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Review

Coupling continuous separation techniques to capillary electrophoresis

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Abstract

One of the weak points of capillary electrophoresis is the need to implement rigorously sample pretreatment because its great impact on the quality of the qualitative and quantitative results provided. One of the approaches to solve this problem is through the symbiosis of automatic continuous flow systems (CFSs) and capillary electrophoresis (CE). In this review a systematic approach to CFS–CE coupling is presented and discussed. The design of the corresponding interface depends on three factors, namely: (a) the characteristics of the CFS involved which can be non-chromatographic and chromatographic; (b) the type of CE equipment: laboratory-made or commercially available; and (c) the type of connection which can be in-line (on-capillary), on-line or mixed off/on-line. These are the basic criteria to qualify the hyphenation of CFS (solid-phase extraction, dialysis, gas diffusion, evaporation, direct leaching) with CE described so far and applied to determine a variety of analytes in many different types of samples. A critical discussion allows one to demonstrate that this symbiosis is an important topic in research and development, besides separation and detection, to consolidate CE as a routine analytical tool.

Keywords: Sample handling; Leaching; Dialysis; Evaporation; Extraction methods; Membranes; Isotachophoresis; Reviews

Contents

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1. Introduction

The analysis of real samples by capillary electrophoresis (CE) requires efficient sample preparation procedures to remove interfering solutes, (in)organic salts and particulate matter. Sample preparation systems reported in the literature are based on chromatographic, electrophoretic, membrane-based procedures, solid-phase extraction (SPE), supercritical fluid extraction (SFE) among others techniques. The combination of automated sample preparation and CE is especially useful if complex samples have to be analysed and helps to improve both selectivity and sensitivity. In this review, the different modes of sample pretreatment will be discussed and an overview of the potential of these procedures will be given.

The number of routine applications of real samples using CE is limited because there still are several problems that have to be solved. No doubt the main problem is that analyte detectability expressed in concentrations units, generally is rather poor because of the low volume loadability of the capillary. Another problem is the presence of particulate matter, which can easily clog the CE system [1]. The emphasis of this review will be on those sample preparation procedures that can be used for the quantitative determination of analytes in real samples.

This review is not intended to provide readers a detailed description of the published papers; rather, it has tried to summarise in different tables more than 450 references which were classified according to the coupling mode, the type of the continuous flow system (CFS) used, the electrophoretic mode, type of sample and analytes. Moreover, tables will help to organise the subject and emphasise the primary events. Only documents published since 1980 up to beginning year 2000 will be considered in this review. The rapid growth in the number of publications for CE has forced this review to limit the number of papers cited and to concentrate primarily on those documents which the emphasis is on the coupling of CFSs with CE.

2. Functions of continuous flow systems

The use of low-pressure continuous flow systems can be viewed as one of the first reliable steps towards real performance improvement in analytical methods through the automation, miniaturisation and simplification of the preliminary operations of analytical methodologies. These systems allow the implementation of one or several (simultaneous or sequential) chemical reactions, as well as reliable separation techniques for increasing sensitivity and selectivity, calibration procedures, etc. Also, CFSs have recently proved to be useful tools for developing rapid response analytical systems such as sensors and screening devices [2].

The favourable influence of CFSs on analytical properties [3] is undeniable. In fact they provide a highly useful means for improving: (a) the productivity of analytical laboratories through increased throughput, personnel safety and comfort, and reduced costs, and (b) the accuracy of the analytical results through increased precision, sensitivity and selectivity. In summary, CFSs can be included among the most outstanding advances in chemical measurement processes.

There are two main ways of combining continuous flow systems and instruments. In the simpler one, the flow system is connected via an appropriate interface to an instrument that is the exclusive source of analytical information. More powerful is the coupling of a flow system furnished with a non-destructive detector to an instrument of a high information level allowing physical or physicochemical discrimination of analytes and providing reliable qualitative and/or quantitative information. The global information furnished by the detector in the continuous flow system can be used for a variety of purposes [2].

3. Continuous flow system–capillary electrophoresis coupling modes

CE is a highly flexible and efficient analytical separation technique that has become a serious competitor for gas, liquid and supercritical column chromatographies. Nevertheless, its intrinsic features make direct sample introduction almost impossible. Sample pretreatment is crucial in order to obtain reliable analytical results. Thus, CFSs linking gaseous, liquid gaseous, liquid or solid samples to the CE system are of great practical importance because they

can automatically implement such operations as dissolution, leaching, filtration, derivatisation, matrix isolation, analyte concentration, solvent exchange and so on. Continuous separation techniques such as SPE, dialysis, etc., play major roles in this context.

Although CFSs and CE are of hydrodynamic nature, there are many technical aspects that make them theoretically incompatible. Nevertheless, several types of interfaces have been described for coupling CFS–CE and it were summarised by our group [2].

Combinations of a CFS and CE equipment can be characterised by the degree of integration between these two units. Four levels of coupling can be distinguished: off-line, at-line, on-line and in-line. The sample preparation modules can be coupled with CE either off-line (manual), at-line (robotic interface) on-line (coupling via a transfer line) or in-line (complete integration between sample preparation and separation system).

4. Leaching coupled to capillary electrophoresis

A few determinations of analytes in solid samples have been find in the literature. In Table 1, five references are summarised.

An automatic system for stepwise treatment of solid samples and application to pollution evaluation by measuring ion lixiviation rates in lichens by capillary zone electrophoresis (CZE) was developed by Arhoun et al. [4]. Lichen (0.4 g) was immersed in 10 ml water in an extraction tube and sonicated in a bath at 30°C for 20 min. The supernatant was filtered and hydrodynamically injected. The cation lixiviation rates are related to their exposure to pollution. Recently, a CE method was developed for the simultaneous determination of a number of major ingredients of green tea. Analysis was carried out after treatment (extraction, filtration and dilution) of the samples in a flow system which was coupled to a commercial CE equipment via a programmable arm [5].

Another example of leaching coupled to CE was reported by Aguilar et al. [6]. Capillary electrophoretic determination of cyanide leaching solutions from automobile catalytic converters was carried out. A CE method was developed for determining Fe(II)–, Cu(I)–, Ni(II)–, Pd(II)– and Pt(II)–cyano complexes and nitrite in the leaching solutions.

5. Dialysis coupled to capillary electrophoresis

Dialysis is normally used to remove particulate material and can be coupled with a wide variety of separation techniques. Microdialysis has emerged as a powerful tool for monitoring the extracellular environment of a variety of organs, tissues and bodily fluids in vivo. Microdialysis is powerful because it can be coupled with a variety of analytical methods; therefore, it can be used to simultaneously monitor a large variety of endogenous and exogenous compounds. Techniques that have been coupled to the dialysis probe include LC, immunoassay, mass spectrometry (MS) and CE, an example is shown in Fig. 1 [7]. The usefulness of the dialysis for the pretreatment of the samples before its introduction into the CE equipments is shown with more than 45 references in Table 2.

A flow-gated, on-line interface between a microdialysis sampling probe and CE with UV de-

Table 1
Coupling continuous leaching systems to capillary electrophoresis

Coupling mode				CFS		Electrophoretic mode	Sample	Analytes	Remarks	Ref.
OFF	IN	ON	OFF/ON	NC	C					
x				x		CZE	Lichen	Cations	Determination of lixiviation rates of K^+, Na^+, Ca^{2+} and Mg^{2+} from the lichen	[4]
		x		x		CE	Tea	Polyphenols	Extraction of analytes with water from a solid sample (tea)	[5]
x				x		CE	Car exhaust gases	PGMs	Analytes recovered from automobile catalytic converters by a leaching process	[6]
x				x		CE	Metal cyanide	Au, Ag, Cu, Ni	Analytes were determined in samples from the leaching of Au numeral and sand	[58]
x				x		CE	Aerosols/rainwater	Iron (II)	Sample was prepared for CE by leaching with 0.2 ml of HCl and 2.0 ml of water	[59]

CFS: Continuous flow system; NC: no chromatography; C: chromatography; CZE: capillary zone electrophoresis; PGMs: platinum group metals.

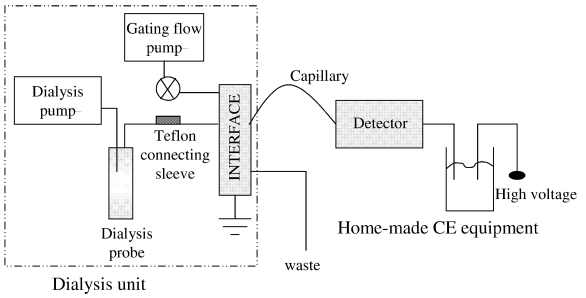

Fig. 1. On-line interface between microdialysis and capillary zone electrophoresis (adapted from Ref. [7]).

tection was characterised and applied. The data presented by Lada et al. [7] demonstrated the utility of the interface for allowing low perfusion flow-rates while allowing temporal resolution of 65–85 s.

An on-line coupling of microdialysis sampling to CE was described by Hogan et al. [8]. The heart of the on-line system is the interface between the microdialysis apparatus and the CE system. The interface must isolate the experimental animal from the high potential of the CE experiment and convert the microliter per minute microdialysis flow into discrete nanoliter volume samples for the CE while not adding to the system's dispersion. This was accomplished by using a commercially available rotary microinjection valve for LC with an injection apparatus designed in the laboratory.

In 1997, an automated method for high temporal resolution monitoring of the neurotransmitters glutamate and aspartate in vivo using CE with laser-induced fluorescence (LIF) detection was developed [9]. Microdialysis probes placed in the striatum of anaesthetised rats were coupled on-line with the CE system by an automated flow-gated interface. Flexible loop microdialysis probes (ESA, Bedford, MA, USA) made from cellulose fibres (M_r 6000 cutoff) with 450 μm O.D. tip diameters and 2 mm tip lengths were used for all sampling experiments. The dialysis membrane had a 210 μm I.D. for an internal volume of 0.12 μl.

In the same year, a CE–electrospray ionisation (ESI) MS interface, based on an electric circuit across a microdialysis membrane surrounding a short capillary segment closely connected to the separation capillary terminus was demonstrated by Severs and Smith [10] to be sensitive, efficient, and rugged.

Kuban and Karlberg [11] described a CE application using a common electrolyte for separation of the most common small inorganic/organic anions and cations. The system uses one capillary and just one detector placed in the centre of the capillary. The technique has successfully been applied to the simultaneous determination of anions and cations in natural water samples. Milk and mud samples pretreated by dialysis have also been analysed. On-line dialysis performed in a flow injection analysis (FIA) system was integrated with a CE system via a specially designed interface, developed by the same authors [12]. Samples were continuously pumped into a dialysis unit and the outgoing acceptor stream containing the analytes is allowed to fill a rotary injector in the FIA part of the system. A discrete, representative volume of the acceptor stream is injected into an electrolyte stream, which continuously passes through the FIA–CE interface into which the end of a capillary has been inserted.

