Coupling of Flow Techniques with Capillary Electrophoresis: Review of Operation Principles, Challenges, Potentials, and Applications

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Abstract

In this review, an overview is given of the up-to-date accomplished analytical systems combining flow techniques and capillary electrophoresis with the main focus on interfacing principles, challenges, potentials, and the resulting operation versatility as well as the incorporation of automated sample treatment prior to the electrophoretic separation. The up-to-date reported coupled systems are classified in tabular form in respect to interface types and analytical applications. An introduction into the different flow techniques and capillary electrophoresis is given further. In consequence, this review is aimed to complement former review articles, whose main focuses were analytical performance of the reviewed systems.

Flow techniques

Introduction to flow techniques

Flow techniques (FT) are used for the automation of the analyst's work. They all have in common the carrying and treatment of a sample in flow within a tube "manifold". Peristaltic, syringe, or solenoid membrane pumps are mainly used for liquid driving; as alternative propulsion techniques, gravimetric flow (1), electro(endo)osmotic flow (EOF) (2), miniature piston (3), or piezoelectric pumps (4) have been reported further. Apart from the in-line addition of reagents in order to enhance the detection sensitivity and selectivity, a variety of laboratory preparative and analytical procedures have been accomplished by FT so far. Among these, dilution, leaching, microwave-, ultrasonic-, UV-irradiation-, and agent-assisted digestion or photo-degradation, pre-concentration and matrix separation by distillation, gas diffusion, pervaporation, dialysis, liquid–liquid and solid-phase extraction (SPE) and co-precipitation can be found (5–20). Their automation included the development of adequate "hardware" such as phase separators, membrane holders, dilution chambers, reactor and detection flow cells, and interfaces for coupling to other analytical instrumentation. Continuous or stop-flow registration of the physical or chemical property of interest leads generally to peak-shaped signals, whose characteristic area, height, width, or increment are correlated with the analyte concentration. Most reported benefits are an increase of the sample frequency, lower consumption of reagents and sample, and reduced cost of analysis and environmental impact. Due to the closed manifold, sample alteration and working area contamination is avoided. Where volumes and flow rates are precisely controlled, a considerable gain of repeatability is possible, which allows taking benefit of reaction kinetics and enabling detection of intermediate reaction species before reaching the steady-state.

Due to these characteristics, analytical flow technique are used widespread where a fast, economic, reliable, and automated analysis of a large number of samples is demanded, such as routine clinical analysis, industrial or biotechnological process control and environmental vigilance.

The different modes of liquid propulsion, sample introduction, operation schemes, flow pattern, and manifold configurations have led to the proposal of distinct FT. Extensive comparisons and introductions into the different techniques can be found in specialist books (17–20) and in numerous reviews and treatises about the different FT and their fundamental aspects. In the following, the FT coupled up-to-date with capillary electrophoresis (CE) are shortly described.

Flow injection analysis and multicommutation flow injection analysis

In flow injection analysis (FIA), proposed by Ruzicka and Hansen (21) and reviewed by the same in 1986 and 2000 (22,23), a sample aliquot is inserted into a non-segmented carrier laminar flow and disperses in radial and axial directions in the laminar flow. Reagents are added to the carrier solution or by confluent mixing. Controlled dispersion and reproducible timing and injection are the key principles and conditions (19). Important advantages of FIA are its high reproducibility, simplicity, and continuous flushing of the manifold, prolonging the lifetime of integrated reactors and detection probes. Various modes have been derived from classical FIA, among these minia-

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turization using microconduits (3), capillaries (24), and lab-on-a-chip systems, mono-segmented flow (25,26), reagent or double injection (27,28), and all injection analysis (29) lowering of solution consumption or reverse-flow (30) and stop-flow (31) amplifying the possibilities of kinetic evaluations.

Multicommutated flow injection analysis (MCFIA), proposed in 1994 (32) and reviewed in 2002 (33,34), is based on the additional use of 3-way solenoid valves (SV) acting as software-controlled multicommutators obtaining a "flow network". SV are used to substitute the classical injection valve, to enable the return of non-required solutions (recycling), deactivation of pumping or manifold lines (system software configuration), and binary sampling schemes known also as zone stacking techniques. A high flexibility is achieved by circumventing physical changes of the manifold but only alteration of the commutation sequence of the SV enabling multi-parameter determinations with one system. The main disadvantage of FIA and MCFIA is the general use of peristaltic pumps, which lead to a reduced robustness in respect to pressure and use of solvents and acids. Typical FIA and MCFIA systems are depicted in Figure 1A and 1B, respectively.

Sequential injection analysis and lab-on-valve

Sequential Injection Analysis (SIA) was proposed by Ruzicka and Marshall in 1990 (35) as an alternative FT to FIA. A typical SIA system consists of a bi-directional syringe pump connected via a holding coil (HC) to the common port of a selection valve. One or more lateral ports are connected to the manifold, consisting at least of a reaction coil and the detection flow cell. All solutions are located on other lateral ports. An intermittent flow scheme is carried out by obligatory computer control. Sample and reagents are aspirated in succession from the selection valve into the holding coil. By flow direction reversal, the stacked solutions are propelled through the reaction coil towards the detection flow cell. The reaction product is formed in the overlapping zones of reagents and sample penetrating each other by dispersion.

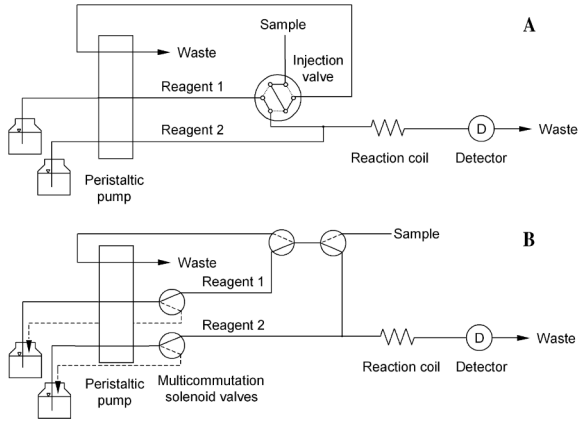

Figure 1. Typical structure of a FIA manifold (A) and MCFIA manifold (B). In FIA, the concept consists in the reproducible insertion of the sample into a non-segmented continuous flow. The reagent product is formed at the penetration zones of sample and reagent or by confluent mixing with the reagent. In MCFIA, the FIA manifold is modified by the introduction of multicommutation solenoid valves allowing the change of flow direction e.g. to reduce reagent and sample consumption.

