

**Capacitively Coupled Contactless Conductivity Detection and
Sequential Injection Analysis in Capillary Electrophoresis and
Capillary Electro-Chromatography**

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Summary

This thesis focuses on the applications of capacitively coupled contactless conductivity detection (C^4D) in capillary electrophoresis (CE) hybridized with high-performance liquid chromatography (HPLC), *i.e.* in capillary electrochromatography and pressure-assisted capillary electrophoresis, as well as on the development and applications of an extension of CE- C^4D with sequential injection analysis (SIA).

At first, the in-house built C^4D was used for electro-chromatographic determinations of different classes of non-UV absorbing cations using monolithic octadecylsilica capillaries. Combined mechanisms of differential interactions and electrophoresis were employed by the application of a high-voltage through a monolithic octadecylsilica structure in order to achieve high separation efficiencies, which otherwise are difficult to obtain with either CE or HPLC alone. CEC conditions were optimized for both home-made and commercial monolithic columns of short lengths of 15 cm for baseline separations of inorganic cations, amines and amino acids. Detection limits were found to be comparable with those obtained from CE with conductivity detection.

C^4D was then utilized for sensitive detection in pressure-assisted capillary electrophoresis with separation columns as slender as 10 μm . The use of such narrow capillaries is required to minimize peak broadening effects caused by the hydrodynamic flow created when a pressure is applied during electrophoresis. UV-radiation detection is impossible in this case due to very limited optical pathlengths offered by capillaries of 10 μm internal diameter. The introduction of a hydrodynamic flow at a specific time during electrophoresis can be employed to achieve high separation efficiencies or short analysis time for improved sample throughput, or to compensate for the electro-osmotic flow (EOF) for electrophoretic separation of anions at high pH without addition of an EOF modifier. The application of pressure also allows converting a monotonous electrophoretic run into a separation scheme with flow gradient akin to HPLC with gradient elution. C^4D was applied for detection of both cations and anions in this hybrid mode of CE.

For monitoring applications, an automated system was designed and constructed based on an extension of conventional CE-C⁴D with a sequential injection analysis (SIA) manifold via an interface. The SI manifold relies on a two-way motor-driven microsyringe pump and a multiport valve with a holding coil in between. The developed system allows automated sampling, separation, detection and data acquisition. Both cations and anions can be analyzed successively by automatic switching of the high-voltage polarity. The system was applied successfully to monitor the variations of concentrations of major ions in a lake in Switzerland during a rainy period. Crosschecking with discrete samples analysed with ion-chromatography gives acceptable deviation, which proves that the system is suitable for unattended long-term monitoring tasks.

To overcome the inherent problem of sensitivity limitation in CE due to small injection volumes, a new configuration of SIA-CE-C⁴D was designed and developed in order to implement an on-line solid-phase-extraction (SPE) preconcentration procedure prior to automated CE separation. The system was designed as an industrial prototype, with all fluidic and electronic parts, as well as all power supplies assembled into a standardized 19" frame for easy transportation and mobile deployment. Some drug residues in water, including ibuprofen, diclofenac, bezafibrate and naproxen, were selected as exemplary analytes to demonstrate the functionality of the system. With preconcentration prior to CE-C⁴D determination, enrichment factors of several hundreds can be obtained and the concentrations of drug residues in water can be monitored down to the nM scale, which is impossible with normal CE setups.

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1. Introduction

1.1. Capillary electrophoresis (CE) and capillary electro-chromatography (CEC)

1.1.1. A brief history of the development of CE

Electrophoresis is defined as the differential migration of charged species by attraction or repulsion under the influence of an externally applied electric field. The term “electrophoresis” was initiated by Michaelis in 1909 during his work for the separation of proteins based on their isoelectric points [1]. The groundwork of electrophoresis, however, was laid by Tiselius in 1937 [2-4]. His pioneering employment of “moving boundary electrophoresis” under a gradient of applied voltage for successful separation of complex protein mixtures triggered the first recognition of the potential use of electrophoretic analysis. For this work, Tiselius was awarded the Nobel Prize in chemistry in 1948. This initial approach to electrophoresis, nevertheless, encountered a unsatisfactory separation efficiency due to band broadening caused by thermal diffusion and convection. Many following efforts, hence, leaned toward the improvement of anti-convective support media for zone electrophoresis, starting from the application of paper as non-gel media, to the employment of starch, agarose gels, cellulose acetate or polyacrylamide gel as stabilizers [5]. The term “zone electrophoresis” was indeed originated by Smithies in 1955 for the electrophoretic separation of serum proteins in a starch gel medium [6]. The application of electrophoresis with polyacrylamide gel as stabilizer, *i.e.* polyacrylamide gel electrophoresis (PAGE) and sodium dodecyl sulphate (SDS)-PAGE, which is best suited for protein characterization and determination of oligonucleotides and protein monomer, is still very popular in biochemistry laboratories nowadays.

The use of the above-mentioned anti-convective support media in electrophoresis, however, poses different drawbacks, such as long analysis time, poor reproducibility, limitation to low electric fields (only 15 – 40 V/cm) due to poor dissipation of Joule heat in slab systems, cumbersome methodology hindering detection and automation. These limitations, together with increasing demands for high resolution, quantitative precision of the analysis of bio-pharmaceuticals, and control of waste management costs led to the appearance of capillary electrophoresis (CE) on the analytical scene. CE, defined as electrophoretic separations performed in narrow-bore tubes or capillaries, was for the first time carried out by Hjertén in 1967 [7]. Much of his work was carried out with a fully automated apparatus that he

constructed in 1959 which allows the rotation of a millimetre-bore quartz-glass capillary coated with methylcellulose along its longitudinal axis to minimize the adverse effects of convection. As early as 1974, Virtanen set a further step in CE with the use of a glass capillary of smaller channel (0.2 mm) to minimize convection problems and to simplify instrumentation [8]. In the late 1970s, Mikkers and coworkers performed another CE work using Teflon capillaries of 200 μm internal diameter [9]. Another milestone in CE was set in the early 1980s by Jorgenson and Lukacs with the pioneering employment of 75 μm inner diameter fused silica capillaries to separate charged compounds and amino acids with on-column fluorescent detection [10-12]. Reduction of zone spreading caused by convection and efficient dissipation of the heat generated by the application of high voltages were observed with the introduction of these narrow separation channels. In modern CE, even narrower capillaries of typically 25 – 50 μm internal diameters are employed. Over the past decades after the first attempt with CE, with the widespread availability of high-quality fused silica capillary tubing, and especially with the introduction of the first commercially available instruments in 1988, the horizons of CE have expanded. The recent trend in CE to which much research has been devoted is miniaturization and automation, whereby injection, separation and detection are carried out on microchannels embedded on planar devices (Lab-on-Chip concept) or automated via incorporation with micro-syringe driven sequential injection analysis (Lab-on-Valve concept). Nowadays, as acknowledged in dozens of monographs, thousands of publications and reviews reflecting its exponential growth, CE proves to be a very powerful separation technique, whose application spectrum covers a variety of disciplines, ranging from bioanalytical, pharmaceutical, clinical to environmental separations.

1.1.2. Basic principles and concepts of CE

1.1.2.1. Basic principle and setup of CE

CE, or electrophoresis in the capillary version, is the separation of charged species in a narrow-bore capillary under an externally applied electrical field. Capillary electrophoresis relies on the principle that ions in a medium, possessing different electrical charges and masses, when exposed to an electrical field will move in different directions and at different speeds within the substrate. The high surface-to-volume ratio of capillaries allows for very efficient dissipation of Joule heat generated from high applied electrical fields. The employment of capillaries offers rapid analysis times associated with the application of high

voltages (typically up to 30 kV), and little consumption of sample (μL range) and buffer reagent (mL range), which in turn makes CE the solution for a diverse number of analytical problems.

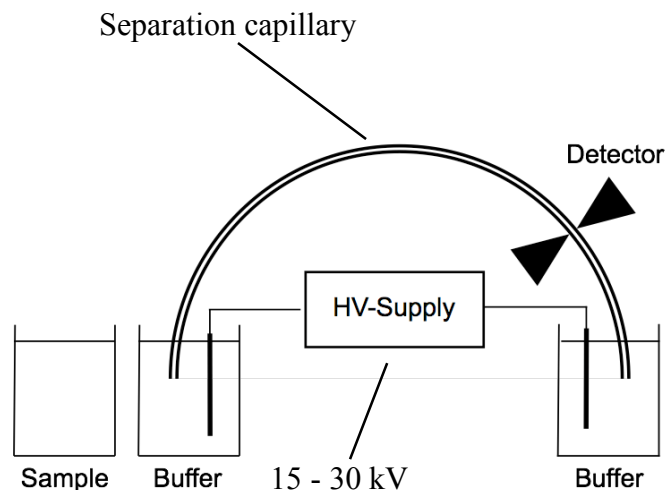


Figure 1: General schematic of a CE instrument

A typical capillary electrophoresis setup, as illustrated in figure 1, consists of a high-voltage power supply of maximum 30 kV with dual polarities, a polyimide-coated fused silica capillary of internal diameter from 25 μm to 100 μm , two buffer reservoirs that can accommodate both the capillary and the electrodes, a detector and data acquisition system. Bare fused silica capillaries are most commonly used in CE. The two ends of the capillary are dipped into two different vials equally filled with an electrolyte buffer solution, in which two electrodes, usually made from platinum and connected to a high voltage supply, are placed. To introduce a small quantity of the sample into the column, one buffer vial at the inlet of the capillary is shortly replaced with the sample vial prior to application of either an electric field (electrokinetic injection) or pressure (hydrodynamic injection) for a specific period (typically in seconds). The source vial containing the buffer is then switched back to the original position before turn-on of the high voltage, which enables the ions to migrate towards the detector where they are visualized. A detector can be positioned either on-column or off-column at the outlet of the capillary for detection of charged species. The output signal is then collected, processed and stored by a data acquisition/analysis system. With the assistance of the commercially available apparatus, this basic setup can be upgraded with more advanced

features, for example incorporation of autosamplers, multi-detectors, multi-injection devices, temperature conditioner *etc.*

1.1.2.2. Electrophoretic mobility

Separations of charged species by CE are based on differences in their velocity under an electric field. The velocity of an ion is described in the following equation:

$$v = \mu_e \times E \quad (1)$$

where v is the ion velocity ($\text{cm}\cdot\text{s}^{-1}$).

μ_e is the electrophoretic mobility ($\text{cm}^2\cdot\text{s}^{-1}\cdot\text{V}^{-1}$), and is constant and specific for a given ion in a given medium.

E is the electric field strength ($\text{V}\cdot\text{cm}^{-1}$), which is a function of the applied voltage and capillary length.

At a steady state during electrophoresis, a molecule with charge q experiences two forces, *i.e.* electric force and frictional force, which are opposite in directions and balance each other. The electric force is a function of q and E , as described as follow:

$$F_e = q \times E \quad (2)$$

The frictional force, or the force induced from viscosity during the movement of an ion in the buffer medium, can be expressed by Stokes' Law for a spherical ion:

$$F_f = 6 \times \pi \times \eta \times r \times v \quad (3)$$

where q is the charge of ion

η is the viscosity of the solution

r is ion radius

v is ion velocity

At a steady state, we have:

$$F_e = F_f \quad (4)$$

or
$$q \times E = 6 \times \pi \times \eta \times r \times v \quad (5)$$

By substituting (1) in (5), the mobility of an ion can be expressed as follow:

$$\mu_e = \frac{q}{6 \times \pi \times \eta \times r} \quad (6)$$

From equation (6), it can be deduced that small, highly charged species have high electrophoretic mobilities, and vice versa for species with larger sizes and lower charge numbers. The apparent mobility of a charged species, however, also depends on other factors, *i.e.* ambient temperature and electroosmotic flow (EOF) (details on EOF can be found in section 1.1.5).

1.1.2.3. Sample Injection in CE

In CE, the batch-type sample injection, as illustrated in figure 2, is classified as hydrodynamic and electrokinetic modes, the former of which is the most widely used method. Hydrodynamic injection can be accomplished either by siphoning action achieved by elevating one capillary end immersed in the sample vial relative to the outlet vial, as always done in manual operation, or by application of pressure at the injection end of the capillary or vacuum at the exit end of the capillary. Conventional instruments rely on the complex application of gas pressure to affect hydrodynamic injection. As studied by Huang *et al.* [13], the sample plug length should account for less than 2 % of the total length of the capillary, corresponding to only some nanoliters of samples, in order to maintain high separation efficiency. With hydrodynamic injection, the quantity of sample loaded is quasi-independent of the sample matrix.

Electrokinetic injection, on the other hand, is implemented by replacement of the injection-end reservoir with the sample vial and then application of high voltage, whose electric field strength is 3 to 5 times lower than that used for separation. In this electromigration injection mode, analytes enter the capillary by both electrophoretic migration and by the dragging action of the electroosmotic flow (EOF). However, sampling bias and ionic discrimination, which in turn lead to poorer injection reproducibility compared to the hydrodynamic counterpart, are often encountered due to strong dependence of sample loading on the electroosmotic flow, matrix composition and mobility of individual solutes. This injection method, nevertheless, is still advantageous when viscous media or gels are employed in the

capillary, stacking effect is to be employed for sensitive detection, or when hydrodynamic injection is ineffective.

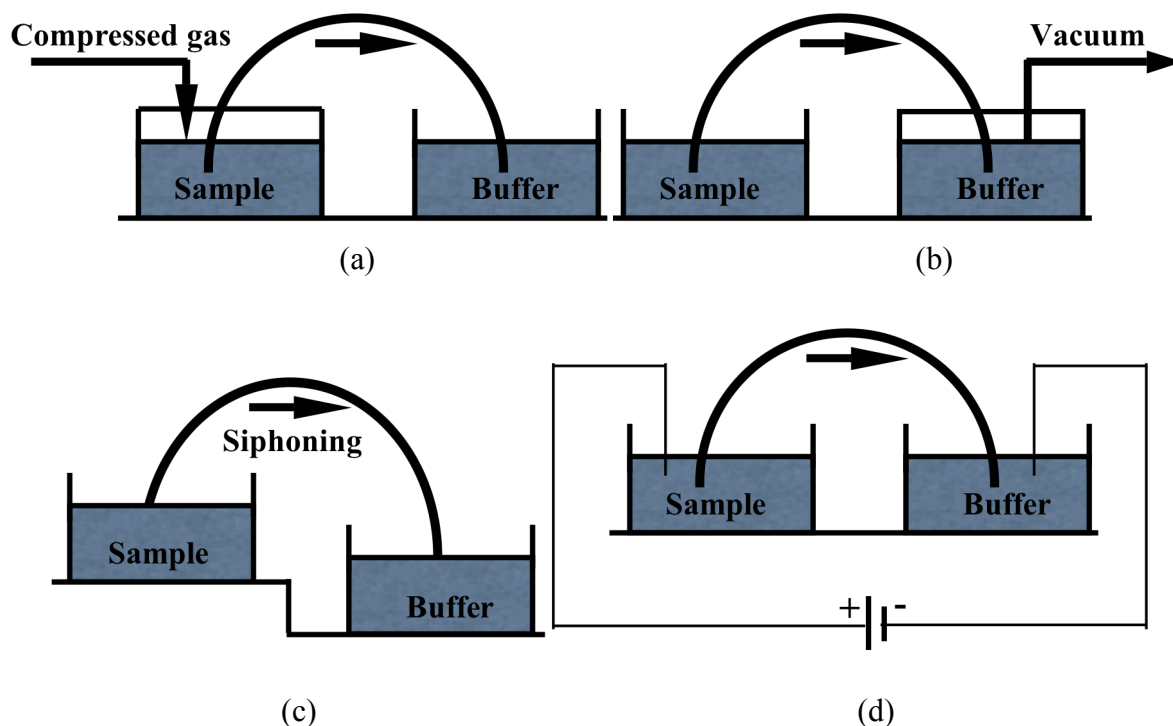


Figure 2: Typical injection methods in CE
 (a), (b), (c): Hydrodynamic injection
 (d): Electrokinetic injection

In any of these above-mentioned injection modes, the capillary is physically transferred from the electrolyte vial into the sample vial and back. These batch-type injection modes, which are now still in use in commercial CE instruments cannot be easily coupled to other on-line sample treatment systems, so sample preparation typically has to be done off-line. The first attempt to automate repeated sample injection in CE was reported by Deml *et al.* in 1985 [14], relying on sample splitting techniques used in HPLC. As early as 1993, Liu and Dasgupta [15] performed for the first time injections from a flowing sample stream into a CE system using an electroosmotically pumped capillary flow technique. In 1995, Kaljurand and co-workers [16] described a pneumatic sampling device that could be used for repetitive injections in CE. In 1997, for the first time, the flow-based injection techniques for CE via special interfaces were introduced independently by Kubáň *et al.* [17] and by Fang *et al.* [18]. In this technique, for the sample to be injected in to the capillary, during the time the flow of sample is delivered passing through an interface in which the CE capillary is positioned,

pressurization of the interface is applied with the aid of a peristaltic or a motor-driven syringe pump. Since then, this interface-based injection technique has been widely applied in coupled systems of flow injection (FI) / sequential injection (SI) – CE.

1.1.2.4. The family of CE modes

Originated and developed from a combination of electrophoretic and chromatographic techniques, capillary electrophoresis is collectively constituted of different specialized modes, among which the most frequently exploited are capillary zone electrophoresis (CZE) (often referred to as free solution CE, or FSCE), micellar electrokinetic capillary chromatography (MEKC), capillary gel electrophoresis (CGE), capillary isoelectric focussing (CIEF) and capillary isotachopheresis (CITP).

CZE, which is the simplest and the most universal of the techniques, relies on solute migration in discrete zones and at different mobilities, governed by the electroosmotic flow, for separation. It is performed in a homogeneous background electrolyte. While cations are accelerated by EOF on the migration toward the cathode, anions, on the contrary, though electrophoretically migrating towards the anode, are swept towards the anode by the bulk flow of electroosmosis. For separation of anions, thus, EOF reversal methods are normally employed to facilitate the migrations of anions toward the detector. In this mode, neutral solutes are co-migrated with EOF and thus are not separated electrophoretically. This renders CZE a method inapplicable for neutral species.

MEKC, a hybrid of CZE and chromatography, is the only technique of electrophoresis that allows concurrent separations of both neutral and charged analytes. This method is based on differential partitioning between micelles (pseudo stationary phase) and running buffer (mobile phase). Separation of neutral species is implemented with the addition of a surfactant (normally sodium dodecyl sulphate SDC) to form charged micelles with the neutral solutes. The different migrations of analytes are due to variations of interaction with the micelles. This method is commonly employed for separation of peptides and proteins.

CGE is an advanced technique of size-exclusion separation that relies on differences in solute size as analytes migrate through the pores of the gel-filled column. This technique is a modified generation of traditional gel electrophoresis. The critical point of this method is the inclusion of a suitable gel to serve as a molecular sieving medium. With this method, compounds of similar charge-to-mass ratios can be separated. Weight analysis of proteins and sizing of DNA fragments are often carried out using this mode of CE.

In CIEF, separation is accomplished based on the isoelectric points (pH values) of the substances to be determined. In this mode, the capillary is filled with buffers containing different ampholytes to create a pH gradient. Upon the application of an external electric field, charged proteins migrate through the pH-gradient medium until they reside at a specific pH region where they become uncharged and thus stop migrating. This will create different steady zones along the capillary. After this focusing step, the zones can be mobilized from the capillary with a pressure-induced flow for subsequent detection. This method is commonly utilized for separation of proteins and peptides, and for determination of pI of unknown proteins.

CITP is performed by confining sample components between leading and terminating electrolytes in a discontinuous buffer system and under an electric field in the constant current mode. The ions in the leading and terminating electrolytes are selected in such a way that their mobilities are higher than that of the fastest migrating ion and slower than that of the lowest migrating ion in the sample, respectively. At the application of a high voltage, a steady-state migrating configuration, in which analytes arrange themselves according to their mobilities in the sandwich zone between the leading and terminating ions, and migrate at the same speed towards the detector, is formed. This method, commonly used as a sample pre-concentration or sample purification step, is, however, not suitable for analysis of unknown samples.

1.1.3. Some landmarks in the development of CEC

CEC, or electro-chromatography in the capillary version, is a variant of HPLC in which the flow of mobile phase is driven through the micro column by an electric field with electro-osmotic flow (EOF) rather than by applied pressure. In 1939, Strain for the first time reported the use of EOF in chromatography to separate dyes in an alumina column [19]. The term

“electro-chromatography”, however, was introduced by Berraz in 1943 [20]. Early work in electrical chromatography was then carried out by Strain and his co-worker in 1951, either in relatively large diameter columns (0.1 mm) or in thin layers for the analysis of neutral, basic and acidic molecules by electro-migration [21]. Nevertheless, after that, the use of electro-osmosis as a pumping mechanism for analytical separations remained undeveloped until the contribution of Pretorius *et al.* in driving solvent through a glass chromatographic column packed with microparticulate silica in the mid 70’s [22]. This group is considered the originator of CEC. Significant progress in CEC began in the 1980s. In 1981, Jorgenson and Lukacs [10] demonstrated the very first attempt to utilize electro-osmosis in capillaries as an alternative to pressure-driven flows to obtain reduced theoretical plate heights. Tsuda then showed that CEC was possible in coated open tubular columns and recognized the factors that control the EOF as well as the importance of practical effects, such as bubble formation, in packed columns [23]. The peak of the resurgence of CEC dates in 1987 with the detailed theoretical analysis done by Knox and Grant [24], followed by practical demonstrations based on slurry-packed and draw-packed capillaries by the same group in 1991 [25]. The next landmark came in 1994 with the recognition of the potential of CEC in the analysis of mixtures relevant to the pharmaceutical industry, using a reversed-phase C18 column [26]. Since 1996, the popularity of CEC has been on the increase, as reflected in the number of publications and reviews [27-35] relating to CEC, reporting a wide range of applications in environmental, chiral, industrial, pharmaceutical and biological separations.

1.1.4. Basic principles and advantageous features of CEC

1.1.4.1. Basic principles of CEC

CEC, as a hybrid technique, combines some of the features of CE and of HPLC. In CEC, both separation mechanisms occur concurrently: the separation process is based on differential interactions between the stationary and mobile phases, whilst the electroosmotic flow, rather than a pressure-driven hydrodynamic flow, transports the mobile phase through the capillary. In other words, transport of the analyte is due to both electro-osmotic and electrokinetic mobility, under the effect of a flat flow profile induced from EOF. CEC differs crucially from CE in that the separation principle is chromatographic partitioning between the liquid and solid phases.

1.1.4.2. Column technologies for CEC

In the first years of its development, CEC was mostly carried out in capillaries (50-100 μm internal diameter) filled with silica packing material as used in chromatographic columns [30, 32]. Silica particles with a variety of surface modifications, enabling CEC separations based on different mechanisms, *i.e.* hydrophilic or hydrophobic interaction, ion-exchange and affinity, were readily available. The silica matrix of such particles usually carries enough surface charge to enable the generation of a substantial EOF. The success of packed capillaries for CEC can be attributed to the increased loading capacity of highly characterized and commercially available packing materials. However, the preparation of such types of columns requires considerable expertise due to many practical difficulties encountered. Moreover, fabrication of robust frits and stable column packing has been fraught with constructional problems [31, 32]. Open-tubular electrochromatography (OT-CEC), in which the stationary phase is coated on the inner wall of the capillary, on the other hand, overcomes these drawbacks. In this mode, due to the slow rate of solute diffusion in the liquid phase, internal column diameters equal to or smaller than 25 μm are typically employed to promote interaction with the immobilized ligands. Separation efficiency with OT-CEC is better compared with that from a packed column, mainly because the eddy diffusion contribution is minimized. However, due to the drawback of having only a single layer of stationary phase, the capacity of OT-CEC is low, which adversely affects detection. And compared to packed-column CEC, the loadability of OT-CEC is very poor, and lower retention factors are often encountered. This method has therefore seen limited use, and the interest in OT-CEC appears to be dwindling [31, 33]. The third trend, to which much research is devoted, is the development of monolith technology for the preparation of CEC columns. As the continuous skeleton is anchored to the capillary wall, retaining frits are not needed. The high porosity of the monolith affords high chromatographic efficiency and allows a higher sample loading. The surface of the monolithic stationary phase can be modified to create tailored sites for interaction and desired charged moieties for the generation of electroosmotic flow. This technique thus overcomes the disadvantages of packed-column CEC and OT-CEC. As a result, much attention has been focused on the *in situ* preparation of different types of monolithic columns [36-38].

A monolithic stationary phase is the continuous unitary porous structure prepared by *in situ* polymerization or consolidation inside the column tubing, and if necessary, the surface is

functionalized to convert it into a sorbent with the desired chromatographic binding properties [39]. With many advantages over packed-column CEC and OT-CEC, monolith technology has triggered interest from the scientific community in the last ten years and great progress has been made, which allows at the same time the control of the surface chemistries for interaction and generation of the EOF, and the control over the porous properties [37, 40]. Monolithic stationary phases can be subdivided into two main categories, *i.e.*, silica- [41, 42] and polymer-based materials [43, 44], which are prepared by polymerization of alkoxy silane precursors or polymerization of organic monomers, respectively. Despite a wide variety of precursors, which allow a nearly unlimited choice of both matrix and surface chemistries in the fabrication of organic polymer-based monoliths, and the ease with which these polymers can be confined in capillaries, the resulting monolithic columns undergo shrinking and swelling in organic solvents, which as a result leads to a lack of stability. Moreover, the polymeric monolithic structure may contain domains of micropores (< 2 nm) whose size hinders the motion of analytes in and out of the pore [45]. This is a reason for deteriorated efficiency and peak symmetry of the column. On the other hand, although the silica-based monoliths may be more tedious to fabricate, the micropores can be very conveniently converted into mesoporous network in the skeleton in a subsequent pore tailoring step [46]. Moreover, the presence of surface silanols provides the reactive sites for attaching a wide variety of surface ligands to achieve different stationary phases and EOF characteristics. For different separation mechanisms, *i.e.* reversed phase, normal phase, ion exchange and chiral separation, different corresponding preparation procedures have been reported for the surface modification of the monolithic structure ([40] and references reported therein).

1.1.4.3. Advanced features of CEC over HPLC and CE

By avoiding the use of pressure, CEC exhibits many strong points over conventional HPLC. First of all, much less instrumental complexity can be achieved with CEC, as high voltage power supplies are much simpler, and less expensive than high-pressure pumps. Secondly, the electrically driven flow, in contrast to pressure-induced hydrodynamic flow in HPLC, is not vulnerable to band broadening associated with pressure-driven parabolic-flow profiles, and is independent of particle diameter and column length so that smaller particles and longer columns can be used, which contribute to considerably higher efficiencies in CEC. And since CEC is normally carried out in narrow-channelled columns, it is viewed as a miniaturized separation technique (like CE), which then translates to low solvent and sample consumption,

high separation efficiency and low operational costs. Combined features of chromatographic interactions and electrophoresis in CEC may be employed to achieve selective separations concurrently for both charged and neutral compounds, which otherwise are difficult to obtain with either CE or HPLC alone.

1.1.5. Electroosmotic flow (EOF)

Electroosmosis is best described as the movement of liquid relative to a stationary charged surface under an applied electric field. EOF is generated at the solid-liquid interface of the support material (in CEC) or the internal wall of the capillary (in CE), which in most cases is silica. In a fused silica capillary, as illustrated in figure 3, the ionization of silanol groups gives rise to a negatively charged surface (SiO^-), which affects the distribution of nearby ions in solution. An excess of ions of positive charge (counter-ions) are attracted to the surface to maintain the charge balance, forming an electrical double layer whilst ions of like charge (co-ions) are repelled. Essentially the counterions are arranged in two regions, including the fixed layer at the surface (Stern layer) and the diffused layer that extends into the bulk of the solution (Gouy-Chapman layer) [47, 48]. Under the influence of an electric field, the solvated cationic species are electrically driven towards the negative electrode. These solvated cationic species drag solvent molecules along as they migrate toward the cathode, generating electroosmotic flow. This bulk movement of liquid sweeps all analytes irrespective of charge in one direction, normally towards the detection end of the capillary. As a consequence, in certain situations where electrophoretic mobilities of anions are smaller than the magnitude of EOF, positively and negatively charged species may be simultaneously determined in one single run, which otherwise is impossible in the absence of EOF. Neutral species co-migrate with EOF, thus are undetectable in the normal CE mode.

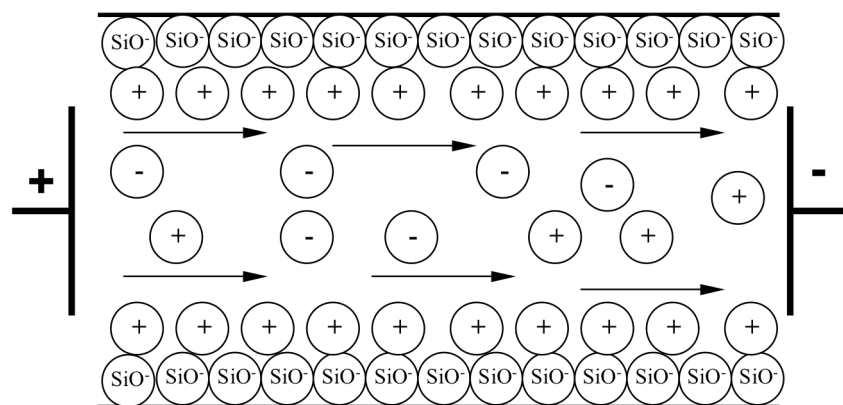


Figure 3. Interior surface of a fused silica capillary filled with electrolytes

The EOF through a channel has a characteristic flat elution profile, in contrast to the parabolic profile typical for pressure-driven systems, as shown in figure 4. The advantage of this plug-like pumping mechanism is that all solutes experience the same delivering velocity induced by EOF regardless of their cross-sectional position inside the capillary. Analytes thus elute as narrow bands, giving sharp peaks of high efficiency.

