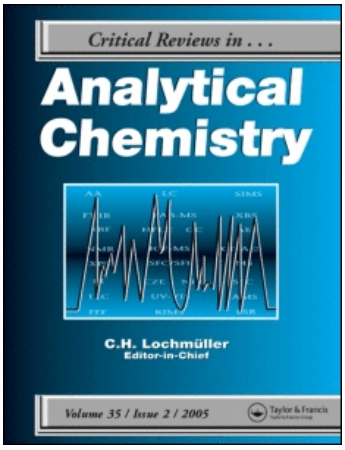

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Nonconventional Samplers in Capillary Electrophoresis

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ABSTRACT: Developments in nonconventional sample introduction in capillary electrophoresis have focused on the possibility of forcing the sample stream to pass the separation capillary inlet. The advantages of such input devices are the absence of a voltage rise/drop time during sampling, ease of operation because no vial manipulations are involved, and ease of automation and computerization. However, besides these the main advantage of such input devices seems to be the fact that they facilitate easy, multiple input from a single sample vial. This opens the possibility of monitoring the concentration changes taking part in inside the vessel — an important task for — analytical biotechnology.

This article describes some possible designs of CE nonconventional samplers and discusses applications of such samplers in sample preparation, in coupling CE with flow injection analysis (FIA) and HPLC, in multiple input experiments (with or without stacking) for reduction of detection limits and for monitoring of reaction kinetics.

KEY WORDS: capillary electrophoresis, nonconventional interfaces, preconcentration, clean-up, monitoring.

I. INTRODUCTION

Capillary electrophoresis (CE) is an attractive separation technique that has received much attention since its introduction more than 2 decades ago.[1] Since the pioneering work by Hjerten,[2] Everaets,[3] and Jorgenson,[4] it has developed into an important analytical tool for the separation of charged compounds, ranging from small inorganic ions to large molecules such as proteins and even viruses, cells, and particles. In contrast to the common slab gel electrophoresis, CE method is versatile, has high resolution power, allows rapid analysis, and needs very small amounts of sample and chemicals. One of the advantages of the method is the ease of computerization. Despite the wide acceptance of the method, and its outstanding advantages over other separation techniques, some aspects of the technique still remain weak. The sample introduction mode and low concentration sensitivity are among those that most often demand further efforts for improvement.[5,6,7]

The sampling methods usually applied in CE are injection with a syringe, injection valves, in an injection block, and dipping one end of the capillary tube into the sample solution, the last two being most common and widely used. In all these cases the sample is introduced by the gravity flow or by electroendosmosis and/or electromigration. Because the task of the sampler in CE is to introduce reproducibly a small amount (usu-

ally several nanoliters) of sample without mixing it with the background electrolyte, the use of a syringe has several disadvantages. These range from reading errors, especially for small amounts of sample, to the perhaps greatest disadvantage — mixing of the sample with the buffer. Automated carousel samplers are more precise and convenient. However, both types of conventional samplers are often inflexible in terms of changes in the operation mode and is difficult to connect new parts into the separation system, for example, units for on-line sample preparation or concentration. Moreover, contemporary sampling devices are not very suitable for monitoring purposes, or do they permit performing fast, multiple injections without high-voltage interruption.

In attempts to extend the field of application of CE adding more flexibility into the sampling and overcoming many disadvantages of common samplers found in commercial instruments, several research groups have focused their attention on the development of nonconventional sample injection devices. Nonconventional sampling devices (or interfaces) are designed to achieve at least one of the following goals in CE:

1. On-line sample clean-up/preparation/preconcentration
2. Hyphenated techniques (CE method is combined with another analytical method to increase the resolution power of the method)
3. Monitoring of the changes in the reaction vessel or *in vivo*
4. The reduction of detection limit by using multiple input techniques
5. High-speed separations

II. EARLY DEVELOPMENTS IN SAMPLING INTERFACES

The very early, lab-made CE instrumentation designed by Hjerten[2] (Verheggen et al.[8]) included quite ingenious and sophisticated sampling. Because of the need to introduce very tiny quantities of sample, many researchers acknowledge that the method of sampling is one of the central instrument problems in capillary electrophoresis. Classically, the sample is introduced manually using mostly electro-osmotic flow or suction/siphoning. In the case of electro-osmotic method, the total amount of each solute injected depends on the nature of the solute. In sample introduction with suction/siphoning, it is difficult to inject repeatedly a precise amount of sample. The reproducibility of experiments is immensely improved when using sampling valve where a known amount of a representative sample solution is introduced without mixing it with the background electrolyte. However, the volumes of CE capillaries are very small and therefore the volume of the loops were usually two to three orders of magnitude higher than it is acceptable in terms of separation efficiency.