A lower consumption of sample and reagent, use of a pulsation-free, pressure-robust syringe pump, and method flexibility by computer control of all operational parameters (timing, volumes, and flow rates) are important advantages of SIA, enabling multiparametric analysis, whereas the impossibility of confluence mixing generally leads to a lower detection sensitivity and higher signal contribution of schlieren effects and the required syringe refilling and sequential aspiration of the solutions to a lower sample frequency. Comprehensive overviews to SIA technique can be found elsewhere (36,37).

In the variant technique Lab-On-Valve (LOV) proposed by Ruzicka in 2000 (38), an integrated microconduit including a multi-purpose detector flow cell and a flow-through port is used as stator on the selection valve, enabling downscaling, higher sample throughput and robustness, and lower consumption of solutions. One of the most noteworthy advantages is the easy adaptation for handling of sorbent beads in flow due to the

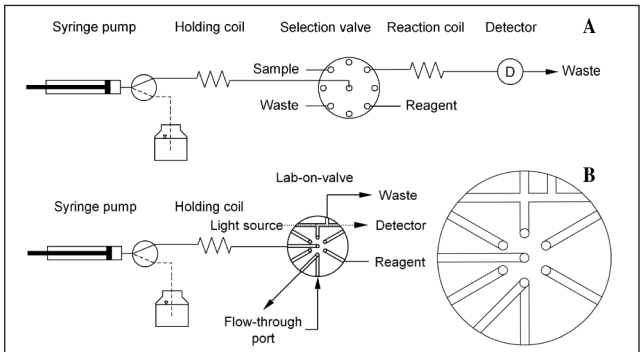

Figure 2. Typical structure of a SIA manifold (A) and LOV manifold (B left) and LOV conduit (B right). The concept consists in the performance of an intermittent flow scheme, during which sample and reagents are aspirated from a multiposition valve into a holding coil and afterwards propelled towards the detection cell passing a reaction coil by flow reversal. In LOV, a special selection valve design with integrated detection cell and flow-through port is used. This facilitates downscaling of the analyzer system and handling of active beads for in-situ preparation of columns with renewable fillings.

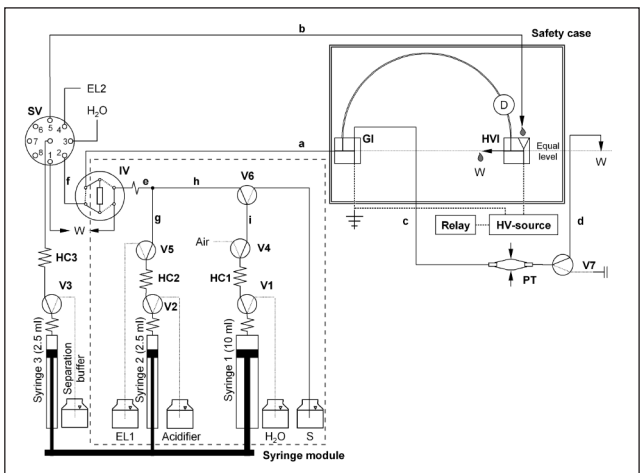

Figure 3. Example of a MSFIA manifold coupled to a homemade CE system (121), divided into a pre-concentration part (circled by broken gray line) and CE part operating in parallel. A similar flow concept as in MCFIA is applied in MSFIA but sequential and bi-directional operations are fundamental of the multisyringe pump. SV: selection valve (Pos1: Waste, Pos2: IV, Pos3: water, Pos4: EL2, Pos5: HVI, Pos6-7: auxiliary solutions), V1–V7 solenoid valves with normally open position (OFF, deactivated) dotted (V3 and V7 of enhanced pressure stability), IV: Injection valve with SPE column, D: detector, HC: holding coils, BGE: separation buffer, S: sample, W: waste.

rigid and short manifold channels (39–41). This so-called bead-injection technique allows the fabrication of renewable enzymatic or pre-concentration columns or electrode surfaces. Typical SIA and LOV systems are depicted in figure 2A and 2B, respectively.

Multisyringe flow injection analysis

Multisyringe flow injection analysis (MSFIA) proposed and described by Cerdà and co-workers in 1999 (42,43) combines the advantages of MCFIA (multichannel, multicommutation, confluent mixing) and SIA (robustness, sequential, bidirectional operation, and volumetric precision) by using parallel moving syringes incorporated into one single pump device as liquid drivers and SV at the syringe head or integrated into the manifold paths for flow networks. The flow rate ratio is configured by selection of different syringe sizes, whereas flow rates, volumes, and manifold configuration are software-controlled. Characteristics, applications, and possible injection modes have been reviewed recently (44). As the main disadvantage, the volumetric limitation of the syringes has to be named. As an example, a MSFIA system coupled to CE is depicted in Figure 3.

Limitations of flow techniques

Selective detectors based on as potentiometry, amperometry, spectrophotometry, and fluorescence spectrometry, atomic absorption or fluorescence spectrometry are mostly applied in FT to overcome this main limitation of FT. This is the low capacity of separation of analytes, which cannot be discriminated by non-chromatographic techniques due to insufficient differences regarding their chemical and physical properties, such as vapor pressure, dissociation constant values, or reactivity (45). In consequence, there is a trend in the investigation area of FT of their coupling with separation techniques such as CE.

Capillary Electrophoresis

Introduction to CE

CE was initiated by Hjerten (46) using millimeter-bore, buffer filled glass capillaries rotated along their longitudinal axis to suppress thermal convection. By reduction of the inner diameter, done by Jorgenson and Lukacs (47), the capillary becomes anti-convective for the first time due to improved heat dissipation and low radial temperature gradient, enabling high electrical fields (100–500 V/cm), and consequently fast separations (< 10 min) with high separation efficiencies ($N > 10^5$).

As Karger stated that "High-performance capillary electrophoresis is expected to be the fastest-growing analytical technique since HPLC" (48), CE is today a major separation technique due to the variety of operation modes. It has been applied to a wide range of analytes including carbohydrates, carboxylic acids, inorganic anions, phenols, metal ions, drugs, explosives, pigments, vitamins, toxins, chiral separations, DNA, pesticides, and peptides, as well as proteins in environmental, forensic, clinical, and food analysis (49). Due to capillary inner diameters of only 10–75 µL and minute sample volume (1–50 nL), even single-cell analysis is possible.

Injection is generally done by electrokinetic or hydrodynamic mode. In electrokinetic injection, the application of voltage with sample at the capillary inlet forces the migration of the analytes into the capillary. Bias results from the different migration velocities of the analytes favor the faster migrated analytes, which become and can be enriched in this operation mode. The ionic strength of the sample further affects the injection performance, requiring the use of internal standards (50,51). In hydrodynamic mode, a sample aliquot is forced into the capillary by generally positive pressure on the injection side. Here, the disadvantage is the unselective introduction of the all matrix components into the capillary.