The linear mean velocity and mobility of the EOF (v_{EOF} and μ_{EOF}) in open tubes (for CE) are expressed according to Smoluchowski's equations:

$$v_{EOF} = \frac{\epsilon_o \times \epsilon \times \zeta}{\eta} \times E \quad (7)$$

$$\text{or} \quad \mu_{EOF} = \frac{\epsilon_o \times \epsilon \times \zeta}{\eta} \quad (8)$$

where ϵ_o is the permittivity of the vacuum; ϵ is the dielectric constant of the solution; η is the viscosity of the bulk solution; E is the applied field strength and ζ is the zeta potential of the wall, which is defined as the voltage drop between the Stern layer and the shear layer caused by an electrical imbalance across the layers. The zeta potential depends on the thickness of the electrical double layer and the charge density of the diffuse layer. In the diffuse layer, this potential falls exponentially to zero. All factors affecting EOF, *i.e.* the solution viscosity, dielectric constant and zeta potential, are variables that can be modified by changing corresponding experimental parameters such as electrolyte concentration, ionic strength, pH, temperature, surface characteristics, and cosolvents used. The concentration of the electrolyte in the mobile phase affects the EOF by varying the thickness (δ) of the electrical double layer, which in turns affects ζ . A decrease in the concentration of the electrolyte leads to an increase in the resulting EOF. The pH of the mobile phase affects the degree of ionization of the surface silanol groups, hence influencing the EOF. As the pH of the mobile phase is elevated, the EOF increases. When pH value of the background solution inside the capillary is higher than 4, EOF becomes significantly strong, and in some cases can drag the solute out of the capillary before the separation is completed.

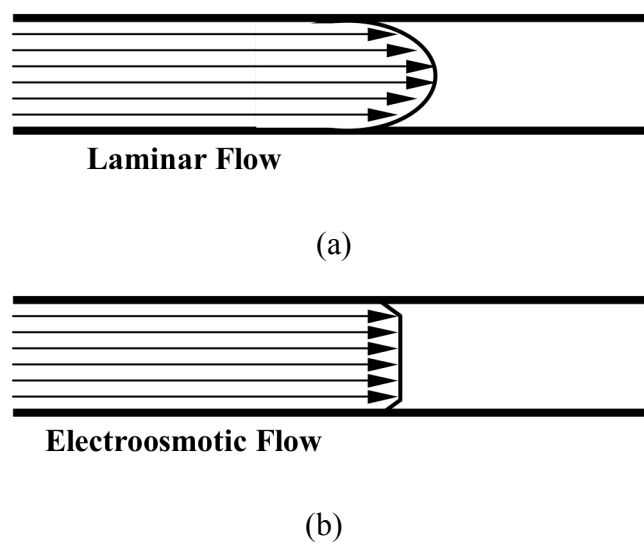


Figure 4. The flow profiles in CE (a) and in HPLC (b)

In CEC, the capillary wall, internal porous material and/or the column packing may carry surface charges that are capable of supporting EOF. In packed columns, EOF mobility in the interstices can be expressed in a way similar to equation (8), with the zeta potential of the wall ζ replaced by the zeta potential at the surface of the packing ζ_s (with the assumptions that the particles have uniform zeta potential and a double layer thin compared to the radius of the pores). And since the EOF in CEC is independent of the channel diameter, EOF velocities through the small intraparticle pores and the larger interstitial channels are quasi equal for similar path lengths. This feature of CEC where the EOF velocity is independent of the channel diameter plays an instrumental role in attaining high plate efficiencies. Compared to a pressure-driven flow, the EOF is more homogeneous and less influenced by differences of the packing or porous material. However, the EOF velocity in a CEC column is most likely to be reduced compared to that in an open tube, on account of the tortuosity and porosity of the packed bed.

1.2. Detection in CE and CEC

In modern CE and CEC, polyimide coated capillaries of 10 μm to 100 μm are typically used. With these narrow channels, detection volumes are very small, typically in nanoliter ranges. Most of CE detection modes, either destructive (*e.g.* mass spectrometry) or non-destructive

(e.g. optical), positioned on-column, end-column or post-column, so far have been adapted from HPLC, of which the most commonly employed will be described.

1.2.1. Optical detection

Detection schemes with optical radiation are quite easy to implement and by far are the most often used methods for detection with CE and CEC. This technique allows on-column detection with UV-transparent fused silica capillaries. Nowadays, absorbance and fluorescence detectors are conveniently incorporated in commercial state-of-the-art CE instruments.

1.2.1.1. UV/Vis

The UV/Vis absorbance detection is a non-destructive and on-column detection technique and is most commonly used in CE/CEC, partially due to its availability from HPLC. This detector is only responsive to UV-absorbing molecules at the wavelength of the light source. For non-UV-absorbing species, such as inorganic ions, amino acids *etc.*, detection is performed in the indirect mode where a chromophore, *i.e.* an ionic UV absorbent, is added into the background electrolyte. The presence of chromophore(s), however, yields lower sensitivity and poorer linearity. The employment of UV/Vis detection requires removal of a small section of the polyimide coating of the capillary for an optical window, which in turn renders the capillary fragile and vulnerable to breakage. The sensitivity of this method is dependent on the optical pathlength defined by the internal diameter of the capillary. For this reason, capillaries of inner diameters less than 50 μm are not preferable for UV/Vis-radiation detection.

1.2.1.2. Fluorescence

Fluorescent detection is implemented for recognition of fluorescent molecules which absorb excitation light at one wavelength and then re-emit it instantaneously at a longer wavelength. Fluorescence detection is by far the most sensitive detection, with detection limits normally one to three orders of magnitude lower than those obtained with UV/Vis absorbance method, mainly due to the high intensity of the incident light and the ability to accurately focus light onto a very narrow channel of a capillary. The drawbacks of this method, however, are expensiveness, restriction of laser excitation wavelengths, and possible photo-degradation of

the analytes caused by the high light intensity. This detector is poorly versatile as many solutes of interest do not exhibit native fluorescent characteristics. Fluorescent detection for the non-native-fluorescent compounds can be carried out alternatively either by incorporation of a fluorophore into the background buffer (indirect detection method) or by fluorescent tagging of the analyte via pre-derivatisation prior to detection.

1.2.2. Mass Spectrometry (MS)

Mass spectrometric detection is a destructive, end-column and highly sensitive detection method. It is a more universal detector than UV-Vis, laser induced fluorescence (LIF) or electrochemical detectors. Additional information of the structures of the analytes is also provided when using MS. MS thus has become a key tool for the characterization of biologically relevant molecules, especially peptides and proteins [49-52]. This detection technique, however, is expensive and complicated in configuration, and poses physical problem of interfacing when coupling with capillary electrophoresis: The outlet electrode in the CE setup must be employed as the interface to MS, which is difficult to realize and is based on some specific interfacing techniques, so-called sheath-flow interface, sheathless interface and liquid-junction interface. Among all coupling modes of MS with CE, electrospray ionization (ESI) is the most frequently applied coupling technique for bioanalysis. This method, nevertheless, requires a specific arrangement where the ESI needle is grounded and the ESI voltage is applied on the MS inlet to circumvent any problems with respect to CE-current-ESI interaction (currents in CE are typically three orders of magnitudes higher than those found in the electrospray). The coupling of CE with MS detection has been explored for quite a number of applications in forensics, environmental analysis, bioanalysis, pharmaceutical analysis and the study of metabolites; see for example [53-59].

1.2.3. Electrochemical detection

1.2.3.1. Potentiometric detection

Potentiometric detection is the most simple and straightforward mode of electroanalytical detection, which relies on a working and a reference electrode in contact with analyte ion(s), and does not require an application of external voltage as in other methods. The signal arises due to a potential drop formed at the working electrode. The working electrode, typically made from a crystalline-, liquid- or glass- membrane, specific and permeable only to the ion

of interest, is accordingly termed ion-selective electrode (ISE). Indeed, miniaturized versions of liquid membrane ion-selective electrodes, which are routinely applied in physiological studies, have been initially employed for this mode of detection in CE. As potentiometric electrodes are responsive only to ions of specific charge number and sign, this detection method is the most selective of all electrochemical detection methods. The Nernst's equation is applied to calculate the potential change between the two electrodes. Principles and applications of potentiometric detection in CE can be found, for instance, in [60-65].

1.2.3.2. Amperometric detection

In amperometry, the redox current induced from oxidation or reduction of ions of interest is measured. Three different electrodes are required, namely a working electrode, a reference electrode and an auxiliary (or counter) electrode. A special electronic circuitry, termed potentiostat, is required for this triple electrode setup. Amperometric detection is based on the application of a fixed potential across a supporting electrolyte between the working and reference electrodes to let electroactive compounds gain (reduction) or lose (oxidation) electrons from/to the electrode. The current resulting from the electron transfer is measured between the working and auxiliary electrodes, and is directly correlated with analyte concentration. This method, however, is only applicable to oxidisable or reducible species, notably catecholamines, phenols and aromatic amines. Recent reviews on amperometric detection in CE can be seen in [66-70].

1.2.3.3. Conductivity detection

Of all types of electrochemical detection, conductivity detection is the most universal, which responds to all charged species. A baseline signal (and noise) is thus always present at the detector as ions are always abundant in the background electrolyte. Unlike potentiometric and amperometric detection, this bulk detector does not rely on electrochemical reactions on the surface of the electrode, but measures the conductance of the solution between the two electrodes. A conductivity detector is based on two inert, typically platinum, electrodes across which a high frequency AC potential is applied. The use of AC instead of DC voltage avoids current limitation and electrolysis reactions on the surfaces of the electrodes. At the electrode surfaces, a double layer is established, in which charges in the electrode are balanced by ions of opposite sign in the adherent solution. Such double layers behave like electronic capacitors,

which are transparent to AC currents and voltages. From the correlation between conductivity and current described in the Ohm's law, the difference in conductance between the analyte and the background co-ion(s) results in a current signal that will be measured. Conductivity detection may be implemented in contact or contactless mode, where detection is performed with or without galvanic contact of electrodes and the electrolyte solution, respectively. There is no fundamental difference between conventional contact conductivity and (capacitively coupled) contactless conductivity detections, as a double layer originating from coulombic attraction of charges can be established through an insulating layer [65]. Working with the contact mode requires an operating frequency of around 1 kHz, whilst in contactless method higher frequencies of several hundreds kHz are typically applied. As detection sensitivity is strongly dependent on background conductivity, it is recommended to select background buffer(s) of low conductivity for this mode of detection. Conductivity detection favours the determination of poor or non-UV absorbing charged species of relatively high specific conductivity, such as inorganic ions, amino acids *etc.* Further details on principles and applications of conductivity detection can be found in [61, 67, 71-76].

One common drawback for electrochemical methods, though when used for direct quantification, is a certain inherent limitation in selectivity. A solution to the selectivity limitation is the combination of these electrochemical quantification methods with a separation step. The electrophoretic separation methods can be considered to be electroanalytical techniques, and therefore match the simplicity of an electrochemical quantification step more closely than the flow-driven chromatography.

1.2.4. Capacitively coupled contactless conductivity detection C⁴D

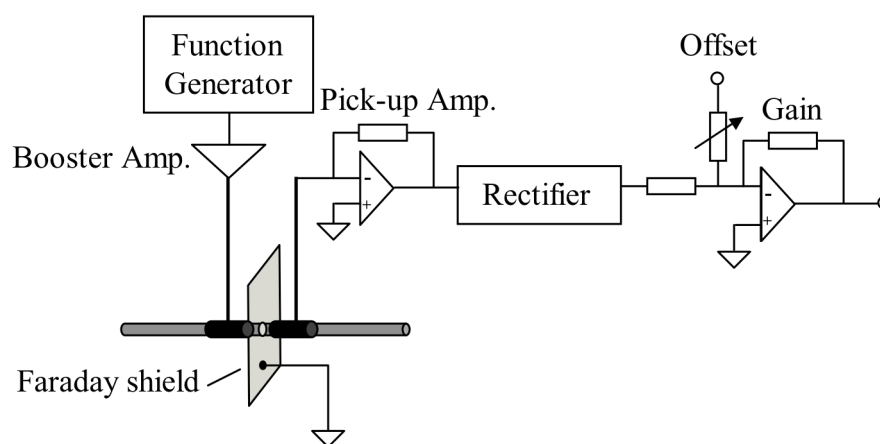
1.2.4.1. Basic principles and configuration of C⁴D

The basic arrangement of an axial C⁴D, which was first introduced independently by Zemann *et al.* [77] and by da Silva and do Lago [78] in 1998, and is still widely used nowadays, is illustrated in figure 5a. Two electrodes of a few millimeter lengths, namely actuator and pick-up electrodes, made from conductive silver varnish or short metallic tubes, which are separated by a gap of typically 1 mm, are placed side by side around the capillary. Cells can be readily made for capillaries of the standard 365 µm outer diameter. Since the two sensing electrodes themselves can couple with each other to give a stray capacitance, leading to an

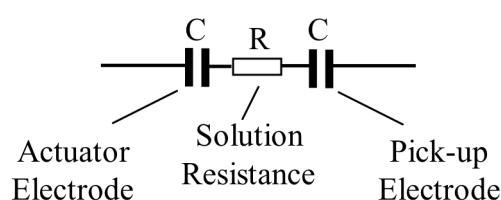
additional background signal, which is not preferable for detection, they are normally surrounded in Faradaic shielding to minimize their direct capacitive coupling. The Faraday shield is typically made of a thin copper foil, on which only one hole is drilled to admit the capillary. The two external electrodes form two capacitors (C) with the solution inside the capillary. The equivalent circuitry of a conventional contactless conductivity cell, as shown in figure 5b, can be represented by an arrangement of two double layer capacitances C connected to the solution resistance R. An AC excitation voltage with high frequency of several hundreds kHz is applied at the actuator electrode. The current (I) passing through such a circuitry is dependent on the applied alternative voltage (V) and frequency (f) as expressed by the following equation:

$$I = \frac{V}{\sqrt{R^2 + \left(\frac{1}{2\pi f C}\right)^2}} \quad (9)$$

According to equation (9), at low frequencies, the current is limited by the double layer capacitances. For the higher frequencies, the current is determined only by the solution resistance, not by the capacitance at the electrodes, resulting in a plateau value of current (I). The fact that the two electrodes are taken out of the solution leads to an increased separation of the charges, resulting in a greater distance between the two plates of a capacitor. This translates into a smaller capacitance, and hence higher required operating frequency. In practice, frequencies higher than 100 kHz are employed, and the value of 300 kHz is deemed optimized [79]. The AC current signal, which is picked up at the second electrode, first has to be transformed into a voltage with a feedback resistor and then rectified to obtain a recordable DC signal that varies with conductivity changes. Typically, the background signal should be suppressed electronically (“offset” or “zeroed”) before amplifying the measured signal to obtain the best resolution of the analog-to-digital converter. For more details on fundamental aspects of C⁴D consult the papers by Kubáň and Hauser [79-81].



(A)



(B)

Figure 5: Schematic drawing of C^4D in an axial arrangement.

(A) Schematic drawing of the electronic circuitry; (B) Simplified circuitry

In C^4D , the electrodes are positioned on the outside of the capillary, leading to ease in setup, and exact alignment of electrodes matching with various diameters of capillaries. With the axial and contactless configuration, many advantages can be obtained, such as avoidance of corrosion of electrodes, prevention of electrode fouling, inherent decoupling from the electric field applied for separation, simple construction of the detector cell and possibility for miniaturization. Moreover, removal of the polyimide coating, which renders the capillary fragile but is required to allow passage of UV radiation in optical detection techniques, is not needed in C^4D . Commercial versions of C^4D have recently become available in the market, fitting not only to CE but also to other analytical techniques, such as IC, HPLC or FIA (www.edaq.com and www.istech.at).

1.2.4.3. Applications of C^4D

With unprecedented simplicity in terms of geometry and electronic circuitry, the construction of a C^4D cell is relatively easy and therefore can be done in-house by different research

groups. Together with the introduction of commercially available C^4D units, this contributes to the extreme popularity of C^4D since its appearance as a robust detection technique for CE in 1998 [77, 78]. Reviews on recent applications of C^4D in CE are gleaned from [72, 82, 83]. Applications of C^4D , moreover, have not been restricted to detection in CE, but have also been extended to the separation methods of ion chromatography [84], HPLC [85-87] as well as to flow-injection analysis [88, 89].

Applications of C^4D in CEC in general have been very limited to date. Hilder *et al.* communicated the determination of several inorganic anions using a column packed with a particulate ion-exchange material as stationary phase [90]. Detection was carried out directly on the column. Kubáň *et al.* gave an account of determination of inorganic cations by OT-CEC using an anionic polymer wall-coating as stationary phase [91]. Nevertheless, till now, the application of C^4D in CEC is still in its immature state.

1.3. Sequential injection analysis coupled to capillary electrophoresis

1.3.1. Sequential injection analysis with Lab-on-Valve (LOV) and Lab-at-Valve (LAV) concepts

Sequential injection analysis (SIA) is a flow methodology for sample handling that allows the automation of manual liquid-phase chemistry procedures based on rapid, precise and efficient aspiration of small volumes of reagents and samples into a single channel. The concept of SIA was invented in 1990 by J. Růžička and G.D. Marshall at the University of Washington [92] in response to an industry-emerged requirement for a more robust automated wet-chemistry technique than the traditional flow-injection analysis (FIA) which was widely accepted in academic analytical chemistry laboratories at the time. They applied a so-called stopped flow method for reproducible chemical analysis of thiocyanates. Further application came as early as 1991 when Růžička and Gubeli determined a proteolytic enzyme using an automated stopped-flow procedure [93]. The very first review on the principles of the sequential injection methodology was reported also by Růžička one year later [94]. Later on, Guzman *et al.* applied SIA with fluorimetric detection for determination of an enzyme named factor thirteen [95]. Not long after, an evaluation of the performance of SIA, as well as recommendations on how to select and configure components of a sequential injection system were outlined by the same research group [96]. Since then, SIA underwent a booming period

where its scope in complex sample-handling procedures, including on-line sample dilution, dialysis and gas diffusion, extraction, enzymatic and immunological assays *etc.*, created an exponential growth of applications [97-101].

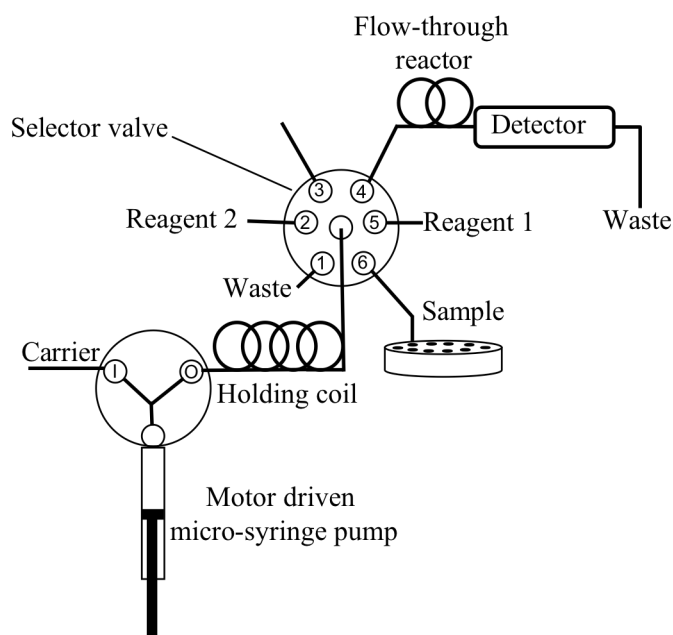


Figure 6: Basic configuration of SIA

SIA is considered as the second generation of FI based technique [94, 102] that can operate multi-tasks in a single channel and can perform most operations of FIA with minimal physical modification of a manifold. The principles upon which SIA is based are reproducible sample handling and controlled partial dispersion, as similar to those of FIA [92]. The basic configuration of SIA is schematically represented in figure 6. The heart of SIA is the multi-port selection valve since it enables the sequential selection of the various solutions and the subsequent redirection towards the detection system. The operation scheme of SIA is based on a multi-position selection valve and the propulsion. The propulsion system typically consists of a peristaltic pump or a piston pump that can provide unattended operation on a 24-h basis. Fluids are manipulated within the manifold between the selection valve and the propulsion by means of a bi-directional pump. The entire operation is computer-controlled and is carried out in a practically single-channelled configuration.

A step forward in miniaturisation of the sequential injection concept has recently been conceived with micro sequential injection lab-on-valve (SI-LOV) configuration [103], which is considered the third generation of flow analysis [104]. In this setup, all analyses are

performed in micro- or nano-liter volumes at the integration of the flow cell for sample processing, chemical reaction and detection in a conduit at a multi-position valve. With this significant downscaling of SIA, an even much lower consumption of reagents and samples is made possible. And as all equipment, *i.e.* selection valve, propulsion device and detector, can be assembled in the same box, this configuration is viewed as the most compact of all the flow methodologies. The employment of SI-LOV can be referred to [104, 105] for environmental analysis, and [106, 107] for bio- and clinical applications.

The advantages of SI-LOV in integrated instrumentation, rapidity, automation, miniaturization, tolerance to dirty samples and low reagent and sample consumption, however, sometimes cannot compensate for its disadvantage of constructional difficulties. For a flow cell and detection unit to be incorporated in SI-LOV, a stator plate of a multi-position selection valve must be replaced with a perfectly machined Perspex block mounted on top of the selection valve and special fibre optics technology must be employed [103], which, very often, raise challenges in equipment support in different laboratories. These drawbacks of SI-LOV, on the other hand, were eliminated by the introduction of a new sequential injection configuration, so-called Lab-at-Valve (LAV) [98, 108]. In this setup, sample processing and detection units are attached or plugged onto ports of a commercial conventional multi-position selection valve without taking apart any component of a purchased valve. The LAV thus can be easily constructed, using ordinary and less precise machine tools, whilst still reserving all advantages of LOV. This simpler approach of SI-LAV was first demonstrated for potentiometric determination of chloride [109]. Consequently, other applications of this SI-LAV format were reported in [108, 110, 111]. Although still in its infancy, SI-LAV has already proved to be an attractive, effective front end for sample treatment and processing prior to detection.

1.3.2. Automation and extension of CE with sequential injection analysis

In SIA, due to the complexity of most of the analyzed samples, and due to poor selectivity of the flow-based methodology itself, a pre-separation step (*e.g.* derivatization with chemicals) or a post-separation step is often required if more than one compound is to be determined. In the case of pre-separation steps typically incorporated, complex manifolds with a multitude of flow channels and detectors are necessary in order to achieve high selectivity for each analyte

in a multi-analyte mixture, thus making this otherwise simple and straightforward flow analysis scheme very complicated. The use of automated SIA as a front end to various analytical protocols, notably liquid chromatography (LC) or capillary electrophoresis (CE), on the other hand, is much more desirable. There is no great technical challenge in coupling SIA with LC as the flow in both systems is generated by pressure. The combination of SIA-LC can be typically done just with a simple chromatographic valve, which facilitates the employment of this setup since the early years of LC. However, this combination always possesses the drawbacks of high back pressure and elevated cost for instrumentation and equipment maintenance associated with LC, which, in many cases, prevent its popularity.

The coupling of SIA based on a syringe pump and a multi-position valve with CE is a relatively new approach that provides simultaneous detection capability to SIA. It is also an attractive and versatile mean to miniaturization, automation and extension of CE. Since CE does not require the implementation of high-pressure pumps, and aggressive solvents are seldom needed, CE is particularly suitable for coupling with flow techniques, notably SIA. Such a coupling is considered the marriage between the powerful separation mechanisms of electrophoresis with the automation concepts of the micro-sequential injection technique. This SIA-CE combination can enjoy both the noteworthy advanced aspects of CE and SIA, *i.e.* high separation efficiency, low sample and electrolyte consumption, experimental simplicity, programmable and precise handling of small liquid volumes, and cost-effectiveness. Nevertheless, commercial CE instruments designed for the laboratory are not well suited for coupling to such external sample handling manifolds as SIA. The use of a syringe pump in SIA-CE systems, moreover, allows simple and reproducible pressurization of the CE capillary inlet for sample injection, capillary rinsing and conditioning without modifying the instrument. It is, on the other hand, easier to construct a CE-separation unit as part of an extended SIA-manifold. Of course, combining pressure-driven flow of SIA with electrophoretic separation methods poses considerable technical challenges because pressure-induced flow is largely incompatible with the typically plug-like flow in CE. The high voltages applied in CE are not compatible with typical flow system manifolds and in some cases can cause malfunctions and failure of the electric controls. Furthermore, flow splitting is needed due to low injection volume in CE (in the nL-range). The coupling of flow-based techniques in general and SIA in particular with CE thus is not trivial, and so far based on special interfaces [17, 18, 112-116] to conduct the flow through the sample preparation

module towards the CE system. These interfaces, some of which are illustrated in figure 7, were designed to be compatible with both the flow mode placed in front and the subsequent CE system. They also facilitate automation with significantly enhanced sampling frequency and precision. A typical arrangement of SIA – CE is shown in figure 8.

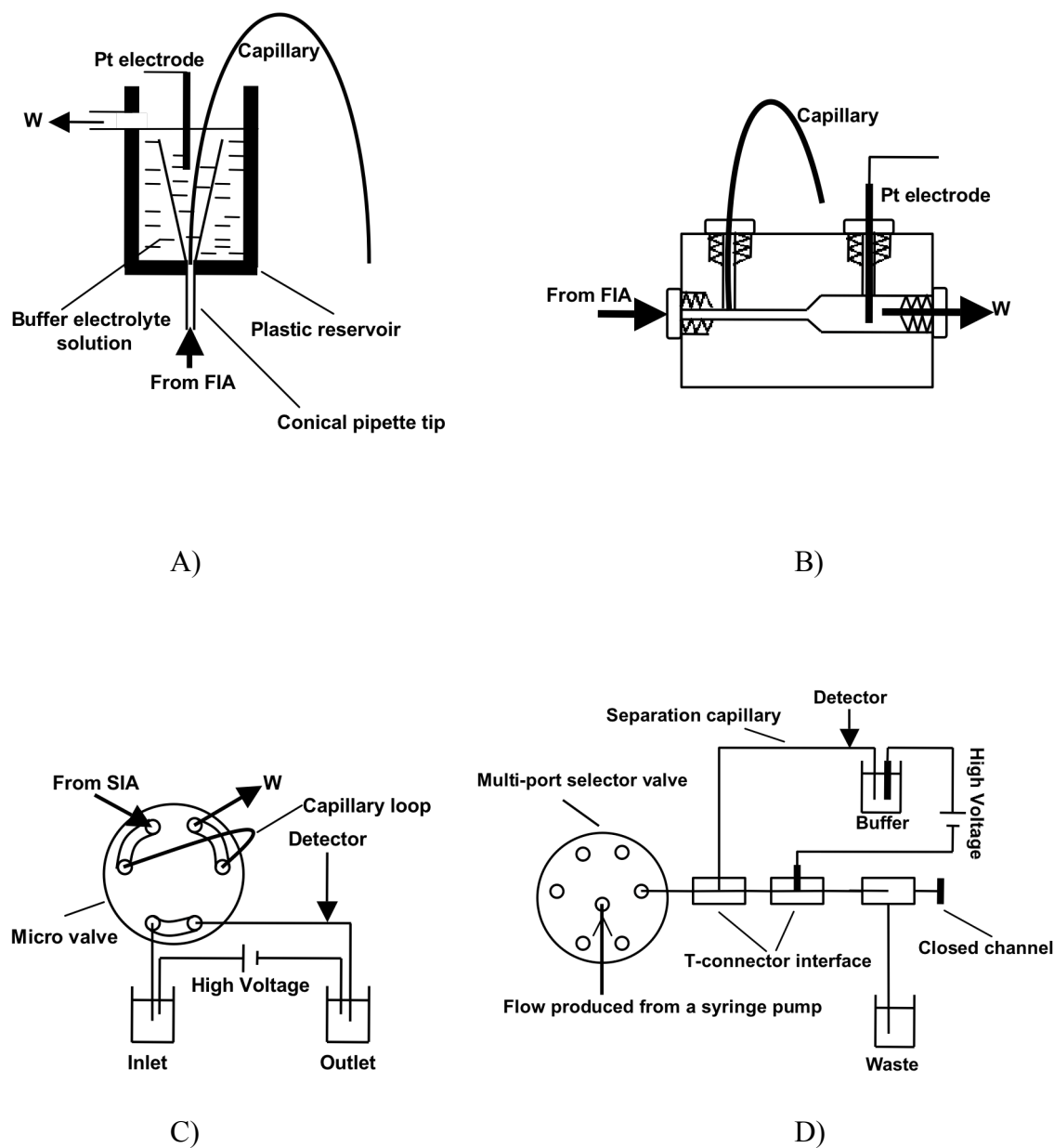


Figure 7. Some split-flow interfaces for coupling of flow-based techniques to CE. A) Plastic tip with embedded Pt electrode; B) Plexiglas interface; C) Micro-valve approach; D) Two T-connector interface. W: waste; Pt: platinum electrode.

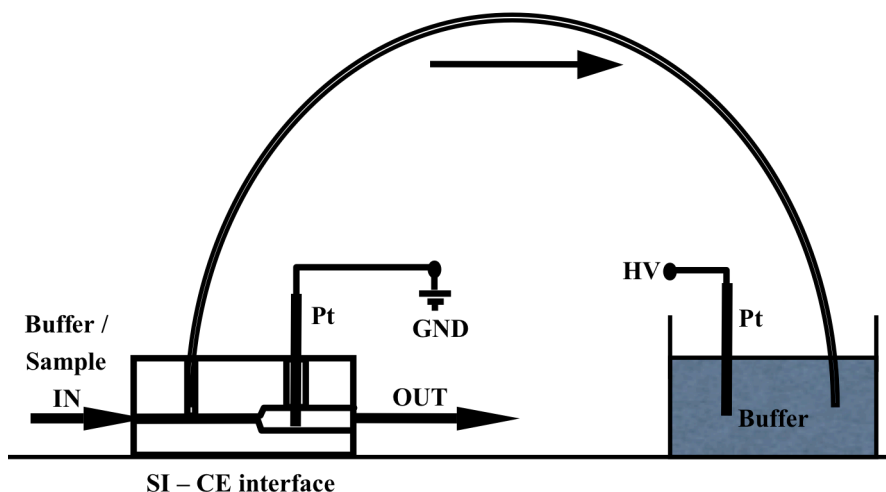


Figure 8. A typical setup of SIA-CE combination

Although two independent works by Kubáň *et al.* [17] and Fang *et al.* [18] in 1997 were considered the milestone for the full exploitation of the marriage between CE and flow-based techniques, the very first attempts to couple SIA with CE were reported by Růžička and co-workers in 2002, for anion separation [118] and for insulin derivatisation and separation [116]. Kulka *et al.* [114] introduced a similar system in 2006 and Horstkotte *et al.* [119, 120] demonstrated the determination of nitrophenols. Zacharis *et al.* [115] designed a SIA-CE instrument employing laser-induced fluorescence for detection. Wuersig *et al.* [121] used an SIA set-up to achieve fast injections into capillaries of only a few centimetres in length and could thus demonstrate the separation of inorganic cations and anions in approximately 10 s. C^4D was employed in the latter case for detection. Many other applications of the coupling of SIA and CE up to 2009 can be found in [117, 122, 123].