Verheggen et al. worked out a coupling interface for narrow bore columns.[8] They used a T-piece to connect preseparation column with final separation column to lower the detector limits. Deml et al. developed a sampler in which a dosing valve. The volume of the valve was about 1 μl. The valve was connected with an electric splitter in order to reduce the amount of sample introduced into the capillary.[9] The concept of the splitter is presented in Figure 1. The apparatus is embodied in a monolithic block of polyester resin. It contained two capillaries: one for dosing, and the other for separation that is, dosing valve, electrolyte chambers, operating valves, and detection cell. The sample was introduced via a dosing valve. The current I1 flows through the dosing capillary and drives the original sample, S_1. This current is then split into I_2, which drives part, S_2, of the original sample into the separation capillary. It is I_3 that leads the rest of the sample, $S_3 = S_1 - S_2$, to the drain. The sample

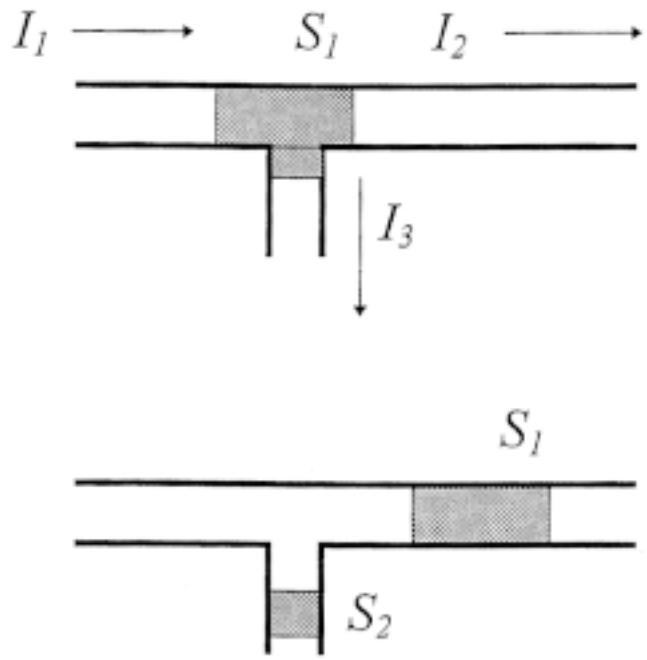


FIGURE 1. Electric sample splitter.

migrates electrophoretically in two electrical circuit paths and the splitting ratio is given by the ratio of the corresponding electric currents. This system enabled to introduce adjustable, very small amount of sample into the separation capillary. The reproducibility of the experiments was not given. If only limited amount of sample is available then the feature of the sampling — only a small part of the applied sample was analyzed with the greater part discarded — could be disadvantageous.

Another approach to improved sampling in CE is to build sample valves with smaller, inner-volume. Tsuda et al. devised a rotary type injector that worked under high voltage. The component parts of the injector were made from fine ceramic and tetrafluoroethylene resins. The inner volume of the rotor was 350 nl. The injector had several advantages: (1) the injection amount was accurate, (2) it was easy to exchange sample solution by refilling a passage with a microsyringe, (3) injection was possible while a high electric field was applied. However, there were also some disadvantages: (1) the injection amount was still bigger then usual in CE, (2) for safety reasons, it was necessary for the injector to be operated automatically, (3) Because the sample solution was kept continuously under a high electrical field, there was a possibility that unstable components may decompose if kept in the flow passage for long periods of time.

A simple sampling device for capillary zone electrophoresis was constructed by Verheggen et al.[11] In this system the capillary was inserted into a block that was connected to electrode compartments. The sampler block involved valves to introduce in and out both background electrolyte and sample solution. The sampling device consisted of a broadened part of the capillary. The sample was introduced directly into this part by means of two feeders that were perpendicular to the capillary tube. The reproducibility of the experiments was less than 2% RSD. The data about detection limits or zone length were not given. The capillaries used were of 250 μm inner diameter.