Limitations of CE

Mostly reported drawbacks of CE are a low detection sensitivity due to the very small detection cell volumes and reproducibility of injection and separation. The latter can be attributed partly to the important influence of the sample matrix (viscosity, ionic strength, macromolecles, etc.) on the local electric field strength and the EOF. The EOF and its control is a fundamental constituent in CE operation and super-imposes the migration of the analytes in the capillary. It results from the zeta-potential at the inner capillary walls and the applied separation voltage. The EOF is therefore affected by matrix components, which adsorb to the inner capillary walls. In consequence, sample clean-up and frequent re-conditioning of the capillary by flushing with separation buffer or auxiliary solutions is crucial for reproducible separation performance.

Detection sensitivity can be improved by derivatization reactions or solid phase supported analyte pre-concentration reviewed comprehensively (52), or by on-capillary gradient-based, stacking and focusing techniques such as electrokinetic enrichment during injection, field amplified sample stacking, transient isotachophoresis, dynamic pH junction, or by quasi-stationary phases (sweeping) (53,54).

Despite their considerable potential to increase the detection sensitivity by several orders of magnitude (55), these techniques are also affected by the sample matrix. Consequently sample clean-up will improve the reproducibility of these methodologies. The integration of a miniature SPE column into the capillary is a powerful approach for on-capillary analyte concentration because all eluted analyte molecules are quantified and only a fraction of the sample volume compared to off-line concentration is required. Here, after column loading, the eluent is injected and transported by the EOF through the column. However, capillary flushing requires a relative high pressure and disturbing matrix components enter the capillary during column loading (55–57).

Potential and Challenges of Coupling CE with FT

Sample clean-up and analyte pre-concentration are generally done by (membrane-supported, micro-) liquid–liquid extraction (LLE), or (micro) SPE (58). Both feature considerable purchase costs, duration time, solvents usage, and waste formation. Automation of these procedures by FT bears the potential of low-

ering these efforts and improving the separation reproducibility, sensitivity, and the application to incompatible matrices, such as samples of high salt or protein contents (55,58). It further offers the ability to overcome the limitations and to combine the advantages of both techniques and provides a potential for new applications, such as monitoring or miniaturization. Because continuous application of pressure is, in contrast to chromatographic techniques, not needed for CE, no further instrumentation than a high voltage power supply is required. Sampling and required sample pre-treatment such as filtration and analyte preconcentration can be automated with higher reproducibility in less time and with less consumption of solvents than by the manual procedure. The combination of FT and CE allows the implementation of uncommon sample pre-treatment techniques, such as gas diffusion and dialysis, and to carry out pre- or post-capillary derivatization reactions in order to enhance detection sensitivity (55,59).

Apart from general objectives such as robustness, simplicity, or easy maintenance, a main challenge of coupling FT with CE is probably the separation of the FT manifold from the high voltage in order to avoid instrumental damage. Therefore, in most FT-CE systems, the manifold is galvanically connected to the grounded capillary end and interface, where injection is performed. In contrast, in commercial systems the detection is generally done on the grounded side, to enable mass spectrometry or electrochemical detection.

If autonomy and applicability of the coupled system to monitoring is the goal, both sides of the capillary have to be accessible by the flow system for cleaning due to the gradual contamination of the capillary outlet buffer reservoir with sample components. Ideally, as a requirement for its long-term use, in-situ flushing of the capillary (i.e., without removal) with separation buffer, water, and auxiliary solutions is possible. However, this task requires the ability of positive or negative pressure application by the flow system (or the applied commercial CE instrument) at one side of the capillary.

Finally, it is of interest in order to achieve a maximal versatility of the system e.g., to enable both electrokinetic as well as hydrodynamic injection mode. Electrokinetic injection of the sample is generally not limited by the flow system but by computer control of the high voltage power supply and optionally, the used pumping devices. However, hydrodynamic injection requires the application of a minor pressure to introduce a sample aliquot into the separation capillary and precision for location of the sample segment at the capillary tip and of the pressure holding time. The ability to perform hydrodynamic injection is further required for different on-capillary stacking or gradient techniques.

Interfacing Modes and Analytical Applications

Preliminary remarks

The different types of interfaces incorporated up-to-date FT-CE systems are described, including a summary of the corresponding analytical applications. A graphical overview of the different interface types is given in figures (Figure 4–12, Letters A–J) cited in the text. At least 10 types can be distinguished, differing in their operation versatility and application potential. An instrumental and operational synopsis and comparison of the reviewed coupled systems of FT and CE is given in Table I.

An overview is also given about the different up-to-date automated sample pre-treatment tasks using FT in coupled systems with CE, showing the outstanding achievement in the automation of analytical sample treatment procedures. A synopsis of applications, specified analytes, automated tasks, matrices and detection techniques is given in Table II. Finally, the reader is also referred to a very comprehensive review of separation techniques coupled to CE (60). Reviews of applications of CE with specific focus on microdialysis (61), biological sample handling (62), chip-based CE (63,64), and off-capillary and on-capillary pre-concentration techniques (52,55,56,58) are given elsewhere as specified.

Coupling of FT with a commercial CE instrument implies some difficulties in order to overcome the safety precautions of the manufacturer, in consequence and for economic reasons, most interfaces do not require a commercial CE instrument.

Injection valves

The simplest way to take advantage of a commercial CE instrument, which can be used to carry out capillary maintenance and buffer replacement and further enables sample injection and enables the classical electrode configuration, is given by the on-capillary interfacing via nanoliter injection valves (Figure 4F) (65). Coupling two of them allowed SPE preconcentration of enkephalin-peptides with injection of the eluent and, in the following, an aliquot of the eluted analytes into the capillary (66). One has been further used in the determination of on-line labeled amino acids (67). In both works, only a fraction of the injection loop volumes were injected electrokinetically in position inject as the capillary loop length corresponded to several centimeters.

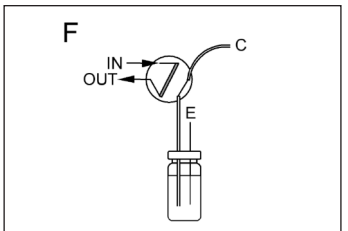

Figure 4. Micro-injection valve with capillary tip controlled by commercial CE apparatus. Electrode (E), capillary (C), flow entrance (IN), outlet (OUT), sample in grew.

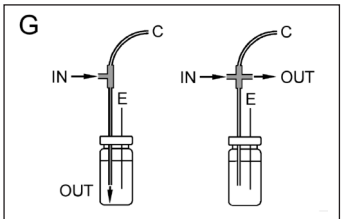

Figure 5. Interfacing via T-connector or cross-connector, respectively. Electrode (E), capillary (C), flow entrance (IN), outlet (OUT), sample in grew.