1.4. Research objectives of the thesis

Five different interrelated projects were carried out in the scope of this dissertation in order to explore the applications of capacitively coupled contactless conductivity detection in capillary electrophoresis and capillary electrochromatography, as well as the extension of capillary electrophoresis with sequential injection analysis. Different system setups were designed and constructed, based on the core arrangement of contactless conductivity detection coupled with capillary electrophoresis, with many modifications and extensions according to different pursued approaches:

- (A) ***Applications of C⁴D in capillary electrochromatography with monolithic octadecylsilica (C18) column.*** After more than one decade since the introduction of C⁴D to CE, hundreds of applications of this detector in CE have been reported, but the potential of this state-of-the-art mode of detection in CEC has still been in its immature state. To our knowledge, the simplicity and applicability of the combination of C⁴D and CEC with monolithic columns have never been explored. Thus, the first project was aimed to demonstrate the separations of inorganic and organic cations based on this combination. The in-house system was constructed, relying on a manual CE-C⁴D setup that had been used in our group. A homemade C18 monolithic column was prepared and together with a commercially available column, they were used for electrophoretic separations of different classes of cations, using C⁴D for detection.
- (B) ***Application of sequential injection analysis (SIA) coupled to CE-C⁴D for extended automated monitoring.*** It is obvious that commercial CE-instruments designed for the laboratory use are not well suited for on-site deployment and for coupling to external sample handling manifolds. It is, on the other hand, relatively feasible to construct a CE-separation unit as part of an extended SIA-manifold. In this contribution, we present a SI-CE-C⁴D system designed for monitoring applications over extended periods and demonstrate its functionality in several days of on-site monitoring of the concentrations of inorganic anions and cations in a creek. The new set-up incorporates a number of improvements compared to previously reported designs in order to achieve high reliability and specific adaptations to allow autonomous operation.
- (C) ***Applications of the SIA-CE-C⁴D system in pressure-assisted capillary electrophoresis.*** The scope of SI-CE-C⁴D was expanded for advanced operation where pressure was incorporated to convert monotonous electrophoresis schemes into pressure-assisted separations with varying superimposed hydrodynamic flows either for separation efficiency improvement or for throughput enhancement. The use of a computer controlled stepper-motor driven syringe pump enables versatile variation of the flow even during a separation run, which can be used to obtain optimized separation profiles akin to gradient elution in HPLC. Theoretical and

application aspects of this advanced approach of CE were comprehensively studied in two sub-projects for both cations and anions.

- (D) *Application of the SIA-CE-C⁴D system in on-line preconcentration prior to automated electrophoretic separation.* In CE, detection sensitivities are generally lower than those in liquid chromatography, mainly due to small injection volumes (in the nano-litre ranges). We thus further worked on designing and development of a new configuration of SIA-CE-C⁴D that allows automated on-line preconcentration before separation in order to greatly improve CE detection limits. Some drug residues were selected as exemplary analytes to demonstrate the applicability of CE-C⁴D in sensitive environmental analysis. It was of interest to further demonstrate its functionality by deployment of the system in Vietnam for monitoring these compounds in water.

2. Results and Discussion

The major parts of the thesis have been published in or submitted to different scientific journals for analytical chemistry. Therefore the chapter of Results and Discussion are presented with a brief summary of the main work of the five projects, together with reprints of three published papers and two manuscripts. The first section (chapter 2.1) is devoted to the application of contactless conductivity detection in capillary electrochromatography with monolithic octadecylsilica column. The work on application of CE-C⁴D coupled to a SIA manifold for unattended monitoring is presented in chapter 2.2. Chapters 2.3 and 2.4 report the employment of the SIA-CE-C⁴D setup in pressure-assisted capillary electrophoresis, for cation and for anion separations, respectively. The last subsection is reserved for presentation of further exploitation of the SIA-CE-C⁴D system in automated electrophoretic procedure with on-line solid-phase-extraction (SPE) preconcentration.

2.1. Capillary electrochromatography with monolithic octadecylsilica columns coupled with contactless conductivity detection for cation separations

Monolithic capillaries with high porosity property are well suited for coupling with a CE unit as only low pressure is required for combined operations. Monolithic capillaries coated with octadecyl groups (C18) were first produced in-house, following the procedures described exhaustively in [36, 124-126]. The homogeneity of the columns was controlled with C⁴D by moving the column through the detector and observing the variation of the magnitude of C⁴D output voltage signal generated from the fraction of the volume taken up by an ion bearing solution inside the capillary. Though the consistency of the homemade column is not quite as good as that of a commercial one, both these columns serve well for separation purposes. The selection of the mobile phase for CEC with conductivity detection is critical, as the requirements for electrophoresis and for the chromatographic process have to be satisfied as well as those for conductivity detection. It must be compatible with the stationary phase, have adequate elution strength and be of low specific conductance in order to allow high sensitivity in conductometric detection and to minimize Joule heating effect. Several buffer systems frequently used for HPLC were briefly tested, namely water/methanol mixtures containing trifluoro-acetic acid, phosphate buffers, tris(hydroxymethyl)-aminomethane, hydrochloric acid, citric acid and formic acid, but they were found not to give stable baseline, presumably in the majority of cases due to Joule heating caused by too high a conductivity. A mobile

phase consisting of acetic acid in a water/methanol mixture was observed to generally give more stable baselines. The optimization of CEC conditions, mainly consisting of finding the best concentration of acetic acid in an appropriate ratio of methanol to water, thus were carried out for both homemade and commercial monolithic columns. For separations of inorganic cations, the buffers composed of 40 mM acetic acid in 50 % (v/v) methanol and 80 mM acetic acid in 55 % (v/v) methanol were found to be optimized for the homemade and commercial columns, respectively. A buffer of 60 mM acetic acid in 40 % (v/v) methanol in water was best suitable for separation of small amines. In the case of amino acid determination, a buffer consisting of 20 % (v/v) acetic acid in 40 % (v/v) methanol in water was found optimal. Quantitative data with these conditions were also collected, and were comparable with those obtained from CE with conductivity detection.

2.2. Capillary electrophoresis with contactless conductivity detection coupled to a sequential injection analysis manifold for extended automated monitoring applications

For monitoring purposes, it is very crucial to set up a system that can implement sampling, separation, detection and data acquisition in an autonomous way. Such a system was designed and constructed based on an extension of conventional CE-C⁴D with a sequential injection manifold via an interface. The SI manifold relies on a two-way motor-driven microsyringe pump and a multiport valve with a holding coil in between. Two major modifications have been made to the set-up compared to earlier designs [119-121]. The first change is the employment of an adjustable needle valve designed for the splitting of small flows into two streams in connection with two solenoid isolation valves for precise hydrodynamic injection, flushing of capillary and renewal of buffer at the two ends of the capillary. The second modification concerns the detection end of the separation capillary where the high voltage is applied. The high-voltage end was enclosed in a safety case to assure electrical isolation. The automated buffer supplementation at the high voltage end was carried out directly through the capillary, instead of using auxiliary tubings [119, 120] to prevent electrical arcing, which can in turn lead to destruction of part of the electronic instrumentation. A special interface of minimal internal volume, with an 'ion-delay' channel with a round cross-section of 0.4 mm diameter and 2 cm length between the end of the capillary and the high voltage electrode was designed in order to allow efficient flushing of the liquid volume at the high voltage electrode through the capillary. The whole analytical procedure, including sampling, flushing of capillary and interfaces, separation, data recording was controlled automatically with a

homemade computer program based on LabView 8.0 software for Windows XP. Cations and anions were determined successively by automatic switching of the high-voltage polarity. To check the operation stability, the system was setup for a supervised test run over a period of 24 h, in which repeated measurements of the standard mixture of the cations and anions of 50 μM at intervals of 30 min were carried out. The maximum deviations are less than $\pm 4\%$, which demonstrates the suitability of the system for automated operation. In order to further evaluate the potential for unattended monitoring, the system was then set up at a pumping station next to the creek Kleine Aa, a tributary to Lake Sempach, Switzerland. Automatic analysis of the water from the creek was then carried out for 5 days during a period of frequent rain (end of April, 2009). Cross checking with discrete samples collected during this period and later analysed in-laboratory with ion-chromatography gives correlation coefficients (r) between 0.826 and 0.989 for all ions. Considering that some deviation can be expected due to possible sampling bias, the results convincingly show that the system is suitable for unattended long-term measuring tasks.

2.3. Pressure-assisted electrophoresis for cation separations using a sequential injection analysis manifold and contactless conductivity detection

The superimposition of a hydrodynamic flow is another potentially useful parameter, besides the injection volume, separation voltage applied and the capillary length, for flexible adjustment of the dwell time of analyte ions in the electric field in order to optimize resolution and/or analysis time. However, pressurization to introduce such a flow during electrophoretic separation has not generally been employed, as the imposition of this laminar flow tends to lead to band broadening. This adverse effect, nevertheless, can be significantly reduced by using capillaries of very small diameters. Experiments with different capillaries of internal diameters from 10 μm to 75 μm with detection by C^4D demonstrated that detection limits are all in the low μM -range, and almost identical for four diameters investigated, with the loss of sensitivity in going to the narrowest capillary being less than a factor of two. At the same time, separation efficiencies were greatly improved when reducing the inner diameter from 75 μm to 10 μm , whether pressurization to introduce a hydrodynamic flow is present or not.

So far, instrumentation for pressure-assisted capillary electrophoresis has been relying on the complex application of gas pressure or vacuum, which is typically equipped in conventional CE systems. The coupling of sequential injection analysis based on a syringe pump and a multi-position valve with CE, which is an attractive and versatile means to miniaturization, automation and extension of CE, is also a very new approach to provide pressure-assistance to introduce a hydrodynamic flow on the fly of electrophoresis. Very fast separation of inorganic cations in a red wine sample was carried out with the application of pressure of 1.6 bar produced from the micro movement of the motor driven pump. Compared to conventional monotonous CE separation, the analysis time was reduced from more than 6 min to less than 2 min, which in turn can greatly enhance sample throughput. The SIA-CE-C⁴D system was then further employed to create pressure gradient for concurrent separation of fast and slow migrating amines. In this complex situation, a constant pressure is not favourable, as a low pressure may be necessary for baseline separation of fast migrating analytes, but may lead to exceedingly long migration times for the slow ions, whilst an elevated pressure can deteriorate separation of the fast moving species. Accordingly, two pressures of 0.9 bar and 3 bar were applied at different times during electrophoretic separation of 9 amines. Salient performance data were also acquired for this operation, with detection limits in the range from 1.5 μ M to 15 μ M, calibration curves up to 300 μ M, and reproducibility for retention time and peak area of 1% and 2-5%, respectively.

The advanced feature of the SIA-CE-C⁴D combination in pressure-assisted CE was further employed to create a hydrodynamic flow to counter-balance the mobility and electroosmotic flow in order to solve the overlapping problem of two adjacent peaks. Ca²⁺ and Na⁺ were chosen as exemplary analytes, with the concentration of Ca²⁺ 20 times as high as that of Na⁺. Baseline separation of these cations, which is not possible with conventional CE conditions, was obtained using this pressure-driven mode with a capillary of an effective length of only 7 cm. Further demonstration of the functionality of the SIA-CE-C⁴D setup was shown in a very complex operation of multi-electrophoresis where 2 hard-to-separate amines are to be separated. Pressurization and application of high voltage are triggered at distinct stages to deliver the sample plug to the proximity of the high voltage end of the capillary and to implement monotonous electrophoresis towards the grounded end of the capillary, respectively. These operations are repeated several times until baseline separation of these amines is obtained at their 10th passage through the detector.

2.4. Anion separations with pressure-assisted capillary electrophoresis using a sequential injection analysis manifold and contactless conductivity detection

After the successful demonstration of the SIA-CE setup for pressure-induced electrophoretic separation of cations, a further study was carried out for the CE separation of anions in bare fused silica capillaries without addition of an EOF modifier. Sample injection was implemented with a new design relying on a simple piece of capillary tubing of defined diameter and length to achieve the appropriate back-pressure for the required split-injection procedure. The dimensions required for the pressurisation tubing can be worked out using the well-known Poiseuille equation, which relates the flow rate with pressure drop and length and diameter of a tubing. Using this approach it was found that for a PEEK tubing of 0.007" i.d. a length of about 35 cm was required in order to inject a 1 cm plug into a capillary of 50 cm length and 10 μm i.d.

This SI-CE arrangement using PEEK tubing for hydrodynamic injection was then first employed for separation of inorganic anions. As most inorganic anions are present in their charged forms even at low pH conditions where the EOF is suppressed, separation of these strong electrolyte anions in CE is often possible without the use of an EOF modifier while applying a positive separation voltage at the detector end. A superimposed hydrodynamic flow was utilized during separation of a range of inorganic anions of fast and relatively slow electrophoretic mobilities to accelerate the movement of slowly migrating anions in order to speed up the analysis. A hydrodynamic flow is not applied at the start of the separation in order to maintain adequate separation of fast moving anions. Rather, the late anions were pushed along by activation of pressure at an exact time in the middle of the electrophoretic separation. If only the more slowly moving anions are of interest, a very fast analysis of the late species can be achieved by a reversal of the applied voltage in combination with employment of pressure to create a hydrodynamic flow to counter the electrophoretic movement of anions.

The separation of weak organic anions, *i.e.* carboxylates with CE was then implemented at a high pH of 8.4, produced from a buffer based on Tris/CHES, in order to assure complete dissociation. A hydrodynamic flow induced from a pressure of 2.8 bar was employed to balance the EOF during the separation (positive voltage applied at the detection end). For

comparison, the separation of the same standard mixture of carboxylates was also carried out using the conventional approach by inclusion of CTAB (0.1 mM) as EOF modifier in the buffer, without application of hydrodynamic flow. Except for some difference in total analysis time (which could be matched by optimization of hydrodynamic flow rate and/or CTAB concentration), very similar results were obtained.

In a more complex situation where a mixture of 16 fast inorganic anions and slow organic anions are to be separated at high pH, EOF compensation with a constant hydrodynamic flow is not appropriate. A small constant pressure, although it can assure baseline separations of slow carboxylates, may lead to unacceptably long analysis time. On the contrary, a high and constant hydrodynamic flow results in significant shortening of the separation time, but at the expense of a loss of baseline resolution for the early peaks. The solution here was to use a change of hydrodynamic flow rate during the separation. With this method, baseline resolution for all analytes was obtained at a relatively short total analysis time. Statistical data were then acquired to evaluate the reproducibility of the pressure assisted method for anion determination and suitability for quantification. The detection limits are in the low μM -range and the reproducibility of retention times and peak areas are about 1-1.5 % and 3-5% respectively, which is comparable to the performance obtained with the conventional approach using an EOF modifier. This method was then successfully applied to determine preservatives in a beverage sample, and to quantify the content of ascorbic acid in vitamin C tablets.

2.5. On-line SPE preconcentration coupled with automated capillary electrophoresis using a sequential injection manifold and contactless conductivity detection

Further exploitation of the extension of the conventional CE-C⁴D with SIA was carried out in automated on-line SPE preconcentration prior to electrophoretic separation. The in-house built C⁴D was employed for sensitive detection with narrow capillaries of 25 μm internal diameter. The system was designed as an industrial prototype, with all fluidic and electronic parts, as well as all power supplies assembled into a standardized 19" frame for easy transportation and mobile deployment. The electronic parts were arranged in two 19" units, one for all power supplies and the other for compartments of electronic circuitry boards, with the controls and switches built on different front panels. These compartments can be easily

taken out for replacement or modification. All fluidic components, including the pump, valves, holding coils, interface and liquid containers, are fixed onto a perlex panel situated above the two electronic units. With this compact all-in-one design, it is very convenient to set-up the system on-site for monitoring applications. Furthermore, as the system is standardized in a 19" arrangement, facile duplication of the system is possible.

Several extensions and modifications have been made to the first SIA-CE-C⁴D design (reported in subsection 2.2) in order to incorporate the preconcentration procedure into the fully automated operation. The essential change is the employment of two holding coils at the same time, one for aspiration of sample (for separation without preconcentration) or eluent (for elution prior to separation) and the other as a reservoir to hold the solution eluted from the preconcentration cartridge before it is pumped to the interface for hydrodynamic injection. The fluid can be diverted either to the preconcentration column or to the SI-CE interface for injection and separation by using a Y-shape intersector and a 3-gate valve in addition to the solenoid isolation valves already used in the previous system. Another modification concerns the on-line acidification of the sample before loading onto the cartridge. The complex setup for on-line acidification, using multi syringes at the same time [120] is replaced with a much simpler approach, using only a graduated needle valve. To eliminate the problem of restriction of loading volume due to the limited capacity of the syringe, repetitive loading was employed.

To demonstrate the functionality of the developed system, four pharmaceuticals, namely ibuprofen, diclofenac, naproxen and bezafibrate, were selected as exemplary analytes. These pharmaceuticals are classified as environmental contaminants due to their high lipophilicity and low biodegradability. These drug residues in water were first preconcentrated on a C18 column. The eluent containing the desorbed drugs was then directly used as a sample for CE-C⁴D separation. The optimized background electrolyte (BGE) is composed of TRIS(hydroxymethyl)aminomethane 36 mM, lactic acid 20 mM and cyclodextrin HP- β -CD 1 mM. With this CE condition, calibration curves were acquired up to 100 μ M with very good correlation coefficient (r more than 0.997), with RSD % for migration times and peak areas of 1 - 1.5 % and 2.5 - 5%, respectively. For SPE preconcentration, selection of SPE cartridge, optimizations of procedures of sample acidification, sample loading and elution were carried out. HCl 0.1 N was used for sample acidification since only a minor amount (less than 1% v/v) of this strong inorganic acid solution is sufficient to keep the pH of the sample under the

pKa of these carboxylated drugs (less than 4), which in turn does not affect much the enrichment factor. It was found that complete elution of the retained drugs from the C18 column was achieved within 20 seconds using 500 μ L of the eluent containing TRIS 9 mM / Lactic acid 5 mM (62.5%) and acetonitrile (37.5%). In order to evaluate the potential for unattended operation, the system was set up for a supervised test run over a period of 10 hours per day over 3 continuous days, in which repeated preconcentrations with an enrichment factor of 30 and CE measurements of the pharmaceuticals (0.5 μ M prepared in DI water) were carried out. The maximum deviation is about ± 8 %, which is deemed acceptable considering that this deviation is the accumulation of minor and inevitable errors in different operations, *i.e.* sample loading, elution, injection and separation. The lifetime of the cartridge was further evaluated by carrying out preconcentrations in more severe conditions, in which solutions of pharmaceuticals were prepared directly in tap water matrix instead of in DI water and enrichment factor was set at 750. It was experimentally found that for good preconcentration performance, a loading volume of 1000 mL should not be exceeded. With preconcentration prior to CE-C⁴D determination, the concentrations of drug residues in water can be monitored down to the nM scale, which is impossible with normal CE setups.

1st project:

Capillary electrochromatography with contactless conductivity detection for the determination of some inorganic and organic cations using short monolithic octadecylsilica columns

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Capillary electrochromatography with contactless conductivity detection for the determination of some inorganic and organic cations using monolithic octadecylsilica columns

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ABSTRACT

A fast separation of alkali and alkaline earth metal cations and ammonium was carried out by capillary electrochromatography on monolithic octadecylsilica columns of 15 cm length and 100 μm inner diameter using water/methanol mixtures containing acetic acid as mobile phase. On-column contactless conductivity detection was used for quantification of these non-UV-absorbing species. The method was also extended successfully to the determination of small amines as well as of amino acids, and the separation selectivity was optimized by varying the composition of the mobile phase. Detection limits of about 1 μM were possible for the inorganic cations as well as for the small amines, while the amino acids could be quantified down to about 10 μM . The separation of 12 amino acids was achieved in the relatively short time of 10 min.

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1. Introduction

Capillary electrochromatography (CEC) as a hybrid technique combines some of the features of capillary electrophoresis (CE) and of liquid chromatography (LC). Both separation mechanisms occur concurrently, and this feature may be employed to achieve selectivities otherwise difficult to obtain. Transport of the analyte is due to electroosmotic and electrokinetic mobility, thus a flat flow profile is obtained, and band broadening is reduced compared to chromatography. A further advantage is the much lower instrumental complexity as high voltage power supplies are much simpler, and less expensive, than high pressure pumps. Since electrochromatography has to be carried out in columns of limited diameter, the amount of consumables (solvent) is also greatly reduced.

CEC may be carried out in capillaries filled with packing material as used in chromatographic columns. However, this requires the employment of frits, and this approach has been fraught with problems [1,2]. Open-tubular electrochromatography (OT-CEC), in which the stationary phase is coated on the inner wall of the capillary, overcomes these problems. However, due to the single layer of stationary phase, the capacity of OT-CEC is low, which adversely affects detection and this method has therefore seen limited use

[2,3]. The third option is to use monolithic columns. As the continuous structure is anchored to the capillary wall, retaining frits are not needed, the high porosity affords high chromatographic efficiency and allows a higher sample loading. This technique thus overcomes the disadvantages of packed-column CEC and of OT-CEC. The surface of the stationary phase may be modified to create tailored sites for interaction and desired charged moieties for the generation of electroosmotic flow.

Detection in CEC is usually achieved by UV-absorbance measurement. However, this method is not suited for all species. For CE capacitively coupled contactless conductivity detection (C^4D) has been gaining popularity in recent years [4] as it allows the determination of any charged species. The contactless approach is possible as external electrodes form an electrical capacitance with the internal electrolyte solution. This allows the coupling of an ac-voltage into and out of the detector cell. Details on the fundamental principles can be found for example in these publications [5–8] and several recent reviews are available [4,9–12]. Applications of C^4D have not been restricted to detection in CE, but have also been extended to the separation methods of ion chromatography [13] and HPLC [14–16] as well as to flow-injection analysis [17,18].

Applications of C^4D in CEC in general have been very limited to date. Hilder et al. communicated the determination of several inorganic anions using a column packed with a particulate ion-exchange material as stationary phase [19]. Detection was carried out directly on the column. Kubáň et al. gave an account of the

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determination of inorganic cations by OT-CEC using an anionic polymer wall-coating as stationary phase [20]. To our knowledge, the application of C^4D to CEC employing a monolithic stationary phase has not yet been reported.

2. Materials and methods

2.1. Chemicals and materials

Tetramethylorthosilicate (TMSO), poly(ethylene glycol) (PEG, $M_w = 10,000$), urea, diethylamine, dimethyloctadecylchlorosilane and methanol were purchased from Fluka (Buchs, Switzerland) and were of puriss grade. 2-Amino-1-butanol, 1-amino-2-propanol were obtained from Lancaster (Eastgate, White Lund, Morecambe, England). 1-Phenyl-ethylamine was purchased from Fluka and of analytical grade. 1,2-Dimethylpropylamine was purchased from Sigma–Aldrich (Buchs, Switzerland). Toluene was from TCI (Zwijndrecht, Belgium). The chemicals for the preparation of background electrolytes (BGE), and the amino acids were of analytical grade and purchased from Fluka. Fused-silica capillaries (100 μm inner, 365 μm outer diameter) were purchased from BGB Analytik AG (Boeckten, Switzerland). The commercial monolithic capillary column, RP-18, end-capped, with a length of 150 mm, an inner diameter of 100 μm , and an outer diameter of 365 μm , was purchased from Merck (Dietikon, Switzerland). All stock and BGE solutions were prepared with deionised water with a resistivity higher than 18 $M\Omega\text{ cm}$. The stock solutions of inorganic cations (5 mM) were prepared from their corresponding chloride salts (Merck, analytical grade). All standard solutions were prepared by diluting the stock solutions to the desired concentrations with the separation buffer. All solutions were filtered through 0.2 μm PTFE membrane filters (Chromafil O-20/15 MS, Macherey-Nagel, Oensingen, Switzerland), and degassed in an ultrasonic bath for 5 min before injection into the capillary.

2.2. Instrumentation

2.2.1. Preparation of self-made monolithic octadecylsilica capillaries

The preparation of monolithic silica gel for capillary HPLC and the factors affecting this process were described exhaustively by Ishizuka et al. [21,22], Guiochon [23] and Nakanishi et al. [24]. The coating process of octadecyl groups (C18) onto the monolithic silica layer was also described previously by Tanaka et al. [25] and Yang et al. [26]. Accordingly, the preparation procedure was carried out as follows: tetramethoxysilane (TMSO, 0.8 mL) was added into a solution of poly(ethylene glycol) (PEG, $M_w = 10,000$, 0.176 g) and urea (0.18 g) in 2 mL acetic acid (0.01 M). The mixture was stirred at 0 °C for 40 min until a homogeneous solution was obtained. This solution was then pumped through a fused-silica capillary tube (i.d. of 100 μm and length of 120 cm) that had already been treated with 1 M NaOH solution for 3 h at 40 °C, and allowed to “age” at 40 °C for 24 h. The monolithic silica column formed was put into an oven at 120 °C for 3 h and then rinsed with water and methanol subsequently. The column was dried by flushing with nitrogen and left in an oven at 70 °C for 3 h. After drying, heat-treatment was carried out at 330 °C for 24 h, followed by a rinse with water and then methanol.

The column produced was then cut into 3 smaller pieces of 40 cm length due to the high backpressure when pumping octadecyldimethyl-N,N-diethylaminosilane (ODS-DEA) solution through a long monolithic capillary. The solution of ODS-DEA was prepared by placing 1 g octadecyldimethylchlorosilane (ODS-Cl) into a mixture of 1 mL diethylamine and 4 mL toluene, followed by stirring continuously at 50 °C for 1 h. The mixture was then passed

through a PTFE 0.2 μm membrane filter to obtain a clear solution of ODS-DEA. ODS-DEA was pumped through a 40 cm long monolithic silica capillary for 3 h at 60 °C. The column was then washed again with methanol and then with water. Both ends of the final capillaries were removed (5 cm at each end), and the remainder cut into 2 columns with a length of 15 cm each.

2.2.2. CEC- C^4D system

A purpose-built CE- C^4D system was used for column checking and all separations. This instrument is based on a high voltage power supply with interchangeable polarity (CZE 1000R) from Spellman (Pulborough, UK). The capacitively coupled contactless conductivity detector used was built in-house, and is based on two tubular electrodes of 4 mm length which are separated by a gap of 1 mm and a Faradaic shield. Details on this detector can be found elsewhere [27–29]. The resulting signal was recorded with a MacLab/4e data acquisition system (AD Instruments, Castle Hill, Australia).

The columns were mounted horizontally on a perspex sheet together with the detector cell and the containers at the two ends which hold the electrodes for application of the high voltage. The cell was mounted 1 cm from the capillary end. In other words, the effective and total lengths for capillaries used were 14 and 15 cm, respectively. For safety, the assembly was placed into a perspex cage, which was fitted with a microswitch to interrupt the high voltage on opening. A voltage of +5 kV was applied for all separations. Standards were injected electrokinetically using a voltage of +1.5 kV for 3 s after stability of the baseline had been ascertained.

3. Results and discussion

3.1. Quality evaluation of the self-made capillary with C^4D

After a preliminary check with a microscope, the longitudinal homogeneity of the self-made monolithic capillary was compared with that of an open capillary and the commercial capillary column, using C^4D , for further quality assessment. The technique had been used previously for checking the homogeneity of the coating applied to a commercial monolithic column [30], and of the uniformity of a packed column [31]. The columns were filled with an aqueous electrolyte solution of 20 mM CH_3COOH and conditioned by applying a high voltage of 5 kV until a stable current was observed. This took about 5 min. The capillaries were then moved through the detector and the magnitude of the output signal of the contactless conductivity detector was recorded every 5 mm along the length. The magnitude of this signal is a measure for the total ionic conductivity between the electrodes which not only depends on the concentration of the ions, but also on the fraction of the volume taken up by the ion bearing solution. For a dry capillary the signal is negligible. The amplitude of the signal therefore gives an indication of the density of the monolithic structures and the approach is thus a facile method to evaluate the porosity of the columns. The results obtained are shown in Fig. 1. Two important conclusions can be drawn from the data. Firstly, it is seen in the figure, that for both monolithic columns the signal is clearly reduced compared to the open capillary, but that the porosity of the commercial column is slightly lower (appr. 76%) than that of the column made in-house (appr. 85%) as the conductivity signal is lower for the former. Secondly, the signal variation along the axis allows conclusions regarding the longitudinal homogeneity of the monolithic structures as the columns were filled with a solution of even ionic concentration. Clearly, the consistency of the in-house made column is not quite as good as that of the commercial one as indicated by the variation in the signal amplitude along the capillary, but

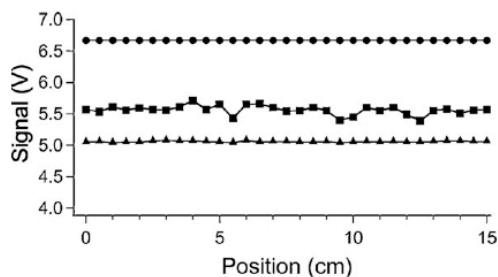


Fig. 1. Homogeneity comparison between the C18-silica monolithic column made in-house (■), commercial monolithic column (▲) and open-tubular (●) capillary. Electrolyte inside the capillaries: 20 mM CH_3COOH in water.

these fluctuations are within a few percent and not considered significant.