Tsuda and Zare also described a split injector for capillary electrophoresis.[12] This sampling system was suitable both for circular and rectangular capillaries. The sampling interface consisted of an automatic rotary valve, delivery tube of fused silica capillary, and a reservoir with a buffer. The buffer reservoir and the injector were connected through the delivery tube. Into the buffer reservoir one electrode and the inlet end of the rectangular or circular capillary was mounted. Between the end of the delivery tube and the inlet end of the separation capillary was a gap whose length varied from 0 to 10 mm. The sample was delivered through the narrow delivery tube into the sampling interface. During whole operation the interface was under high voltage. At the interface the flow was split — small part of the flow was carried by electromigration into the capillary, most of the sample left through the opened part of the interface and was removed. The split-injector had its advantages and disadvantages. One of the advantages was that the device enabled sampling without high-voltage interruption therefore being a pioneer in this area. The sampling method also permitted using large cross-section capillaries — particularly rectangular capillaries. The separation efficiency of the method was rather sufficient. The biggest disadvantages were again the wasting and dilution of the sample.

A fully automatic mechanical sampler based on siphoning was devised by Honda et al.[13] The sample operated according the following principle: the cathode vessel (where the outlet end of the capillary was inserted) remained at the fixed position throughout an analysis, but the anode vessel was on a turntable. Its level could be changed automatically by a microcomputer-controlled motor. During the changing anode vessel for sample vessel the anodic end of the capillary was held at the original level by means of a small rubber stopper. During sampling the turntable was raised to an higher level. The sampler enabled reproducible introduction of a sample (the coefficient of the variation was 0.5 to 3% depending on the length of the sampling time). The reproducibility of the sampling was 3 to 5 times higher than in the case of using manually operating sampler based on siphoning. The sampler that served as a prototype of the following autosamplers had some disadvantages. It was not flexible enough for changes and provided no benefits in terms of detection limits or sample clean up. However, the sampling reproducibility of the method was significantly better.

The samplers discussed so far were designed for capillaries with an internal diameter (i.d.) of 200 to 300 μm. Using capillaries of larger diameter permits introduction of a bigger amount of sample. However, due to Joulian heating, capillaries with i.d. larger than 100 μm were not very useful because of the decreased efficiency of the separation. For capillaries with i.d. less than 100 μm, the amount of sample introduced by above described sampling interfaces is too large. Therefore, eventually it was found that sample introduction into smaller capillaries can be performed by tipping the end of the capillary into sample vessel and applying subsequently either electric field or gravity flow to move sample into the end of the capillary. Rose and Jorgenson introduced an autosampler enabling one to perform both electrokinetic and hydrodynamic sampling.[14] The autosampler enabled the comparison of electromigration and hydrodynamic sample introduction methods in terms of reproducibility, zone dispersion, and preferential introduction of sample components. The autosampler was computer controlled, and it was possible to automate whole sample introduction process.

The sampler consisted of a sample tray with places for 20 sample vials and 2 buffer vials. To select the desired vial a stepper motor was used to rotate the tray. The inner diameter of the fused silica capillaries was either 75 μm i.d. or 50 μm i.d. The autosampler was software controlled and able

to perform a sequence of steps to introduce sample into the end of the capillary. First the buffer vial was removed into the tray and then the desired sample vial was rotated and raised up to the capillary/electrode assembly to the same level as the previous buffer reservoir was been. In the case of sample introduction by electromigration, high voltage was applied to inject sample into the inlet end of the capillary. If hydrodynamic sample introduction was carried out, the sample vial was lifted until the desired height difference between sample and outlet buffer vial levels was achieved. The sampling time was determined by a programmable timer. After the sampling time the sample vial was again changed for buffer reservoir by rotating the tray. The inlet buffer reservoir was lifted to the same level with the outlet buffer reservoir. Then the high voltage was applied and the experiment started.

Experiments with manual sample introduction were carried out simultaneously to compare the reproducibility of results in both cases. The automatic sampler gave significantly better results both in the case of electrokinetic and hydrodynamic sample introduction mode. This was apparently due to the greater reproducibility of experimental conditions (e.g., the sampling time, the height of sample liquid levels, and applied voltage). Comparing automatic electrokinetic sampling with hydrodynamic sample introduction showed slightly better results in the case of hydrodynamic sampling mode. The study of the influence of sample mode (e.g., electrokinetic of hydrodynamic) on sample zone efficiency showed no significant differences.

