[125] G. Nouadje, H. Rubie, E. Chatelut, P. Canal, M. Nertz, P. Puig, F. Couderc, J. Chromatogr. A 717 (1995) 293.
[126] P. Rada, S. Tucci, L. Teneud, X. Paez, J. Perez, G. Alba, Y. García, S. Sacchettoni, J. Del Corral, L. Hernandez, J. Chromatogr. B 735 (1999) 1.
[127] S. Tucci, C. Pinto, J. Goyo, P. Rada, L. Hernández, Clin. Biochem. 31 (1998) 143.
[128] G. Thorsén, J. Bergquist, J. Chromatogr. B 745 (2000) 389.
[129] A. Becker, E. Scheuch, U. Bode, U. Jaehde, Electrophoresis 23 (2002) 2457.
[130] M.J. Lu, T.C. Chiu, P.L. Chang, H.T. Ho, H.T. Chang, Anal. Chim. Acta 538 (2005) 143.
[131] C. Wang, S. Zhao, H. Yuan, D. Xiao, J. Chromatogr. B 833 (2006) 129.
[132] P.L. Chang, T.C. Chiu, H.T. Chang, Electrophoresis 27 (2006) 1922.
[133] M.M. Hsieh, C.E. Hsu, W.L. Tseng, H.T. Chang, Electrophoresis 23 (2002) 1633.
[134] C. Bayle, N. Siri, V. Poinsot, M. Treilhou, E. Causse, F. Couderc, J. Chromatogr. A 1013 (2003) 123.
[135] B. Arvidsson, N. Johannesson, A. Citterio, P.G. Righetti, J. Bergquist, J. Chromatogr. A 1159 (2007) 154.
[136] A. Hiraoka, J. Akai, I. Tominaga, M. Hattori, H. Sasaki, T. Arato, J. Chromatogr. A 680 (1994) 243.
[137] D. Leveque, C. Gallion, E. Tarral, H. Monteil, F. Jehl, J. Chromatogr. B 655 (1994) 320.
[138] H.H. Yeh, Y.H. Yang, S.H. Chen, Electrophoresis 27 (2006) 819.
[139] Y.W. Chou, Y.H. Yang, J.H. Chen, C.C. Kuo, S.H. Chen, J. Chromatogr. B 856 (2007) 294.
[140] A. Klekner, A. Ga'spa'r, S. Kardos, J. Szabo, G. Cse'csei, J. Neurosurg. Anesthesiol. 15 (2003) 249.
[141] F.O. Paias, V.L. Lanchote, O.M. Takayanagui, P.S. Bonato, Electrophoresis 22 (2001) 3263.
[142] E. Svenungsson, M. Andersson, L. Brundin, R. Van Vollenhoven, M. Khademi, A. Tarkowski, D. Greitz, M. Dahlström, I. Lundberg, L. Klareskog, T. Olsson, Ann. Rheum. Dis. 60 (2001) 372.
[143] D. Sadamitsu, Y. Kuroda, T. Nagamitsu, R. Tsuruta, T. Inoue, T. Ueda, K. Nakashima, H. Ito, T. Maekawa, Crit. Care Med. 29 (2001) 77.
[144] G. Žunic, S. Spasić, Z. Jelić-Ivanović, J. Chromatogr. B 727 (1999) 73.
[145] B. Madea, Forensic Sci. Int. 151 (2005) 139.
[146] F. Tagliaro, G. Manetto, F. Cittadini, D. Marchetti, F. Bortolotti, M. Marigo, J. Chromatogr. B 733 (1999) 273.
[147] F. Tagliaro, F. Bortolotti, G. Manetto, F. Cittadini, V.L. Pascali, M. Marigo, J. Chromatogr. A 924 (2001) 493.
[148] G. Bocaz-Beneventi, F. Tagliaro, F. Bortolotti, G. Manetto, J. Havel, Int. J. Legal Med. 116 (2002) 5.