3.2. Determination of some inorganic cations

The selection of the mobile phase for CEC with conductivity detection is critical as the requirements for electrophoresis and for the ion pair chromatographic process have to be satisfied as well as those for conductivity detection. It must be compatible with the stationary phase, have adequate elution strength and be of low specific conductance in order to allow high sensitivity in conductometric detection and to minimize Joule heating. Note, that conductometric detection is more sensitive to heating effects than other detection methods because of the relatively high temperature coefficient of ionic conductivity. Several buffer systems frequently used for HPLC were briefly tested, namely water/methanol mixtures containing trifluoro-acetic acid, phosphate buffers, tris(hydroxymethyl)aminomethane, hydrochloric acid, citric acid and formic acid, but these were all found not to give stable baselines, presumably in the majority of cases due to Joule heating caused by too high a conductivity. A buffer based on 2-(*N*-morpholino)ethanesulfonic acid and histidine (MES/His), which is widely used in CE with conductivity detection, was also found to be problematic as it tended to cause blockage of the monolithic column. This is thought to be caused by precipitation of histidine occurring on evaporation of the solvent mixture at the ends of the columns during the inevitable periods when they need to be handled outside of the buffer containers. A mobile phase consisting of acetic acid in a water/methanol mixture was observed to generally give more stable baselines. The optimization for the separation of inorganic cations thus consisted of finding the best concentration of acetic acid in an appropriate ratio of methanol to water. However, it was still found necessary to carefully control the applied voltage as evidenced by Ohm's plot studies. The voltage applied to the 15 cm long columns when using acetic acid based electrolyte solutions had to be restricted to a maximum of about 5 kV in order to prevent instability due to thermal effects, but the exact limit depended on the buffer composition.

The proportion of methanol in the mobile phase was found to strongly affect the retention time of the analytes. As seen in Fig. 2, for separations carried out on the monolithic C18-column made in-house, the analytes are more strongly retained for the higher percentages of methanol, and therefore also the separation is improved. However, the peak areas were also found to be dependent on the methanol content. Note, that the first separation shown in the figure was carried out with only half the concentrations of the cations of the subsequent runs. For the highest methanol content in the mobile phase, the peaks even practically disappeared as seen in electropherogram (d). The change in conductivity for the analyte peaks is governed by the Kohlrausch regulating function (which in

turn is dependent on the mobility of all ionic species involved) as well as the degree of dissociation of acetic acid, and therefore not intuitively predictable for the partly aqueous medium. At a fixed concentration of acetic acid, when the proportion of methanol is increased, the degree of dissociation of acetic acid is decreased. This must be responsible for the change in peak area, but also leads to a reduction of background conductivity as evidenced by the decrease in current through the capillary from 3 to $0.4 \mu\text{A}$ for the change of methanol content from 20 to 70%. The baseline drift in electropherogram 2(a) illustrates the effect of excessive Joule heating on detection caused by too high a background conductivity. This is due to the higher susceptibility of $\text{C}^{4\text{D}}$ to thermal drifts compared to other methods of detection. The experimental data of Fig. 2 indicates that a high fraction of methanol is not favourable for detection without adjusting the concentration of acetic acid.

A further investigation was thus carried out by varying the concentration of acetic acid for different proportions of methanol. Three electropherograms obtained for 40, 50 and 60% methanol which represent the optimum concentrations of acetic acid for these methanol levels in terms of separation are shown in Fig. 3. Note that Fig. 3(a) is identical to Fig. 2(b) but is reproduced here to facilitate a direct comparison in terms of migration times and peak separation. It is evident, that all tested cations, including NH_4^+ and K^+ , can be well separated using a mobile phase consisting of 40 mM acetic acid in a 50% (v/v) methanol/water-mixture. However, the sensitivity is not at the maximum for these conditions. For simple samples with few of the ions present, different conditions which give higher sensitivity, or faster analysis times, may be suitable.

A further investigation of the column made in-house is documented in Fig. 4. For comparison, the separation was carried out by electrophoresis alone, in an open capillary with the identical length of 15 cm, and by equally applying a voltage of 5 kV. As shown in Fig. 4(a), the separation in an aqueous background electrolyte by electrophoresis alone under these conditions is inadequate, as almost complete overlaps of the peaks for the NH_4^+/K^+ and $\text{Na}^+/\text{Mg}^{2+}$ pairs was found. When carrying out the electrophoretic separation in the same partly methanolic acetic acid solution as used for the CEC experiment, see electropherogram 4(b), the peaks are found to be delayed compared to the purely aqueous solution, presumably due to a reduction of the electroosmotic flow, but again the separation is only partial.

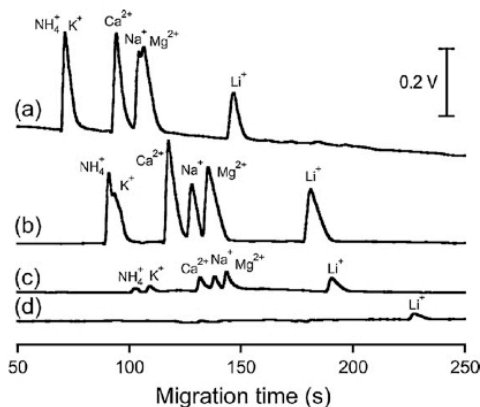


Fig. 2. Influence of the concentration of CH_3OH in the background electrolyte solution containing 20 mM CH_3COOH on the separation of inorganic cations. Capillary: self-made C18-silica monolithic column (15 cm total length, 14 cm to detector \times 100 μm i.d.); separation voltage: 5 kV; electrokinetic injection: 3 s/1.5 kV. (a) 20% (v/v) CH_3OH , 50 μM cations, $V=5$ kV, $I=3.0 \mu\text{A}$. (b) 40% (v/v) CH_3OH , 100 μM cations, $V=5$ kV, $I=1.5 \mu\text{A}$. (c) 60% (v/v) CH_3OH , 100 μM cations, $V=5$ kV, $I=0.7 \mu\text{A}$. (d) 70% (v/v) CH_3OH , 100 μM cations, $V=5$ kV, $I=0.4 \mu\text{A}$.

Table 1
Performance parameters for determination of amines with the commercial column.

	Calibration range ^a (μM)	Correlation coefficient r	LOD ^b (μM)	Reproducibility peak area ^c %RSD	Reproducibility retention time ^c %RSD
Methylamine	5–100	0.9996	1.5	1.4	0.46
Dimethylamine	5–100	0.9989	2.5	2.9	0.44
Trimethylamine	10–100	0.9992	3.0	2.8	0.67
Diethylamine	5–100	0.9991	2.5	4.0	0.30
1-Amino-2-propanol	5–100	0.9979	1.0	3.4	0.47
1,2-Dimethyl-propylamine	5–100	0.9975	2.0	4.6	0.26
2-Amino-1-butanol	5–100	0.9992	1.0	2.4	0.24
1-Phenyl-ethylamine	5–100	0.9987	1.5	1.9	0.61

^a 5 concentrations.

^b Based on peak heights corresponding to 3 times the baseline noise.

^c Intra-day, $n = 3$.

The remaining two traces of Fig. 4 represent a comparison of the CEC separation of the 6 cations on the two different monolithic columns available. It was found that the two separation columns behaved quite differently, even though they were both monolithic C18-columns of identical length. Part of the reason must be the differences in the monolithic structures (density and homogeneity) as documented in Fig. 1. It can also be assumed that the density of the C18-coating on the monoliths differed. An independent optimization of the buffer composition was carried out for the commercial column as described above, and the two traces of Fig. 4(c) and (d) represent CEC separations for conditions individually optimized for best separation on the purpose made and commercial columns respectively. Complete baseline separation was possible by CEC for the 6 cations tested for the column made in-house, while for the commercial column a partial overlap between Ca^{2+} and Na^+ could not be completely resolved even for the best conditions. Nevertheless, the results clearly indicate the potential of monolithic CEC with C^4D for achieving fast separations which are not possible by electrophoresis alone (compare electropherograms 4(a) and (b)) under similar conditions.

Quantitative data was acquired for the self-made column using the buffer consisting of 40 mM acetic acid in 50% (v/v) methanol in water. Calibration curves were determined in the range from 5 to 50 μM for NH_4^+ and K^+ , from 5 to 100 μM for Na^+ and Ca^{2+} and from 2 to 100 μM for Mg^{2+} and Li^+ . Linear correlation coefficients, r , from 0.9975 to 0.9991 were obtained. Limits of detection (LODs), based on peak heights corresponding to 3 times the baseline noise, were determined for two of the ions, namely Mg^{2+} and Li^+ and found

to be 0.5 and 1 μM , respectively. These values are close to results obtained with a similar detector in CE using open tubings [28].

3.3. Determination of small amines

Preliminary trials on the use of CEC- C^4D for the determination of organic ions were carried out using the mobile phase employed for the inorganic cations with methyl-, dimethyl- and trimethylamine as model substances. These species are present in protonated form under the conditions used, and separation and detection were successful for both columns. A more thorough investigation was thus conducted by including 1-amino-2-propanol, 2-amino-1-butanol, 1,2-dimethylpropylamine, diethylamine, and 1-phenyl-ethylamine in the standard mixture. The compounds are often used as intermediates in the synthesis of pharmaceutical drugs. In order to achieve best separation of the 8 species, an optimization of the composition of the mobile phase was again carried out systematically by adjusting the methanol to water ratio and the level of acetic acid as discussed above for the inorganic cations. The results for optimized conditions are illustrated in Fig. 5. As can be seen, the majority of the compounds can be separated rapidly with both columns. However, complete baseline separation of all ions, namely the distinction between 1,2-dimethylpropylamine and 2-amino-1-butanol, can again only be achieved with one of the monoliths, the commercial column in this case. Note, that again the optimized conditions differ for the two columns. Calibration data

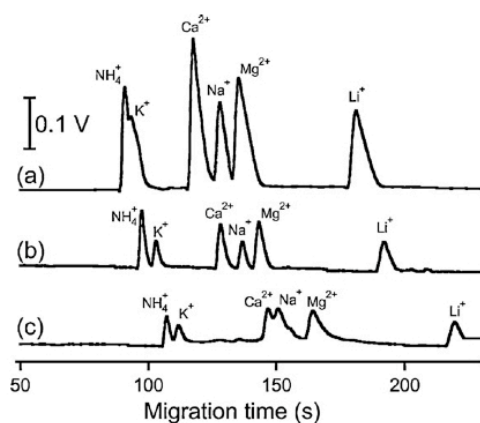


Fig. 3. Separation of inorganic cations (100 μM) at different concentrations of acetic acid and methanol. (a) 20 mM CH_3COOH , 40% (v/v) CH_3OH . (b) 40 mM CH_3COOH , 50% (v/v) CH_3OH . (c) 80 mM CH_3COOH , 60% (v/v) CH_3OH . Other conditions as for Fig. 2.

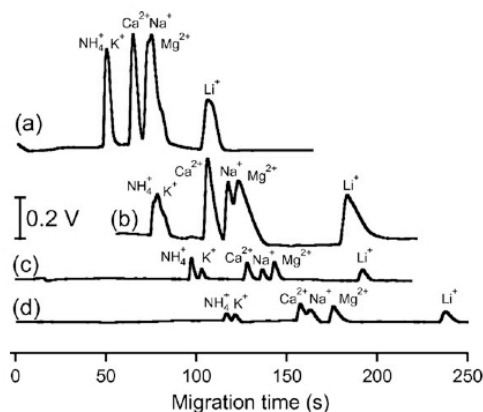


Fig. 4. Separation of inorganic cations by CE and CEC, using (a) an open-tubular capillary of 15 cm length and 40 mM CH_3COOH in water, (b) an open-tubular capillary of 15 cm length and 40 mM CH_3COOH in 50% (v/v) CH_3OH , (c) the self-made C18-silica monolithic column of 15 cm length and 40 mM CH_3COOH in 50% (v/v) CH_3OH , (d) commercial monolithic column of 15 cm length and 80 mM CH_3COOH in 55% (v/v) CH_3OH . Other conditions as for Fig. 2.

Table 2
Performance parameters for determination of amino acids with the commercial column.

	Calibration range ^a (μM)	Correlation coefficient <i>r</i>	LOD ^b (μM)	Reproducibility peak area ^c %RSD	Reproducibility retention time ^c %RSD
Lysine (Lys)	40–500	0.9954	7.5	3.0	0.24
Arginine (Arg)	40–500	0.9988	7.5	2.9	0.22
Histidine (His)	40–500	0.9975	7.5	2.6	0.21
Glycine (Gly)	62.5–500	0.9973	10	2.9	0.37
Alanine (Ala)	62.5–500	0.9941	10	2.7	0.35
Valine (Val)	62.5–500	0.9966	15	2.3	0.46
Leucine (Leu)	62.5–500	0.9987	10	2.6	0.39
Serine (Ser)	62.5–500	0.9988	15	5.4	0.60
Threonine (Thr)	62.5–500	0.9916	15	5.0	0.58
Phenylalanine (Phe)	62.5–500	0.9985	20	5.6	0.66

^a 5 concentrations.

^b Based on peak heights corresponding to 3 times the baseline noise.

^c Intra-day, *n* = 3.

was acquired for all compounds for the commercial column using the buffer consisting of 60 mM acetic acid in 40% (v/v) methanol in water and the results are summarized in Table 1. Quantification of the 6 species which could be resolved on the in-house made column was also carried out using the latter, and the results obtained were very similar to those for the commercial product. As can be seen, the detection limits of approximately 1 μM achieved for these small organic ions match those for the inorganic cations.

3.4. Determination of amino acids

The use of conductivity detection for the quantification of amino acids is attractive as most of these important analytes cannot be detected by direct optical means. The determination of amino acids by CE-C⁴D [32–34] as well as HPLC-C⁴D, using either packed columns [14,15] or a monolithic capillary [30], has been reported. The best quantification with C⁴D is achieved in a mobile phase containing acetic acid at a pH-value around 2 to ensure that the amino acids are present in their fully protonated states and can thus be determined as cations [32]. Optimization for CEC was thus done with acetic acid at a low pH-value with different proportions of methanol, using the commercial monolithic column. The results obtained for a standard mixture of 12 amino acids with the best conditions arrived at are shown in Fig. 6, together with a purely

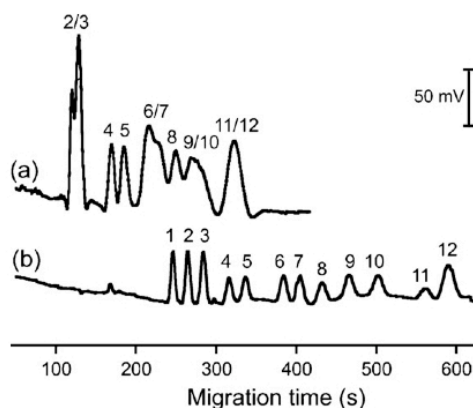


Fig. 6. Separation of 12 underivatized amino acids with the commercial monolithic column and an open-tubular capillary. (a) Open-tubular capillary of 15 cm length, 2 M CH_3COOH in water (pH 2.25); 125 μM for all amino acids except for Tyr and Asp (250 μM). (b) Commercial monolithic column, 20% (v/v) CH_3COOH in 40% (v/v) CH_3OH (pH 2.25); 500 μM for all amino acids except for Tyr and Asp (1 mM). Other conditions as for Fig. 2. Peak denotation: (1) Lys; (2) Arg; (3) His; (4) Gly; (5) Ala; (6) Val; (7) Leu; (8) Ser; (9) Thr; (10) Phe; (11) Tyr; (12) Asp.

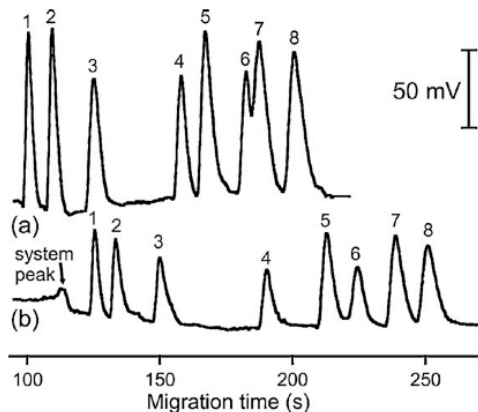


Fig. 5. Separation of 8 amines (50 μM) with the self-made and commercial monolithic columns at their optimized conditions. (a) Self-made column, 20 mM CH_3COOH in 40% (v/v) CH_3OH (pH 3.5). (b) Commercial column, 60 mM CH_3COOH in 50% (v/v) CH_3OH (pH 3.4). Other conditions as for Fig. 2. Peak denotation: (1) methylamine; (2) dimethylamine; (3) trimethylamine; (4) diethylamine; (5) 1-amino-2-propanol; (6) 1,2-dimethylpropylamine; (7) 2-amino-1-butanol; (8) 1-phenyl-ethylamine.

electrophoretic separation with an open capillary shown for comparison.

Clearly, the CEC-approach can resolve the selectivity limitation apparent for the purely electrophoretic separation in the short capillary employed. Although the separation of all 20 essential amino acids is possible by CE-C⁴D, a significantly longer analysis time of about 30 min is required [32]. The quantitative data determined for 10 of the amino acids using the commercial column and a buffer consisting of 20% (v/v) acetic acid in 40% (v/v) methanol in water is given in Table 2. The detection limits for these species were found to be within a concentration interval from 7.5 to 50 μM . These values are about half an order of magnitude higher than detection limits obtained in HPLC with the same detector [14]. It is assumed that the reason for these values being higher than for the other analytes reported herein, is the fact that a higher concentration of acetic acid had to be used, leading to a higher background signal, and hence a more significant noise level.

4. Conclusions

Contactless conductivity detection for electrochromatography conducted in monolithic capillary columns was explored; to our knowledge for the first time. A complete validation of quantitative aspects was not intended. The results demonstrate the potential of

the method. The separation of inorganic cations, as well as small amines and amino acids was found possible on octadecylsilica monoliths, and the method is deemed to be generally useful for applications where the fast determination of non-UV-absorbing species is desired but purely electrophoretic separation does not have adequate efficiency or is not fast enough. It is presumed that these benefits can also be obtained for inorganic and organic anions using appropriate conditions.

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2nd project:

Capillary electrophoresis with contactless conductivity detection coupled to a sequential injection analysis manifold for extended automated monitoring applications

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Capillary electrophoresis with contactless conductivity detection coupled to a sequential injection analysis manifold for extended automated monitoring applications

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ABSTRACT

A capillary electrophoresis (CE) instrument with capacitively coupled contactless conductivity detection (C⁴D) based on a sequential injection analysis (SIA) manifold was refined. Hydrodynamic injection was implemented to avoid a sampling bias by using a split-injection device based on a needle valve for precise adjustment. For safety and reliability, the integrity of the high voltage compartment at the detection end was fully maintained by implementing flushing of the high voltage interface through the capillary. With this set-up, extended fully automated monitoring applications are possible. The system was successfully tested in the field for the determination of the concentration levels of major inorganic cations and anions in a creek over a period of 5 days.

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1. Introduction

Instrumentation for capillary electrophoresis (CE) is much more simple than for column chromatography as the separation is achieved by the relatively straightforward application of voltages. High pressure pumps and eluents are not needed and the consumption of chemicals is very low. A significant further simplification was also brought about by the introduction of contactless conductivity detection (C⁴D), which, with the exception of a simple measuring cell based on a pair of short tubular electrodes, is fully electronic and thus less demanding in construction and power consumption than the common optical detection methods employing UV-radiation. For reviews see for example [1,2]. Field portable CE-instruments employing C⁴D have therefore been developed in our research group [3,4] and Hutchinson et al. [5] have demonstrated that a portable instrument may be employed for the identification of post-blast residues of IEDs (improvised explosive devices).

Capillary electrophoresis furthermore has potential for extended on-site measurement applications, such as in environmental monitoring or in process control. The coupling of conventional CE-instruments with flow-injection analysis (FIA) manifolds for sample handling ahead of the separation step has

indeed been reported for such applications. Arce et al. [6] have reported a system with analyte preconcentration for use in a water purification plant using a commercial CE-Instrument and indirect optical detection, and Sirén et al. [7] have reported an assembly for monitoring use in a paper mill.

Nevertheless, commercial CE-instruments designed for the laboratory are not well suited for on-site deployment and coupling to external sample handling manifolds. It is, on the other hand, relatively easy to construct a CE-separation unit as part of an extended FIA-manifold and such systems have been constructed by several researchers (see the recent review by Kubáň and Karlberg [8]). The use of C⁴D is also attractive for such a set-up and FIA-CE-C⁴D instruments have been reported [9–11]. Sprung et al. [10] detailed the construction of a system for on-line measurements, and Kubáň et al. [11] have demonstrated the on-line field monitoring of the drainage of a pasture for some inorganic anions and cations. The use of a sequential injection analysis (SIA) system, based on a syringe pump and a multi-position valve, instead of an FIA-manifold typically using a peristaltic pump, has several advantages, such as allowing sample pretreatment and automated flushing of the separation capillary. This combination was introduced by Růžička and coworkers in 2002 [12,13] who used UV-detection. Kulka et al. [14] reported a similar system in 2006 and Horstkotte et al. [15,16] demonstrated the determination of nitrophenols. Zacharis et al. [17] designed an SIA-CE-instrument employing laser-induced fluorescence for detection. Wuersig et al. [18] used an SIA set-up to

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achieve fast injections into capillaries of only a few centimeters in length and could thus demonstrate the separation of inorganic cations and anions in approximately 10 s. $C^{4}D$ was employed in the latter case for detection.

In this contribution we present an SI–CE– $C^{4}D$ system designed for monitoring applications over extended unattended periods and demonstrate its functionality in several days of on-site monitoring of the concentrations of inorganic anions and cations in a creek. The new set-up incorporates a number of improvements compared to previous designs in order to achieve high reliability and specific adaptations to allow autonomous operation.

2. Experimental

2.1. Chemicals and materials

All chemicals were of reagent grade. Deionised water (Millipore, Bedford, MA, USA) was used throughout the experiments. Stock solutions (5 mmol L^{-1}) of chloride, nitrate, sulfate, nitrite, fluoride and phosphate were prepared from their potassium or sodium salts. Stock solutions (5 mmol L^{-1}) of ammonium, potassium, calcium, sodium, magnesium and lithium ions were prepared from their chloride salts. All chemicals were purchased from Fluka (Buchs, Switzerland) or Merck (Darmstadt, Germany). All multi-ion standards were prepared from these stock solutions. The separation buffer consisted of 12 mM L-histidine and 2 mM 18-crown-6 adjusted to pH 4 with acetic acid. The capillary was preconditioned with 1 M NaOH for 10 min and deionised water for 10 min prior to flushing with electrolyte solution (for 1 h). The capillary was then used continuously for successive analyses.

2.2. Instrumentation

The SIA manifold is based on a syringe pump (Cavro XLP 6000) and a 6-port channel selection valve (Cavro Smart Valve) (both purchased from Tecan, Crailsheim, Germany). The SIA–CE interface consists essentially of two consecutive T-junctions for connecting the capillary and electrophoretic ground electrode to the liquid channel. It was machined in a perspex block ($2 \text{ cm} \times 2 \text{ cm} \times 3 \text{ cm}$); details have been given previously [19]. The micro-graduated splitting valve was obtained from Upchurch Scientific (P-470, Oak Harbor, WA, USA) and the isolation valves for pressurization from NResearch (HP225T021, Gümligen, Switzerland). All fluid connections to the selector valve were made with 0.02 in. inner diameter (id) and 1/16 in. outer diameter (od) Teflon PFA tubing (Upchurch Scientific) with the exception of the connection between the selector valve and the SIA–CE interface where 0.01 in. id and 1/16 in. od PEEK tubing (4 cm) was used in order to minimize dispersion.

The electrophoresis section is based on a dual polarity high voltage power supply (Spellman CZE2000, Pulborough, UK) with $\pm 30 \text{ kV}$ maximum output. Polyimide coated fused silica capillaries of 50 μm id and 363 μm od from (Polymicro, Phoenix, AZ, USA) were used for separation. The detection end of the capillary was connected with a fitting to a perspex block, which contains a channel of 0.4 mm id and 2 cm length at the end of which the high voltage electrode is placed. This assembly was isolated in a safety cage made from perspex, which was equipped with a microswitch to interrupt the high voltage on opening. Detection was carried out with a $C^{4}D$ -cell built in-house, and is based on two tubular electrodes of 4 mm length, which are separated by a gap of 1 mm and a Faradaic shield. Details on this detector can be found elsewhere [20]. The resulting signal was recorded with an e-corder 201 data acquisition system (eDAQ, Denistone East, NSW, Australia) connected to the USB-port of a personal computer.

For the field test the instrument was set up in a pumping station located next to the creek Kleine Aa, a tributary to Lake Sempach, Switzerland. A submersible pump conveyed a constant stream of water from the creek ($\sim 2 \text{ L s}^{-1}$) into an overflowing bucket, from where a small part was diverted with a peristaltic pump to a beaker of 20 mL which was also overflowing (the excess was collected with a funnel underneath and led to a drain). Samples were aspirated into the SI-manifold from this beaker through an inlet filter (10 μm pore size, Supelco, Buchs, Switzerland) in order to remove suspended matter. Occasional water samples were collected, filtered with 0.45 μm membrane filters, and inorganic anions and cations analyzed later in the laboratory by ion-chromatography (IC, Metrohm, Switzerland) within 2 days.

2.3. System control

The system was controlled with the personal computer using an RS232-serial connection to the syringe pump. The multi-position valve is daisy-chained to the syringe pump. Auxiliary TTL-output ports on the two units allow switching of the stop-valves, of the high voltage, the polarity of the high voltage and triggering of the recording of electropherograms with the help of a purpose built electronic interface. The two solenoid operated isolation valves were controlled via a special driver board obtained from the supplier of the valves (CoolDriver, 225D5X12, NResearch). The programming package LabVIEW (version 8.0 for Windows XP, from National Instruments, Austin, TX, USA) was used to write the control code for the SIA–CE system. Different modules were written to independently carry out tasks such as injection, flushing, separation etc. All modules were then assembled together to produce the instruction protocol for the entire analytical method. The program can be modified easily during the optimization steps or during setting up of the system.

3. Results and discussion

3.1. System design

A schematic drawing of the system is depicted in Fig. 1. The SI-manifold consists of the standard arrangement based on a two-way syringe pump and a multiport valve with a holding coil between the two units. This is used for the initial conditioning of the capillary by flushing with sodium hydroxide solution, rinsing the system with the separation buffer, and aspiration of a plug of the sample solution and passing this volume to the capillary inlet. Injection proper is carried out hydrodynamically. The volumes injected in capillary electrophoresis are in the nanoliter range and too small for direct handling with the SI-manifold. Thus only part of the dispensed sample plug is injected into the separation tubing by pressurization of the interface while pushing the sample plug past the capillary inlet. This is more difficult to implement than electrokinetic injection, but a sampling bias, which would arise with the latter mode, is avoided. Separation is carried out by applying the high voltage from the detection end, the second electrode in the SI–CE interface is grounded.

Two modifications have been made to the set-up compared to earlier designs [15,16,18]. The first of these concerns the method used for pressurization of the SI–CE interface for injection and flushing of the capillary. Previously, a length of flexible tubing was connected to the outlet of the interface, the end of which was closed with a valve for injection. Solution would thus be pumped into the expanding piece of tubing leading to a gradually increasing pressure. The performance of this set-up would depend on the length and the condition of the tubing. However, predictability and reproducibility are poor, and only one setting is possible which has to be

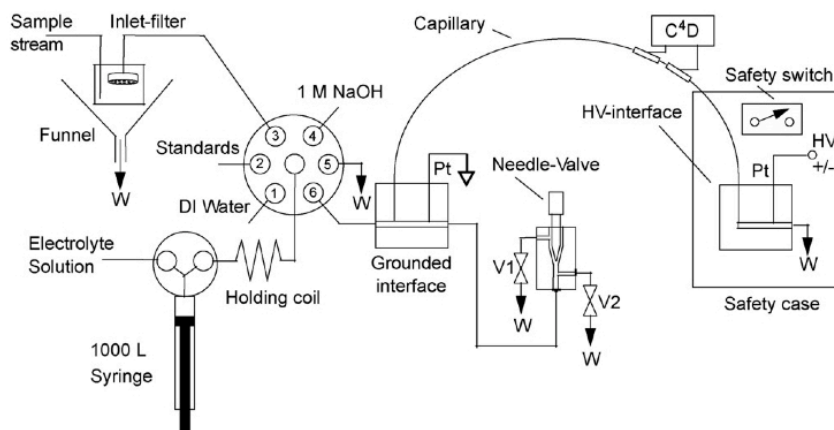


Fig. 1. Schematic drawing of the SI-CE-C⁴D system (not to scale). C⁴D: capacitively coupled contactless conductivity detector; HV: high voltage power supply; W: waste; Pt: platinum electrode; E: electrolyte solution. The high voltage interface is flushed through the capillary.

used for the two different tasks of injection and capillary flushing. The arrangement was thus replaced with an adjustable needle valve designed for the splitting of small flows into two streams (see Fig. 1). A graduated micrometer screw allows precise and reproducible setting of the splitting ratio and thus the backpressure created for injection on closing the solenoid operated isolation valve 1 (designated as V1 in the figure). Simultaneous closure of both valves (V1 and V2) allows fast capillary flushing.