Common electrokinetic/hydrodynamic samplers met in commercial instruments have been designed for handling "reasonable" amount of sample. Most of the cases there is sufficient sample volume available for experiments in CE, especially because the volumes needed for CE analyses are small. However, in some cases of biological samples (for example, if the blood diseases of small birds are under the study or single cell analysis) the amount of sample can be extremely limited. In electromigration introduction the sample volume must be large enough to allow both the capillary and electrode to dip into the sample solution. In the case of very limited sample volumes, this is an important limitation. A "double-barrelled" microinjector for CZE has been constructed to relax this limitation. The drawbacks of this type of injector were the problems with electric coupling between the barrels and band broadening that was caused by connections between injector and capillary. The other problem, which is specific to electromigration sampling if sampling from very small sample volumes, is the creation of electrochemical reaction products as during the period of high voltage applied to the sample solution. The applied electric field may cause damage or contamination of the sample solution.

If the sample is introduced hydrodynamically, the needed amount of sample must be sufficient to accommodate the tip of capillary and generally no sample contamination problems occur. Still throughout the studies of the hydrodynamic sampling it showed that the amount of sample in the vial influenced the amount of sample introduced into the capillary (the injection time was kept constant). When reducing the external sample volume the amount of sample introduced into the capillary also decreased. The reduction of peak area was significant. Therefore, in the case of quantitative analysis a need exists for an internal standard both in the case of electrokinetic and hydrodynamic injection.

III. SAMPLERS INVOLVING ON-LINE SAMPLE CLEAN-UP/ PRECONCENTRATION

It is generally agreed that improvements in on-line sample preparation and reduction

of limits of detection of the conventional UV absorbance detection mode in CE method are needed. It is especially true in the case of biological samples. Laser-induced detection is very sensitive and may even allow the detection of counted number of molecules, but this detection mode is neither always directly applicable nor achievable. With regard to sample pretreatment, CE has the requirements originating from the fused silica capillary properties (ionizable inner surface where hydrophobic macromolecules may easily absorb) and very narrow inner diameter; therefore, the sample must be cautiously filtered, etc. In some cases the high concentration of matrix components may severely interfere with the determination of the analyte or destroy the separation. However, the need of sample pretreatment can be a problem if very diluted samples are analyzed because the concentration of trace analytes may be affected easily when pretreatment manipulations are carried out. Sample pretreatment can also be laborious and time consuming; therefore, several interfaces are designed to enable on-line sample pretreatment in CE.

A. Membranes

One of the most widespread approaches to facilitate sample pretreatment in CE is the usage of different kind of membranes. Membrane interfaces are capable of interface transfer, matrix isolation and preconcentration. Membranes have been applied to different modes of chromatography and also to flow injection analysis.[16] Considering of pressure limitations, membrane interfaces have to be coupled on the loading channel of a loop type sample injector in a chromatograph. As in a CZE system high pressures are not used, the membrane interface can be connected in-line.

Bao and Dasgupta demonstrated effective membrane interfaced CZE system utilizing porous, permeative, and dialysis membranes and showed real sample applications ranging from the determination of atmospheric trace gases to the analysis of wastewater and constituents in blood plasma. In the interface a membrane (porous, silicone rubber, or dialysis) tube united two segments of silica capillary tubing. (see Figure 2). The first segment had to be short and of larger bore to minimize voltage drop across it