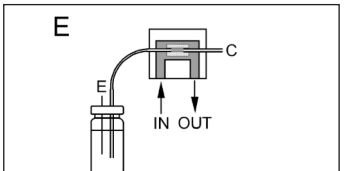

Figure 6. Membrane based on-capillary interface. Electrode (E), capillary (C), flow entrance (IN), outlet (OUT), sample in grew.

Confluent connectors

Similar, but simpler and with less effort, confluent T- or cross-connectors (Figure 5G) can be used as an interface between the flow manifold and the commercial CE instrument. Using a T-connector, the injection volume was given by the short piece of capillary between the connector or sample entrance

and the grounded buffer or drain vial as sample outlet (68). Electrical isolation of the employed sample preparation unit with SPE pre-concentration of quinolones was established by a 2-position valve positioned in the sample transfer capillary.

Using a cross-connector, the additional flow channel is used as sample outlet to waste and the injected volume is given only by the cross-section volume of the connector, allowing the simple injection of volumes as small as 30 nL without the requirements of precise pressure application and timing (69). T- and cross-connectors present an ideal interface for µFIA, and are further commonly applied in microchip CE. Using a T-connector at the grounded outlet of the separation capillary, Rayleigh scattering was used as off-capillary detection technique, mixing the capillary effluent on the coupled FIA system with rhodamine B and potassium iodide for the quantification of oxidative anions (70).

Membrane interfacing

Another type of on-capillary interfacing, which allows taking advantage of buffer reservoir control on both sides of the capillary by a commercial CE instrument, is based on the use of a short membrane tube, which connects two parts of the capillary, encased in a flow cell, and rinsed from the outside with sample (Figure 6E). Depending on the permeability of the membrane tube, gas diffusion, dialysis, or microfiltration can be carried out with the enrichment of the analytes in the inner volume of the membrane tube by simultaneous exclusion of matrix components with molar weights beyond the membrane-specific cut-off. The usefulness of these approaches has been successfully demonstrated in by Bao and Dasgupta in a pioneering work of coupling FIA and CE for gas-sampling, phenolic com-pounds, and proteins, respectively (71). The disadvantage of the incorporation of a membrane tube in the capillary is that rinsing of the capillary by pressure is possible only between one capillary tip and the membrane interface.

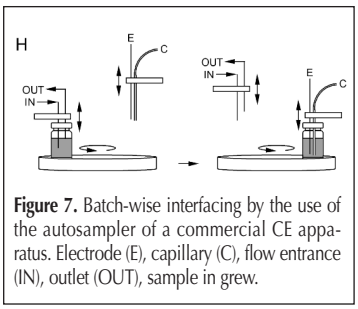

Figure 7. Batch-wise interfacing by the use of the autosampler of a commercial CE apparatus. Electrode (E), capillary (C), flow entrance (IN), outlet (OUT), sample in grew.

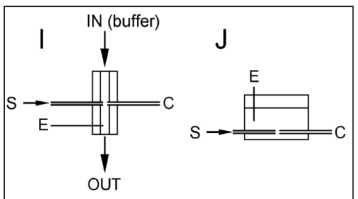

Figure 8. Gap interface (I) and flow gated interface (J), both with transfer capillary (S), Electrode (E), capillary (C), flow entrance (IN), outlet (OUT), sample in grew.

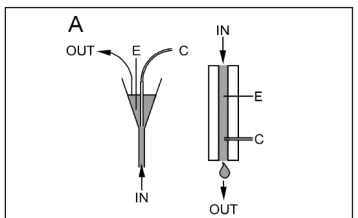

Figure 9. Vertical, atmospherically open flow interface (conical and from H-chip design). Electrode (E), capillary (C), flow entrance (IN), outlet (OUT), sample in grew.

Batch-wise coupling

Batch-wise coupling of flow technique and CE was done by the use of the autosampler of a commercial CE instrument (Figure 7H) was reported. Firstly, a vial is flushed intermediately with sample on an inactive sampler position, and secondly, it is transported to the injection position. Because the flow manifold is not physically connected with the CE apparatus, there is no limitation in respect of electrode configuration, applied injection mode, and detection technique, or on-capillary analyte concentration techniques. In consequence, coupling of the grounded capillary outlet to mass spectrometry detection allowed the highly sensitive quantification or biogenic amines and drugs, respectively (72,73). The discrete sampling further allows parallel operation of analyte SPE pre-concentration on the flow system prior to CE separation, such as demonstrated for amines (74), inorganic ions (75), pesticides (76), chlorphenols (77), drugs (78–80), and aflotoxines (81). Complex cleaning and re-conditioning of the capillary is carried out by the commercial CE instrument as it has been required for the determination of polyphenols in green tea (82). The advanced sample pre-treatment automated for the determination of sulfonamides should be highlighted (83). Analyte content of the sample was screened by in-line protein precipitation and elimination by filtration, following CE with MS detection, applying a screening CE buffer leading non-separative conditions for the analytes. In the case of positive results, SPE pre-concentration of the off-line deproteinized sample was done and the eluted analytes were separated applying another CE buffer composition and likewise MS detection.

Limitations of this batch-wise interfacing mode are the instrumental requirement of a commercial CE instrument available with at least two autosampler positions accessible by robotic arms and the relatively large dead volume and resulting sample dilution. On the other side, this fact has facilitated the FIA-automated preparation of standard solutions (84) or the re-absorption of on-line volatilized and headspace sampled amines by the water in the sample vial (85).

As far as it becomes clear from the respective publication, Palmarsdottir et al. (86) applied likewise batch-wise coupling but using a commercial injection system in combination with supported liquid membrane for enrichment of bambuterol and physostigmine from human plasma and in capillary double stacking prior to CE separation.

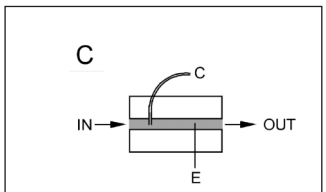

Figure 10. Horizontal, lockable split-flow interface. Electrode (E), capillary (C), flow entrance (IN), outlet (OUT), sample in grew.

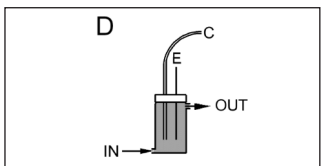

Figure 11. Flushed vial interface. Electrode (E), capillary (C), flow entrance (IN), outlet (OUT), sample in grew.

Gap and flow-gated interfaces

The required sample volume for injection can be highly reduced by using a transfer capillary to provide the sample to the separation capillary. For this, the ends of both capillaries have to be positioned oppositely with a sub-millimeter gap. The assembly is either submerged in the grounded buffer

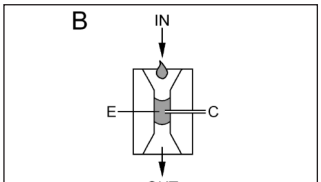

Figure 12. Falling-drop interface, Electrode (E), capillary (C), flow entrance (IN), outlet (OUT), sample in grew.

reservoir, denoted "gap interface" (Figure 8J) (87–89) or in a flow channel, housing the grounded electrode, where the sample transfer can be interrupted by the cross-flow of separation buffer, denoted "flow-gated interface" (Figure 8I) (90,91). In both cases, sample injection is based on electrokinetic injection and EOF traction.