The second modification concerns the detection end of the separation capillary where the high voltage is applied. In CE in general, when the separation voltage is applied, electrolysis occurs at the electrodes at both ends of the capillary, thus changing the ionic composition of the adjacent solutions. As this electrolyzed buffer can move through the capillary by electrokinetic and electroosmotic flow, it is frequently necessary not only to rinse the capillary but also to change the solutions in the containers at both ends. When carrying out long-term unattended operation, these steps need to be automated. At the grounded injection end, buffer replacement is trivial to implement with the SIA set-up, but this is not so easy at the high voltage end. In the previous system reported by our research group [18], which was not intended for long-term unattended operation, the buffer solution was simply changed manually. Horstkotte et al. [15,16] implemented automated flushing at the high voltage end by leading a separate tube from the multi-selection valve of the SI-manifold to the high voltage side and, in order to achieve electrical isolation, buffer solution was passed to the interface by letting it fall dropwise into it from above. In preliminary experiments, a similar system was set up in our laboratory. However, at least in our hands, such an arrangement was found not to be entirely satisfactory. While the configuration would work fine for some time, unpredictable electrical arcing was found to occur at times in dependence on air humidity, conditions of sur-

faces and the voltage level employed. As the electrolyte filled tubing to the selection valve constitutes a low conductivity electrical path, such arcing was then found to lead to the destruction of part of the electronic instrumentation. It was thus deemed essential to completely isolate the interface at the high voltage end of the capillary by enclosing it on all sides and maintaining complete integrity of this cage in order to be able to use high separation voltages. A special interface with minimal internal volume was thus designed in order to allow efficient flushing of the liquid volume at the high voltage electrode directly through the capillary. The arrangement essentially consists of an 'ion-delay' channel with a round cross-section of 0.4 mm diameter and 2 cm length between the end of the capillary and the high voltage electrode. The greatly enlarged cross-section compared to the internal channel of the capillary caused minimal field strength in this section of the separation manifold, and migration of any constituents from the solution surrounding the electrode back to the detector was not achieved within the time scale of a single separation. For flushing of the capillary and the high voltage interface, both outlets of the splitting valve were blocked by actuating both isolation valves (V1 and V2) in order to push all of the dispensed fluid through the separation tubing. In order to be able to carry this out relatively rapidly, isolation valves which can hold pressures up to 100 psi (~6 bar) were employed. Excess liquid was collected within the safety cage.

3.2. Operation

An overview of a typical sequence of the operations is given in Table 1. The protocol starts with an uptake of electrolyte solution from the reservoir. Rinsing steps follow for the flushing of the capillary and the high voltage interface. Note, that the flushing through the capillary has to be carried out at a relatively low flow rate in

Table 1
Typical operation sequence.

Step	Operation	Position of selection valve	Volume dispensed (μL)	Flow rate (μLs^{-1})	V1	V2
1	Buffer aspiration	–	900	20	Closed	Closed
2	Flushing of the SI-CE interface	6	100	20	Open	Open
3	Flushing of capillary and HV-interface	6	25	0.2	Closed	Closed
4	Backflush of the inlet filter	3	400	117	Open	Open
5	Pick up of sample	3	15	20	Closed	Closed
6	Sample injection	6	15	4	Open	Closed
7	Flushing of the SI-CE interface	6	100	20	Open	Open
8	Electrophoretic separation	6	Variable	67	Open	Open
9	Flushing of the SI-CE interface	6	50	20	Open	Open

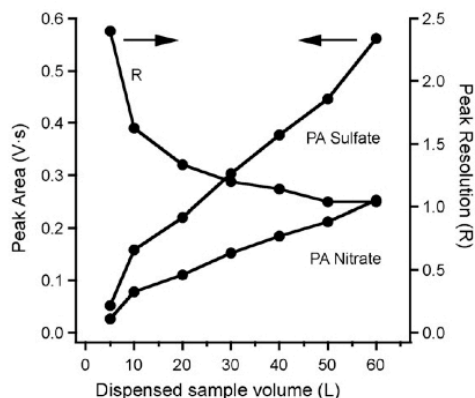


Fig. 2. Effect of dispensed sample volumes on peak area and resolution. Analytes: NO_3^- , SO_4^{2-} 50 μM in deionised water. Background electrolyte: His 12 mM, 18-crown-6 2 mM adjusted to pH 4 with acetic acid. Pumping rate: 4 $\mu\text{L s}^{-1}$. CE conditions: capillary 50 μm id, $l/l = 35 \text{ cm}/60 \text{ cm}$, $U = 20 \text{ kV}$.

order not to exceed the holding pressure of the valves, but can be carried out in just over 2 min. Subsequently, a backflush of the inlet tube and filter is performed removing previous sample from the tube and particles left on the outer surface of the inlet filter, thus preventing blockage due to accumulation of solids. Backflush of the filter is not needed for aspiration of standards, which were passed to the system via an alternate port of the selection valve. For sequential analysis of anions and cations, all steps in the table are repeated, with the polarity of the applied high voltage automatically switched prior to step 1 of each sequence.

The most critical step is the controlled and reproducible injection of sample into the capillary. The volume injected has to be optimized and a suitable compromise has to be found between sensitivity (peak area) and selectivity (peak resolution). Large volumes lead to high peak areas, but may also cause an overlap of peaks. The amount injected is determined by the pressure, length of the sample plug passing through the SI–CE interface and the flow velocity in the interface. These parameters in turn are controlled by the pumping rate of the syringe, the backpressure as adjusted by the splitting valve, and the dispensed sample volume. Thus an optimization has to be carried out by variation of these partially interdependent parameters. For this task, a standard of two inorganic anions (NO_3^- , SO_4^{2-}) at 50 μM and a separation buffer consisting of 12 mM histidine adjusted to a pH-value of 4 with acetic acid were used. First, the setting of the splitting valve and the flow rate was established by variation of these parameters for a fixed dispensed plug of 15 μL of the standard mixture. It was found that a flow rate of 4 $\mu\text{L s}^{-1}$ and a setting of the valve which gave a nominal splitting ratio of 1.5:100 (this is the splitting ratio measured without the backpressure from the capillary and corresponds to a numerical setting on the adjustment scale of the valve of 0.10) gave peaks which were neither of inadequate sensitivity nor overly broadened, and did not lead to excess backpressure (as otherwise indicated by a leakage of the valve). In a second step, a fine adjustment was carried out by variation of the length of the dispensed sample plug. The results in terms of peak area and peak resolution are shown in Fig. 2. As expected, the resolution between NO_3^- and SO_4^{2-} decreases with an increase in peak areas as the volume of the sample plug is increased. The actual setting to be chosen depends on the application at hand. As the resolution is dependent on the concentration, for relatively high concentrations smaller dispensed volumes will be necessary to avoid peak overlaps, while for low concentrations, larger volumes may be injected in order to achieve low limits of detection.

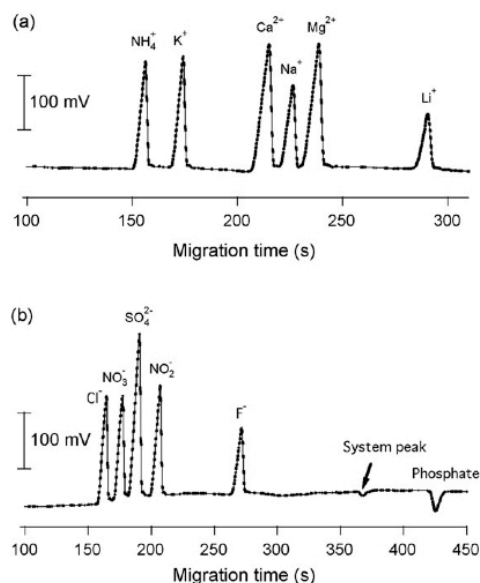


Fig. 3. Analysis of standard solutions containing either inorganic cations or inorganic anions prepared in deionised water. (a) Cations (50 μM), dispensed volume: 40 μL . (b) Anions (50 μM), dispensed volume: 60 μL . Other conditions as for Fig. 2.

3.3. Performance

A separation buffer system based on histidine and acetic acid previously reported to be suitable for the determination of inorganic cations as well as of anions by CE- C^4D [11,21], was adapted for the separation of the cations K^+ , NH_4^+ , Na^+ , Ca^{2+} , Mg^{2+} , Li^+ and the anions Cl^- , NO_3^- , SO_4^{2-} , NO_2^- , F^- and phosphate by subsequent electrophoresis with switched polarity and without using electroosmotic flow reversal. Optimization of the buffer composition was carried out by varying the concentration of histidine and of the pH-value by changing the amount of acetic acid added. Low histidine concentrations were found to give poor resolution of the anions Cl^- , NO_3^- , SO_4^{2-} and NO_2^- ; whereas high concentrations of histidine could not be used with low pH (high concentration of acetic acid), as the electrophoretic baseline was then not stable due to Joule heating. The best compromise electrolyte composition for both anion and cation separations was found to be 12 mM histidine and 2 mM 18-crown-6, adjusted to pH 4 with acetic acid. The crown ether improves the separation of some of the cations, but does not have an effect on the anion separation.

An example of the subsequent analysis of a standard mixture of cations and anions at 50 μM is shown in Fig. 3 using dispensed volumes of 40 and 60 μL respectively. As can be seen, baseline resolution is possible under these conditions. The calibration data obtained using the optimized conditions is given in Table 2. The detection limits achieved for the conditions are in the order of 0.5–1 μM and calibration curves were acquired from about 2 to 100 μM . For higher concentrations peak overlap would occur. The reproducibility of the measurement of peak areas was found to be between about 2 and 4%.

The system was then set up for a supervised test run over a period of 24 h, in which repeated measurements of the standard mixture of the cations and anions of 50 μM at intervals of 30 min were carried out. The results for the peak areas are shown in Fig. 4. The maximum deviations are less than $\pm 4\%$, which demonstrates the suitability of the system for automated operation.

Table 2

Calibration ranges, detection limits (LODs) and reproducibility for the determination of inorganic cations and anions with large dispensed volume injections.

Ion	Dispensed volume (μL)	Range (μM) ^a	Correlation coefficient r	LOD ^b (μM)	RSD% MT ^c (n=6)	RSD% PA ^d (n=6)
NH_4^+	40	2.5–100	0.9995	1.0	0.6	3.3
K^+	40	2.5–100	0.9994	1.0	0.6	3.6
Ca^{2+}	40	2.0–100	0.9997	0.7	0.8	3.9
Na^+	40	5.0–100	0.9995	1.0	0.6	4.0
Mg^{2+}	40	2.0–100	0.9998	0.7	0.9	3.7
Li^+	40	5.0–100	0.9995	2.0	0.9	3.0
Cl^-	60	2.5–100	0.9997	0.5	0.4	1.8
NO_3^-	60	2.5–100	0.9996	0.5	0.5	2.4
SO_4^{2-}	60	2.0–100	1.0000	0.5	0.5	3.3
NO_2^-	60	2.5–100	0.9996	1.0	0.7	3.2
F^-	60	5.0–100	0.9978	2.0	1.0	4.1
Phosphate	60	15.0–100	0.9979	5.0	1.8	5.8

^a 5 concentrations.^b Based on peak heights corresponding to 3 times the baseline noise.^c Migration time.^d Peak area.

3.4. Field test

In order to further evaluate the potential for unattended monitoring the system was set up at a pumping station next to the creek Kleine Aa, a tributary to Lake Sempach. It was found that the concentrations of some of the ions were higher than the conditions reported above allowed, and thus the dispensed sample volumes had to be reduced to 5 and 20 μL for the cations and anions respectively in order to prevent peak overlap due to overload. On the

other hand, some of the ions which had been included in the standard mixture could not be found in the natural water system at detectable levels, even for large volume injections. Automatic analysis of the water from the creek was then carried out at intervals of around 35 min for 5 days during a period of frequent rain (end of April, 2009). Cations and anions were determined sequentially by changing the polarity of the separation voltage as described above. As there was a large amount of suspended matter in the sample stream, filtering with automated backflushing was essential

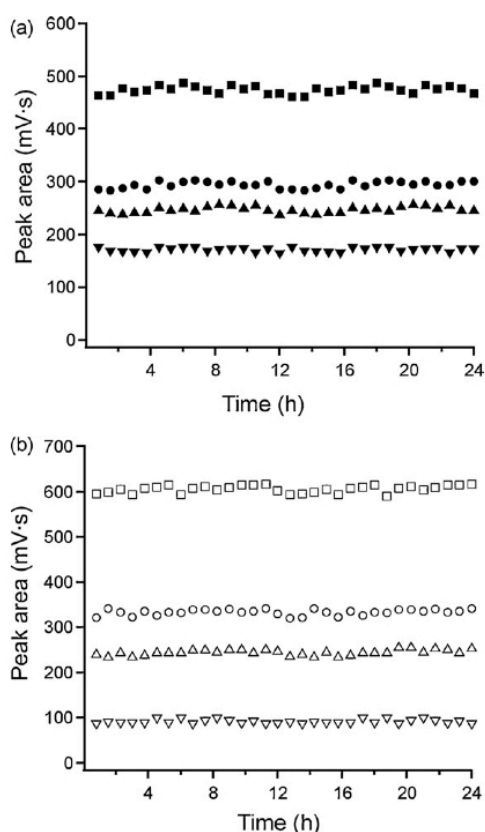


Fig. 4. Reproducibility of peak areas for some inorganic ions over a continuous 24 h run. (a) Cations 50 μM : Ca^{2+} (■), K^+ (●), Na^+ (▲) (---) Li^+ (▼). (b) Anions: 50 μM : SO_4^{2-} (□), NO_3^- (○), F^- (△), and phosphate (▽). Other conditions as for Fig. 3.

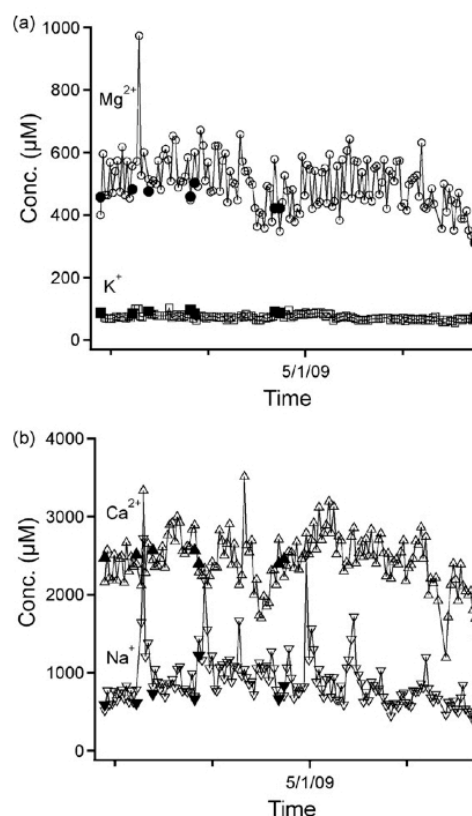


Fig. 5. Variations of some cation concentrations in the Kleine Aa during a rainy period. The solid symbols represent reference measurements for discrete samples carried out later in the laboratory by ion-chromatography.

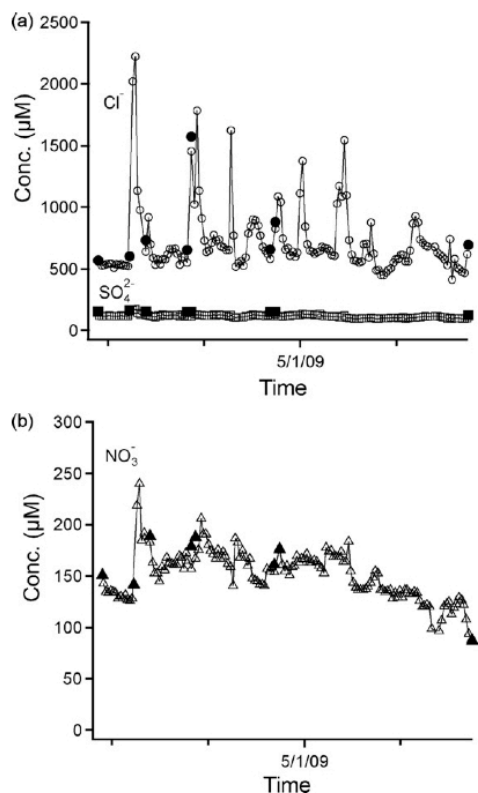


Fig. 6. Variations of some anion concentrations in the Kleine Aa during a rainy period. The solid symbols represent reference measurements for discrete samples carried out later in the laboratory by ion-chromatography.

in order to maintain the integrity of the system. The results for the monitoring test are shown in Figs. 5 and 6 for the cations and anions respectively, and convincingly show that the system is suitable for unattended long-term measuring tasks. Also included are the results for discrete samples, which were analyzed later in the laboratory using ion-chromatography, demonstrating the accuracy of the data obtained. The correlation coefficients (r) between the concentrations obtained with the SI-CE system at the time of sampling of the discrete batches and the results obtained for these by ion-chromatography are 0.989, 0.921, 0.894 for Cl^- , NO_3^- , SO_4^{2-} and 0.845, 0.891, 0.927, 0.826 for K^+ , Ca^{2+} , Na^+ , Mg^{2+} respectively. The correlation coefficients between the results from SI-CE-C⁴D and from IC are deemed acceptable considering that some deviations can be expected due to possible sampling bias.

4. Conclusions

The SI-CE-C⁴D system built in-house was found to be suitable for extended and unattended monitoring application. The use of both large and small dispensed sample volumes allows to quantify samples with very wide concentration ranges. Its flexibility and broad applicability to ion analysis allows easy adaptation to different tasks. The data in our trial was evaluated after completion of the monitoring run, but by developing appropriate software routines, real time concentration reporting should also be possible. Furthermore, connection of the instrument to the Internet for remote querying of data can be envisaged.

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3rd project:

Pressure-assisted capillary electrophoresis for cation separations using a sequential injection analysis manifold and contactless conductivity detection

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Pressure-assisted capillary electrophoresis for cation separations using a sequential injection analysis manifold and contactless conductivity detection

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ABSTRACT

Pressure assisted capillary electrophoresis in capillaries with internal diameters of 10 μm was found possible without significant penalty in terms of separation efficiency and sensitivity when using contactless conductivity detection. A sequential injection analysis manifold consisting of a syringe pump and valves was used to impose a hydrodynamic flow in the separation of some inorganic as well as organic cations. It is demonstrated that the approach may be used to optimize analysis time by superimposing a hydrodynamic flow parallel to the electrokinetic motion. It is also possible to improve the separation by using the forced flow to maintain the analytes in the capillary, and thus the separation field, for longer times. The use of the syringe pump allows flexible and precise control of the pressure, so that it is possible to impose pressure steps during the separation. The use of this was demonstrated for the speeding up of late peaks, or forcing repeated passage of the sample plug through the capillary in order to increase separation.

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1. Introduction

In capillary electrophoresis the performance in terms of separation efficiency, detection limits, and analysis time is generally optimized by varying the injected amount, the separation voltage applied and the capillary length. These parameters are interdependent, in that the injected amount affects both, separation and sensitivity, and separation voltage and capillary length determine field strength as well as residence time of the analytes. The product of the latter parameters is largely responsible for separation efficiency. Of the three variable parameters, only the injection volume and separation volume can be optimized via automated, electronic control; the adjustment of the capillary length requires mechanical manipulations by the operator and possible reconditioning. A potentially further useful parameter is the superimposition of a hydrodynamic flow in order to modify the residence time, either for improved separation or for faster analysis. However, pressurization has not generally been employed as the imposition of a hydrodynamic flow tends to lead to extra bandbroadening (see Section 2). Reports on pressure assisted capillary electrophoresis (PACE) have therefore been largely limited to counterbalancing the electroosmotic flow in order to increase the residence time and hence separation [1–3], and to special applications such as CE coupled to a mass spectrometer

via electrospray ionization and capillary electrochromatography [4–12].

On the other hand, band broadening due to the effect of the laminarity of a hydrodynamic flow can be reduced by using capillaries of very small diameters (see Section 2). While the commonly employed UV-absorption method is not well suited for detection in very narrow capillaries due to its direct dependence on the optical pathlength, Zemann and co-workers [13] showed that capacitively coupled contactless conductivity detection (C⁴D) may be used for capillaries with internal diameters as small as 10 μm . A later report by Wuersig et al. [14] furthermore indicated that good sensitivity should be possible with C⁴D in such slender channels. The increasingly popular method relies on two tubular electrodes placed on the outside of the separation capillary, and is thus very simple and robust and in principle suitable for the determination of any ion. More details can be found in recent reviews [15–17] as well as in fundamental studies [18–21].

The coupling of sequential-injection analysis (SIA) based on a syringe pump and a multi-position valve with CE is a relatively new approach which provides simultaneous detection capability to SIA. On the other hand it is also an attractive and versatile means to miniaturization, automation and extension of CE. Conventional instruments rely on the more complex application of gas pressure or vacuum to effect injection or flushing of capillaries. Some SIA–CE systems with optical detection have been reported by several research groups [22–27] and Wuersig et al. used an SIA–CE–C⁴D system to achieve fast separation of inorganic ions in approximately 10 s [14]. Recently, Mai et al. demonstrated the use of an automated

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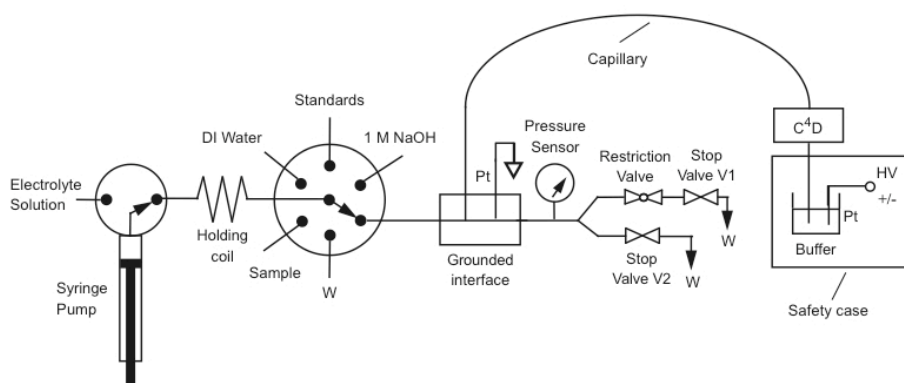


Fig. 1. Schematic drawing of the SIA–CE–C⁴D–system for pressure-assisted capillary electrophoresis. C⁴D: contactless conductivity detector; HV: high-voltage power supply; W: waste.

SIA–CE–C⁴D system for long-term unattended on-site monitoring [28]. Herein, the investigation of the use of an SIA-manifold for pressurization of a CE–C⁴D system in order to superimpose a hydrodynamic flow for the optimization of separation and/or analysis time of cations is reported.

2. Theoretical aspects

Studies of the effect of an imposed laminar flow on dispersion and thus on electrophoretic efficiency have been reported [29–31]. Grushka [31] expressed the dependence of the theoretical plate height (H) on the hydrodynamic flow velocity (v_p) as follows:

$$H = \frac{2D}{v_{\text{tot}}} + \frac{d^2 v_p^2}{24D v_{\text{tot}}} \quad (1)$$

D is the diffusion coefficient and d is the inner capillary diameter. v_{tot} is the total average velocity of the analyte ion, which is given by $v_a + v_p$ when the hydrodynamic and electrophoretic flows are in the same direction (v_a , velocity of the analyte ion) and by $v_p - v_a$ if they are in the reverse direction. For cation separations v_a is given by v_e (electrophoretic velocity of the analyte ion) + v_{EOF} (electroosmotic flow velocity). The first term in the equation relates to longitudinal diffusion while the second term is due to the parabolic flow profile induced by the laminar flow. For parallel-pressure induced CE where $v_{\text{tot}} = v_p + v_a$, an increase in v_p results in a larger value of v_{tot} , leading to a smaller value of $2D/v_{\text{tot}}$ but an increased value of $d^2 v_p^2 / 24D v_{\text{tot}}$.

Eq. (1), however, does not include a further consideration. In an unrelated study by Liu et al. [32] (and in works cited therein) it was found both theoretically and experimentally that a significant contribution to band broadening may also be due to thermal effects caused by Joule heating due to the application of the separation voltage. The contribution of Joule heating to the plate height in CE is displayed in the second term of the following equation (a modified version of the equation given by Liu et al. [32], which does not take into account any possible hydrodynamic flow):

$$H = \frac{2D}{v_a} + \frac{\tau^2 \lambda^2 v_e^2}{k^2 D} \left(K_1 \frac{E^4 d^6}{v_a} + K_2 \frac{E^6 d^8}{LD} \right) \quad (2)$$

E is the electric field strength, L the effective length of a capillary, λ the specific conductance of the solution, k the thermal conductivity of the buffer, τ the thermal coefficient of the solute mobility, and K_1 and K_2 are experimental coefficients. Mayrhofer et al. [13] indeed attributed an improvement of plate numbers found in CE–C⁴D for capillaries with increasingly smaller internal diameters to a reduced effect of Joule heating.

According to these equations both effects are thus strongly dependent on the internal size of the capillaries, and a reduction of the diameter can be expected to lead to an improvement of resolution (corresponding to a low H) even in the absence of hydrodynamic flow.

3. Experimental

3.1. Chemicals and materials

All chemicals were of analytical or reagent grade and purchased from Fluka (Buchs, Switzerland) or Merck (Darmstadt Germany) except for 2-amino-1-butanol and 1-amino-2-propanol which were obtained from Lancaster (Morecambe, England). Stock solutions of 5 mM were used for the preparation of the standards and those of the inorganic cations were prepared from their respective chloride salts. The separation buffer consisted of 12 mM L-histidine adjusted to pH 4 with acetic acid in all cases, unless otherwise stated. Before use, the capillary was preconditioned with 1 M NaOH for 10 min and deionised water for 10 min prior to flushing with electrolyte solution (for 1 h). Deionised water purified with a system from Millipore (Bedford, MA, USA) was used for the preparation of all solutions. The sample of red wine was purchased from a local shop and was prepared by filtering through a 0.02 μm PTF membrane filter (Chromafil O-20/15 MS, Macherey-Nagel, Oensingen, Switzerland), then diluted with deionised water followed by ultra-sonicating for 10 min for degassing. The dilution was carried out immediately prior to use.

3.2. Instrumentation

A simplified diagram of the instrument is given in Fig. 1. The SIA section consists of a syringe pump (Cavro XLP 6000) fitted with a 1 mL syringe and a 6-port channel selection valve (Cavro Smart Valve) (both purchased from Tecan, Crailsheim, Germany). To connect the SIA manifold to the CE part, a purpose-made interface based on two consecutive T-junctions was used. Details of this interface have been given previously [33]. The micro graduated needle valve (restriction valve) and the isolation valves used for pressurization were obtained from Upchurch Scientific (P-47 Oak Harbor, WA, USA) and from NResearch (HP225T021, Gümligen, Switzerland), respectively. A dual polarity high voltage power supply (Spellman CZE2000, Pulborough, UK) with ± 30 kV maximum output voltage and polyimide coated fused silica capillary of 365 μm OD (from Polymicro, Phoenix, AZ, USA) were used for all CE experiments. One end of the capillary was connected to the grounded SIA–CE interface, the other end was placed in a

filled with background electrolyte (BGE), in which the high voltage electrode is placed. A safety cage, which was equipped with a microswitch to interrupt the high voltage on opening, was used to isolate the high voltage assembly. Detection was carried out with a C^4D -system built in-house, details can be found elsewhere [34]. The cell currents are strongly dependent on the capillary diameter and therefore different feedback resistors were fitted to the pick-up amplifier which converts the signal to a voltage, for details see [20]. Resistors of 220 k Ω , 270 k Ω , 1 M Ω and 3.9 M Ω were fitted for capillaries of 75 μm , 50 μm , 25 μm and 10 μm , respectively. An e-corder 201 data acquisition system (eDAQ, Denistone East, NSW, Australia) was used for recording the detector signals. The fluidic pressure was monitored in-line with a sensor from Honeywell (24PCFFM6G, purchased from Distrelec, Uster, Switzerland). The programming package LabVIEW (version 8.0 for Windows XP, from National Instruments, Austin, TX, USA) was used to write the control code. Further detail on the instrument can be found in our previous publication [28].

3.3. Operation

The SIA-manifold allows automated capillary conditioning, flushing as well as hydrodynamic sample aspiration and injection. For capillary flushing both stop-valves (designated as V1 and V2 in Fig. 1) are closed while pumping solution at a low flow rate. Injection is carried out by pumping a defined sample plug past the capillary inlet in the SIA-CE interface while partially pressurizing the manifold by closing only V2. This procedure is necessary as it is not possible to create sample plugs of appropriate small size for complete injection. More details on the typical procedures can be found in the previous publication [28]. Separation is carried out by application of the high voltage of appropriate polarity from the detector end, while the injection end remains grounded at all times. This is contrary to conventional set-ups, but C^4D is not affected by this arrangement. Pressurization of the capillary during separation was achieved by closing both stop-valves while advancing the stepper motor driven syringe pump by the smallest increment possible (corresponding to 0.02 μL). To obtain constant pressure the increment was repeated at appropriate time intervals (typically 10 s) to compensate for its slow decrease due to the passing of the solution. Pressure gradients could be established by adjusting the time intervals and/or the volume increments and the use of the pressure sensor allowed a precise monitoring and adjustment. The resulting hydrodynamic flow velocities were experimentally determined by pumping a small plug of water through the capillary filled with background electrolyte (in the absence of an applied voltage) and determining the time until passage through the detector.

4. Results and discussion

4.1. Dependence of the sensitivity and separation efficiency on the internal diameter of the capillary

The first experiments concerned an investigation of the premise that C^4D is indeed compatible with narrow capillaries. Separations were carried out, initially without application of pressure, in capillaries of IDs from a standard size of 75 μm down to 10 μm . The use of smaller IDs was attempted, but was found not to be readily possible because of the excessive pressures required for flushing of the narrower capillaries. A sample plug of 0.8 cm length was injected in each case, which corresponds approximately to 2% of the effective capillary length (37 cm) as was suggested by Huang et al. [35] as optimum. Mixtures of the three cations, K^+ , Na^+ and Li^+ , were injected at different concentrations and the detection limits, defined as the concentrations which give peak heights correspond-

Table 1

Detection limits for the determination of some inorganic cations with capillaries of different internal diameters. CE conditions: 12 mM His adjusted to pH 4 with acetic acid; $l_{\text{eff}} = 37$ cm; $E = 400$ V/cm.

Cation	LOD ^a (μM)			
	75 μm	50 μm	25 μm	10 μm
K^+	1.3	1.0	1.3	2.3
Na^+	1.8	1.5	1.8	3.0
Li^+	2.3	2.0	2.0	3.0

^a Based on peak heights corresponding to 3 times the baseline noise.

ing to three times the baseline noise level, were determined for the different capillary diameters. As can be seen from the data of Table 1, the LODs determined are all in the low μM -range, and almost identical for the 4 diameters investigated, with the loss of sensitivity in going to the narrowest capillary being less than a factor of two for all three ions. This interesting feature of C^4D is deemed to be due to the fact that the device is a bulk detector, that is, it responds to a general solution property, rather than being analyte specific. Thus when decreasing the cell size (by reducing the capillary diameter) not only the signal for the analyte is reduced, but also the background signal. The reduction of the noise associated with the latter must lead to the observed behaviour. Clearly, the use of narrow capillaries down to 10 μm ID is possible with C^4D without incurring a significant penalty in detection limits as would be the case with optical detection.