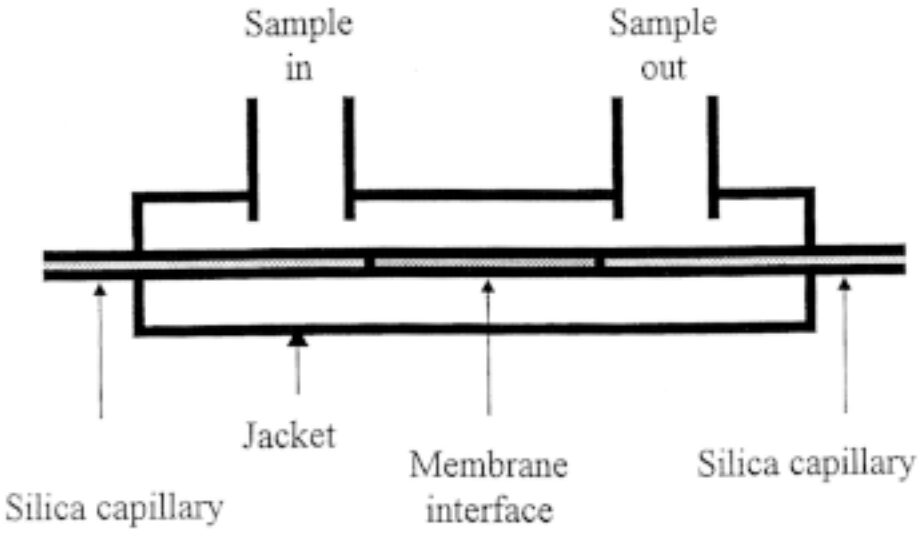


FIGURE 2. Membrane interface.

(sample is introduced after this). The active length of the membrane was 1 to 1.5 mm.

The sampler enabled to analyze mixtures of gases (if porous membrane was used) without any pretreatment. The detection limits were quite good, and the reproducibility of measurements was moderate. In some cases particular tailing occurred. The reason for that could be that during gas flow through a stagnant liquid acceptor phase microbubbles could form at the inner surface of the membrane. This leads to inefficient washout pattern that is probably responsible for the tailing. The other problem to be recognized was that water vapor penetration through the membrane, supported by Joulian heating of the liquid phase, can lead to condensation in the surrounding jacket. Unless the condensed water is removed before subsequent sampling, sample gases are removed by the liquid. Dry gas flow is used to prevent this condensation.

The sampler was effective for measurement and concentration of both ionizable and nonionic compounds. For that purpose, a silicone rubber membrane was used. Preconcentration of ionizable compounds via a membrane interface needs just to adjust pH values in the donor and acceptor phases so that the compound would be unionized in donor solution (in this form an analyte is permeable through a silicone membrane) and ionized in acceptor solution (the charged form does not penetrate through the membrane) and an one way transfer path is established. This system does not work with nonionic compounds. The creators of the described sampler established a mechanism for preconcentration of neutral molecules using a micelle solution as the acceptor phase because the partition constant for a typical neutral molecule from a water solution into a micellar solution can be quite large. To minimize peak tailing and rising of the baseline, the jacket volume was washed with aqueous acetonitrile solution after sampling. The observed LODs for analytes (e.g., 8, 150, and 240 ppb for *o*-nitrophenol, *m*-cresol, and phenol, respectively) were acceptable for conventional UV absorptiometric detection in CZE. Silicone membrane interfaced systems can handle real world samples bearing particulate matter, oil, and grease, etc. The recoveries of measured analytes spiked into wastewater ranged from 30 to 96%, depending on the particular wastewater sample. Comparative studies showed that the plate numbers of peaks decreases significantly when using membrane conduits.

Authors did not address the problem of permeation rate of different sample components. It is well known that permeation rates of different compounds differ a lot depending mainly of the molecular weight of the compound. Thus, the membrane at the capillary inlet can be a powerful discrimination factor, which may be disadvantage or advantage depending on the problem. In this light the recoveries reported above for the membrane interface (96%) seems to be rather optimistic. It was also not clear shown how much the LODs received depended on the preconcentration step. A complication of the method was also the need for continuous pH adjustment, because the pH of the acceptor and donor solutions influenced the permeation process of analytes. This was especially disadvantageous if the analytes had very different pKa values. Reproducibility of the experiments ranged from 3.2 to 6.6 in RSD being reasonable but not very high.

The dialysis membrane interface was used for presampling purposes only, to measure low-molecular-weight constituents of blood plasma. In this mode the acceptor concentration of analytes cannot be higher than that of the original sample. In this case no specific studies on the reproducibility, the response of the sample contact time, or LODs were made. Using the above-described membrane/capillary interface, sample pretreatment and preconcentration was performed; however, with a cost of quite remarkable loss in efficiency.