A fully automated method is presented for the determination of acidic drugs in urine and serum using on-line dialysis–SPE–CE [13]. This system is fully automated and 250–500 analyses can be carried out without any maintenance or exchange parts.

In 1999, a rapid determination of aspartate enantiomers in tissue samples by microdialysis coupled on-line with CE was developed by Thompson et al. [14]. The microdialysis probe was inserted into a homogenised tissue sample, which allowed generation of a continuous, sample stream that was filtered and deproteinated. Values of D- and L-aspartate in different tissues agreed well with those obtained by a high-performance liquid chromatography (HPLC) procedure that required protein precipitation, centrifugation, and extraction. The speed and compatibility with automation of the microdialysis–CE method may make it a general approach for a variety of applications involving high-throughput analysis or sensorlike operation.

Finally Crowder et al. [15] developed a method for the determination of phosphoamino acids with indirect photometric detection. The samples were hydrolysed in HCl, aliquots of the cooled hydrolysis solution were dialysed for 3 h in M_r 100 cutoff Spectra/Por Biotech cellulose ester dialysis tubing. A portion of this solution was injected into the CE instrument.

Table 2
Coupling continuous dialysis systems to capillary electrophoresis

Coupling mode				CFS		Electrophoretic mode	Sample	Analyte	Remarks	Ref.
OFF	IN	ON	OFF/ON	NC	C					
		x		x		CE	Rat brain	Ascorbic acid	Microdialysis sampling probe	[7]
		x		x		CE	Rats	Pharmacokinetics products	Cellulose fibre dialysis	[8]
		x		x		CE	Rats	Glutamate, aspartate	Microdialysis probes	[9]
		x		x		CE	–	Proteins	Interface based on microdialysis membrane	[10]
x				x		CE	Water, milk, mud	Cations and anions	CE laboratory-made	[11]
		x		x		CE	Water, snow, mud,	Small anions	Microdialysis CE laboratory-made	[12]
		x		x		CE	Serum, urine	Acidic drugs	Cellulose acetate membrane	[13]
		x		x		CE	Tissue	Aspartate enantiomers	Microdialysis coupled with CE	[14]
x				x		CE	–	Phosphoamino acids	–	[15]
x				x		CZE	Royal jelly	Proteins	–	[60]
x				x		MEKC	Polymers	Alkylphenyl ketones, alkyl benzoates	Products were purified by dialysis	[61]
		x		x		IEF	–	Proteins	Microdialysis sleeve tubing	[62]
				x		CE	Plasma	Drug–protein	Review	[63]
				x		CE	Tissues, organs	Pharmacokinetic	Review	[64]
		x		x		CE	Live animal	Nicotine	Rotary switching valve	[65]
		x		x		CE	Cerebrospinal fluid	Gabapentin (I)	Microdialysis probe	[66]
		x		x		CE	Blood serum	Sulfonamides	Dialysis–SPE device	[67]
		x			x	CE	–	Collagen crosslinks	Three detection modes	[68]
x				x		CE	–	Ethoxylated polymers	Cellulose ester dialysis tube	[69]
		x		x		IEF	Pharmalyte 5-8	Carbonic anhydrase	Plexiglas microdialysis chamber	[70]
x				x		CE	Serum	Oligonucleotides	Membrane filters (cellulose)	[71]
x				x		CE	Blood plasma	Oligonucleotides	Drop dialysis	[72]
		x		x		CE	–	Myoglobin, C anhydr.	Piece of dialysis tubing	[73]
x				x		CZE	Rat brain	Glutamate	–	[74]
		x		x		CE	–	Benzenesulfonamides	Sleeve of polysulfone dialysis	[75]
						CE	–	–	Review	[76]
x				x		CE	PCR products	Nucleic acids	Membrane dialysis	[77]
x				x		CE	Smoked fish	*Clostridium botulinum*	Membrane dialysis	[78]
x				x		CGE	DNA	*Clostridium botulinum*	Membrane dialysis	[79]
x				x		CGE	Human plasma	Phosphorothioate oligonucleotides	Millipore VS membrane	[80]
x				x		CE	Living rat brain	γ-Aminobutyric acid	In vivo microdialysis	[81]
x				x		CE	Blood	α-Difluoromethylornithine	Microdialysis	[82]
x				x		CE	Proteins	–	Dialysis memebrane	[83]
		x		x		IEF	Proteins	–	Hollow dialysis fibre	[84]
x				x		CE	Blood serum	Organic anions	Dialysis fluids	[85]
x				x		CE	Animals	Aspartate and glutamate	Microdialysis sampling	[86]
x				x		CE	Peas	Soyasaponin I	–	[87]
x				x		CE	DNA	Polymerase chain reaction	–	[88]
x				x		ITP–CZE	Kidney cells	Antithrombin III	Samples purified by dialysis	[89]
x				x		CE	Brain rats	Glutamic acid	Microdialysis probe sampling	[90]
x				x		CE	Brain	Glutamate I	Microdialysis coupled to CE	[91]
x				x		CE	Rats	Aspartate, glutamate and alanine	Microdialysis	[92]
x				x		CE	Rats	L-dopa	In vivo microdialysis	[93]
x				x		CGE–ITP	Roasted coffee	Total titratable acid	–	[94]
x				x		CE	Brain	Glutamate	Microdialysis and CE	[95]
		x		x		CE	Rat caudate nucleus	Primary amines	Microdialysis coupled by a flow-gated interface to CE	[96]

CFS: Continuous flow system; NC: no chromatography; C: chromatography; CZE: capillary zone electrophoresis; CE: capillary electrophoresis; MEKC: micellar electrokinetic chromatography; IEF: capillary isoelectric focusing; CGE: capillary gel electrophoresis; IEF: capillary isoelectric focusing; PCR: polymerase chain reaction; SPE: solid-phase extraction; ITP: capillary isotacophoresis.

6. Evaporation coupled to capillary electrophoresis

In the classical analyte extraction protocols from real samples, a great number of organic solvent are used. The analytes dissolved in theses solvents are in almost all cases incompatible with the buffer used in the CE method. Due to this reason is compulsory the evaporation of these solvents and the redisolution in solvent compatible with the CE methodology. Some examples are shown in Table 3.

The determination of methotrexate and its major metabolite, 7-hydroxymethotrexate using CE and LIF detection was carried out by Roach et al. [16]. Serum was mixed with 0.2 *M* sodium acetate (pH 5.1) and the mixture was cleaned up on a Sep-Pak C_{18} cartridge with elution with methanol. The eluate was evaporated to dryness and the residue was dissolved in 1 *M* 2-(*N*-morpholino)ethanesulfonic acid (I) and 0.35% $KMnO_4$ was added to oxidise the analytes.

Micellar electrokinetic capillary electrophoresis (MEKC) provides rapid and efficient separation and detection of organic gunshot and explosive constituents [17]. Samples for MEKC analysis were obtained by ultrasonic agitation of the swab in 500 μl of ethanol. The ethanol was evaporated down to a 2–3 μl under a stream of nitrogen and then diluted with 50 μl of the running buffer.

Zhang and Hjerten [18] developed a micro method for concentration and desalting utilising a hollow fibre with special reference to CE. Aqueous sample, e.g., K_2CrO_4 or protein, respectively, was introduced into a hollow fibre and concentrated by spontaneous or forced evaporation of water through the fibre wall or by Donnan transport of water into a polymer solution surrounding the fibre. The concentrated solutions were diluted if required and subjected to CE (procedures described). The possibility of using

Table 3
Coupling continuous evaporation systems to capillary electrophoresis

Coupling mode				CFS		Electrophoretic mode	Sample	Analyte	Remarks	Ref.
OFF	IN	ON	OFF/ON	NC	C					
x				x		CZE	Serum/blood	Methotrexate and its 7-hydroxy metabolite	Eluate was evaporated to dryness	[16]
x				x		MEKC	Swabs	Organic gunshot	Ethanol evaporated under a stream of N_2	[17]
		x		x		CE	Low or high molecular mass	–	Hollow fibre, forced evaporation	[18]
x				x		CE	Urine	Tramadol and its main metabolites	Residue evaporation	[97]
x				x		CE	Human plasma	Doxorubicin (I)	Evaporation of organic phase	[98]
x				x		CZE	Corn	Tosylated polyamines	Alkaline solution evaporated	[99]
x				x		CE	–	Dansyl-D-L-glycine/valine	Solvent evaporation	[100]
x				x		CZE	*Magnolia officinalis*	Magnolol and honokiol	Evaporation of ethanol	[101]
x					x	GPC	Vesicle	Dextrans in lipid	Reversed-phase evaporation	[102]
x				x		CE	Organic solvents	Acidic species	Evaporation of volatile solvents	[103]
x				x		CE	–	–	Evaporation of buffer solvent	[104]
x				x		MEKC	Citrus seeds	Limonoid glucosides	Soxhlet extracted	[105]
x				x		CZE	Butter	Free fatty acids	–	[106]
x				x		MEKC	Soil samples	PAHs	Solvent evaporation	[107]
x				x		CE	Glycoproteins	Oligosaccharides	Evaporation of excess reagent	[108]
x				x		CZE	Serum	Polyamines	Evaporation of acetone	[109]
x				x		MEKC	Plasma, human milk	Thiocyanate, iodide, nitrate and nitrite	Solvent evaporation	[110]
x				x		CE	Glycoproteins	Oligosaccharides	Solvent evaporation	[111]
x				x		MEKC	Seeds and plants	Glucosinolates	Evaporation from samples	[112]
x				x		CZE	–	Amino acids	–	[113]

CFS: Continuous flow system; NC: no chromatography; C: chromatography; CZE: capillary zone electrophoresis; CE: capillary electrophoresis; MEKC: micellar electrokinetic chromatography; GPC: high-performance gel exclusion chromatography; PAHs: polycyclic aromatic hydrocarbons.

the method for on-line preconcentration for capillary electrophoresis is discussed.

7. Supercritical fluid extraction coupled to capillary electrophoresis

The application of multidimensional chromatography to the analysis of complex matrices helps to minimise sample pretreatment steps. Still, when the matrix to be analysed is not totally soluble in a particular solvent, such as for example plant tissue, a preliminary step is necessary to obtain a solution suitable for subsequent introduction into the preliminary separation stage. Supercritical fluids offer potential advantages over liquid solvents to meet the sample preparation requirements. SFE was used in conjunction with thin-layer chromatography and conventional LC. The on-line coupling of SFE to gas chromatography (GC) is experiencing rapid growth and will continue to be studied as a simplified method of sample preparation and analysis [19]. To our knowledge only 12 articles used SFE to extract the analytes which were separated by CE (see Table 4).