The second critical aspect is the question if indeed it is possible to introduce hydrodynamic flow without serious deterioration of separation efficiency when using CE- C^4D with narrow capillaries. Thus the theoretical plate numbers (N) were determined from electropherograms obtained for the injection of 100 μM Na^+ into capillaries of different internal diameter and for superimposed hydrodynamic flow velocities in the range from 0.025 to 0.27 cm/s. Note, that for the field strength used, the velocity of sodium ions due to the electrophoretic mobility would be 0.16 cm/s and the electroosmotic flow would be 0.025 cm/s. The data is shown in Fig. 2. It is, first of all, clearly evident that the separation efficiency is strongly dependent on capillary diameter, even when no hydrodynamic flow is imposed. The application of pressure leads to a lowering of separation efficiency, but the relative deterioration in plate numbers is indeed much less pronounced for the smaller

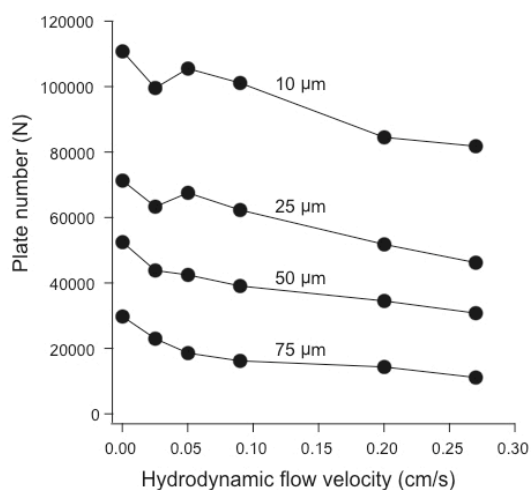


Fig. 2. Plate number versus superimposed hydrodynamic flow velocity for different capillary IDs. Analyte: Na^+ 100 μM in deionised water. Separation: $l_{\text{eff}} = 37$ cm; $E = 300$ V/cm.

diameters. For the largest capillary of 75 μm ID, the deterioration of about 50% from about 30,000 to 15,000 is significant, while the lowering of about 25% from the high initial level of 110,000 for the 10 μm capillary can easily be tolerated. Note the slight increase in plate numbers for capillaries of small IDs (10 μm and 25 μm) when going from flow rates of 0.025 cm/s to 0.05 cm/s. This phenomenon was also described by Grushka [31] and was ascribed to the fact that at small flow rates and with narrow capillaries, the increase in laminar flow induced dispersion is only small when increasing the flow rate, and is more than compensated for by a decrease in longitudinal diffusion.

Hydrodynamic pumping is thus well possible with capillaries of 10 μm ID as high separation efficiency ($N > 80,000$) can always be maintained at any of the flow rates tested which encompass a range relevant for modification of the mobilities of the ions due to electrophoretic and electroosmotic migration, and, as shown above, the loss in sensitivity is negligible.

4.2. Separations with hydrodynamic flow in the same direction as the electrophoretic mobility

4.2.1. Optimization of analysis time of inorganic cations

When the resolution between analytes in CE is found to be more than adequate ($R \geq 2$), an optimization of analysis time, and hence sample throughput, is possible. This can in principle be achieved by an increase of the separation voltage or by a shortening of the capillary. The first approach may however not be possible if the upper limit of the available voltage range is already used or Joule heating is problematic, and the second method requires mechanical manipulations which can only be reversed by installing and conditioning a new capillary. Using a hydrodynamic flow to push the ions through is an alternative, flexible and easily reversible approach. The electropherograms for the three inorganic cations K^+ , Na^+ and Li^+ obtained subsequently without and with parallel pumping are shown in the two parts of Fig. 3 along with the recorded pressure profiles. As can be seen, the pressurization allows optimization of the analysis time on the fly, the separation time is reduced to less than half, while baseline resolution is still preserved.

For a further demonstration, the method was applied to the separation of inorganic cations in a sample of red wine. The electropherograms of a standard mixture containing 6 cationic species, as well as those of a diluted red wine sample, with and without pressure assistance, are shown in Fig. 4. To determine the cations present in the red wine sample, each peak was identified by comparing the migration time with that of the standard mixtures. It is seen that K^+ , Ca^{2+} , Mg^{2+} and Na^+ are present in abundant amounts and the complete passage through the detector with more than adequate baseline resolution was observed after 5 min without the application of pressure (electropherograms a and b). By employing a pressure of 1.6 bar from the beginning of electrophoresis the running time could be reduced to around 2 min while still obtaining baseline resolution (electropherograms c and d). Thus the sample throughput could be significantly improved. Note that a number of additional peaks were detected, but no effort was made to identify these species.

4.2.2. Concurrent separation of fast and slow migrating amines

A related, but slightly more complex situation are separations of mixtures of fast and slowly migrating analytes. Optimization of separation then has to be done for the fast ions, but this can lead to exceedingly long migration times for the slow ions. The separation of a range of 9 amines, separated in their protonated cationic form, shown in the electropherogram of Fig. 5a illustrates the situation. The first 6 ions are separated within about 7 min while the passage of the slow ions (with negative going peaks) requires more than 20 min. The situation is also familiar from HPLC and is the

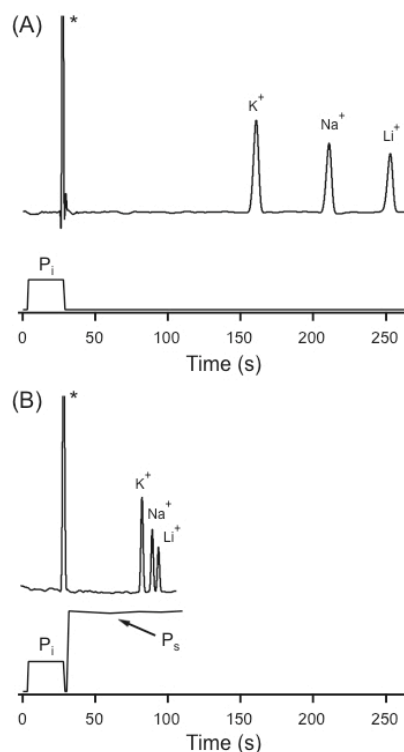


Fig. 3. Optimization of electrophoresis time with hydrodynamic flow. Analytes: 100 μM . Separation: 10 μm ID capillary with $l_{\text{eff}} = 37$ cm; $E = 400$ V/cm. The pressure was recorded on-line during hydrodynamic injection and (pressure-assisted) electrophoretic separation. (A) Separation without pressure; (B) separation with pressure. P_i : pressure applied for injection; P_s : pressure applied for separation; *Voltage pulse occurring when HV is turned on, indicating the start of the electrophoresis process.

reason why for this method usually gradient elution is employed. However, this approach is not possible in capillary electrophoresis. As more than adequate baseline resolution was obtained with the conditions employed, a significant overall shortening of the analysis time to 4 min is possible by superimposing a hydrodynamic flow using an applied pressure of 0.9 bar as illustrated in electropherogram of Fig. 5b. A further improvement is possible by increasing the applied pressure to 3 bar after passage of the faster ions, as shown in Fig. 5c. Note that a constant pressure of 3 bar from the beginning of the separation would not allow resolution of any of the analytes. The calibration data obtained under parallel-flow driven CE with moderate pressure of 0.9 bar is given in Table 2. The detection limits achieved for the conditions are in the range from 1.5 μM to 15 μM and calibration curves were acquired up to 300 μM . As the reproducibility data for retention time (approximately 1%) and for peak area (between 2 and 5%) also given in Table 2 shows, the precision obtained in the approach is not deteriorated compared to conventional CE without pumping.

4.3. Separations with hydrodynamic flow against the electrophoretic mobility

4.3.1. Separation of high mobility inorganic cations

In CE- C^4D fast migrating cations may not be resolved under given conditions, and this is more pronounced when the concentrations are high. Overlaps can generally be minimized by reducing the injected volume, or by dilution of the sample, but this approach is

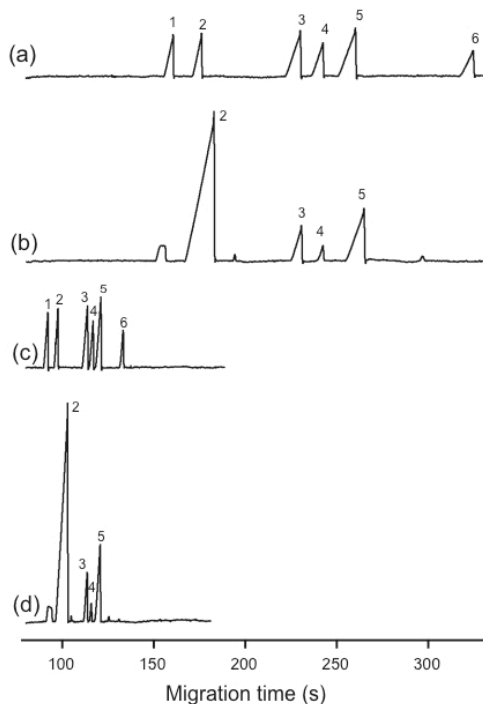


Fig. 4. Separation of inorganic cations in a red wine sample. (a) Solutions of standards, 200 μM , $P=0$ bar; (b) diluted red wine sample, $P=0$ bar; (c) solution of standards, 200 μM , $P=1.6$ bar; (d) diluted red wine sample, $P=1.6$ bar. CE conditions: 10 μm ID capillary with $l_{\text{eff}}=43$ cm; $E=400$ V/cm; BGE: His 12 mM and 18-Crown-6 2 mM adjusted to pH 4 with acetic acid. Analytes: (1) NH_4^+ ; (2) K^+ ; (3) Ca^{2+} ; (4) Na^+ ; (5) Mg^{2+} ; (6) Li^+ .

not possible when one of the adjacent peaks is at low concentration. This situation is illustrated by electropherogram (a) of Fig. 6. The relatively small signal for the sodium ion is completely obscured by the large and tailing peak for calcium ions. As demonstrated by electropherogram (b) of Fig. 6, it is possible to resolve the peaks by increasing the residence time of the ions via the introduction of a hydrodynamic flow against the electrophoretic and electroosmotic migration. This requires a reversal of the applied voltage. The analytes then migrate electrophoretically towards the injection end, but are slowly pushed hydrodynamically to the detector end. This leads to a swapping of the peak order and the more slowly migrating Na^+ is now arriving at the detector first. The separation was achieved in a short capillary of only 7 cm length. The triangular peak shapes are a common feature of capillary electrophoresis

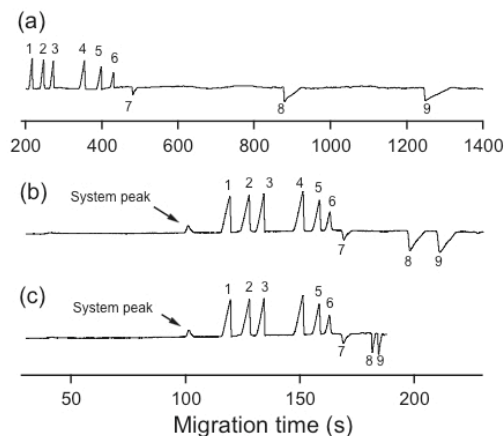


Fig. 5. Concurrent separation of fast and slowly migrating amines. Analytes: (1) methylamine (100 μM); (2) dimethylamine (100 μM); (3) trimethylamine (100 μM); (4) 1-amino-2-propanol (200 μM); (5) 2-amino-1-butanol (200 μM); (6) 1-phenyl-ethylamine (200 μM); (7) 3,5-dimethylaniline (200 μM); (8) 2,6-dimethylaniline (100 μM); (9) 2,6-diisopropylaniline (100 μM). Separation: 10 μm ID capillary with $l_{\text{eff}}=40$ cm; $E=400$ V/cm. (a) No pressure applied; (b) $P=0.9$ bar from $t=0$ s; (c) $P_1=0.9$ bar from $t=0$ s and $P_2=3$ bar from $t=175$ s.

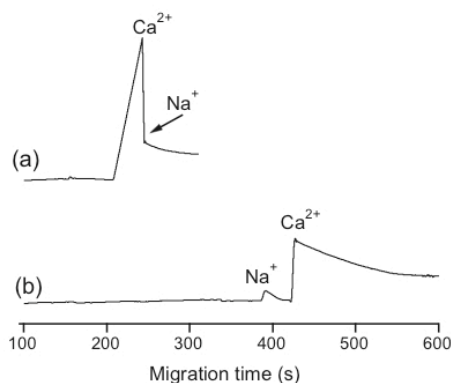


Fig. 6. Separation of Ca^{2+} (2000 μM) and Na^+ (100 μM). (a) Normal CE, 10 μm ID capillary with $l_{\text{eff}}=40$ cm; $E=400$ V/cm; (b) counter-pressure assisted CE, 10 μm ID capillary with $l_{\text{eff}}=7$ cm; $E=400$ V/cm, $P=0.9$ bar.

due to electrodispersion which occurs because of differences in the electrophoretic mobilities (μ) of analyte and buffer ions, and this effect is more pronounced for higher concentrations. For conductivity detection a certain mismatch is necessary for good detection

Table 2
Calibration ranges, detection limits (LODs) and reproducibility for the determination of amines with pressure-assisted CE.

Amine	Range (μM) ^a	Correlation coefficient, r	LOD ^b (μM)	RSD% MT ^c ($n=4$)	RSD% PA ^d ($n=4$)
Methylamine	5–200	0.9992	1.5	0.7	2.2
Dimethylamine	5–200	0.9995	1.5	0.7	2.6
Trimethylamine	5–200	0.9993	1.5	0.8	2.4
1-Amino-2-propanol	10–300	0.9991	3.0	0.8	2.9
2-Amino-1-butanol	10–300	0.9992	3.0	0.9	3.1
1-Phenyl-ethylamine	15–300	0.9993	5.0	0.8	3.0
3,5-Dimethylaniline	40–400	0.9980	15.0	0.9	3.9
2,6-Dimethylaniline	5–200	0.9993	2.0	1.0	4.2
2,6-Diisopropylaniline	5–200	0.9989	2.0	1.2	4.6

^a 5 concentrations.

^b Based on peak heights corresponding to 3 times the baseline noise.

^c Effective migration time.

^d Peak area.

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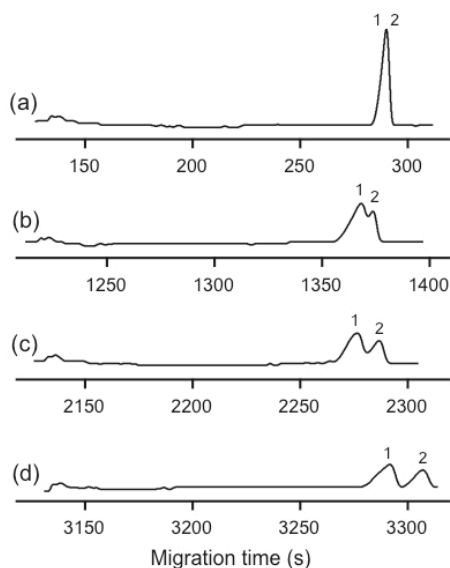


Fig. 7. Separation of diethylamine (1) and 1-amino-2-propanol (2) (300 μ M) by normal CE, but repeated several times by using hydrodynamic flow to return the sample to the starting point before each run. (a) 1st electrophoresis run; (b) 4th run; (c) 7th run; (d) 10th run. 10 μ m ID capillary with $l_{\text{eff}} = 7$ cm; $E = 400$ V/cm. Fluidic pressure for sample delivery: 5.5 bar.

sensitivity as mobility is directly related to ionic conductivity (λ) according to $\lambda = \mu F$, where F is the Faraday constant.

4.3.2. Separation of organic cations of moderate electrophoretic mobility

Previous work in our group showed that the two amines, 1-amino-2-propanol and diethylamine can be separated in capillary electrochromatography (CEC) carried out in monolithic columns with contactless conductivity measurements [36], but no success was obtained when trying to achieve baseline separation under normal CE conditions in open capillaries with conductivity detection. Therefore the use of a hydrodynamic flow to counter-balance the mobility and electroosmotic flow, as described above for the fast inorganic cations, was also investigated for this pair of hard to separate species. It was found that this was more challenging than for the inorganic cations in that a much longer residence time was required. This poses a difficulty in that it was also found hard to accurately balance the electroosmotic flow for extended periods, as this depends on the surface condition of the capillary and is not perfectly stable over time. A different approach was therefore chosen. First, the sample plug is delivered close to the high voltage end of the capillary by using hydrodynamic pumping while the separation voltage is off. During this process no separation is expected. Pressurization is then ended by opening the stop valves and the anodic separation voltage turned on. The two amines then move towards the grounded end of the capillary electrophoretically. As soon as the amines reach the proximity of the grounded end, the separation voltage is turned off, and pressurization triggered again to force the two amines to move back to the HV-end by hydrodynamic flow. These steps are repeated several times until baseline separation of the two amines is obtained. Note that the detector is positioned near the grounded end of the capillary in this case. The process is illustrated by the electropherograms of Fig. 7,

which show the detector response following increasing numbers of passages. Complete separation is achieved at the 10th round.

5. Conclusions

Pressure assisted capillary electrophoresis can be carried out without significant penalty in terms of resolution and sensitivity in capillaries of 10 μ m internal diameter when contactless conductivity detection is employed. The use of hydrodynamic flow as an additional parameter leads to increased flexibility in the optimization of separations and can be implemented on the fly for different tasks at hand without requiring mechanical changes to the system geometry. This then allows separations which otherwise are difficult to achieve. The use of an SIA manifold for pressurization was found to be straightforward, allows precise control, and is highly flexible.

Acknowledgement

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4th project:

Anion separations with pressure-assisted capillary electrophoresis using a sequential injection analysis manifold and contactless conductivity detection

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Anion separations with pressure-assisted capillary electrophoresis using a sequential injection analysis manifold and contactless conductivity detection

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Keywords: capillary electrophoresis (CE), pressure-assisted capillary electrophoresis (PACE), sequential injection analysis (SIA), anions, electroosmotic flow compensation, capacitively coupled contactless conductivity detection (C⁴D).

Abstract

It is demonstrated that a hydrodynamic flow superimposed on the mobility of analyte anions can be used for optimization of analysis time in capillary zone electrophoresis. It was also found possible to use the approach for counter-balancing the electroosmotic flow (EOF) and this works as well as the use of surface modifiers. In order to avoid any band-broadening due to the bulk flow narrow capillaries of 10 µm internal diameter were employed. This was enabled by the use of capacitively coupled contactless conductivity detection (C⁴D) which does not suffer from the downscaling and detection down to between 1 and 20 µM for a range of inorganic and small organic anions was found feasible. Precisely controlled hydrodynamic flow was generated with a sequential injection (SI) manifold based on a syringe pump. Sample injection was carried out with a new design relying on a simple piece of capillary tubing to achieve the appropriate back-pressure for the required split-injection procedure.

1 Introduction

In capillary zone electrophoresis, electrophoretic separation and/or analysis time can be optimised by adjustment of the applied voltage and/or the capillary length. However, there are limits due a restriction of the high voltage range, Joule heating effects, and the possible need for manual mechanical manipulations. The electroosmotic flow (EOF) is another parameter that usually needs to be controlled by using buffers of appropriate pH and ionic strength and often an additive is included for dynamic coating of the capillary wall to achieve passivation or reversal of the surface charges. Adjustments require careful reconditioning of the capillaries. Much effort has been spent for development of such coating procedures for modification of the EOF [1]. Semi-permanent [2] and permanent [3-5] coating procedures are used but are elaborate and time consuming, and necessitate an exchange of capillaries when requirements change.

In principle, the incorporation of a hydrodynamic flow can be used as an additional variable which may be used for control of the residence time in order to improve the separation efficiency and/or analysis time, as well as for compensation of electroosmotic flow. This does not require a modification of the composition of a buffer and the associated capillary reconditioning and may be easily controlled and reversed electronically. However, despite its potential, other than for some specialised applications using pressurised systems such as coupling CE to mass-spectrometry and for CEC, there are only a few reports on employing hydrodynamic flow for controlling the residence time [6-8]. The reason for this is the fact that the laminar flow introduced by conventional pumping tends to lead to additional bandbroadening. The high separation efficiencies that can be obtained with CE are indeed attributed precisely to the absence of laminar flow.

For anions, without the use of a modifier to reverse the electroosmotic flow, the influences of longitudinal diffusion and laminar flow induced dispersion on separation efficiency (given as theoretical plate height, H) can be expressed as the first and second terms of the following equation respectively, which is an extension of the original version proposed by Grushka [9]:

$$H = \frac{2D}{v_{EP} - v_{EOF} + v_{HD}} + \frac{d^2 v_{HD}^2}{24D(v_{EP} - v_{EOF} + v_{HD})} \quad \text{equation (1)}$$

D is the diffusion coefficient of the analyte and d the inner capillary diameter; v_{EP} , v_{EOF} , v_{HD} are the electrophoretic velocity of the analyte ion, the electroosmotic flow velocity and hydrodynamic flow velocity respectively. Little quantitative experimental data is available, but Kutter and Welsch reported a study on the use of counterpressure to prevent UV-absorbing auxiliary reagents from reaching the detector [10], which confirmed that for capillaries of 50 and 75 μm internal diameter the imposition of a hydrodynamic flow generally results in a significant decrease in theoretical plate numbers.

Electrodispersion, arising from differences in electrophoretic mobility between analyte ions and buffer ions is another factor causing band broadening. If this is the dominating contribution, the triangular peak shapes typical for capillary electrophoresis are the result. Detailed studies on electromigration dispersion have been reported by different authors [11-16]. This dispersion effect can be described by the following equation, which was first developed by Mikkers *et al.* [11, 14] and later simplified by Erny *et al.* [16]:

$$\delta(t) = 2 \times \sqrt{c \times \alpha \times \Delta l_o \times \mu_A \times E \times t} = 2 \times \sqrt{c \times \alpha \times \Delta l_o \times \mu_A \times E \times \frac{l_{eff}}{v_{EP} - v_{EOF} + v_{HD}}} \quad \text{equation (2)}$$

$\delta(t)$ is the peak width, comprising both the sharp and the tailing sides of the peak, in an electropherogram for the electrophoretic migration of an analyte of concentration c under an

electrical field strength E for a residence time interval t ; l_{eff} is the effective length of the capillary; μ_A is the ionic mobility of the analyte; Δl_o is the injection length; and α is a parameter dependent on the differences in mobilities of the analyte, counterion and co-ion.

The separation efficiency may thus be dependent on the magnitudes of longitudinal diffusion or electromigration dispersion, which in turn depends on the balance of velocities, or, if hydrodynamic flow is present, on the flow rate and the inner diameter of the capillary. Due to the quadratic contribution of the diameter in the second term of equation (1) it can be expected though, that any effect of laminar flow can be significantly reduced by using very narrow capillaries. This however, is not readily possible with the standard detection technique of optical absorption as the accompanying reduction in optical pathlength leads to a significant loss in sensitivity and the required reduction in aperture would increase detector noise and pose significant challenges in the manufacturing and alignment of a cell.

On the other hand, it has been shown that capacitively coupled contactless conductivity detection (C^4D) can be used with narrow capillaries of 10 μm without severe penalty in sensitivity [17, 18]. The construction of such a measuring cell is also much less demanding than that of an optical cell as the external tubular electrodes need to be aligned with the outer diameter (typically 365 μm) only, not with the inner diameter of the capillaries. A discussion of the various applications of C^4D for CE can be found in recent reviews [19-23], whereas fundamental details may be gleaned from [20, 24-29]. It has indeed been demonstrated very recently by the authors that for the separation of cations in a CE- C^4D -system using 10 μm capillaries the superimposition of hydrodynamic flow may be used with advantage [30]. By pumping with the mobility of the ions the analysis time may be shortened, or by pumping against the mobility of the ions their residence time in the field may be extended, and thus the

separation be improved. The detection limits were not significantly lower than those obtained with larger diameter capillaries while the separation efficiency was strongly improved for the 10 μm capillary compared to capillaries of a more standard diameter of 75 μm , regardless of whether pumping was applied or not [30]. This is attributed to a reduction in Joule heating, and its reduced additional effect on band broadening, for the smaller capillary.

For controlled creation of hydrodynamic flow a sequential-injection analysis (SIA) manifold based on a syringe pump and a multi-position valve was employed [30]. This is an attractive means for automation, extension and miniaturization of CE. Applications of this SIA-CE combination are summarized in [31]. Recently Mai *et al.* also used an SIA-CE- C^4D system for unattended monitoring applications [32]. Herein, a study of pressurization of a CE- C^4D system in the analysis of inorganic and small organic anions using an SIA-manifold and a 10 μm capillary is reported.

2 Experimental

2.1 Chemicals and Materials

All chemicals were of analytical or reagent grade and purchased from Fluka (Buchs, Switzerland) or Merck (Darmstadt, Germany). Stock solutions of 10 mmol/L were used for the preparation of the standards of inorganic and organic anions, using their respective sodium salts, except for ascorbate, which was prepared directly from ascorbic acid. Before use, the capillary was preconditioned with 1 M NaOH for 10 min. and deionised water for 10 min. prior to flushing with buffer. Deionised water purified using a system from Millipore (Bedford, MA, USA) was used for the preparation of all solutions. A sample of a carbonated softdrink containing some fruit juice and a vitamin C supplement tablet were purchased from local shops in Basel, Switzerland. The beverage sample was prepared by filtering with 0.02

μm PTFE membrane filters (Chromafil O-20/15 MS, Macherey-Nagel, Oensingen, Switzerland), then diluting with deionised water and ultra-sonicating for 10 min. The same sample pre-treatment procedure was also applied to the vitamin tablet that had been dissolved in deionised water.

2.2 Instrumentation

The instrument was a slightly modified version of a previous design and more details may be found in the earlier publication [32]. A simplified diagram is given in Fig. 1. The SIA section consisted of a syringe pump (Cavro XLP 6000) fitted with a 1 mL syringe and a 6-port channel selection valve (Cavro Smart Valve) (both purchased from Tecan, Crailsheim, Germany). A purpose made interface is used for connection of the capillary to the SIA system. The stop valves at the outlet of the interface were obtained from NResearch (HP225T021, Gümligen, Switzerland). The fluidic pressure was monitored in-line with a sensor from Honeywell (24PCFFM6G, purchased from Distrelec, Uster, Switzerland). A dual polarity high voltage power supply (Spellman CZE2000, Pulborough, UK) with ± 30 kV maximum output voltage and polyimide coated fused silica capillaries of $365 \mu\text{m}$ OD and $10 \mu\text{m}$ ID (from Polymicro, Phoenix, AZ, USA) were used for all experiments. Detection was carried out with a C^4D -system built in-house, details can also be found elsewhere [33]. An e-corder 201 data-acquisition system (eDAQ, Denistone East, NSW, Australia) was used for recording the detector signals.

2.3 Operation

All operations, including capillary conditioning, flushing, hydrodynamic sample aspiration and injection, pressurisation as well as separation and data acquisition were implemented automatically. The programming package LabVIEW (version 8.0 for Windows XP, from

National Instruments, Austin, TX, USA) was used to write the control code. Details on the typical procedures can be found in the previous publication [32]. Briefly, for creating a hydrodynamic flow through the capillary during separation and for flushing both stop-valves (designated as V1 and V2 in Fig. 1) are closed while advancing the stepper motor driven syringe pump by appropriate increments. Hydrodynamic injection is carried out by pumping a defined sample plug past the capillary inlet in the SIA-CE interface while partially pressurising the manifold by closing only V2. Flushing of the interface is achieved by opening V1 (or both stop valves). Separation is performed by application of the high voltage of appropriate polarity at the detector end, while the injection end remains grounded at all times. The C⁴D is not affected by this reversal of the usual arrangement.

3 Results and Discussion

3.1 Pressurisation for hydrodynamic injection

The transfer of a sample plug into the capillary is carried out hydrodynamically in order to avoid a sampling bias which would be inherent with the more easily implemented electrokinetic injection method. However, the sample volumes employed in CE are in the nanoliter range, which is too little for direct handling with the SI-manifold. Therefore only part of the dispensed sample plug is injected into the separation tubing using a split-injection procedure carried out by creating a backpressure in the interface while pumping the plug past the capillary inlet. Previously a micrograduated valve was used for controlled partial pressurisation [32]. A new and simpler approach was developed, which is, as shown in Fig. 1, based on the use of a piece of tubing of defined diameter and length to set the backpressure. The dimensions required for the pressurisation tubing can be worked out using the well known Poiseuille equation, which relates the flow rate with pressure drop and length and diameter of a tubing. Knowing the length of the sample plug passed from the SI-manifold

through the interface and its flow rate, as well as the length and diameter of the separation capillary the pressure required for injection of a desired length of a secondary sample plug into the capillary can be calculated. As the pressure at the inlet is determined by the backpressure created by the flow through the pressurisation tubing (the flow through the separation capillary itself can be neglected because of the large splitting ratio) a second application of Poiseuille's equation leads to the required dimensions. Using this approach it was found that for a PEEK tubing of 0.007" i.d. a length of about 35 cm was required in order to inject a 1 cm plug into a capillary of 50 cm length and 10 μm i.d. Note that the presence of a pressure sensor at the SI-CE interface allows to monitor not only the injection but also the application of any hydrodynamic flow during separation as the resulting flow can always be calculated using Poiseuille's equation. A verification can be obtained by injection a plug of water into the separation buffer as this will lead to a signal in C^4D . Note that subsequently pressure values are quoted instead of flow rates for some of the procedures, as this is the more directly measurable experimental parameter.