The high advantages of both interfaces are their simplicity and the very small dead volume. The last characteristic was a basic requirement for the monitoring of pharmaceuticals sampled by microdialysis in vivo (rats) (87,89,90) or in tissue samples (88) carried out with FT with following speciation and separation by CE. As important advantage over the gap interface, the separation buffer in the flow-gated interface can be replaced; however, an additional pump is required for this task. Optical gating was achieved with a gap interface by photodecomposition of the fluorophore, which was used for pre-capillary derivatization of amino acids. Injection was accomplished by shutting the respective laser beam, which allowed passing a small sample volume with unaffected fluorescence activity (88). Due to the near-to-zero sample dispersion and very small gap dimensions, well-defined and very short sample segments can be injected. In consequence, shorter capillaries can be used and highly efficient and fast separations can be performed (62) demonstrated by the separation of inorganic ions, amino acids, carbohydrates, and pharmaceuticals (91). Gap interfacing also allows the de-location of the electrode from the capillary tip in order to accomplish off-capillary injection by EOF traction via the transfer capillary (92) or off-capillary detection at the end of the transfer capillary acting as bridge between the buffer reservoir and the detection cell (93).

Nevertheless, in-situ capillary maintenance, hydrodynamic injection, and on-capillary concentration techniques, which all require hydrodynamic injection of a large sample volume, are not applicable.

Table I. Instrumental Comparison of Coupling Flow Techniques Via Flow Interfaces with CE*

Flow technique	Type of interface	Buffer reservoirs connected to manifold	Injection location	Electrokinetic injection	Hydrodynamic injection	In-situ conditioning	Pressure adaptation	Comments	References
FIA	A	G	G	+	–	–	–		(96–98)
FIA	A	G	G	+	–	–	–	Chip design	(100,101,103–105)
SIA	A	G	G	+	–	–	–	Chip design	(99,102)
FIA	B	H	H	+	–	–	–	Chip design	(126,128,129)
FIA	B	G,H	H	+	–	–	–	Chip design	(127)
FIA	C	G	G	+	–	–	–		(51,106–110)
FIA	C	G	G	+	+	–	–	Gravimetric flow	(1)
SIA	C	G	G	+	+	+	–		(118)
SIA	C	G	G	+	+	+	+		(119)
SIA	C	H	H	+	+	+	+		(122)
LOV	C	G	G	+	+	+	–		(116,117)
FIA	C	H	H	+	–	–	–	Interface adapted to SPME	(123)
FIA	C	G	G	+	+	–	+		(113)
FIA	D	G	G	X†					(124,125)
FIA	E	X†	OC	X†					(71)
SIA	F	X†	OC	X†					(66,67)
μFIA	G	G	G	X†				T-connector	(69)
μFIA	G	G	G	X†				X-connector	(68)
FIA	G	H	G	X†				T-connector to post-capillary FIA	(70)
SIA	H	G	G	X†					(86)
FIA	H	X†	H	X†					(72–85)
SIA	I	G	G	+	–	–	–		(90,91)
SIA	J	G	G	+	–	–	–		(87–89)
FIA	A,J	G	OC	+	–	–	–	Injection by EOF-traction	(92)
SIA	C,B	G,H	G	+	+	+	+		(120)
MSFIA	C,B	G,H	G	+	+	+	+		(121)
FIA	C,C	G,H	G,H	+	+	–	–	Gravimetric flow & dual side injection	(111,112)
FIA	C,C	G,H	G	+	–	+	+	Direct connection of pump and HV	(115)
FIA	C,C	G	G	+	+	+	+	Negative pressure by vacuum pump	(114)

* A, vertical, atmospherically open split flow interfaces (conical and from H-chip design); B, falling drop interface; C, horizontal, lockable split flow interface; D, flushed vial; E, membrane interface; F, micro-injection valve on commercial CE instrument; G, T- or cross-connector interface; H, batch-wise interfacing using different autosampler positions; I, flow gated interface; J, flow gap interface; G, on grounded side; H, on high voltage side; OC, on capillary.
† X = Via commercial CE instrument.

Split-flow interfaces

Non-lockable split-flow interfaces

In split-flow interfaces, one single pumping device is used for sample and separation buffer provision in contrast to gap and flow-gated interfaces, where an additional sample transfer capillary is required. The separation capillary and electrode are positioned in an interface, which is intermittently or continuously flushed by separation buffer. By injection of the sample into the separation buffer, it passed the capillary tip for a well-defined time.

Fang et al. (94) proposed a conical-shaped, atmospherically open flow interface (Figure 9A). Sample and separation buffer enter the interface from below and are drawn off simultaneously from the liquid surface by a second pumping device. The interface allowed electrokinetic injection and was applied to the analysis of different drugs (94–96), two including on-line SPE preconcentration (97,98). Using an additional on-capillary naphion-membrane supported gap interface (J), off-capillary sample injection via EOF-traction at the transfer-capillary inlet and bias-free injection of inorganic anions was achieved (92).

A chip design, so-denoted H-channel configuration, was proposed by Fang et al. (99) coupled to SIA used for the separation FITC-labeled amino acids. The interface worked on a similar principle as the conical split-flow interface (A), but capillary and electrode were positioned perpendicular to the flow direction, which lowered the effect of the carrier flow on the injected volume and separation efficiency. While in the first work and in three later FIA systems (100–102) separation buffer and sample entered the interface from above and drop-out below, in other FIA systems, the solutions enter either from below and are drained-off by an additional pump (103–105). Using an 16-way injection valve with three loops, head-column field amplified stacking was carried out by the passing of injected volumes of water, sample, and water achieving an 15-fold sensitivity improvement (105). The H-channel configuration chip was mainly applied to the separation of different drugs used as model analytes, but was further applied to high throughput separtion of inorganic cations by contactless conductivity detection (CCD) (101) and determination of inorganic and mono-methyl-mercury by coupling the capillary outlet to atomic fluorescence spectroscopy (100). In another work, separation of ethidiumbromide labeled oligonucleotides was accomplished using the gel-filled separation capillary as liquid-core waveguide. In order to handle a sample volume of only 3 µL with minimal carry over and dispersion, the concept of mono-segmentation (25,26) was applied and the sample was intercalated between two segmentation air bubbles of 5 µL (102).