In the last 2 years, our team has worked on the coupling SFE–CE. An automatic method for the determination of phenols in liquid samples (river water and human urine) using co-electrosmotic CE coupled via a laboratory-mechanical arm to a supercritical fluid extractor, was developed and validated. Samples were preconcentrated onto C_{18} sorbent and carefully transferred to extraction cartridges for extraction with supercritical CO_2. The analytes were collected in a diol-trap and eluted with methanol, which is synchronically fed to the CE vial by the mechanic arm, controlled via an electronic interface [20].

In 1996 solid–liquid extraction and SFE carried out the determination of carbamate residues in tobacco samples. The results were analysed by CE and the results compared, demonstrating the advantages of using SFE to reduce time, expense, hazardous wastes and enhance extraction power [21].

In the same year, a separation method using cyclodextrin-modified CE has been developed for analysis of the polycyclic aromatic hydrocarbons (PAHs) in contaminated soils by Brown et al. [22]. Contaminated soil was extracted using CO_2 supercritical fluid. The diluted extracted was analysed using fluorescence detection.

Finally, Wang and Chang [23] described an off-line SFE–CE procedure for the determination of four parabens in cosmetics samples.

Table 4
Coupling continuous supercritical fluid extraction systems to capillary electrophoresis

Coupling mode				CFS		Electrophoretic mode	Sample	Analyte	Remarks	Ref.
OFF	IN	ON	OFF/ON	NC	C					
		x		x		CE	Phenols	Water and urine	Laboratory mechanical arm	[20]
x				x		CZE	Residues in tobacco	Carbamates	Supercritical CO_2 modified	[21]
x				x		CE	Contaminated soils	PAHs	Cyclodextrin-modified	[22]
x				x		CZE	Cosmetic products	Parabens	Supercritical CO_2 modified	[23]
x				x		CE	–	Ethoxylated polymers	Excess reagent was removed by SFE	[69]
x				x		MEKC	Blood stains	Smokeless powder residues	–	[114]
x				x		MEKC	Food	Vitamins	–	[115]
x				x		CE	Water	Carboxylic acids	Supercritical water	[116]
						CE	Environmental	–	Review (solid matrices)	[117]
x				x		CZE	Water	Phloxine B and uranine (II)	SFE was used for spiked water samples	[118]
x				x		CE	*Stevia rebaudiana*	Steviol glycosides	Subcritical fluid extraction using CO_2	[119]
x				x		CE	Antioxidative	Plant beverages	Leaf extracts were prepared by SFE	[120]

CFS: Continuous flow system; NC: no chromatography; C: chromatography; CZE: capillary zone electrophoresis; CE: capillary electrophoresis; MEKC: micellar electrokinetic chromatography; PAHs: polycyclic aromatic hydrocarbons; SFE: supercritical fluid extraction.

8. Solid-phase extraction coupled to capillary electrophoresis

SPE can be used to simultaneously enrich the trace analytes and remove potentially interfering compounds. As can be seen in Table 5, it can be combined with CE in several ways that vary from off-line to in-line approaches. To circumvent poor CE limit of detection off-line sample pretreatment and analyte concentration could be achieved. However, if possible, this should be avoided for dilute analyte solutions since losses to exposed surfaces (e.g., walls of Eppendorf tubes, pipette tips, solid extraction phases, etc.) can be substantial. To avoid these problems, minimal sample handling is advisable. This can be achieved using an analyte concentrator [24,25] on-line with the CE capillary. These devices usually consist of an adsorptive phase at the inlet of the CE capillary and serve to enrich trace levels of analytes, as well as allow on-line sample clean up prior to component separation by CE.

In 1997, a sensitive method for the determination of PAHs by solid-phase microextraction (SPME) coupled with cyclodextrin-modified CE using UV detection has been developed [26]. A glass fibre was prepared and used for absorbing 16 US Environmental Protection Agency (EPA) priority PAHs from diluted samples until equilibrium was reached. After the glass fibre was connected to a separation capillary via an adapter, the absorbed analytes were directly released into the CE buffer stream. Very satisfactory reproducibility with respect to migration time and peak area was obtained for repetitions using the same separation capillary and adapter, where only the extraction fibre was discarded after each analysis.

One year later, an on-column interface, coupling the SPME sampling technique with CE was constructed [27]. This interface facilitates the direct insertion of a thin silica fibre into the inlet end of a separation capillary and, therefore, the zero-dead volume connection requirement for hyphenation between SPME and CE was fully realised. The performance of the interface was evaluated by SPME–CE analysis of the priority pollutant phenols using a laboratory-made 40 μm O.D. poly(acrylate) (PA)-coated silica fibre connected to a 75 μm I.D. separation capillary. The results clearly demonstrated that the interface was effective for on-line coupling SPME with CE.

In the same year, a separation of chiral biodegradation intermediates of linear alkylbenzenesulfonates by CE was carried out by Kanz et al. [28]. After the enrichment on graphitised carbon black material, the extracts were analysed by HPLC and CE.

In our laboratory, a CZE method was developed for the determination of heterocyclic aromatic amines in meet and fish sample. SPE (the Gross methodology) was tested for isolating the amines [29]. The same authors [30] developed also a method for the determination of chlorophenols (CPs) in human urine by using MEKC coupled via a mechanic arm to an on-line automatic clean up and preconcentration unit for urine samples. The coupling of both systems allows the expeditious, reproducible, sensitive, and inexpensive determination of CPs in human urine with acceptable precision and accuracy. In the same laboratory, different flow injection (FI) systems furnished with different minicolumns (C_{18} and Chelex-1000) were used to clean up the samples and preconcentrate analytes. The analytes eluted from the solid phases were driven from the FI systems to the autosampler of the CE equipment by a programmable arm [31–34], an example is shown in Fig. 2. This interface can be considered as a general approach to make discrete analytical equipment (which they commonly use sample turntables) compatible with the continuous flow system.

The FI–SPE–CZE system described by Chen and Fang [35] was shown to be applicable to the automated preconcentration, separation and determination of trace amounts of drug constituents in blood plasma, as demonstrated by the quantitation of pseudophedrine in plasma down to the 50 $\mu g\ l^{-1}$ level. The FI–SPE system not only concentrated the analyte but also modified the sample medium to create the low conductivity required for further sensitivity enhancement by electro-stacking.

On-line ion-exchange preconcentration, performed in a FIA system, has been integrated with laboratory-made CE equipment via a specially designed interface. A sensitive and selective method for the determination of nitrite, nitrate, bromide and iodide

Table 5
Coupling continuous solid-phase extraction systems to capillary electrophoresis

Coupling mode				CFS		Electrophoretic mode	Sample	Analytes	Remarks	Ref.
OFF	IN	ON	OFF/ON	NC	C					
x				x		CE	Serum and urine	Acidic drugs	Dialysis unit/SPE column	[13]
		x		x		CE	Blood serum	Anti-IgE mAb	Preconcentration–CE using an analyte concentrator	[24]
		x		x		CE	–	Methylamphetamine	Use of concentration step to collect analytes	[25]
		x		x		CE	–	PAHs	SPME with a optical fibre	[26]
		x		x		CE	Natural water	Phenols	SPME fibres	[27]
x				x		CE	Waste solids	Alkylbenzenesulfonates	SPE with Carbopack B	[28]
x				x		CZE	Fried meat and fish	Heterocyclic aromatic amines	Clean up in cartridges	[29]
			x	x		MEKC	Chlorophenols	Human urine	MEKC coupled to a clean up system	[30]
			x	x		CE	Wine	Biogenic amines	C_{18} adsorbent minicolumn	[31]
			x	x		CE	Wines	*trans*-Resveratrol/polyphenols	C_{18} SPE column	[32]
			x	x		CE	Inorganic cations	Water	Chelex-100 minicolumn incorporated in the CFS	[33]
			x	x		MEKC	Pesticides	Water	C_{18} SPE minicolumn was used	[34]
		x		x		CZE	Pseudoephedrine	Human plasma	Combination of flow injection SPE and CZE	[35]
			x	x		CE	Inorganic anions	Water	On-line ion-exchange in a flow injection–CE	[36]
		x		x		CE	Serum and urine	Sulfonamides	Dialysis–SPE	[67]
		x		x		CE	–	Collagen crosslinks HP and LP	SPE–dialysis LC system	[68]
x				x		CGE	Human plasma	Phosphorothioate oligonucleotides	SAX cartridge	[80]
						CE	Environmental	–	Review	[117]
		x		x		CE	Aqueous samples	Environmental pollutants	Review	[121]
x				x		CZE	Plasma	*E*-5-(2 Brvinyl)-2′deoxyuridine	SPE column	[122]
x				x		CE	Urine	Catecholamines	SPE alumina cartridge	[123]
		x		x		CE	–	–	Membrane preconcentration	[124]
x				x		CE	Water	Phenylurea herbicides	C_{18} SPE	[125]
x				x		CE	Tap and river water	Atrazine, terbutylazine ...	LiChrolut EN cartridges	[126]
x				x		MEKC	Human urine	Glucuronides of entacapone	Cartridges of Sep-Pak Vac C_{18}	[127]
			x	x		CE	Serum and urine	Sulfonamides	Cartridges packed	[128]
		x		x		CZE	–	Fluorescein isothiocyanate	SPE by immunoaffinity	[129]
x				x		CZE	Soy, lupin and pea protein	Polyphenols	SPE with polyamide cartridges	[130]
x				x		CE	Soil	Imidazoline herbicides	C_{18} cartridge	[131]
	x			x		CE	Proteins	Peptides	Miniaturised reversed-phase C_{18}	[132]
x				x		CE	Wines	*cis*- and *trans*-resveratrol	SPE cartridges	[133]
x				x		CE	Seawater	Hydroxamate-type siderophores	SPE column	[134]
			x	x		CE	Urine	Phenprocoumon (I)	SPE column	[135]
x				x		CE	Human plasma and urine	Acetylsalicylic acid	SPE column	[136]
x				x		CE	Blood and urine	Common illicit drug	GDX301 SPE	[137]
		x		x		CZE	*Bauhinia purpurea* lectin	Peptides and glycoprotein	Adsorption preconcentration	[138]
		x		x		CZE	Tap water	Haloacetic acids	Four types of adsorbents	[139]
x				x		CE	Rat urine	Bupivacaine and its metabolites	–	[140]
			x	x		CE	Serum and urine	Non steroid anti-inflammatory drugs	LC-18 cartridges	[141]
x				x		CE	Peptides substrate	Chymotrypsin	Streptavidin–agarose beads	[142]
x				x		CE	Potatoes and onions	Maleic hydrazides	C_{18} SPE cartridge	[143]
x				x		CE	Pea plant	Indole-3-acetylaspartic acid	C_{18} SPE cartridge	[144]
x				x		CE	Water	Aromatic sulfonates	LiChrolut EN SPE column	[145]
x				x		CZE	Plasma	Midazolam and its metabolites	Bond Elut C_{18} cartridge	[146]
x				x		CZE	Plasma	Methotrexate and leucovorin	Bakerbond C_{18} SPE column	[147]
x				x		CGE	Blood and plasma	Antisense oligonucleotides	Cationic nanoparticles for SPE	[148]
x					x	CE	Biological tissues-M	Substances P	Combination SPE–HPLC–CE	[149]
x				x		CE	Haemophilus	Pathogenic lipopolysaccharides	C_{18} column and membrane	[150]