3.2 Effect of hydrodynamic flow on peak width

The influence of the hydrodynamic flow on the peak shape of a small anion, namely oxalate, is illustrated in Fig. 2. The running buffer used for this experiment is composed of tris(hydroxymethyl)aminomethane (Tris) and 2-(cyclohexylamino)ethanesulfonic acid (CHES), has a pH 8.4, and is found to be suitable for detection with C^4D . A positive separation voltage was applied at the detector end and no EOF modifier was added. At the relatively high pH a strong EOF is therefore present towards the injection side, while the oxalate anion migrates electrophoretically towards the detector end of the capillary. As can be seen from trace (a) of the figure, without the imposition of hydrodynamic flow, oxalate arrives very late at the detector as it is strongly retarded by the electroosmotic flow going in

the opposite direction. The fact that the peak shows a pronounced triangular shape indicates that electrodispersion is the predominant factor responsible for peak broadening. The other traces of Fig. 2 were recorded with increasing increments of hydrodynamic flow towards the detector end. The triangular peak shapes are retained, which clearly shows that for the conditions employed the bulk flow imposed does not lead to any significant added band broadening due to laminarity. It is also evident that the introduction of hydrodynamic flow does not only cause the peak to reach the detector earlier, but also leads to significantly sharpened peaks. One may argue that this is simply due to a faster movement of the peak through the detector.

For a more detailed examination, the same experiments were also carried out with chloride (fast electrophoretic mobility) and formate (electrophoretic mobility smaller than that of oxalate). The numbers of theoretical plates (N) were then calculated from the peaks as a numerical measure for peak width for different superimposed hydrodynamic flow velocities. The quantitative data is shown in Fig. 3. Note that in the absence of hydrodynamic flows and for flow rates smaller than 0.015 cm/s, formate is not detected as the electroosmotic flow rate towards the injection end is larger than its electrophoretic velocity, thus no data could be obtained. For all three anions, poor efficiencies are observed for no hydrodynamic flow or small flow rates below 0.1 cm/s whereas significant improvements can be achieved at higher velocities. The curves show a maximum, indicating that the effect is not merely due to a faster movement of the ion plugs through the detector cell, but is most likely also partially caused by a reduction of electrodispersion through the shortening in residence time (compare equation (2)).

3.3 Separation of fast inorganic anions

Most inorganic anions are present in their charged forms even at low pH-value. In fused silica capillaries the EOF is small under acidic conditions. This means that the separation of strong electrolyte anions in CE is often possible without the use of an EOF modifier while applying a positive separation voltage at the detector end. In this case a superimposed hydrodynamic flow may be utilized during separation to accelerate the movement of anions of relatively slow mobilities in order to speed up the analysis. In Fig. 4 the separation of a range of inorganic anions of fast and relatively slow electrophoretic mobilities under an EOF-suppressed condition at pH 4 and no superimposed hydrodynamic flow is shown. As can be seen from trace (a) of part A, 5 of the ions are just baseline separated in a relatively short time, while two of the ions, namely dihydrogenphosphite and dihydrogenphosphate arrive late while being well separated from each other and the other ions. Note, that the negative going peak for phosphate is a normal feature of C⁴D. In part B of Fig. 4 the pressures as measured at the injection end of the capillary during separation are shown, and remained at 0 bar for measurement (a). The application of a hydrodynamic flow right from the start of the separation would in this case not be possible as then the 5 fast ions could not be separated adequately. However, the SIA manifold allows precisely controlled addition of hydrodynamic flow at any time during the separation, and as shown in electropherogram (b) it is thus possible to push along the late peaks by activation of pressure at 125 s (see Fig. 4B) in order to achieve a significant reduction in analysis time.

If only the more slowly moving anions are of interest a different mode of operation is also possible. A very fast analysis of the two late species can be achieved by a reversal of the applied voltage in combination with employment of pressure to create a hydrodynamic flow to counter the electrophoretic movement of anions. This situation is illustrated in

electropherogram (c) of Fig. 4A. The analytes, though migrating electrophoretically towards the injection end, are pushed hydrodynamically to the detector. With application of an appropriate pressure, only the more slowly migrating anions are pushed towards the detector while the faster ones are lost towards the injection end. Note that the peak order is swapped.

3.4 Separation of slow organic anions

The separation of weak organic anions, such as carboxylates, with CE has to be implemented at a relatively high pH in order to assure complete dissociation. Under those conditions the EOF is strong, and an EOF modifier is usually added in order to obtain parallel electrophoretic and electroosmotic flows. Otherwise unduly slow separations would result where the anions are swept towards the detector by the EOF against their electrophoretic mobility. As shown by electropherogram (a) of Fig. 5, it is perfectly well possible to employ a hydrodynamic flow to balance the EOF. A buffer based on Tris/CHES at pH 8.4 was employed and a pressure of 2.8 bar was applied during the separation (positive voltage applied at the detection end). For comparison, the separation of the same standard mixture of carboxylates was also carried out using the conventional approach by inclusion of CTAB (0.1 mM) as EOF modifier in the buffer, without application of hydrodynamic flow. Except for some difference in total analysis time (which could be matched by optimization of hydrodynamic flow rate and/or CTAB concentration), very similar results were obtained.

3.5 Concurrent separation of inorganic and organic anions using a pressure step

In the separation of mixtures of fast and slow anions with EOF reversal by using an additive in the buffer, or by EOF compensation with a constant hydrodynamic flow, the situation can arise that the peaks for the fast ions are close to each other, but those for the slow ions are unduly extending the analysis time. In other words, slow organic acids require stronger

measures to adequately overcome the EOF than inorganic anions with fast electrophoretic mobilities. This situation is illustrated by electropherogram (a) of Fig. 6 for a mixture of 16 inorganic and organic anions. There is a similarity to the circumstances represented by electropherogram (a) of Fig. 4, but here EOF compensation by applying a constant pressure of 1.7 bar is already in place to an extent that will give an analysis time as short as possible without compromising resolution. As can be seen from electropherogram (b) of Fig. 6, a higher hydrodynamic flow at 2.4 bar will lead to significant shortening of the separation time, but at the expense of a loss of baseline resolution for the early peaks for nitrate and nitrite. The solution is to use a change of hydrodynamic flow rate during the separation. Optimised conditions with a pressure increase from 1.7 bar to 2.4 bar after 240 s led to the electropherogram given as trace (c) of Fig. 6 which gives baseline resolution for all peaks at a relatively short total analysis time.

3.6 Quantification and samples

The reproducibility of the pressure assisted method for anion determination and suitability for quantification was then evaluated. This was carried out by acquiring statistical data for a standard mixture consisting of 15 anions (as for the previous section, but omitting nitrite) and using a fixed hydrodynamic flow at 2.4 bar. The data is summarised in Table 1. The detection limits achieved for the conditions are in the low μM -range and the reproducibility of retention times and peak areas are about 1-1.5 % and 3-5 % respectively, which is comparable to the performance obtained with the conventional approach using an EOF modifier.

In Fig. 7 the electropherograms obtained for a beverage sample and the solution of a vitamin C supplement tablet are shown. Appropriate dilutions were carried out to avoid overloading. The beverage contains a large amount of citric acid as well as smaller amounts of compounds

which would have been added as preservatives such as benzoate and sorbate. The vitamin supplement has a stated content of 150 mg vitamin C (851 μM), the amount determined by comparison of the peak area with a calibration curve is 835 μM , which matches well the indicated value.

4 Conclusions

It was found that for the conditions used hydrodynamic flow could be imposed without significant band-broadening due to laminar flow. In fact, the number of theoretical plates was increased for anions on application of a bulk flow. The technique was thus found to be a highly useful tool for flexible adjustment of the residence time of analyte ions in the electric field in order to optimize resolution and/or analysis time. In the separation of anions the balancing of EOF by this purely mechanical means is particularly attractive as it eliminates the need for dynamic or permanent chemical modification of the capillaries. Furthermore, the use of a computer controlled syringe pump enables versatile variation of the flow even during a separation run, which can be used to obtain optimized separation profiles akin to gradient elution in HPLC.

Acknowledgements

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Table 1: Calibration ranges, detection limits (LOD) and reproducibility for the determination of anions with pressure-assisted CE. Conditions: $l_{\text{eff}} = 35$ cm; $E = 400$ V/cm; BGE: His 90 mM and MES 90 mM; $P = 2.4$ bar.

Anions	Range (μM)^a	Correlation coefficient, r	LOD^b (μM)	RSD% separation time (n = 4)	RSD% peak area (n = 4)
Cl ⁻	3-200	0.9989	1.3	1.0	3.4
NO ₃ ⁻	3-200	0.9994	1.3	1.1	3.3
SO ₄ ²⁻	1.5-100	0.9998	0.6	1.0	3.8
Oxalate	1.5-200	0.9991	0.6	0.9	3.7
Formate	6-200	0.9990	2.5	0.9	3.8
Malonate	6-200	0.9998	2.5	1.0	4.1
Succinate	6-200	0.9996	2.8	0.9	4.0
Citrate	3-200	0.9993	1.3	1.3	3.9
Acetate	6-200	0.9992	2.5	1.3	3.5
Lactate	6-200	0.9996	2.3	1.2	3.5
Salicylate	12-200	0.9989	5.0	1.5	4.5
Benzoate	12-200	0.9997	5.3	1.4	4.9
Sorbate	12-200	0.9995	5.5	1.4	4.9
Ascorbate	50-800	0.9967	20	1.6	5.6
Gluconate	50-800	0.9976	15	1.6	5.5

^a 5 concentrations

^b Based on peak heights corresponding to 3 times the baseline noise

Figure captions:

- Fig. 1. Schematic drawing of the SIA-CE-C⁴D-system for pressure-assisted capillary electrophoresis. C⁴D: contactless conductivity detector; HV: high-voltage power supply; W: waste; V1, V2: stop valves.
- Fig. 2. Electropherograms of oxalate (200 μ M) obtained with different hydrodynamic flow velocities at relatively high pH. a) Flow rate = 0 cm/s; b) Flow rate = 0.030 cm/s; c) Flow rate = 0.062 cm/s; d) Flow rate = 0.105 cm/s; e) Flow rate = 0.314 cm/s. CE conditions: $l_{\text{eff}} = 35$ cm; $E = 400$ V/cm; BGE: Tris 70 mM and CHES 70 mM, pH 8.4. Negative high voltage applied at the detector end.
- Fig. 3. Number of theoretical plates vs. superimposed hydrodynamic flow velocity for different anions. Analytes (200 μ M): chloride, oxalate and formate in deionised water. Other conditions as for Fig. 2.
- Fig. 4. Separation of inorganic anions with normal and pressure-assisted CZE. A) Electropherograms, B) Pressure at the injection end of the capillary. a) Normal CZE ($P_a = 0$ bar); b) CE with pressure-assistance (P_b) and with negative voltage applied at the detector end; c) CE with pressure assistance (P_c) and with reversed applied voltage. CE conditions: $l_{\text{eff}} = 25$ cm; $E = 400$ V/cm; BGE: His 12 mM adjusted to pH 4 with acetic acid. Anions: 1) Cl^- (100 μ M); 2) $\text{S}_2\text{O}_3^{2-}$ (100 μ M); 3) NO_3^- (100 μ M); 4) SO_4^{2-} (100 μ M); 5) NO_2^- (100 μ M); 6) H_2PO_3^- (400 μ M); 7) H_2PO_4^- (400 μ M).

Fig. 5. Separations of organic anions. a) Pressure-assisted CZE with $P = 2.8$ bar. b) Normal CZE using CTAB (0.1 mM) in the BGE as EOF modifier. Anions: 1) Oxalate; 2) Malonate; 3) Formate; 4) Succinate; 5) Carbonate; 6) Acetate; 7) Lactate; 8) Salicylate; 9) Benzoate; 10) Sorbate; 11) Gluconate (all 200 μM). Other conditions as for Fig. 2.

Fig. 6. Concurrent separation of fast and slow anions using a pressure step. a) $P = 1.7$ bar from $t = 0$ s; b) $P = 2.4$ bar from $t = 0$; c) $P_1 = 1.7$ bar from $t_1 = 0$ s, $P_2 = 2.4$ bar from $t_2 = 240$ s. CE conditions: $l_{\text{eff}} = 35$ cm; $E = 400$ V/cm; BGE: His 90 mM and MES 90 mM. Anions (200 μM): 1) Chloride; 2) Nitrate; 3) Nitrite; 4) Sulphate; 5) Oxalate; 6) Formate; 7) Malonate; 8) Succinate; 9) Citrate; 10) Acetate; 11) Lactate; 12) Salicylate; 13) Benzoate; 14) Sorbate; 15) Ascorbate; 16) Gluconate.

Fig. 7. Determination of anions in samples using pressure-assisted CE. a) Soft drink; b) Vitamin C supplement. CE conditions: $l_{\text{eff}} = 35$ cm; $E = 400$ V/cm; BGE: His 90 mM and MES 90 mM; $P = 2.4$ bar. Anions: 1) Chloride; 2) Nitrate; 3) Oxalate; 4) Citrate; 5) Acetate; 6) Benzoate; 7) Sorbate; 8) Ascorbate.

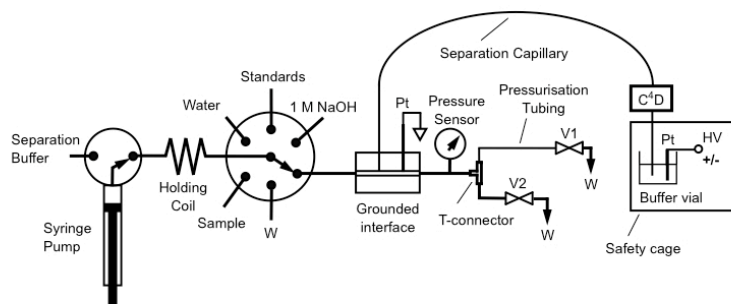


Figure 1

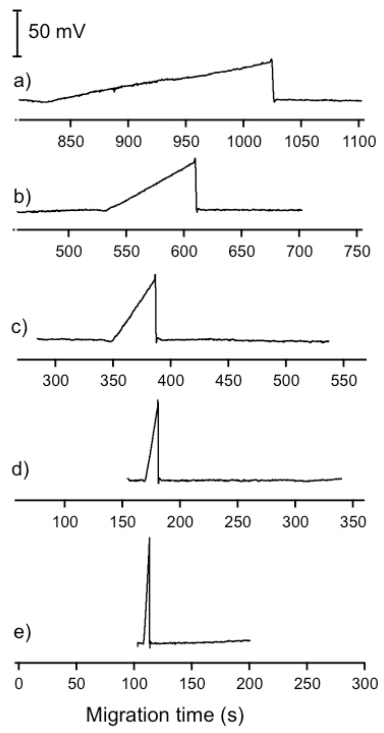


Figure 2

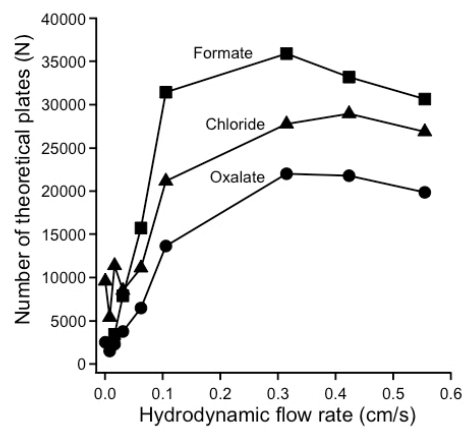


Figure 3

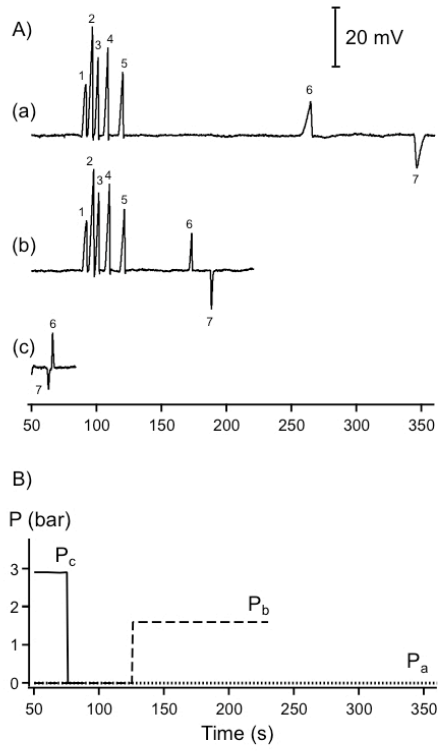


Figure 4

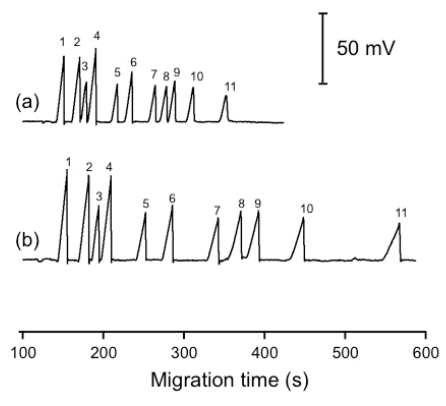


Figure 5

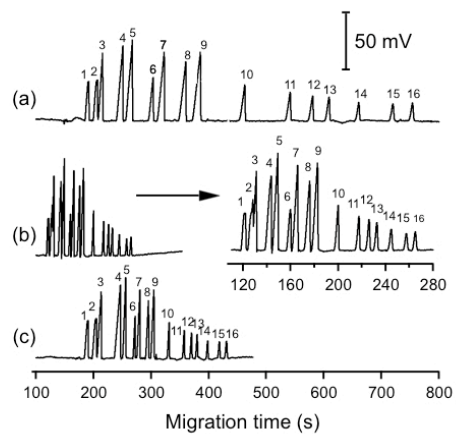


Figure 6

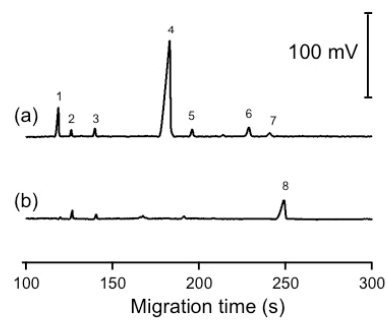


Figure 7

5th project:

Automated capillary electrophoresis with on-line preconcentration using a sequential injection analysis manifold and contactless conductivity detection

Draft of manuscript in preparation

Automated capillary electrophoresis with on-line preconcentration using a sequential injection manifold and contactless conductivity detection

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Keywords: Solid phase extraction (SPE), capillary electrophoresis (CE), sequential injection analysis (SIA), capacitively coupled contactless conductivity detection (C⁴D), drug residues.

Abstract

An extension of a capillary electrophoresis (CE) instrument with a sequential injection analysis (SIA) manifold was developed for automated CE measurements with on-line solid phase extraction (SPE) preconcentration. An in-house built capacitively coupled contactless conductivity detector (C⁴D) was employed for sensitive detection with narrow capillaries of 25 µm internal diameter. The SIA-CE-C⁴D system was assembled into standardized 19" frames and racks for easy transport and mobile deployment. The system can be left running unattendedly without any manual intervention with good operation stability. For application of the system, sensitive determination of drug residues in water was carried out with enrichment factors up to several hundreds. Detection of the drug residues in water down to nM scale was found possible.

1. Introduction

In capillary electrophoresis, detection sensitivities are generally lower than those in liquid chromatography, mainly due to small injection volumes (in the nano-litre ranges). To improve CE sensitivity, several preconcentration strategies have been applied, of which the most commonly employed are electrophoretic-based techniques (ITP, stacking, sweeping and dynamic pH junction) [1] and solid-phase extraction (SPE) methods [2-4]. The electrophoretic-based method, though having the advantage of simplicity, has such limitations as restriction of the injection volume, injection discrimination between analytes having different electrophoretic mobilities, and the need of a matrix clean-up step beforehand for complex samples. Chromatographic-preconcentration techniques, based on analyte sorption onto a solid-phase material, on the other hand, allows the loading of multiple capillary volumes of samples onto a sorbent and the elution in a small amount of solvent, resulting in much lower detection limits. In addition of increased sensitivity, chromatographic techniques also efficiently remove unretained matrix interferences, which are useful for analysis of complex matrices. Of all types of coupled SPE-CE, including off-line, at-line, in-line and on-line [5, 6], the latter design, which allows a direct stream of liquid between the SPE column and CE capillary via an interface [7], offers the highest sample throughput due to a minimum of manual and laborious sample handling and the possibility for automation.

So far, most of the applications of coupled SPE-CE have been carried out with UV, LIF or MS detection [2-4]. With conventional UV-radiation detectors, however, when the inner diameter of the capillary has to be decreased if required for better electrophoretic separation efficiency, sensitivity in CE is often deteriorated, mainly due to insufficient optical path lengths. Improved sensitivity in such cases can be accomplished by using more sensitive detection methods, such as LIF and MS. Those methods of detection, however, are very

expensive and too complicated for a compact and mobile set-up for monitoring purposes. On the other hand, with the introduction of the capacitively coupled contactless conductivity detection (C⁴D), sensitive detection is now possible even with separation channels as narrow as 10 μm [8-10]. Based on a simple measuring cell using a pair of short tubular electrodes placed on the outside of the separation capillary, C⁴D is fully electronic and less demanding in construction and power consumption than the common optical detection methods employing UV-radiation. Applications of C⁴D for CE can be found in recent reviews [11-15] whereas fundamental studies can be referred to [16-18]. The employment of C⁴D in coupled SPE-CE for enhancement of sensitivity factor, so far, however, is very limited and only in the off-line mode, which is very laborious and time-consuming [6, 19].

Liquid stream delivery in on-line SPE-CE till now has been mostly based on a LC pump or a flow injection setup [2-5, 7, 20], which is not preferable for full automation and system miniaturization. The coupling of sequential-injection analysis (SIA) based on a syringe pump and a multi-position valve with CE, on the other hand, is an attractive mean to miniaturization, automation and extension of CE. Applications of this SIA-CE combination are summarized in [21]. Recently Mai *et al.* used a SIA-CE-C⁴D system for unattended monitoring applications [22] and for pressure-assisted CE [10]. However, to our knowledge, SIA was employed for on-line SPE-CE only by Horstkotte *et al.* [23], using a multi-syringe setup with UV detection. Herein, the development and application of a CE-C⁴D system coupled with a SIA-manifold for automated SPE preconcentration and determination of some drug residues in water is reported.

2. Experimental

2.1. Chemicals and Materials

All chemicals were of analytical or reagent grade and purchased from Fluka (Buchs, Switzerland) or Merck (Darmstadt, Germany). Stock solutions of ibuprofen, diclofenac, naproxen and bezafibrate in the form of sodium salts (1 mmol/L) were used for the daily preparation of the standards. Before use, the capillary was preconditioned with 1 M NaOH for 10 min. and deionised water for 10 min. prior to being flushed with the BGE solution at appropriate pH (for 1 hour). Deionised water purified using a system from Millipore (Bedford, MA, USA) was used for the preparation of all solutions. For sample preparation, tap water or river water was filtered with 0.02 µm PTFE membrane filters (Chromafil O-20/15 MS, Macherey-Nagel, Oensingen, Switzerland), then spiked (if need be) with the selected drug residues and ultra-sonicated for 2 min.

2.2. Instrumentation

A dual polarity high voltage power supply (Spellman CZE2000, Pulborough, UK) with ±30 kV maximum output voltage and polyimide coated fused silica capillaries of 365 µm OD and 10 µm ID (from Polymicro, Phoenix, AZ, USA) were used for all experiments. Detection was carried out with a C⁴D-system built in-house; details can be found elsewhere [24]. An e-corder 201 data acquisition system (eDAQ, Denistone East, NSW, Australia) was used for recording the detector signals. The SIA section consists of a syringe pump (Cavro XLP 6000) fitted with a 5 mL syringe and a 9-port channel selection valve (Cavro Smart Valve) (both purchased from Tecan, Crailsheim, Germany). The isolation and 3-gate valves used were obtained from NResearch (HP225T021 and HP225T031, Gümligen, Switzerland). SPE cartridges packed with silica particles (56 µm) coated with C-18 group (52602-U, Supelco, Buchs, Switzerland) were fitted into the system with the help of two adapters for sample reservoirs (57020-U, Supelco). The programming package LabVIEW (version 8.0 for

Windows XP, from National Instruments, Austin, TX, USA) was used to write the control code. Further details on the instrumentation can be found in [22].

3. Results and Discussion

3.1. System design and operation

A simplified diagram of the instrument is given in Fig. 1. Several extensions and modifications have been made to the earlier SIA-CE-C⁴D design [22] in order to incorporate the preconcentration procedure into the fully automated operation. For electrophoretic separation without preconcentration, the previously reported system [22] relies on a combination of a motor-driven 2-way syringe and a multi-port selector valve for delivery of solution(s), and on a SI interface as well as some isolation valves for hydrodynamic injection and flushing of the capillary. For operation with SPE preconcentration, the essential change is the inclusion of the second holding coil (HC2) between the multi-port valve and the interface. Two holding coils play different roles in the task of liquid handling. The conventional holding coil (HC1), situated between the pump and the multi-selector valve, is utilized for aspiration of sample (for separation without preconcentration) or eluent (for elution prior to separation) whereas the other (HC2) serves as a reservoir to hold the solution eluted from the cartridge before it is pumped to the interface for hydrodynamic injection. A Y-shape intersector is employed to divert the fluid to HC2 either from the multi-selector valve (for non-preconcentration operations) or from the cartridge (for elution step in the preconcentration procedure). The employment of a 3-gate valve positioned after the cartridge allows the passing solution to be flowed either to waste (during loading of sample, flushing and generation of the cartridge) or to HC2 during elution.

Another modification concerns the on-line acidification of the sample before loading onto the cartridge. Previously, many syringes were required to acidify the sample and at the same time deliver the acidified sample to the cartridge [23]. This arrangement, however, requires so many controls of the multi syringes and valves, and is cumbersome, thus preventing the whole system to be miniaturized. This complex setup for on-line acidification is therefore replaced with a much simpler approach, using only a graduated needle valve (shown in Fig 1). The normal and splitted inputs of this needle valve are connected to the sample and to an acid solution, respectively. By adjusting the splitting ratio of the needle valve to vary the proportions of the sample and acid, a desirable pH of the aspired solution can be achieved.

To eliminate the problem of restriction of loading volume due to the limited capacity of the syringe, which was encountered in the earlier design [23], repetitive loading was employed. In this mode, the system operates in a manner that the sample is first aspired into the syringe and then pumped to the preconcentration column. The emptied syringe is then again filled with the sample for the subsequent loading. These steps are repeated many times until the expected sample volume (typically much larger than the fixed volume of the syringe) is completely loaded onto the cartridge. With this setup, there is no upper limit of the loading volume.

A photograph of the in-house built system, designed as an industrial prototype, is shown in Fig. 2. For easy transportation and deployment of the system, all the fluidic and electronic parts were assembled into a standardized 19" frame. The electronic parts were arranged in two 19" racks, one for all power supplies and the other for compartments of electronic circuitry boards, with the controls and switches built on different front panels. These compartments can be easily taken out for replacement or modification. All fluidic components, including the pump, valves, holding coils, interface and liquid containers, are fixed onto a perplex panel

situated above the two electronic racks. With this compact all-in-one design, it is very convenient to set-up the system on-site for monitoring applications. Furthermore, as the system is standardized in a 19" arrangement, facile duplication of the system is possible.

An overview of a typical sequence of the operations with preconcentration procedure is given in Table 1. The protocol starts with rinsing of the cartridge with water (step 1) prior to loading of sample onto the preconcentration column (step 2). The loaded cartridge is then rinsed again with water (step 3). Flushing of the SI-CE interface (step 4) is subsequently carried out by pumping the buffer through the interface on the simultaneous opening of both stop-valves (designated as V1 and V2 in Fig. 1). The capillary is then flushed (step 5) by advancing the stepper-motor driven syringe pump while both V1 and V2 are closed in order to push all of the dispensed fluid through the separation tubing. After these non-preconcentration steps, elution is implemented (step 6) by conducting the eluent through the cartridge while the 3-gate valve is at the C position. Once the eluted solution is already collected inside the holding coil HC2, hydrodynamic injection takes place (step 7), followed by electrophoretic separation (steps 8, 9). Hydrodynamic injection is carried out by pumping a defined sample plug past the capillary inlet in the SIA-CE interface while partially pressurizing the manifolds by closing only V2. Separation is implemented by application of the high voltage of appropriate polarity from the detector end, with the injection end remained grounded. Rinsing of the manifolds, interface and capillary with buffer (steps 10-12) is then carried out at the completion of electrophoretic separation. Finally, the cartridge is generated and flushed thoroughly (steps 13 to 16) so that it is ready for the next preconcentration operation. Separations with or without preconcentration can be selected from the computer. For separation without preconcentration, the sample is transferred directly to the SI-CE interface instead of being

loaded onto the cartridge and the steps 1, 2, 3, 4, 6, 13, 14, 15 and 16 are excluded from the protocol.

3.2. CE optimization for determination of some drug residues

Nowadays, many pharmaceuticals are classified as environmental contaminants due to their high lipophilicity and low biodegradability, and due to the fact that their bioactive properties might lead to adverse effect on human beings and ecosystems [25-28]. Determination of many drug residues in water with CE has been reported, with detection by MS [29], contactless conductivity [30] or UV, with and without stacking preconcentration [31-33]. In this study, four widely used pharmaceuticals, namely ibuprofen, diclofenac, naproxen and bezafibrate, were selected as exemplary analytes for demonstration of the functionality of the developed system. These drug residues in water were first preconcentrated on a C18 column. The eluent containing the desorped drugs was then directly used as a sample for CE separation.

Background electrolyte (BGE) composition, which was optimized based on the study reported by Quek *et al.* [30], composes of TRIS(hydroxymethyl)aminomethane, acid lactic and cyclodextrin HP- β -CD. The optimized concentrations of the BGE components indeed differ from those reported in [30] because instead of using water as the sample matrix, the eluent in SPE preconcentration containing a certain proportion of an organic solvent will be employed as the sample matrix. It is shown in Fig. 3 the electrophoretic separations of these 4 pharmaceuticals in different matrices with conductivity detection. In the case where water is employed as the sample matrix, due to very low conductivity of water compared to that of the BGE, stacking effect is provoked at the beginning of electrophoresis, resulting in sharp and high peaks, as reflected in electropherogram (a) of Fig. 3. These sharp peaks in turn favoured sensitive detection, which is exactly the situation reported in [30]. However, when samples are prepared in the SPE eluent comprising TRIS, acid lactic and acetonitrile, high

conductivity of the matrix diminishes the stacking effect, and the analytes are visualized at the detector as very broadened and low peaks, as shown in Fig. 3b. To maintain the stacking effect, the concentrations of TRIS and lactic acid in the BGE were increased by four times, while keeping their relative proportions unchanged. By doing so, sharp peaks were obtained for all four analytes and resolutions were not degraded, as demonstrated in Fig. 3c.