Lockable split-flow interfaces

Kuban et al. proposed a tubular but horizontal split-flow interface (Figure 10C), where the capillary and the electrode are positioned perpendicular to the flow direction, which lowered the effect of the separation efficiency by the flow in the interface and enabled multiple electrokinetic injection during a running separation of small organic and inorganic anions (106). The interface allowed the easy integration into the FIA manifold and high robustness due to tube connections and stress-free fixation of the separation capillary by screw fittings.

Applying electrokinetic injection, FIA-CE systems with horizontal split-flow interfaces have been used for the determination of inorganic anions with ion-exchange pre-concentration carried out by FIA (107), determination of U.S. Environmental Protection Agency (EPA) priority phenols with electrokinetic sample stacking achieving a pre-concentration factors up to 2000 (108), determination of small organic and inorganic anions with sample clean-up on the FIA system by gas diffusion (109)

Table IIA. Overview of Analytical Applications and Accomplished Tasks by Flow Techniques in the CE-Coupled Analytical Systems

Small in- and organic ions	Tasks carried out by flow system*	Sample matrix†	Detection‡	Refs.
K^+, Na^+, NH_4^+, Ca^{2+}, Mg^{2+}		Surface water	CCD	(101)
Hg^{2+}, Met-Hg		Natural waters	AFS	(100)
Cl^-, F^-, NO_2^-, SO_4^{2-}		Test system	indirect UV	(92)
Cl^-, NO_3^-, SO_4^{2-}		Test system	Indirect UV	(51)
Cl^-, F^-, NO_2^-, SO_4^{2-}		Test system	CCD	(1)
Cl^-; CO_3^{2-}, SO_4^{2-}, formate, oxalate	Maintenance	Pulp waters	indirect UV	(115)
K^+, Li^+, Na^+, Rb^+, Ba^{2+}, Ca^{2+}, Mg^{2+},				
Br^-, Cl^-, ClO_4^-, NO_2^-, NO_3^-, SO_4^{2-}		Test system	CCD	(91)
K^+, Na^+, NH_4^+, Ca^{2+}, Cd^{2+}, Mg^{2+}, Mn^{2+},	SPE (Chelex), filtration	Waste water	indirect UV	(75)
Pb^{2+}, Fe^{3+}, Al^{3+}, Cl^-, NO_2^-, NO_3^-, SO_4^{2-}, HPO_4^{2-}				
NO_2^-, NO_3^-, Br^-, I^-	SPE (anion exchange)	Natural waters	UV	(107)
Cl^-, F^-, HCO_3^-, NO_3^-, OH^-, HPO_4^{2-}, SO_3^{2-}, $S_2O_3^{2-}$,	Dialysis	Natural waters,	UV	(110)
formate, acetate, propionate, benzoate, lactate		milk, juice		
Cl^-, F^-, HCO_3^-, NO_3^-, HPO_4^{2-}, SO_4^{2-},	Multiple injection	Natural waters	UV	(106)
$S_2O_3^{2-}$, citrate, acetate				
K^+, Na^+, NH_4^+, Ca^{2+}, Co^{2+}, Mg^{2+},	Dual-side end injection	Natural waters	CCD	(111,112)
Mn^{2+}, Cl^-, NO_2^-, NO_3^-, HPO_4^{2-}, SO_4^{2-}, oxalate				
Ca^{2+}, Mg^{2+}, Sr^{2+}, Ba^{2+}	HD by negative pressure	Test system	CCD	(114)
Na^+, Li^+, Ca^{2+}, Mg^{2+}	HD	Natural waters,	UV	(113)
Cl^-, F^-, HCO_3^-, NO_2^-, NO_3^-, HPO_4^{2-},		soft drinks		
SO_4^{2-}, $S_2O_3^{2-}$, acetate, citrate,	HD, maintenance	Test system	indirect UV	(116)
K^+, Na^+, NH_4^+, Ca^{2+}, Mg^{2+}, Cl^-, NO_3^-, SO_4^{2-}	HD, maintenance	Test system	CCD	(119)
ClO^-, MnO_4^-, $Cr_2O_7^{2-}$	Post-capillary reaction	Test system	Rayleigh scattering	(70)
HCO_3^-, HS^-, $S_2O_3^{2-}$, acetate, formate	Gas diffusion	Beverages	UV	(109)
HCl, HNO_3, HCOOH, HONO (gases)	Gas diffusion	Air	indirect UV	(71)

* It is supposed that in all systems, the flow part accomplish at least sample aliquotation, provision to the CE part and post-injectional cleaning of the interface: HD, hydrodynamic injection; maintenance, refers to the re-conditioning of the capillary in-situ using the flow system; PreCapDerv, pre-capillary derivatization; SLM, pre-concentration by supported liquid membrane; SPE, pre-concentration by solid-phase extraction; SPME, pre-concentration by solid-phase microextraction; NQS, 1,2-naphthoquinone-4-sulfonate; DTAF, dichlorotriazinylaminofluoresceinm; OPA/BME, o-phthaldialdehyde/β-mercaptoethanol.

† "Test-System" refers to cases where no real samples were analyzed but the systems performance was tested using model analytes.

‡ AFS, atomic fluorescence spectrometry; Amp, amperometry; CCD, contactless conductivity detection; EGCL, electrogenerated chemiluminescence; FL, fluorescence spectrometry; LIF, laser-induced fluorescence detection; MS, Mass spectrometry (quadropole); Pot, Potentiometry; UV, ultraviolet spectrometry; Vis, photometry.

and by dialysis (110), and the determination of inorganic anions with CCD. In the last work, the carrier and sample were driven by gravimetric, pulsation-free flow achieving a repeatability of injection of 0.8% relative standard deviation (RSD) (1). Gravimetric liquid propulsion was applied for dual-opposite end injection, where split-flow interfaces on both capillary ends allowed the electrokinetic injection of both anions on the negative HV side and cations on the grounded detection side detected by CCD (111,112).

The important difference to the split-flow interfaces described in the former section is that positive pressure build-up is possible by an intermediate closure of the interface outlet due to the tight connections of capillary, electrode, and flow lines in the split-flow interface enabling hydrodynamic injection. This injection mode was firstly applied using a split-flow interface and FIA by Kuban et al. applied to the separation of inorganic cations. A pinch valve was used to shut the interface flow outlet for 0.3 s in order to build up pressure for hydrodynamic injection in the moment when the peak maximum of the injected sample passed the capillary inlet (113). Likewise, hydrodynamic injection was enabled with split-flow interfaces at each capillary end by negative pressure application at the capillary outlet (detection side) using a vacuum pump and applied to the separation of alkaline earth metal cations (114). Sirén et al. (115) used flow interfaces on both capillary ends of the hydrodynamic injection accepting the galvanic contact of the FIA manifold and HV applied to small organic and inorganic anions in a pulp mill process. The concept of hydrodynamic injection was applied further to the syringe pump based FT, where pressure build-up is done by a discrete dispense with the interface closed. The high advantage of syringe pumps over peristaltic pumps is a higher pressure robustness. By this, the possibility to increase the pressure further in order to rinse the capillary for cleaning and re-conditioning, which is a crucial requirement for achieving high reproducibility over an extended operation period. However, continuous pump operation with flow rates of a few microliter per minute is required for flushing. Such direct coupling was done by Wu et al. on a LOV-CE system for the separation of small organic and inorganic anions (116) and peptides performing pre-capillary derivatization and simultaneous fluorescence and UV spectrometric detection (117). Relatively large injection volumes resulted from the applied injection pressure and head column field stacking on-capillary concentration was performed. Likewise, Kulka et al. used an SIA-CE system for the separation of myoglobin and nucleotides as model analytes (118).