Table 5. Continued

Coupling mode				CFS		Electrophoretic mode	Sample	Analytes	Remarks	Ref.
OFF	IN	ON	OFF/ON	NC	C					
x				x		CE	Drinking water	Pesticides	C_{18}-bonded SPE cartridge	[151]
x				x		CE	Plasma	Oligodeoxyribonucleotides	–	[152]
x				x		MEKC	Milk	Cyclopiazonic acid	Sep-Pak plus silica gel SPE	[153]
x				x		CZE	Foodstuffs	Histamine	Clean up of samples by SPE	[154]
	x			x		CZE	Proteins, proteomes	–	SPE C_{18} cartridge	[155]
	x			x		CE	Insulin	Somatomedin C, IGF I and IGF II	Concentrator tips	[156]
x				x		CE	Soybeans	Multiple herbicides	SPE with Alumina-N dichloromethane	[157]
x				x		MEKC	Urine	Nitrazepam, flunitrazepam, triazolam	SPME	[158]
x				x		CGE	Human blood plasma	Phosphorothioate oligonucleotides	Cationic polystyrene nanoparticles	[159]
x				x		CE–MEKC	Paddy water	Pesticides/Insecticides	SPE cartridges	[160]
x				x		CE	Ground water	Sulfonated azo dyes	Cartridges of Isolute ENV	[161]
x				x		CE	Groundwater	Fluorescein (I)	Styrene–divinylbenzene disks	[162]
x				x		CZE–CEC	Human urine	Metabolites of paracetamol	C_{18} SPE	[163]
x				x		CE	Proteins	Trypsin	–	[164]
x				x		CZE–ITP	Proteins	Tryptic digests of bovine serum albumin	Reversed-phase resins for SPE	[165]
x				x		MEKC	Water	Sulfonated azo dyes	Cartridges Isolute ENV	[166]
x				x		CE	Water	Nitrophenols	C_{18} membrane discs	[167]
x				x		CE	River water	Naphthalene sulfonates	Preconcentration by SPE	[168]
x				x		CE	Human serum	Pentazocine enantiomers	Phenyl SPE cartridges	[169]
x				x		CZE	Coffee cherries	Phloxine B and uranine	SPE column	[170]
x				x		CZE	Potable water	Hydroxymetabolites of atrazine	Adsorbent cartridge	[171]
x				x		MEKC	Ground water	Hexazinone and its metabolites	Cartridge of Supelclean ENVI-Carb	[172]
x				x		CE	Laundry detergent	Alkylbenzenesulfonates	Clean up by SPE	[173]
x				x		CE	Serum	*R*(+)-, *S*(−)-pentobarbital	C_{18} SPE column	[174]
x				x		CE	Human serum	Bile acids	Cartridges of Whatman ODS-3	[175]
	x			x		CE	Sheep liver	Metallothioneins	Divinylbenzene resin SKP	[176]
x				x		MEKC	Urine	Corticosterone	SPME discs cartridges	[177]
x				x		CZE	Juice	Yellow and red safflower pigments	ODS-4 cartridge	[178]
x				x		CZE	Human urine	β-Blocker atenolol	Bond Elut certify SPE columns	[179]
						CE	Proteins	–	Review	[180]
						CE	–	Anions	Membrane based SPE discs	[181]
x				x		CE	Catfish	Antibiotic oxytetracycline	Sep-Pak C_{18} SPE cartridges	[182]
x				x		CE	Rat serum	D-Pen 2,5 enkephalin	SPE cartridges	[183]
x				x		CE	Plant tissue	Indole-3-acetic acid	SPE C_{18} Bakerbond cartridges	[184]
x				x		MEKC	Human serum	Cortisone, dexamethasone	SPE C_{18} cartridges	[185]
x				x		CE	Human urine	Debrisoquine, 4-hydroxydebrisoquine	C_{18} cartridges	[186]
x				x		CE	Urine	Enantiomers 4-hydroxydebrisoquine	Isolute ENV cartridges	[187]
x				x		CE	Milk	Tetracyclines antibiotics	SPE MP1 micro-column	[188]
x				x		CE	Human serum	Lipoproteins	C_{18} Sep-Pak	[189]
x				x		CE	Human plasma, urine	EDTA	Anion-exchange disc	[190]
x				x		CE	Water	Sulfonylurea herbicides	Anion-exchange cartridges	[191]
x				x		CZE	Blood serum	Proteins	Beckman Paragon SPE system	[192]
x				x		CZE	Skin	Fatty acid	Silica gel+LiChroprep	[193]
x				x		CE	Oral solid dosage	Betamethasone (I), ergotamine tartrate	Empore C_{18} SPE discs	[194]
x				x		CE	River water	Naphthalenesulfonates	–	[195]
x				x		CZE	Plasma	Linear pentapeptide dolastin	Sep-Pak C_{18} SPE column	[196]
x				x		CZE	Clinical urine	Orotic acid	RP-18 SPE column	[197]
x				x		CZE	Beet, tobacco, wheat	Cytokinins	SPE on C_{18} cartridges	[198]
x				x		CE	Human serum	*S*(+)-, *R*(−)-ondansetron	SPE on cyanopropyl cartridge	[199]
x				x		CE	Urine and serum	Barbiturates	SPME device	[200]
	x			x		CE	Tryptic digest from bovine serum albumin	Proteins	Fused silica column C_{18} silica	[201]

Table 5. Continued

Coupling mode				CFS		Electrophoretic mode	Sample	Analytes	Remarks	Ref.
OFF	IN	ON	OFF/ON	NC	C					
x				x		CE	Shellfish tissues	Domoic acid	LC-SAX cartridge	[202]
x				x		CE	Plant tissue	Indol-3-yl acetic acid	C_{18} SPE column	[203]
x				x		CE	Water	Aliphatic amines	Cartridges and discs	[204]
x				x		CE	Environmental water	Ethylenediaminetetraacetic acid	Anion-exchange disc	[205]
x				x		CE	Human or rat urine	Paracetamol and phenacetin	IST C_{18} bonded cartridges	[206]
x				x		CZE	Human urine	Enantiomers of methadone (I)	SPE on Bond Elut cartridge	[207]
x				x		CE	Water	Phenolic compounds	ENVI-Chrom P cartridge	[208]
x				x		CZE	Neonatal urine	Lactate, pyruvate, organic acids	C_{18} SPE cartridges	[209]
x				x		CZE–MEKC	Blackcurrant bud extract	Flavonoids, cinnamic, phenolic acid	Celite SPE column	[210]
x				x		MEKC	Human plasma	Fluconazole	Bakerbond C_{18}	[211]
x				x		MEKC	Equine urine	Morphine and meclofenamic acid	Bond Elut C_2 column	[212]
	x			x		AGE	Human serum	Proteins	Prepoured REP-agarose gel	[213]
x				x		CE	Biological matrices	Drug identification	Accubond Evidex cartridge	[214]
x				x		MEKC	Jelly and juice	Anthraquinone pìgments	–	[215]
x				x		CZE	Plant tissues	Ascorbate	C_{18} SPE cartridge	[216]
		x		x		CE	–	Metal ions	SPE cartridge	[217]
x				x		CZE	Tobacco	Carbaryl and carbofuran	Florisil SPE cartridge	[218]
x				x		CZE	Rats	[D-penicillamine 2,5]enkephalin in	C_{18} SPE column	[219]
x				x		CZE	–	Eleven priority phenols	Styrene–divinylbenzene	[220]
x				x		CE	Human serum	Benzodiazepines	Cleaned-up by SPE	[221]
x				x		CZE–ITP	Urine	Adenosine	Sep-Pak SPE column	[222]
x				x		CE	Water	Trialkylstannane derivates	XAD-2 column	[223]
		x		x		CE	–	Peptides	C_{18} coupled to silica capillary	[224]
		x		x		CZE	Drugs	Hypoglycaemic sulfonylurea	Bakerbond C_{18} SPE silica	[225]
x				x		CE	Rat liver	*Lycopus europaeus* L.	Bakerbond Phenyl column	[226]
x				x		CE	Urine	Enantiomers of amphetamines	Bond Elut certify cartridge	[227]
x				x		CZE	Rain water	Heterocyclic aromatic amines	Bond Elut C_{18} SPE cartridge	[228]
x				x		MEKC	Human urine	Hypoglycaemic drugs	Samples extracted by SPE	[229]
						CE	Biological fluids	Neuropeptides	Review	[230]
x				x		CZE	Water	Aromatic amines	Cartridge of copolymer	[231]
x				x		CE	Urine	Ibuprofen, fluriprofen, aspirin	C_{18} SPE cartridge	[232]
x				x		CE	Paper	Anion	SPE as a sample clean up	[233]
x				x		CE	Soil	Sulfonylurea herbicides	C_{18} SPE columns	[234]
x				x		CZE	Human plasma	Cimetidine	Supelclean LC-18 SPE	[235]
x				x		CZE	Household detergents	Alkylbenzenesulfonates	ODS C_{18} SPE column	[236]
x				x		CE	Rat liver microsomes	Theophylline and their metabolites	SPE on C_{18} columns	[237]
		x		x		CE	Bovine serum albumin digests	Peptides	Reversed-phase C_{18} packing	[238]
x				x		CE–ITP	Plasma	Heterocyclic peptides	SPE cartridge	[239]
x				x		CE	Biological fluids	Organic anions	–	[240]
x				x		CZE	Urine	Organic acids	C_{18} SPE cartridge	[241]
x				x		CE	Wines	Organic acids	C_{18} SPE cartridge	[242]
x				x		CE	DNA adduct	Nucleotides	Analytes separated by SPE	[243]
x				x		CZE	Urine	Racemethorphan and racemorphan	BakerBond Octadecyl HC	[244]
x				x		CZE	Water	Phenoxy acid herbicides	C_{18} membrane extraction (Overview)	[245]
x				x		MEKC	Urine	9-Tetrahydrocannabinol-9-carboxylic acid	–	[246]
x				x		ITP	Blood serum	γ-Aminobutyric acid	Separcol Si-C_{18} L minicolumn	[247]
x				x		MEKC	Serum	Cimetidime	Reversed-phase C_{18}	[248]
x				x		CE	Human serum	Cytarabine	C_{18} SPE cartridges	[249]
x				x		MEKC	Tablets	Water soluble vitamins	Octadecylsilane SPE column	[385]
x				x		CE	Lung tissue	Angiotensins	C_{18} extraction cartridge	[387]

CFS: Continuous flow system; NC: no chromatography; C: chromatography; CZE: capillary zone electrophoresis; CE: capillary electrophoresis; MEKC: micellar electrokinetic chromatography; SPE: solid-phase extraction; SPME: solid-phase microextraction; CGE: capillary gel electrophoresis; CEC: capillary electrochromatography; ITP: isotachophoresis; AGE: agarose gel electrophoresis.