Cyclodextrin HP- β -CD was added into the BGE as a complex reagent for separation of ibuprofen and diclofenac. It possesses a neutral charge, which does not contribute to the conductivity of the BGE. Its presence in the BGE thus was kept fixed at the optimized concentration of 1 mM. The salient performance data are given in table 2. The achieved detection limits are in the order of 0.8-1.5 μ M and the calibration curves were acquired from about 2 to 100 μ M. For higher concentrations peak overlap would occur for naproxen and diclofenac. The reproducibility of the measurement of peak areas and migration time was found to be 2.5 – 5% and 1 – 1.5%, respectively.

3.3. Optimization of SPE preconcentration

3.3.1. Selection of SPE cartridge

For optimization of the flow through the sorbent, different shapes and sizes of the preconcentration columns were tested. Self-made columns using tubings of 1/8" internal diameter (ID) packed with C18 material were first tried, but were found not suitable due to backpressure elevated over time and inconvenience in replacement of the used material. Commercial cartridges of 3mL, though eliminating the above-mentioned drawbacks, encounter another problem of bubble formation. The optimal type of preconcentration column was then found to be the commercial cartridge of 1mL. This cartridge is packed with 100 mg absorbing material, with 2 frits 20 μ m positioned at both end of the cartridge to confine the

material in between. For renewal of the sorbent, it is sufficient to plug a new cartridge into the adapters already fixed between the multi-selector valve and the 3-gate valve.

3.3.2. Sample acidification

The four investigated drug residues are carboxylates, which are negatively charged in water of neutral pH. For complete adsorption onto C18 sorbent particles, these compounds must be uncharged under acidic condition prior to being loaded onto the column. HCl 0.1N was found to be suitable for sample acidification since a minor amount (less than 1% v/v) of this strong inorganic acid solution is sufficient to keep the pH of the sample under the pKa of these carboxylates (less than 4). The amount of HCl 0.1N added can be considered negligible compared to the sample amount to be loaded onto the cartridge and therefore does not affect the enrichment factor. Note that higher concentrations of HCl were avoided to prevent corrosion of the graduated needle valve.

3.3.3. Optimization of sample loading and elution

As a large volume of sample (hundreds of millilitres) has to be loaded onto the sorbent for preconcentration, optimization of the loading speed is very crucial for enhancement of sample throughput. The configuration of the stepper-motor driven pump in principle allows a variation of flow rate from some $\mu\text{L/s}$ to more than 1000 $\mu\text{L/s}$, depending on the syringe capacity. However, due to the employment of very slender tubings of only 0.02" ID to minimize diffusion and dilution during the delivery of solution(s), the actual flow rate is limited to only 417 $\mu\text{L/s}$ so that the stepper motor can function properly. At faster transferring velocity, leakage of liquid induced from excess backpressure was observed at the multi-selector valve. Optimization of the loading speed thus was carried out within this restricted range. Slow loading velocities significantly augment the operation time, whilst too high flow

rates through the cartridge can lead to formation of bubbles and incomplete retaining of the analytes on the sorbent. Accordingly, the optimal loading speed was found to be 140 $\mu\text{L/s}$.

For efficient desorption of the retained analytes, the flow rate and the volume of elution were investigated. Since the sorbent particles packed in the commercial column occupy about one fourth of the cartridge volume, *i.e.* 250 μL , the elution volume must be larger than this value in order for all the particles to be in contact with the eluent. To minimize any dilution of the eluent plug along the delivery from HC1 through the cartridge to HC2, a volume of 500 μL was used for elution. Larger amounts of eluent were avoided to maintain high enrichment factors. The elution speed was then optimized for the fixed elution volume of 500 μL , and was found to give complete desorption of the retained analytes at 25 $\mu\text{L/s}$. In other words, optimal elution occurs within 20 seconds.

3.3.4. Optimization of the eluent composition

For complete elution of all four protonated drug compounds retained on the C18 cartridge, two requirements must be satisfied. First, electrolytes in the eluent must produce a pH much higher than pKa(s) of the retained compound(s) in order to make them deprotonated, which in turn facilitates desorption from the C18 material. Second, a suitable organic solvent miscible in water must be added into the eluent to a certain percentage since desorption depends much on the affinity of the dissolved organic component with the retained compounds. In addition, because the eluent containing the eluted analytes is used directly as a sample for electrophoretic separation, the eluent composition should contain only the components present in the background electrolyte in order to avoid any unexpected and undefined peak(s) in the electropherogram with conductivity detection. Accordingly, a solution composed of TRIS 9 mM and lactic acid 5 mM was employed for elution. This eluent has a pH of 8, which is

exactly the pH of the BGE. Different organic solvents frequently used in HPLC, namely methanol, acetonitrile and tetrahydrofuran, were then tested for the TRIS/lactic acid eluent. Note that the organic proportion must be kept as low as possible to prevent bubble formation caused by evaporation of the organic solvent under a high electric field (the boiling points of these tested organic solvents are lower than that of water) and thus to assure a stable baseline during electrophoretic separation. Methanol, due to its characteristics of weak reversed phase, must be used at very high concentrations (more than 50% v/v) for good elution.

Tetrahydrofuran, which possesses strongest reversed phase property among all commonly used organic solvents miscible in water, though offering very efficient desorption even at low concentrations (less than 25 % v/v), disturbs the resolution of the peaks of naproxen and ibuprofen. Acetonitrile, which locates between methanol and tetrahydrofuran in the scale of reversed phase strength, was therefore chosen as the organic solvent for elution. Effect of the concentration of acetonitrile on elution efficiency is shown in Fig. 4. Four retained drugs are eluted with different efficiencies, with diclofenac as the easiest compound to be desorbed. Complete elution occurs at acetonitrile concentration of 37.5 %v/v in an eluent of TRIS 9 mM and lactic acid 5 mM. Note that no significant elution was observed when only acetonitrile in water was employed as the eluent even at concentrations more than 50 % v/v.

3.3.5. Cartridge generation

A solution of TRIS 9 mM and acid lactic 5mM mixed with acetonitrile (50 %v/v) was used as the generating solution. A large generation volume of 5 mL was employed to assure efficient removal of organic species (if any) retained on the cartridge after preconcentration. After being flushed with the generation flow, the cartridge was subsequently rinsed with 5 mL of HCl 0.1M to dissolve precipitate(s) (if any) formed on the surface of the sorbent particles

during precedent operation(s) at high pH. Finally, the cartridge was rinsed with deionised water (5mL) before being used for the next preconcentration.

3.3. Performance

In order to evaluate the potential for unattended operation, the system was set up for a supervised test run over a period of 10 hours per day in 3 continuous days, in which repeated preconcentrations with an enrichment factor of 30 and CE measurements of the pharmaceuticals (0.5 μM prepared in DI water) were carried out. The results for peak areas are shown in Fig. 5. The maximum deviation is about $\pm 8\%$, which is deemed acceptable considering that this deviation is the accumulation of minor and inevitable errors in different operations, *i.e.* sample loading, elution, injection and separation. Note that even without preconcentration procedure, deviations up to $\pm 4\%$ were still observed for automated CE separations [22]. No bias of peak areas was recorded after more than 50 continuous runs in 3 days, which demonstrates the suitability of the system for unattended operation.

The operation performance was further evaluated by carrying out preconcentrations in more severe conditions, in which solutions of pharmaceuticals were prepared directly in tap water matrix instead of in DI water. Loading of 375 mL of tap water spiked with pharmaceuticals (0.01 μM) and desorption with 0.5 mL eluent were repeated several times with the same cartridge. The enrichment factor in this case is 750, which is relatively large for SPE preconcentration. The results for peak areas are shown in Fig. 6. It is observed that after 3 successive preconcentrations, peak areas of all pharmaceuticals decrease significantly, among which those of naproxen and bezafibrate are dramatically reduced. It may be due to the fact that when a huge sample volume is flushed through the cartridge, part of the C18 group coated on silica sorbent particles is permanently covered with irremovable species previously

present in the matrix in minor quantities. For good preconcentration performance, a loading volume of 1000 mL thus should not be exceeded.

4. Conclusions

With the in-house built SIA-CE-C⁴D system, designed as an industrial prototype, automation of CE measurements with on-line SPE preconcentration was made possible. The compact all-in-one arrangement allows the system to be easily transported and deployed. All operations, especially the preconcentration procedure are carried out in an unattended manner, which otherwise are very time consuming and laborious with manual involvement. With preconcentration prior to CE-C⁴D determination, the concentrations of drug residues in water can be monitored down to the nM scale, which is impossible with normal CE setups. The application scope of this system can be expanded to various monitoring tasks and for sensitive determination of different charged species present in water in trace quantities.

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Table 1: Typical operation sequence with preconcentration

Step	Description	Position of Selection Valve	Volume Dispensed (μL)	Flow rate ($\mu\text{L/s}$)	V1	V2	3-gate valve
1a	Pick up of water	5	5000	140	Open	Open	O
1b	Flushing of the cartridge with water	8	5000	140	Open	Open	O
2a ⁿ	Pick up of sample	9	5000	140	Open	Open	O
2b ⁿ	Dispensing of sample to cartridge	8	5000	140	Open	Open	O
3a	Pick up of water	5	5000	140	Open	Open	O
3b	Flushing of the cartridge with water	8	5000	140	Open	Open	O
4a	Buffer aspiration	-	3000	167	Open	Open	O
4b	Flushing of syringe and HC1 with buffer	6	3000	167	Open	Open	O
5a	Buffer aspiration	-	3000	167	Open	Open	O
5b	Flushing of the SI-CE interface	7	1000	167	Open	Open	O
5c	Flushing of the capillary	7	25	8	Closed	Closed	O
6a	Pick up of eluent	4	500	140	Open	Open	O
6b	Dispensing of eluent through cartridge to HC2	8	500	25	Open	Open	C
7	Sample injection	7	500	167	Open	Closed	O
8	Flushing of the SI-CE interface	7	500	167	Open	Open	O
9*	Electrophoretic Separation	7	0	-	Closed	Closed	O
10	Flushing of the SI-CE interface	7	500	167	Open	Open	O
11	Flushing of the capillary	7	25	167	Closed	Closed	O
12	Empty syringe	6	-	167	Open	Open	O

13a	Pick up of generating solution	3	5000	140	Open	Open	O
13b	Dispensing of generating solution to cartridge	8	5000	140	Open	Open	O
14a	Pick up of water	5	5000	140	Open	Open	O
14b	Flushing of the cartridge with water	8	5000	140	Open	Open	O
15a	Pick up of HCl 0.1N solution	2	5000	140	Open	Open	O
15b	Dispensing of HCl 0.1N to cartridge	8	5000	140	Open	Open	O
16a	Pick up of water	5	5000	140	Open	Open	O
16b	Flushing of the cartridge with water	8	5000	140	Open	Open	O

n: the steps 2a and 2b are repeated n times until the desired sample volume is completely loaded onto the cartridge.

*: High voltage is turned on at step 9.

Table 2: Calibration ranges, detection limits (LOD) and reproducibility for the determination of some drug residues in water. Conditions: capillary 25 μm ID, $l_{\text{eff}} = 41$ cm; $E = 400$ V/cm; BGE: TRIS 36 mM / Lactic acid 20 mM / HP- β -CD 1 mM.

Compound	Range (μM)^a	Correlation coefficient r	LOD^b (μM)	RSD% MT^c (n = 4)	RSD% PA^d (n = 4)
Ibuprofen	2-100	0.9976	0.8	1.2	2.7
Bezafibrate	2.5-100	0.9986	1.3	1.1	4.3
Diclofenac	2.5-100	0.9993	1.5	1.5	5.4
Naproxen	2.5-100	0.9987	1.5	1.6	4.9

^a 6 concentrations

^b Based on peak heights corresponding to 3 times the baseline noise

^c MT: migration time

^d PA: Peak area

Figure captions:

- Fig. 1. Schematic drawing of the SIA-CE-C⁴D-system for automated electrophoretic separation with on-line preconcentration. C⁴D: contactless conductivity detector; HV: high-voltage power supply; Pt: Platinum electrode; W: waste; V1, V2: Stop valves; HC: Holding coil.
- Fig. 2. Photograph of the in-house built SIA-CE-C⁴D system, designed as an industrial prototype.
- Fig. 3. CE separations of pharmaceuticals in different matrices. (a) Matrix: water; buffer: TRIS 9 mM / Lactic acid 5 mM / HP- β -CD 1mM; (b) Matrix: TRIS 9 mM / Lactic acid 5 mM (62.5 % v/v) and Acetonitrile (37.5 % v/v); Buffer: TRIS 9 mM / Lactic acid 5 mM / HP- β -CD 1mM; (c) Matrix: TRIS 9 mM / Lactic acid 5 mM (62.5 % v/v) and Acetonitrile (37.5 % v/v); Buffer: TRIS 36 mM / Lactic acid 20 mM / HP- β -CD 1mM. Other CE conditions: U = 400 V/cm, feedback resistance for C⁴D = 1 M Ω , capillary of 25 μ m inner diameter and 41 cm effective length. 1) Ibuprofen; 2) Bezafibrate; c) Diclofenac; 4) Naproxen.
- Fig. 4. Effect of acetonitrile concentration on elution efficiency. The eluent is prepared by addition of acetonitrile into a solution of TRIS 9 mM / Lactic acid 5 mM. Peak areas were obtained from electropherograms with following CE conditions: U = 400 V/cm, feedback resistance for C⁴D = 1 M Ω , capillary of 25 μ m inner diameter and 41 cm effective length, BGE composed of TRIS 36 mM / Lactic acid 20 mM / HP- β -CD 1mM.

Fig. 5. Reproducibility of peak areas for the preconcentrated pharmaceuticals during continuous runs of 10 hours per day in 3 successive days. For each operation, 15mL solution of standard mixtures 0.5 μ M in DI water was loaded onto a C18 cartridge followed by an elution with 0.5 mL eluent of TRIS 9 mM / Lactic acid 5 mM (62.5%) + CH₃CN (37.5%). Peak areas were obtained for a preconcentration factor of 30. Other CE conditions as in Fig. 4.

Fig. 6. Reproducibility of peak areas for the preconcentration and CE determination of the pharmaceuticals spiked in tap water. For each operation, 375 mL solution of standard mixtures 0.01 μ M prepared in filtered tap water was loaded onto a C18 cartridge followed by an elution with 0.5 mL eluent of TRIS 9 mM / Lactic acid 5 mM (62.5%) + CH₃CN (37.5%). Peak areas were obtained for a preconcentration factor of 750. Other CE conditions as in Fig. 4.

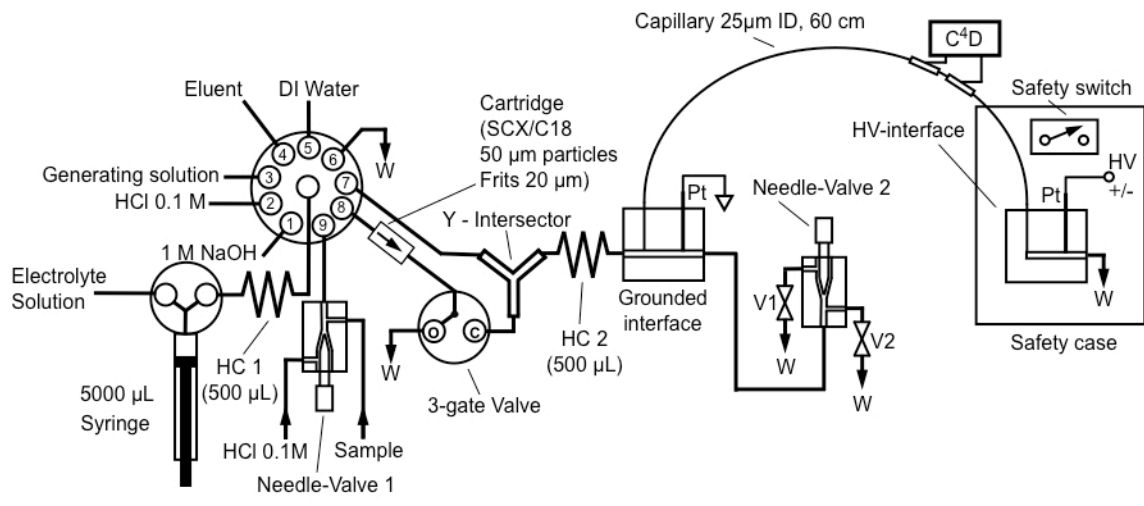


Figure 1

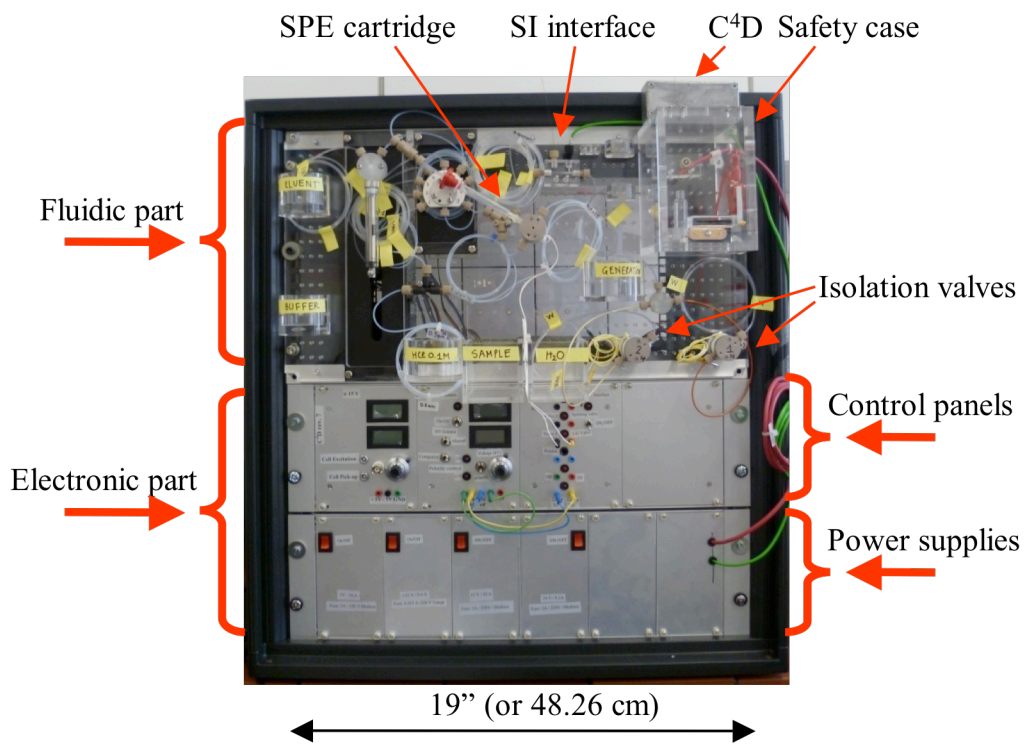


Figure 2

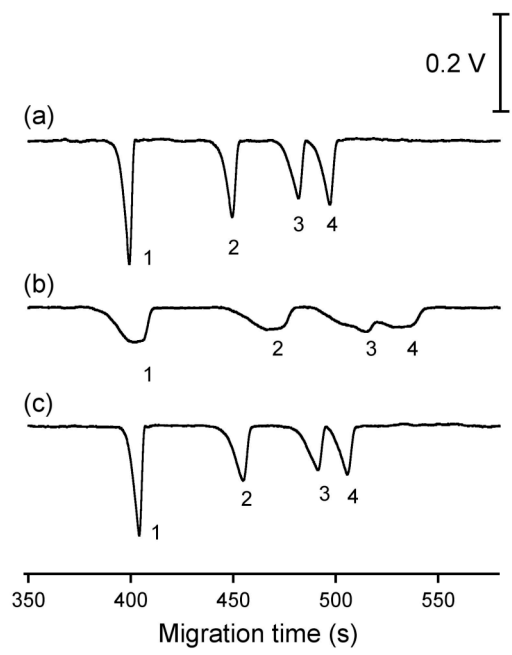


Figure 3

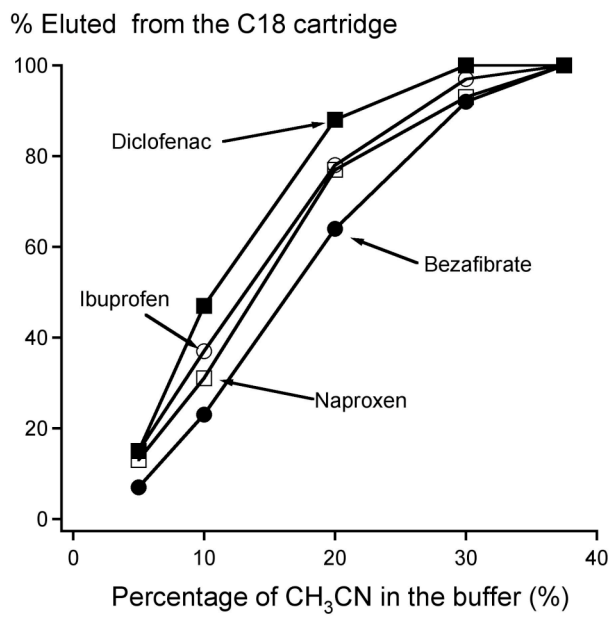


Figure 4

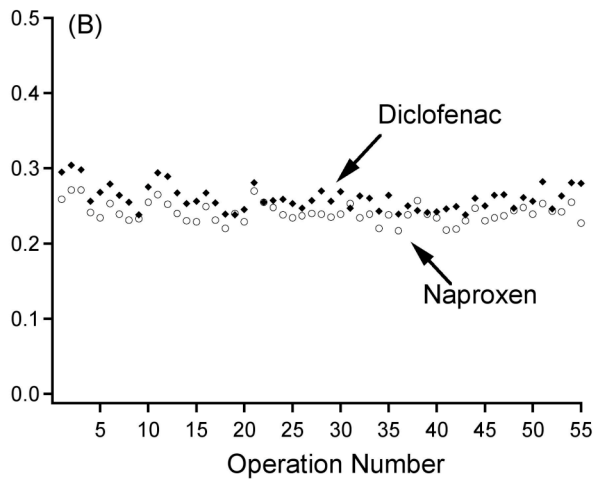
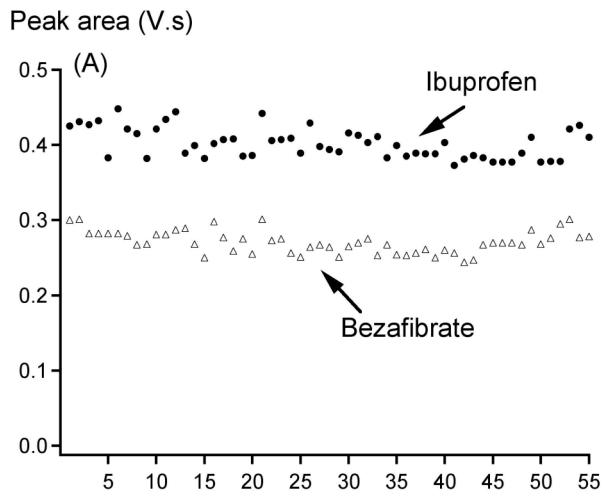


Figure 5

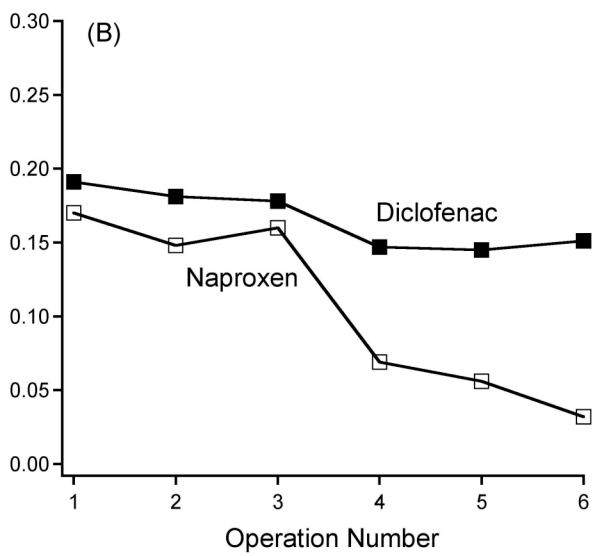
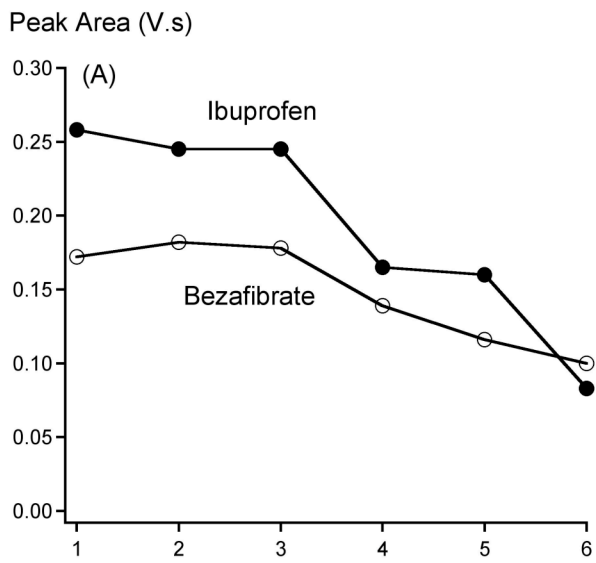


Figure 6

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4. Curriculum Vitae

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EDUCATION & QUALIFICATIONS

- 2009 - 2011** PhD student at the University of Basel (Switzerland).
Specialty: Analytical and Bioanalytical Sciences
- 2007 - 2009** Postgraduate at the University of Basel; holder of a Swiss Federal Scholarship.
- 2005 - 2007** Master of Science in Hanoi University of Science - Vietnam National University.
Specialty: Environmental Chemistry
- 01 - 06 /2005** Master student at the Technical University of Denmark (DTU)
- 2001 - 2005** Bachelor of Science in Hanoi University of Science (Vietnam National University);
Specialty: Chemical Technology. *Grade: Very good (average marks 8.9 / 10)*
Holder of Toyota (Japan) scholarship in 2002
- 2001** Baccalaureate, *grade: very good*
University-entrance examination attendance:
Vietnam National University: marks 28.5 / 30
University of Foreign Trade: marks 29 / 30, first-rated

WORK EXPERIENCE

- 2011** Key member of a research project financed by National Foundation for Science and Technology Development (Nafosted, Vietnam)
<http://nafosted.gov.vn/funded/2010.php?mid=104&year=2010>
- 2005 - present** Staff of the Research Centre for Environmental Technology and Sustainable Development (CETASD) – Hanoi National University
(http://www.hus.edu.vn/tienganh_ver/institues_centres/institutes_centres1.htm)
- 2007** Member of a survey project supported by the Swiss Federal Institute of Aquatic Science and Technology (EAWAG – Switzerland)
(<http://sozmod.eawag.ch/gallery.php?id=2>).
Project's name: Testing strategies for the adoption of techniques for As removal for households in Vietnam.
- 2004 -2007** Member of VietAs Project – Water Resources Research in Vietnam, a Danida Enreca Project (<http://vietas.er.dtu.dk/>).

LANGUAGE & COMPUTER COMPETENCES

- Languages:** English: *excellent (Toefl score: 607)*
German: *intermediate (Goethe Certificate, level B1)*
French: *intermediate*
Vietnamese: *mother language*
- Informatics :** Win XP, Microsoft Office, Excel and some other special advanced softwares, such as Labview, Surfer.

OTHER INFORMATION

- 2001 – 2005:** Prefect of the university course
- Hobbies:** Sports, reading news, cooking, traveling (already been to Denmark, France, Holland, Sweden, Germany, Austria, Switzerland, Italy, China, Thailand)

5. List of Publications and Posters

4.1. Publications

1. **Thanh Duc Mai**, Hung Viet Pham, Peter C. Hauser
Capillary electrochromatography with contactless conductivity detection for the determination of some inorganic and organic cations using monolithic octadecylsilica columns.
Analytica Chimica Acta, 653, (2009), 228-233
2. **Thanh Duc Mai**, Stefan Schmid, Beat Mueller, Peter C. Hauser
Capillary electrophoresis with contactless conductivity detection coupled to a sequential injection analysis manifold for extended automated monitoring applications
Analytica Chimica Acta, 665, (2010), 1-6 (featured article)
3. **Thanh Duc Mai**, Peter C. Hauser
Pressure-assisted capillary electrophoresis for cation separations using a sequential injection analysis manifold and contactless conductivity detection
Talanta, 84, (2011), 1228-1233 (special issue)
4. **Thanh Duc Mai**, Peter C. Hauser
Anion separations with pressure-assisted capillary electrophoresis using a sequential injection analysis manifold and contactless conductivity detection
Submitted to Electrophoresis, (2011)

4.2. Posters and presentation

1. **Thanh Duc Mai**, Peter C. Hauser

Capillary electrophoresis with a contactless conductivity detector as a versatile simultaneous detection technique for sequential injection analysis

**16th International Conference on Flow Injection Analysis, Pattaya, Thailand
April 2010**

2. **Thanh Duc Mai**, Peter C. Hauser

Sequential injection technique coupled with contactless conductivity detection for automated and pressure-assisted capillary electrophoresis

Anakon 2011, Zuerich, Switzerland, March 2011

3. Marko Stojkovic, Worapan Pormsila, Benjamin Bomastyk, **Thanh Duc Mai**,
Thitirat Manhim, Peter C. Hauser

Contactless Conductivity Detection with conventional and micro-chip electrophoresis

25th International Symposium on Microscale Bioseparations. Prague, Czech Republic, March 2010