[149] J. Grimshaw, A. Kane, J. Trocha-Grimshaw, A. Douglas, U. Chakravarthy, D. Archer, Electrophoresis 15 (1994) 936.
[150] G. Cowdrey, M. Firth, R. Moss, A. Karim, G. Thompson, G. Firth, Exp. Eye Res. 67 (1998) 449.
[151] E. Rohde, A.J. Tomlinson, D.H. Johnson, S. Naylor, J. Chromatogr. B 713 (1998) 301.
[152] E. Rohde, A.J. Tomlinson, D.H. Johnson, S. Naylor, Electrophoresis 19 (1998) 2361.
[153] M.J. Lu, J.S. Pulido, C.A. McCannel, J.E. Pulido, R.M. Hatfield, R.F. Dundervill III, S.A. Shippy, Exp. Diabet. Res. 2007 (2007) 1.
[154] L. Gao, J.S. Pulido, R.M. Hatfield, R.F. Dundervill III, C.A. McCannel, S.A. Shippy, J. Chromatogr. B 847 (2007) 300.
[155] M.L. Cheng, T.Z. Liu, F.J. Lu, D.T.Y. Chiu, Clin. Biochem. 32 (1999) 473.
[156] K.E. Ferslew, A.N. Hagardorn, W.F. McCormick, J. Forensic Sci. 40 (1995) 245.
[157] D.N. Tzovolou, F. Lamari, E.K. Mela, S.P. Gartaganis, N.K. Karamanos, Biomed. Chromatogr. 14 (2000) 301.
[158] S.M. Ghazi-Nouri, J. Lochhead, A.A. Mearza, M.A. Qureshi, G.M. Thompson, G. Cowdrey, M. Firth, R. Moss, A.K. Karim, G. Firth, Clin. Exp. Ophthalmol. 31 (2003) 40.
[159] M.A. Huestis, J.M. Oyler, E.J. Cone, A.T. Wstadik, D. Schoendorfer, R.E. Joseph Jr., J. Chromatogr. B 733 (1999) 247.
[160] B.M.R. Appenzeller, C. Schummer, S.B. Rodrigues, R. Wennig, J. Chromatogr. B 852 (2007) 333.
[161] T. Hirokawa, H. Okamoto, Y. Gosyo, T. Tsuda, A.R. Timerbaev, Anal. Chim. Acta 581 (2007) 83.
[162] M. Jin, Q. Dong, R. Dong, W. Jin, Electrophoresis 22 (2001) 2793.
[163] D.L.P. da Silva, H.H. Rüttinger, Y. Mrestani, W.F. Baum, R.H.H. Neubert, Electrophoresis 27 (2006) 2330.

[164] O. Schmut, J. Horwath-Winter, A. Zenker, G. Trummer, Graefe's Arch. Clin. Exp. Ophthalmol. 240 (2002) 900.
[165] A. Zuberovic, S. Ullsten, U. Hellman, K.E. Markides, J. Bergquist, Rapid Commun. Mass Spectrom. 18 (2004) 2946.
[166] C.J. Stocking, J.M. Slater, R. Unwin, S. Walter, E. Folkerd, Kidney Int. 56 (1999) 338.
[167] N.J.R. Faria, H. Dobbie, J.M. Slater, D.G. Shirley, C.J. Stocking, R.J. Unwin, Kidney Int. 67 (2005) 357.
[168] N.N. Davydova, R. Gotti, W.J. Welch, I.W. Wainer, J. Chromatogr. B 728 (1999) 143.
[169] G. Capasso, R.J. Unwin, A. Pica, L. Quagliuolo, A. Giovane, Kidney Int. 62 (2002) 1068.
[170] A. Hu, C.T. Chen, P.J. Tsai, Y.P. Ho, Anal. Chem. 78 (2006) 5124.
[171] M. Petsch, B.X. Mayer-Helm, R. Sauermann, C. Joukhadar, E. Kenndler, J. Chromatogr. A 1081 (2005) 55.