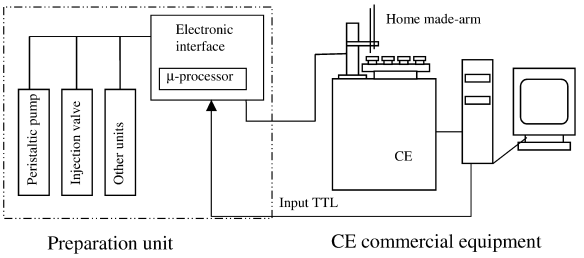

Fig. 2. Continuous flow system–capillary electrophoresis, commercial equipment (adapted from Ref. [34]).

using direct UV absorbance detection was developed to demonstrate the usefulness of this arrangement (see Fig. 3) [36].

9. Preconcentration membrane systems coupled to capillary electrophoresis

Poor concentration limits of detection (LODs) of CE methods often preclude their use for the analysis of dilute analyte mixtures. This limitation was addressed by the development of analyte concentrator and membrane preconcentration cartridges. The development of membrane preconcentration was undertaken to decrease or remove all the limitations observed in studied of solid-phase preconcentration [37,38]. The membranes have been used in more than 90 references shown in Table 6.

Using a suitably coated/impregnated membrane it is possible to minimise the bed volume of adsorptive phase at the inlet of the preconcentration capillary. The membrane is installed in a cartridge that is

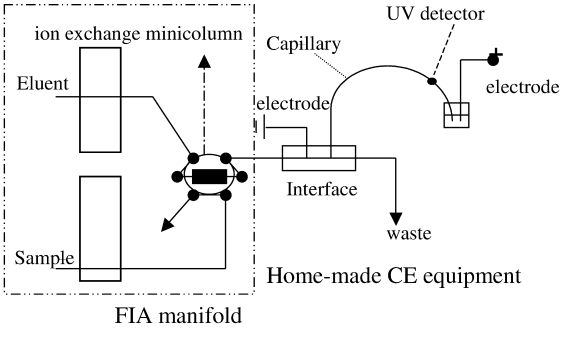

Fig. 3. Flow system–capillary electrophoresis, laboratory-made (adapted from Ref. [36]).

usually prepared from PTFE tubing. In addition to analyte preconcentration, membranes can also be used to effect sample clean up. This is particularly important for physiologically derived samples such as blood, urine, etc., where the presence of high salt concentrations can dramatically effect analyte separation by CE.

Small lengths of narrow-bore tubular membrane [39] can be interposed in the separation capillary in CE separation systems. These membrane segments can be used as sampling interfaces; a jacket is built outside the membrane, and the sample is introduced by diffusion/permeation through the membrane. Various examples are shown; the determination of gaseous samples through a porous membrane, the determination of ionisable/nonionic solutes by permeation through a silicone rubber membrane, and the separation of low-molecular-mass constituents in blood plasma by transport through a dialysis membrane.

In 1996, Szostek and Koropchak [40] described two means for interfacing condensation nucleation light scattering detection to CE. With the first method, a fused-silica capillary was used for the separation and the CE was grounded through a Nafion membrane that also connected the system to a microconcentric pneumatic nebuliser.

In the same year, a CE post-column radionuclide detector was developed that uses a commercial phosphor-imaging detector and was optimised for low-energy β emitters. Eluent from the separation capillary was deposited on a membrane. Emission from radioactive analytes on the membrane was integrated using the phosphor-imaging detector system. Results from the phosphor-imaging system were converted to conventional electropherograms [41].

A hollow fibre miniaturised supported liquid membrane (SLM) device for sample preparation is connected on-line with CE and used for determination of a basic drug, bambuterol, in human plasma. The analyte was extracted from the outside of the hollow fibre (donor) through the liquid membrane (pores of the fibre impregnated with organic solvent) into the acceptor solution in the fibre lumen. Very clean extracts of low ionic strength are obtained from the SLM treatment, making this sample pretreatment method compatible with the CZE double-stacking procedure, which in turn makes it possible to inject

Table 6
Coupling continuous membrane systems to capillary electrophoresis

Coupling mode				CFS		Electrophoretic mode	Sample	Analytes	Remarks	Ref.
OFF	IN	ON	OFF/ON	NC	C					
		x		x		CE	–	Proteins	Interface based on microdialysis memebrane	[10]
x				x		CE	–	Peptides	Preconcentration cartridge contained a membrane	[37]
		x		x		CE		Peptides	Membrane preconcentration	[38]
		x		x		CZE	Blood plasma	Low-molecular-mass analytes	Membranes were used as sampling interfaces	[39]
		x		x		CZE	–	Acids and peptides	Capillary grounded electrically through a membrane	[40]
		x		x		CE	–	Radionuclides	Eluate was deposited on to a membrane	[41]
		x		x		CZE	Human plasma	Basic drugs	Supported liquid membrane	[42]
		x		x		CE	Beverage	Carbonate	Gaseous products trough a membrane	[43]
x				x		CZE	–	Amino acids	Concentration using liquid membranes	[44]
						CE	Blood plasma	Total and unbound drug	Review	[63]
		x		x		IEF	Pharmalyte 5-8	Carbonic anhydrase	Separated by cellulose membrane	[70]
x				x		CE	Serum	Oligonucleotides	Membrane filters	[71]
x				x		CE	Blood plasma	Oligonucleotides	Dialysis using a cellulose membrane	[72]
x				x		CE	Polymerase chain reaction products	Nucleic acids	Membrane dialysis was a sample clean up	[77]
x				x		CE	Smoked fish	*Clostridium botulinum*	Membrane dialysis before analysis	[78]
x				x		CE	DNA fragments	*Clostridium botulinum*	Products were desalted by membrane dialysis	[79]
x				x		CGE	Plasma	Phosphorothioate	Dialysis with a Millipore VS membrane	[80]
x				x		CE	Living rat brain	4-Aminobutyric acid	Polycarbonate membrane dialysis probe	[81]
		x		x		CE	–	–	Membrane preconcentration	[124]
x				x		CE	Seawater	Siderophores	Membrane filter	[134]
		x		x		CE	Haemophilus	Pathogenic lipopolysaccharides	On-line preconcentration	[150]
						CE	Proteins	–	Review	[180]
x				x		CE	–	Anions	SPE discs comprise PTFE membrane	[181]
x				x		CE	Paper	Anion	Membrane based on SPE as a sample clean up	[233]
x				x		ITP	–	Gaseous sulfur dioxide	Membrane collector	[250]
x				x		CE	–	Trace metals	Hollow fibre membrane	[251]
		x		x		CZE	–	Inorganic anions	Membrane separated	[252]
		x		x		CIEF	–	–	Membrane sample preparation	[253]
x				x		CE	Rat	Monoamine and their metabolites	Polycarbonate ether membrane	[254]
x				x		IEF–CE	Pharmaceutical	Erythropoietin glycoforms	Centrifuging through a membrane	[255]
		x		x		CE–FI	Drug dissolution	Trimethoprim, sulfamethoxazole	On-line membrane filter	[256]
x				x		CZE	Blood plasma	Human IgG	Affinity membrane cartridge	[257]
x				x		CGE	Plants	RNA	Positively charged nylon membrane	[258]
x				x		MEKC	Pharmaceutical	Amphotericin B	Filtration through membrane filters	[259]
x				x		CE	–	Organic ions, alkylamines	Poly(vinyl chloride) membranes	[260]
x				x		CE	Cellulose solutions	Supercoiled plasmid DNAs	Float membrane	[261]
x				x		CE	–	Proteins	Membrane preconcentration	[262]
x				x		CZE	Feed solutions	Humic acids	Polyethersulfone cut-off membrane	[263]
x				x		CE	Medicines	Magnolol and honokiol	Filter membrane	[264]
x				x		CE	–	DNA sequence	Ultrafiltration trough a polymembrane	[265]
	x			x		CE	–	Proteins	Membrane preconcentration	[266]
x				x		CE	–	Dansylated amino acids	Membrane collection	[267]
x				x		CE	Tumour peptide	Particular antigen	Membrane mounted in a length of silica tubing	[268]
x				x		CE	–	Peptides	Membrane preconcentration	[269]
x				x		CE	Cod muscle tissue	M_r 41 000 protein	Membrane filter	[270]
x				x		CE	–	Amino acids	Liquid membrane extraction	[271]
x				x		CIE	Pharmaceutical	Inorganic and organic anions	PVDF membrane filters	[272]
x				x		CE	Pericarpium	Alkaloids	Filter membrane	[273]
		x		x		CZE	–	Phenylacetate inositol	Separated by cellulose membrane	[274]
x				x		CE		Four electrophore DNA	Dried sample spot on a membrane	[275]