Due to the rigidity of the manifold tubes and syringe pump, the back-pressure increases quite abruptly in these systems, leading to large injection volumes, whereas the expandable peristaltic pumping tubes used in FIA lead to partial absorption of the applied pressure pulse. In order to achieve smaller injection volumes, Wuersing et al. inserted a small piece of silicone tube between the split-flow interface and the outlet valve (119), enabling high injection reproducibility for the separation of inorganic ions. At the same time, the authors proposed a similar construction for a SIA (120) and later MSFIA system (121), where a silicone tube was not only used to decrease the pulse pressure height but was used also as "pressure reservoir". The pressure, prior build-up in the silicone tube by inflation, allowed flushing of the separation capillary without the need of continuous pump operation. In consequence, operations in background were enabled, such as cleaning of the HV buffer reservoir or SPE pre-concentration of mono-nitrophenols as the analytes of interest.

In FIA, in-situ capillary conditioning could be performed using a restriction coil instead of a full closure of the interface outlet in order to avoid excessive pressure increase (122). In this work, the system pressure of a commercial CE instrument, connected to the flow-split interface, was used for hydrodynamic injection for the analysis of amines using mass spectrometry detection.

Santos et al. (123) modified a split-flow interface for its application to solid-phase microextraction. For this, the already loaded microfiber was inserted via an injection opening into the interface. The eluent (injected into the separation buffer flow) passed through the interface outlet and led to the des-

Table IIB. (Continued) Overview of Analytical Applications and Accomplished Tasks by Flow Techniques in the CE-Coupled Analytical Systems

Amines, proteins, and nucleotides	Tasks carried out by flow system*	Sample matrix†	Detection‡	Ref.
Arginine, phenylalanine, glycine (fluorescein isothiocyanate-labelled)		Test system	LIF	(99)
Arginine, phenylalanine, glycine (fluorescein isothiocyanate-labelled)		Test system	FL	(128)
Aspartate enantiomers	Dialysis, PreCapDerv with OPA/BME	Rat organ tissues	LIF	(88)
Proline, phenylalanine, valine	Post-capillary addition of reagent	Test-System	EGCL	(127)
Serine	Dialysis (in-vivo)	Rat cerebrospinal fluid	LIF	(90)
Methionine, leucine, threonine,		Test system	CCD	(91)
Glyrine, serine, threonine, des-Tyr-[Met]-enkephalinamide	PreCapDerv with DTAF	Test system	LIF	(67)
Amino acids (19)	PreCapDerv with NQS	Pharmaceuticals, feed	UV	(125)
Amines	HD, maintenance, standard preparation	Test system	MS	(122)
Amines (biogenic)	Filtration	Wine	MS	(72)
Amines (biogenic)	SPE (C18), filtration	Wine	indirect UV	(74)
Amines (volatile)	Alkalinization, head space sampling	Fish tissue	indirect UV	(85)
Amines	PreCapDerv with propylamine, process monitoring	Production process	UV	(124)
Enkephalin-peptides	SPE (C18)	Test system	UV	(66)
Myoglobin	HD, maintenance	Test system	UV	(118)
Insulin, proinsulin, c-peptide	PreCapDerv, HD, maintenance	Celular perfusion liquid	FL & UV	(117)
Substance P, [Met]-enkephalin, bradykinin, neurotensin		Test system	ESI-TOF-MS	(69)
Proteins	Dialysis	Blood plasma	UV	(71)
Oligonucleotides		Test system	FL	(102)
Adenosine, AMP	HD, maintenance	Test system	UV	(118)

* For footnote see Table IIA.

orption of the analyte and electrokinetic injection was carried out. The system was applied to the determination of tetracycline, oxytetracycline, and doxycycline in soil samples.

Flow-through vial

A flow-through vial (Figure 11D) allows the implementation of the split-flow interfacing principle on the autosampler of a commercial CE instrument. Such interfacing allowed Dantan et al. (124) to monitor amines in chemical production process by applying a pre-capillary quenching reaction in non-aqueous medium in order to avoid the further reaction of the analytes. Latorre et al. (125) used a flow-through vial for the separation of amino acids with pre-capillary derivatization with 1,2-naphthoquinone-4-sulfonate. In contrast to the discrete connection by batch-wise interfacing (H), the vial is permanently connected to the FT manifold. Likewise for the conical interface from Fang, or batch-wise interfacing, two pumps were required for the in- and a slightly higher outflow of the vial. Stopping the outlet flow pump but due to the closed vial, pressure control was possible in both works, applied for hydrodynamic injection and acceleration of the separation procedure, respectively.

Due to the high versatility, split-flow interfaces are one of the most frequently used in FT-CE systems. However, using split-flow interfaces, the FT manifold has steady galvanic contact with the CE system, and in case of manifold connection to the HV side of the CE system (e.g., in order to enable end-of-capillary MS detection) a segment of distillated water or air has to be introduced into the connecting tube to obtain a maximal electrical resistance (122). Another possibility is the use of peristaltic pumps since the pumping tube walls present a sufficient resistance (115) or gravimetric flow (1,111,112), omitting any galvanic contact to further electrical devices.