Table 6. Continued

Coupling mode				CFS		Electrophoretic mode	Sample	Analytes	Remarks	Ref.
OFF	IN	ON	OFF/ON	NC	C					
x				x		CE	Water	Selenium and arsenic	Gaseous hydrides trough membrane	[276]
x			x			CZE	Minced fish	Histamine	Filtered through a membrane	[277]
x			x			CZE	Blood plasma	Inositol phosphates	Separated by two membranes	[278]
x			x			CE	–	Substance P and its metabolism	Membrane reactor	[279]
x				x		CGE	Plasma	Proteins	Nylon membrane	[280]
x				x		CZE	Vegetables	Ascorbic acids	Filtered through a membrane filter	[281]
x				x		CZE	Blood serum	Proteins	Comparison between CZE and CAME	[282]
x				x		CZE	Synthetic food	2-Nitrophenol	Movement of the membrane	[283]
x				x		CE	Drinking water	Arsenic compounds	Membrane gas–liquid separator	[284]
x				x		CE	Peat	Fulvic acids	Dialyzed with a cellulose ester membrane	[285]
x				x		CE	Blood plasma	Paracetamol, salicylic acid	Plasma was filtered through a membrane filter	[286]
			x		x	CZE	Plasma	Bambuterol	Sample enriched by passing through a SLM	[287]
x				x		CE	Labile cyanide	Metallocyanides	Concentration on supported liquid membranes	[288]
		x		x		CZE	Liquors	Adenosine triphosphate	Electrodialysis system separated by a membrane	[289]
		x		x		CE	Biological materials	Peptides and proteins	Membrane preconcentration	[290]
x				x		MEKC	Human blood	Creatinine	Centrifuged through a membrane	[291]
x				x		CZE	Vegetables	Free calcium	Filtered through a membrane	[292]
x				x		CE	Blood plasma	Bambuterol	Extracted through a porous PTFE membrane	[293]
	x			x		ITP	–	Peptides	Preconcetration cartridge incorporating a membrane	[294]
	x			x		CE	–	Organic acids	Liquid membrane phases	[295]
x				x		CZE	Human milk	Organometallic compounds	Milk was ultrafiltrated through a membrane	[296]
x				x		CGE	–	Proteins	Miniature ultrafiltration sampling probe (membrane)	[297]
		x		x		CE	EL-4 cells	Peptides	Membrane preconcentration	[298]
	x			x		CE	Biological material	Biological compounds	Membrane installed in the cartridge of a capillary	[299]
x				x		CE	Food	Histamine	Membrane filtration analysis before analysis	[300]
x				x		CE	Hepatocytes	3-Phenylaminopropane	Membrane was used for sample concentration	[301]
x				x		MEKC	Cheddar cheese	Caseins and large peptides	Buffer fractionated through a cutoff membrane	[302]
x				x		CZE	Plasma	Basic drugs	Supported liquid membrane technique	[303]
		x		x		CE	–	Haloperidol/peptides	Membrane preconcentration	[304]
		x		x		CE	–	Peptides	Preconcentration membrane	[305]
						CE	–	Peptides	Solid-phase reactor for preconcentration	[306]
x				x		CE	Biological fluids	Phosphorothioate oligonucleotide	Portions of the HPLC fractions were dialysed	[307]
x				x		CE	Red blood cells	Glutathione	Solution was filtered through a membrane	[308]
		x		x		CE	–	Peptides and amino acids	Eluent was directed on to peptide-binding membrane	[309]
x				x		CE	Rat retina	Enkephalin	The samples were centrifuged through a membrane	[310]
x				x		CE	–	Proteins	Blotting membrane micro-preparation	[311]
	x			x		CZE	Wheat flour	Calcium and magnesium	Liquid membrane (borax, ethylene glycol, EDTA)	[312]
						CZE	Environmental	Phenoxy acid herbicides	Use of C_{18} membrane extraction discs (overview)	[245]
	x			x		CE	–	Cations and anions	Suppressors were of ion-exchange membrane	[313]
x				x		CZE	Foodstuffs	Additives	Filtered through a micromembrane	[314]
		x		x		CE	Food	Anions	Capillary is linked to a cation-exchange membrane	[315]
		x		x		CE	–	Proteins	Membrane assembly at the exit of a capillary	[316]
	x			x		CE	Blood serum	Proteins	Fractions were collected on a moving membrane	[317]
	x			x		ITP	Flowers	Flavonoids	The filtrate was injected through the inlet membrane	[318]
x				x		CZE	Serum	Lithium	Samples were deproteinised with a filter membrane	[319]

CFS: Continuous flow system; NC: no chromatography; C: chromatography; CZE: capillary zone electrophoresis; CE: capillary electrophoresis; SPE: solid-phase extraction; MEKC: micellar electrokinetic chromatography; CGE: capillary gel electrophoresis; ITP: isotachophoresis; IEF: capillary isoelectric focusing; CIE: capillary ion electrophoresis; SLM: supported liquid membrane.

large volumes of sample onto the separation capillary [42].

On-line gas diffusion was coupled to a laboratory-made CE system via a specially designed interface [43]. The sample was merged with a modifying solution, e.g., a strong acid, in a flow system to transform the analytes of interest into their respective gaseous forms. These transformed, gaseous analytes permeate through a PTFE membrane into an acceptor stream comprising of a Tris buffer.

Finally, among others, basic studies of a procedure for extraction of amino acids using a supported liquid membrane were presented by Wieczorek et al. [44]. The extractions were made from an aqueous donor phase with pH 3 to a more acidic acceptor phase and the mass transfer was driven by the proton gradient between these phases. Both acceptor solutions were analysed by CE.

10. Extraction coupled to capillary electrophoresis

Medina et al. [45] developed a method for speciation of organomercurials in marine samples using CE. Organomercurials were extracted from the samples by the Westoo procedure. The cysteine–organomercury complexes formed were separated by CE. The mercury species, ethylmercury, methylmercury, phenylmercury and inorganic mercury were well resolved in 12 min. The procedure was applied to the analysis of dogfish muscle certified reference material, mussel, cockle, clam and tuna and the results were compared with those obtained by GC.

Liu and Sheu [46] carried out the determination of the six major flavonoids in Scutellariae Radix by MEKC. Scutellariae Radix (SR) is the root of *Scutellaria baicalensis* Georgi and is commonly used in Chinese herbal drugs. Pulverised SR (0.1 g) was extracted with 7.5 ml of aq. 50% ethanol by reflux for 30 min and centrifuged (1500 *g*) for 5 min. Extraction was repeated three times. The solution was analysed on a fused-silica capillary.

Humic acid was separated into two fractions (A and D) by CZE by Rigol et al. [47]. Humic acids are normally extracted from the soil using diluted aqueous alkaline solutions. Sodium hydroxide has been widely used, but there are undesirable features such as autoxidation or condensation of organic constituents. These changes can be minimised by performing extraction under an N_2 atmosphere (see Table 7).

11. Isotachophoresis coupled to capillary electrophoresis

Electrophoresis-based techniques such as isotachophoresis (ITP) can also be used for sample preparation prior to CE analysis (see Table 8).

ITP as an on-line concentration pretreatment technique in CE was described by Stegehuis et al. [48]. On-line coupling of ITP with CE was studied as a means of lowering the range of application for the latter technique. Detection limits could be improved by $\geq$2 orders of magnitude and the additional selectivity of the combined system gave promising results concerning biological samples. For proteins, the electromigration characteristics are hardly changed by isotachophoretic pretreatment. The potential of the combined system was illustrated by separation ion of phthalaldehyde and fluorescein isothiocyanate derivatives of amino acids. A limitation of the coupled system is that a compromise concerning the buffer is needed between analysis time and resolution in each part of the system.

Foret et al. [49] carried out a trace analysis of proteins by CZE with on-column transient isotachophoretic preconcentration. The sample was concentrated by ITP using 0.01 *M* acetic acid as terminating anode electrolyte for 4 min. Sharp and well-resolved peaks were detected due to the initial ITP focusing. On-column transient and coupled-column isotachophoretic preconcentration of protein samples in CZE was developed by the same authors [50]. By this method, the conventional single-column instrument could be used for isotachophoretic sample preconcentration of 50-fold or more without any modification. The same protein mixture was used to demonstrate a coupled-column system, which provides more freedom in selecting capillary zone electrophoretic running conditions, the possibility of injecting higher sample volume, effective sample clean up and selective ion analysis.

Analyte focusing in CE using on-line ITP was carried out by Stegehuis et al. [51]. Derivatised

Table 7
Coupling continuous extraction systems to capillary electrophoresis

Coupling mode				CFS		Electrophoretic mode	Sample	Analytes	Remarks	Ref.
OFF	IN	ON	OFF/ON	NC	C					
x				x		CZE	Lichens	Cations	Lichen immersed in an extraction tube	[4]
			x	x		CE	Tea	Polyphenols	Sample preparation: extraction+filtration ...	[5]
x				x		MEKC	Swabs	Organic gunshot	Extraction was by ultrasonic agitation	[17]
x				x		CZE	–	Amino acids	Extraction of the amino acids from the donor	[44]
x				x		CE	Marine samples	Speciation of organomercury	Extraction by the Westoo procedure	[45]
x				x		MEKC	Chinese medicines	Flavonoids	Extraction with ethanol	[46]
x						CZE	Soil	Humic acid	Extraction with NaOH and $Na_4P_2O_7$	[47]
x				x		CE	Urine	Tramadol	SPE	[97]
x				x		MEKC	Seed meals of citrus	Limonoid glucosides	Soxhlet extracted to remove oils	[105]
						CE	Environmental samples	–	Review (techniques for pretreatment)	[117]
x				x		CE	Antioxidative	Plant beverages	Soxhlet extraction	[120]
x				x		CE	Pea plant	Indole-3-acetylaspartic acid	Analytes were extracted during 1 h.	[144]
x				x		CE	Body fluids	Midazolam and its metabolites	Liquid–liquid extraction and SPE	[146]
x				x		CE	Foodstuffs	Histamine	Clean up by liquid–liquid extraction	[154]
x				x		CE	Soya bean	Herbicides	Pressurised liquid extraction in acetonitrile	[157]
x				x		CZE	Beet, tobacco, wheat	Cytokinins ribosides	Extraction with *n*-butanol and SPE	[198]
x				x		CE	Water	Aliphatic amines	Ion pairing with extraction discs	[204]
x				x		CE	Water	EDTA	Strong anion-exchange extraction disc	[205]
x				x		MEKC	Human plasma	Fluconazole	Liquid–liquid extraction with CH_2Cl_2	[211]
x				x		CE	Human serum	Benzodiazepines	Clean up by solvent extraction and SPE	[221]
						CE	Biological fluids	Neuropeptides	Review (sample preparation method)	[230]
x				x		CZE	Water/soil	Aromatic amines	SPE–liquid–liquid extraction	[231]
x				x		CE	Biological fluids	Organic anions	Six zwitterionic free acids used for extraction	[240]
x				x		CE	Sclerotia	Ergot alkaloids	Liquid extraction (twice)	[246]
x				x		CE		Amino acids	Liquid membrane extraction	[271]
x				x		CE	Plasma	Bambuterol	Supported liquid membrane for pretreatment	[293]
x				x		CZE	Plasma	Basic drugs (bambuterol)	Extraction in supported liquid membrane	[303]
x				x		CE	Plasma/urine	*E*-5-2-Bromovinyl-2′-deoxyuridine	Extraction buffer for determination of analyte	[320]
						CE	Drug	–	Overview (FI solvent extraction system)	[321]
x				x		CE	Wheat flour protein	Puroindolines	Extraction of soluble proteins	[322]
x				x		MEKC	Food	Biogenic amines	Amines were extracted with an acid solution	[323]
						CE	Food/beverages	Inorganic ions	Review (discussion of extraction method)	[324]
x				x		CZE	Roast coffee	Hydroxycinnamic acids	Extraction with organic solvent	[325]
x				x		CZE	Lettuce	Vitamin C and inorganic cations	Extraction with water and oxalic acid	[326]
x				x		CGE	Blood	Apolipoprotein E genotypes	DNA was extracted by two different methods	[327]
						CE	Food	Contamination analytes	Review (examples of extraction techniques)	[328]
x				x		CE	Medicines	Aconitine alkaloids	Extraction with different solvents	[329]
x				x		CE	Foetal sheep liver	Metallothionein isoforms	Samples subjected to a two step extraction	[330]
x				x		CE	Drugs	Naphazoline, dexamethasone	Sample was analysed after acetone extraction	[331]
x				x		CZE	Human erythrocytes	Enzyme activities	Diethyl ether extraction	[332]
x				x		MEKC	Paper food	Biocides	Extraction with hot water	[333]
		x		x		CE–CGE	DNA	HIV-I diagnosis	DNA extraction from samples on line	[334]
x				x		CE	Scleratia	Ergot alkaloids	Extraction with a mixture of solvent	[335]
x				x		CZE	Water	Haloacetic acids	Preconcentration by liquid–liquid extraction	[336]
						CE	–	Metallothionein isoforms	Review	[337]
x				x		CE	Water	Phenols	Adsorption of phenols on organo-clay	[338]
			x	x		CE	Soil	Anions	Automated extraction/filtering	[339]
x				x		CE	Puerariae radix	Herbal medicinal components	Threefold methanol extraction	[340]
x				x		MEKC	Liver and kidney	Naproxen (I)	Liquid extraction	[341]
						CE	Forensic	DNA	Experimental details for sample extraction	[342]

Table 7. Continued

Coupling mode				CFS		Electrophoretic mode	Sample	Analytes	Remarks	Ref.
OFF	IN	ON	OFF/ON	NC	C					
x				x		CE	–	Sulfonated metallophtalocyanines	Soxhlet extraction	[343]
x				x		CE	Wheat flour	Gliadins	Sample was extracted with buffer solution	[344]
x				x		CE	Soil	Platinum	Extraction of platinum after static extraction	[345]
x				x		CE	DNA	Nucleic acids/deoxyribo adducts	Review (extraction as sample preparation)	[346]
x				x		CE	Fish and crab meat	Methylmercury	Pretreatment with different organic solvent	[347]
x				x		CE	Environmental samples	Chromate/aromatic hydrocarbons	Extraction media buffer	[348]
x				x		CE	Hair	Amphetamine derivatives	Liquid–liquid extraction	[349]
x				x		CE	Cocaine	Isomeric truxillines	Liquid–liquid extraction	[350]
x				x		CE	Enantiomers mianserin	Blood plasma	Liquid–liquid extraction	[351]
x				x		CE	Inks	–	Optimisation of the extraction conditions	[352]
x				x		CE	Plasma	Phenylacetic acid	Simultaneous extraction and preconcentration	[353]
x				x		CE	Pharmaceutical	Oxytetracycline (I)	Extraction with *N*-methylformamide (15 min)	[354]
x				x		CE	Body fluids	Toxic drugs	Liquid–liquid extraction	[355]
x				x		CZE	Wool	Dyes	Dyes were extracted from wool	[356]
x				x		CZE	Urine	Methylenedioxymethamphetamine	Hydrolysis was carried out prior extraction	[357]
x				x		CE	Water	Whitening agent (tinopal)	Styrene–divinylbenzene extraction discs	[358]
x				x		CE	Blood/serum	Flunixin	Extraction of flunixin on C_{18} cartridges	[359]
x				x		CGE	Plasma/urine	Phosphorothioate oligonucleotides	Extraction with phenol–$CHCl_3$	[360]
x				x		MEKC	Algal scum	Toxins	10 ml SFE vessel	[361]
x				x		CE	–	Enantiomers of propranolol	Soxhlet extraction	[362]
x				x		CE	Urine	Dimethindane	Extraction with cyclohexane–ethyl acetate	[363]
x				x		MEKC	Biological fluids	Paclitaxel	–	[364]
x				x		CE	–	Nucleic acids	Genomic DNA was extracted from lung	[365]
x				x		MEKC	Human urine	Antidepressants	Extraction was performed with hexane	[366]
x				x		MEKC	Cozaar tablets	Losartan potassium drug	Robotic extraction with buffer	[367]
x				x		CE	Freeze–dried tuna	Methylmercury	Sample was extracted with acetone–water	[368]
x				x		CE	Environmental samples	Methylmercury	Extracted with acetone and toluene	[369]
x				x		CE	Dissolution test	Clenbuterol and levothyroxine	Extraction discs	[370]
x				x		CE	DNA	Benzo[*a*]pyrene diol epoxide	Unconsumed diol was removed by extraction	[371]
						CE	–	–	Review of on-line sample extraction ...	[372]
x				x		CE–MEKC	Soil samples	Inorganic ions	Description of the extraction of analytes	[373]
x				x		CE	Amniotic fluid	DNA markers (Down's syndrome)	Phenol–$CHCl_3$ extraction	[374]
x				x		CZE	DNA	Styrene oxide adducts	Unreacted products removed by extraction	[375]
x				x		CZE	Cotton and wool fibres	Black dyes	Extraction with NaOH (25 min+100°C)	[376]
x				x		CZE	Grass pea	3-*N*-Oxalyl-diaminopropanoic acid	Extraction twice by shaking (45 min)	[377]
x				x		CZE	Natural products	Nitrate, nitrite, amino acids ...	Extraction of anti-cancer natural products	[378]
x				x		MEKC–CE	Urine	Drugs of abuse	Extraction scheme	[379]
x				x		CE	Chinese medicine	Six bioactive components	Sample was extracted with methanol	[380]
x				x		CZE	Milk	Chloroamphenicol	Extraction with ethyl acetate	[381]
x				x		CE	Urine/plasma	7-Hydroxycoumarin	Extraction with diethyl ether (10 min)	[382]
x				x		CE	Organic solvent	Cationic and anionic	Ions were concentrated by extraction	[383]
x				x		MEKC	Water	Monosulfonated dyes	Dyes were isolated by extraction	[384]
x				x		MECC	Pharmaceutical	Anabolic steroids	A one step quantitative extraction procedure	[386]
x				x		MEKC	Human serum	Drugs	Solvent extraction procedures	[388]
x				x		CE	Hair	Cocaine	Extraction with Toxi-Tubes A	[389]
x				x		CE	Rat hair	Amino acids	Extraction with $CHCl_3$–propan-2-ol	[390]
x				x		MEKC	Serum	Amino acids	Extraction with pentane	[391]
x				x		CE–ITP	–	Anionic, cationic and drugs	Liquid–liquid extraction	[392]
x				x		CE	Foods	Glycine, 5-hydroxymethylfurfural	Aldehydes were removed by extraction	[393]
x				x		CZE–MEKC	Soil/water	Aromatic organic acids	Water was extracted with extraction disks	[394]
x				x		CZE	Orange juice	Ascorbic and dehydroascorbic acid	Extraction described in the article	[395]

Table 7. Continued

Coupling mode				CFS		Electrophoretic mode	Sample	Analytes	Remarks	Ref.
OFF	IN	ON	OFF/ON	NC	C					
x				x		CZE–MEKC	Human urine	Morphine 6-glucuronide	Extraction improved the LOD	[396]
						MEKC	Foodstuffs	Mycotoxins	Review (emphasis of sampling, extraction)	[397]
x				x		CE	Dystrophin gene	Nucleic acids	The extraction was with phenol–chloroform	[398]
x				x		CZE	Sugar cane	Flavonoids	Acetonitrile–water extraction prior to CZE	[399]
x				x		CZE	Blood/serum	Pentobarbitone	Extraction gave an increase in sensitivity	[400]
						MEKC	Biological fluids	Drugs	Overview	[401]
x				x		CZE	Rat urine	Cimetidine	Extraction with ethyl acetate–light petroleum	[402]
x				x		CE	Coptidis Rhizoma	Quaternary alkaloids	Extraction was repeated three times	[403]
x				x		CE	Blood/plasma	Cicletanine	Extraction was with diethyl ether	[404]
x				x		MEKC	Residues	Gunshot	Extraction with ethanol	[405]
x				x		MEKC	Human serum/plasma	Thiopental (thiopentone)	Liquid–liquid extraction	[406]
x				x		CE–MEKC	Human serum/urine	Barbiturates	Extraction with cartridges	[407]
x				x		CZE	Organic soils	Caesium	Extraction with NaOH under N_2	[455]

CFS: Continuous flow system; NC: no chromatography; C: chromatography; CZE: capillary zone electrophoresis; CE: capillary electrophoresis; MEKC: micellar electrokinetic chromatography; ITP: isotachophoresis; SPE: solid-phase extraction; LOD: limit of detection; FI: flow injection;CGE: capillary gel electrophoresis; HIV: human immunodeficiency virus; SFE: supercritical fluid extraction.

sample solutions were injected into the ITP system comprising a PTFE separation capillary and a fused-silica detection capillary with on-line UV detection. The ITP and CE systems were coupled via a Plexiglass interface block.

In 1996, Sadecka and Polonsky [52] carried out the determination of some cardiovascular drugs in serum and urine by capillary ITP. The analytical column was connected to a preseparation column. Amiloride and β-blockers were separated by cationic isotachophoresis in a 10 m*M* sodium morpholinoethanesulfonate buffer (pH 5.5)–5 m*M* glutamic acid system. Frusemide was separated in the anionic electrolyte system 10 m*M* histidine hydrochloride buffer (pH 6.2)–morpholinopropanesulfonic acid.

12. High-performance liquid chromatography coupled to capillary electrophoresis

The coupling of LC and CE was described by Bushey and Jorgenson [53]. As CE operates under fundamentally different separation mechanisms, the combination with LC represents a true orthogonal system. A reversed-phase LC (RPLC) system was used in the first dimension, and eluting fractions were introduced and further separated on a CE system, which was used to separate peptide standards and fluorescently labelled peptide fragments from a tryptic digest of ovalbumin. The coupling HPLC–CE is not yet widely used, 13 references are included in Table 9.