Falling drop interfacing

Fang and coworkers proposed the falling-drop principle (Figure 12B) for interfacing a flow system to the HV side of the separation capillary without the former mentioned safety requirements. Here, the separation buffer and sample are dropped from above into the funnel-like interface and flow out by gravity. An aliquot of the solutions remains stuck in the flow cannel between the capillary tip and the HV electrode due to cohesion and acts as buffer reservoir. This principle allowed amperometric (126), electrogenerated chemiluminescence (127), and fiber-coupled fluorimetric (128) end-of-capillary detection of sugars, amino acids, and FITC labeled amino acids, respectively. The possible grounding of the outlet of the capillary facilitated further the flushing of the respective buffer reservoir and the equilibration of the pressure

Table IIC. (Continued) Overview of Analytical Applications and Accomplished Tasks by Flow Techniques in the CE-Coupled Analytical Systems

Drugs and food components	Tasks carried out by flow system *	Sample matrix†	Detection‡	Ref.
Amlodipine enantiomers		Interaction study	UV	(103)
2-Amino-1-(p-nitrophenyl-1,3-propanediol) enantiomers		Test system	UV	(96)
3-Amino-1,2,4-benzotriazine 1,4-di-N-oxide, 3-amino-1,2,4-benzotriazine 1-N-oxide	Dialysis (in-vivo)	Rat blood	LIF	(87)
Bambuterol, physostigmine	SLM	Human plasma	UV	(86)
Magnolol, benzoic acid		Test system	UV	(94)
Ephedrine, doxylamine, dextomethorphane		Pharmaceuticals	CCD	(91)
Pseudoephedrine, ephedrine	Field amplified stacking	Pharmaceuticals	UV	(105)
Pseudoephedrine	SPE (C18)	Blood plasma	UV	(97,98)
Tolmetin, ibuprofen, fenbufen, ketoprofen, indomethacin, acetylsalicylic acid	SPE (C18)	Human urine	UV	(78)
Quinolone antibiotics	SPE (C18)	Serum	UV	(68)
Sulfamethoxazole, trimethoprim		Pharmaceuticals	UV	(95,104,129)
Sulfonamides	SPE (C18), precipitation, filtration, screening	Milk	MS	(83)
Tetracycline, oxytetracycline, doxycycline	SPE (DVB-polymer)	Natural waters	UV	(79)
Tetracycline, oxytetracycline, doxycycline	SPE (carbon nanotubes)	Urine, water	MS	(73)
Tetracycline, oxytetracycline, doxycycline	SPME desorption	Soil	MS	(123)
Dopamine, hydrochinone, DOPAC, nicotine	Dialysis (in-vivo)	Rat skin	Pot	(89)
Myo-inositol phosphates	SPE (anion exchanger)	(Hazel)nuts, almonds, lentils	UV	(80)
Caffeic, p-coumaric, and gallic acid, theophylline	Standard preparation	Test system	UV	(84)
Caffeine, theobromine, theophylline		Beverages	UV	(92)
Polyphenols	Filtration	Green tea	UV	(82)
Fructose, glucose, galactose, sucrose		Test system	CCD	(91)
Sucrose, glucose		Test system	Amp	(126)

* For footnote see Table IIA.

Table IID. (Continued) Overview of Analytical Applications and Accomplished Tasks by Flow Techniques in the CE-Coupled Analytical Systems

Analytes of Environmental Interest	Tasks carried out by flow system*	Sample matrix†	Detection‡	Ref.
Aflotoxins B1, B2, G1, G2, ochratoxins A, B	SPE (C18), screeening	Feed	UV	(81)
DMSO, benzoic acid		Test system	UV	(92)
Phenols (EPA priority)		Test system	UV	(108)
Phenols	Permeation	Water	UV	(71)
Chlorophenols	SPE (C18)	Human urine	UV	(77)
Nitrophenols	HD, maintenance	Waste water	Vis	(120)
Nitrophenols	SPE (C18), HD, maintenance	Leachates	Vis	(121)
Pesticides	SPE (C18)	River waters	UV	(76)

* For footnote see Table IIA.

on both ends of the capillary in a chip-approach for the separation of sulphamethoxazole and trimethoprim (129). A falling drop-interface was further used to access the HV connected detection sided buffer reservoir for cleaning. It was combined with a split-flow interface on the grounded injection side, allowing pressure application for capillary rinsing and hydrodynamic injection. This was achieved by a maximal operational stability for the separation of mono-nitrophenols in waste waters and leachates (120,121).

Versatility of Coupled FT-CE systems

In this section, the versatility of different FT for the coupling with CE will be discussed. It should be pointed out that considerations about the performance of separation or detection are not possible to overview due to the application of the systems to different analytes and the use of different detection techniques in the respective works. It is regrettable, that a critical discussion of instrumental performance and limitations is sometimes neglected, although this information is highly useful for further research and development in this area.

In general, repeatability and sample frequency is superior in FIA and MCFIA compared to SIA and MSFIA, where the aspiration of sample and reagents and syringe refilling is a limitation. On the other side, SIA and MSFIA show a lower flow pulsation, higher pressure robustness, and allow a precise and bi-directional handling of very small volumes. Remote manifold and procedure configuration enabled by computer control is typical for MCFIA, SIA, and MSFIA. Finally, multichannel FT (FIA, MCFIA, MSFIA) enable confluent mixing of sample and reagents with the potential of higher signals compared to monochannel FT due to a lower required sample dispersion. In consequence, similar characteristics are found for coupled FT-CE systems. By example, the reproducibility of peak heights applying hydrodynamic injection are 2–5% in SIA-CE systems [e.g. 3.8% n = 8 (114), 5.7% n = 6 (122), 5.4% n = 5 (118), 4% (120), 1.6% n = 5 (119)] and 2–3% in FIA-CE systems [e.g. 3%, n = 10 (111), 3%, n = 8 (113)].

As discussed before, control of both buffer reservoirs, in-situ flushing of the capillary, and hydrodynamic injection are important in order to achieve a high robustness and stability of the system, to apply it to samples with matrix components, which could affect the separation efficiency by absorption on the inner capillary walls and changing the EOF, and to perform all in-capillary sample stacking techniques. From Table I, it becomes clear that these tasks are mostly achieved when syringe-pump flow techniques were used for automation or when the capillary maintenance was done by a commercial CE instrument.

The MSFIA technique coupled to CE might present an ideal tool as the multichannel concept as well as the high pressure robustness of syringe pumps are combined, which has allowed the parallel execution of SPE pre-concentration to fully capillary maintenance and CE separation (121). The respective system is depicted in Figure 3. In this system, the pre-concentration part, operated with two syringes, and the CE system, operated by a further syringe, where connected only by the SPE column, placed on an injection valve. In position LOAD, in-line sample acidification, pre-concentration, and column maintenance was carried out; in position INJECT, elution to the CE system was done. A similar versatility was only achieved by systems where the flow manifold and a commercial CE instrument where continuously connected as achieved by the interface type H.

Conclusions

A comprehensive overview of interfacing flow techniques and capillary electrophoresis has been given including a synopsis of up-to-date reported analytical applications and on-line automated sample pre-treatment. The versatility and limitations of the manifold and ingenious interfacing modes and resulting operation potentials of coupling flow techniques and capillary electrophoresis were discussed. Automated analytical systems combining preparative flow techniques and separative techniques such as capillary electrophoresis are gaining importance in various fields of analytical chemistry with high potential in process control, kinetic and pharmacokinetic studies, and bio- and molecular-biochemistry research.

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