Larman Jr. et al. [54] developed a two-dimensional separations of peptides and proteins by comprehensive LC–CE. Tryptic digest of horse heart cytochrome *c*, labelled with fluorescein isothiocyanate, was subjected to two-dimensional RPLC and fast CZE (FCZE). Eluates passed through an untreated fused-silica capillary and interface tee which led to waste (to lower the pressure) and into the second dimension FCZE system with sampling controlled by an argon ion laser gating beam.

Moore Jr. and Jorgenson [55] developed a comprehensive three-dimensional separation of peptides using size-exclusion chromatography–RPLC–optically gated CZE. The LC column was linked to the top of a vertical fused-silica CZE capillary, with a high-speed optical gating injection system.

A transparent flow gating interface for the coupling of microcolumn LC with CZE in a comprehensive two-dimensional system was developed by Hooker and Jorgenson [56]. The new interface was based on the original flow gated design developed in their laboratory but is now made from a clear plastic which allows for the direct observation and routine manipulation of the micro-HPLC and CZE capillaries. To evaluate the reproducibility of the inter-

Table 8
Coupling continuous isotachophoresis systems to capillary electrophoresis

Coupling mode				CFS		Electrophoretic mode	Sample	Analytes	Remarks	Ref.
OFF	IN	ON	OFF/ON	NC	C					
		x		x		CE	Proteins	Derivatives of amino acids	ITP as concentration pretreatment technique in CE	[48]
		x		x		CZE	–	Eight proteins	CZE with on-column transient ITP preconcentration	[49]
		x		x		ITP–CZE	–	Protein	On-column transient and coupled-column ITP	[50]
		x		x		CE	–	Derivative of angiotensin III	Analyte focusing in CE using on-line ITP	[51]
		x		x		ITP	Serum/urine	Cardiovascular drugs	Analytical and preseparation column connected	[52]
						CE	Aqueous samples	Environmental pollutants	Overview	[121]
						CE	–	–	Membrane preconcentration system	[124]
	x			x		CZE	Urine	Adenosine	CZE with oncolumn ITP preconcentration	[222]
	x			x		CZE	Blood plasma	Heterocyclic peptides	Improved sensitivity by on-line ITP preconcentration–CZE	[239]
	x			x		CE	–	Peptides	On-line transient ITP	[269]
	x			x		CE	Urine	Peptides and proteins	Analytes can be submitted to stacking or ITP	[290]
x				x		CE	–	Peptides	ITP conditions for membrane preconcentration–CE	[294]
	x			x		CZE		Peptide mixtures	CE–MS in conjunction with transient ITP	[304]
	x			x		CZE	Antimuscarinic drugs	Anionic and cationic	ITP preconcentration for CZE in a single capillary	[392]
	x			x		CZE–ITP	Human erythrocytes	Adenosine deaminase activity	On-column capillary ITP–CZE	[410]
	x			x		CE	Nuclear fission product	Rare-earth metals	ITP permits stacking of large injection volumes	[411]
		x		x		CZE	–	Sulfanilic and hippuric acids	CZE with on-line ITP sample pretreatment	[412]
		x		x		CZE	Antimuscarine drugs	–	Counter-flow ITP–CZE	[413]
	x			x		CZE–ITP	Pseudomonas aeruginosa	Oligosaccharides	Transient ITP preconcentration coupled to CZE	[414]
	x			x		CE	Biological cells	Antisense oligonucleotides	On-capillary ITP and CE polymer sieving	[415]
	x			x		CE	–	Cholinesterase inhibitor	ITP was used to concentrate before CZE	[416]
	x			x		CE	Fission products	^{137}Cs, ^{152}Eu and lanthanide	ITP enabled large volume samples to be analysed	[417]
		x		x		CE	Blood serum	Hippuric acid	On-line ITP–CZE	[418]
	x			x		CE	Drugs	Amitriptyline and metoprolol	ITP preconcentration with CZE in a single capillary	[419]
						CE	–	–	Review (combined technique of capillary ITP–CZE)	[420]
		x		x		CE	Natural, mineral water	EDTA–iron(III) complex	On-line coupling of capillary ITP and CZE	[421]
x				x		CZE	Water	Nitrophenols	Off-line isotachophoretic sample pretreatment	[422]
		x		x		CZE	Mayonnaise	EDTA	On-line coupled capillary ITP–CZE	[423]
x				x		CE	–	Oligonucleotides	On-column transient capillary ITP and CE	[424]
	x			x		CZE	–	Nitrophenols	To enhance the sample load capacity: ITP–CZE	[425]
x				x		CZE	Basic proteins	Basic peptides	On-line ITP–CZE with hydrodynamic counterflow	[426]
	x			x		CGE	X174/HaeIII and PCR product of 118 bp	DNA	ITP preconcentration in capillary gel electrophoresis	[427]
		x		x		CZE	–	–	ITP (preseparation and concentration) and CZE	[428]
	x			x		CZE	–	–	Review (single capillary coupled ITP–CZE techniques)	[429]
		x		x		CZE	β-Blocker drugs	–	Combined LLE–ITP for loadability in CZE	[430]
	x			x		CZE	–	Inorganic anions	On-line coupled capillary ITP–CZE	[431]
	x			x		CZE	–	Neostigmine and others	Combined LLE and ITP as fast on-line focusing step in CE	[432]
	x			x		CZE	Calf urine	β-Agonists	On-capillary ITP for loadability enhancement in CZE	[433]
	x			x		CZE	–	Neostigmine/propantheline	In-line ITP focusing of large injection volumes for CZE	[434]
		x		x		CZE	–	Amino acids	Automated on-capillary ITP followed by CZE.	[435]
	x			x		CZE	–	Brilliant acid green	Correlation between velocity and current in ITP–CZE	[436]
	x			x		CZE	Tap or lake water	Paraquat and diquat	ITP sample pretreatment and determination by CZE	[437]
	x			x		CZE	Biological samples	Enzyme inhibitor	On-column ITP focusing in CZE	[438]
	x			x		CE	Protein	–	On-column transient capillary ITP preconcentration in CE	[439]
		x		x		CZE	Urine	Sulfanilate/3,5-dinitrosalicylate	CZE of mixtures with on-line ITP sample pretreatment	[440]
		x		x		CZE	–	–	Options in electrolyte systems for combined ITP–CZE	[441]
		x		x		CZE	Dilute sample	*p*- and *m*-chlorobenzoic acids	Analysis by CZE with ITP preconcentration	[442]
		x		x		CE	–	–	On-line coupling of capillary ITP–CE	[443]
	x			x		CZE	Feedstuffs	Halofuginone	Combination of ITP–CZE in a column-switching system	[444]
		x		x		CZE	Blood	Anionic/cationic, thiamine	ITP preconcentration for enhancement of detectability in CZE	[445]
	x			x		CZE	–	Cytochrome	CZE of dilute samples with ITP preconcentration	[446]
		x		x		CZE	–	Nitrophenols/labelled amino acids	On-line coupling of capillary ITP with CZE	[447]
	x			x		CZE	–	Polypeptides and proteins	CZE and ITP–mass spectrometry	[448]
						ITP	–	–	Review	[449]
	x			x		ITP	Water	Permethrin and tetramethrin	Double extraction	[450]
	x			x		ITP	Non-aqueous	Cationic metal chelates	Capillary-tube ITP–solvent extraction	[451]

CFS: Continuous flow system; NC: no chromatography; C: chromatography; CZE: capillary zone electrophoresis; CE: capillary electrophoresis; ITP: isotacophoresis; LLE: Liquid–liquid extraction.

Table 9
Coupling continuous HPLC systems to capillary electrophoresis

Coupling mode				CFS		Electrophoretic mode	Sample	Analytes	Remarks	Ref.
OFF	IN	ON	OFF/ON	NC	C					
					x	CE	–	–	Review (multi-dimensional separation systems)	[19]
		x			x	CE	Ovalbumin	Peptides	2D HPLC–CE	[53]
		x			x	CZE	Horse heart cytochrome	Peptides and proteins	2D separations by LC–CE	[54]
		x			x	CE	Hen ovalbumin	Peptides	Three-dimensional separation: SEC–LC–CE	[55]
		x			x	CZE	Urine	Amino acids	Coupling of microcolumn LC with CZE	[56]
		x			x	CE	–	Benzoic acids	On-line chromatographic pretreatment of samples for CE	[57]
x					x	CZE	Plasma	Drugs	Micro-CLC as an interface between SLM extraction and CZE	[287]
x					x	CE	Endoproteinase Lys-C	Thirty glycoforms	CE resolved fractions from the LC	[408]
					x	CE	–	Metallothioneins	Review (coupling of separation techniques)	[409]
					x	ITP	Soil	Herbicide asulam	HPLC as a sample prep. method	[452]
		x			x	CZE	Proteins	–	Transverse flow gating interface for the coupling of LC–CZE	[453]
		x			x	CZE	–	Peptides	Analytes were separated by 2D HPLC and CZE	[454]
		x			x	CE	–	PAHs	Application of 2D statistical theory	[456]
		x			x	CEC	Energetic materials standards	Nitro compounds	Simple interface for gradient CEC, supplies mobile phase from LC	[457]

CFS: Continuous flow system; NC: no chromatography; C: chromatography; CZE: capillary zone electrophoresis; CE: capillary electrophoresis; SPME: solid-phase microextraction; MEKC: micellar electrokinetic chromatography; CGE: capillary gel electrophoresis; CEC: capillary electrochromatography; ITP: isotachophoresis; PAHs: polynuclear aromatic hydrocarbons; SEC: size-exclusion chromatography; CLC: column liquid chromatography; SLM: supported liquid membrane; 2D: two-dimensional.

face, 400 consecutive CZE separations of a mixture of fluorescein 5-isothiocyonate (FITC)-labelled phenylalanine and glutamic acid were performed.

The direct injection of samples with high salt concentrations in CE results in peak splitting and/or serious band broadening. These problems were not encountered when using a liquid chromatography-type of sample pretreatment coupled on-line with a CE system. To demonstrate the feasibility of this approach, the separation of three model compounds (benzoates) in water containing up to 400 m*M* of sodium chloride was studied [57].

13. Conclusions

CE has many weak points that can be attributed to the sample preparation step. As has been described in this review, a lot of ingenious CFSs have been designed to solve a variety of problems encountered in CE analysis. Even though it is not easy to predict the future of all those smart inventions (will they survive and become widely accepted or not) they all enrich the field of CE. In summary, the different techniques presented in this review can overcome the current limitations of the LODs in CE and reduce the need for exhaustive off-line sample preparation prior to analysis by CE. This technique is slowly gaining popularity as a tool with great potential for routine analysis in the laboratory.

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