Kuban and Karlberg designed an elegant interface that connected an on-line dialysis unit with capillary electrophoresis. (The dialysis part was built from parts of flow injection analysis (FIA) system connected via PTFE tubing. Cuprophan dialysis membranes were used in commercial dialysis unit. Two peristaltic pumps were used in the system. CE system used common fused silica capillary (i.d. 50 μm), high-voltage (HV) supply, and UV spectrophotometric detector.) The dialysis part was connected to the interface through the programmable injector via PTFE tubing (see Figure 3). The interface body was made of Plexiglas®. Into the Plexiglas® box a channel was drilled. The inlet end of the capillary and one electrode were inserted into the channel. In normal mode one peristaltic pump delivered fresh running buffer to the channel, the other pump pumped the acceptor solution (sample) from the dialysis unit to the fill the loop of the injector. In the injection mode the injector was switched so that the running buffer flowed through the sample loop and swept the sample plug from the loop and carried it to the interface's channel. The channel serves both as the sample vial and a buffer reservoir. As the HV was applied, part of the sample was sucked into the capillary while the sample plug was passing the inlet end of the capillary. Time periods for filling and injection were determined by respective positions of the injector's thumb wheel settings.

The system described was effective for on-line clean up for real samples. Samples of tap-water, road snow, mud, milk, and juice were analyzed directly or after minimal pretreatment (road snow samples were melted and introduced twice into the dialysis system; mud samples were dispensed and mixed in water before introduction into the system). The system perform injections without HV interruption, which enables to rise the speed of analysis and makes the interface suitable for monitoring purposes. Unlike common CE samplers requesting interruption of high voltage during sampling, the described FIA sampler operated under continuously applied high voltage. Therefore, no irreproducibility was inherited from HV rise/drop time. As the fresh running buffer flowed continuously through the channel, no sample contamination occurred. The system

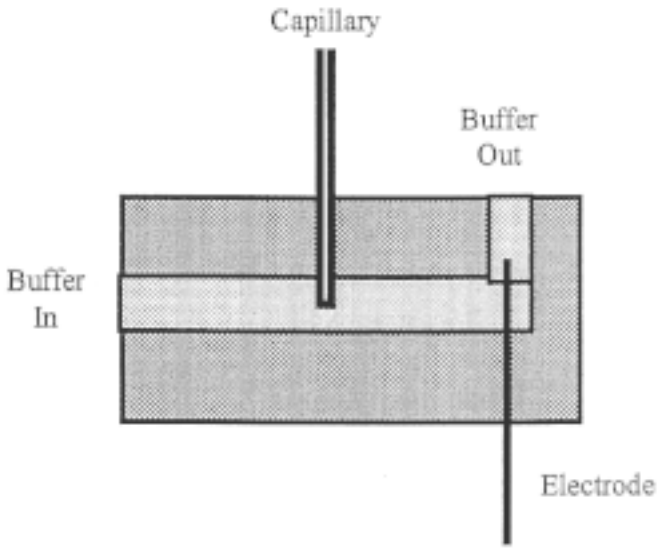


FIGURE 3. FIA-CE interface.[19]

would be easy to build in every laboratory where FIA equipment is available.

The repeatability of the system ranged from 0.7 to 3.3 and 1.6 to 6.7% R.S.D. (being different for different ions) in terms of peak height and peak area, respectively. The concentration sensitivity of the system was moderate (0.1 to 0.3 ppm for model samples) for UV detection of anions. The data of detection limits in real samples were not presented. The factors decreasing concentration sensitivity in the case of present method are probably the following: (1) dialysis unit used has diluting effect on the sample; (2) sample band was submitted to physical dispersion while migrating through the connecting tubing. The concentration sensitivity increased if the flow rate of the running buffer was decreased. The result was expected for then the sample band was longer before the inlet end of the capillary and the real sampling time was therefore longer. However, flow rates lower than 0.8 ml/min caused distortions on peak effects.

Kuban et al. developed the interface further adding a timer controlled valve to the sampling device that allowed performing hydrodynamic injections and therefore enabled more representative sample injecting.[21] The reproducibility and the detection limits of the method were similar to the above-described device.[20]

Dialysis membrane interfaces are not very demanding in construction sophistication terms and can be easily manufactured in house. As an another example of the membrane interface for the CE, the authors of this paper have also developed a CE system where a dialysis unit was connected to the pneumatic sampler (will be described later in Section VI). This interface is presented in Figure 4. The dialysis unit was made from a Plexiglas block. In this block a cylindrically shaped acceptor reservoir was drilled. Two channels leading in and out of this reservoir were also drilled into the block. The acceptor reservoir has a thread that enables one to fix and tighten a sheet of Spectrapor® mem-

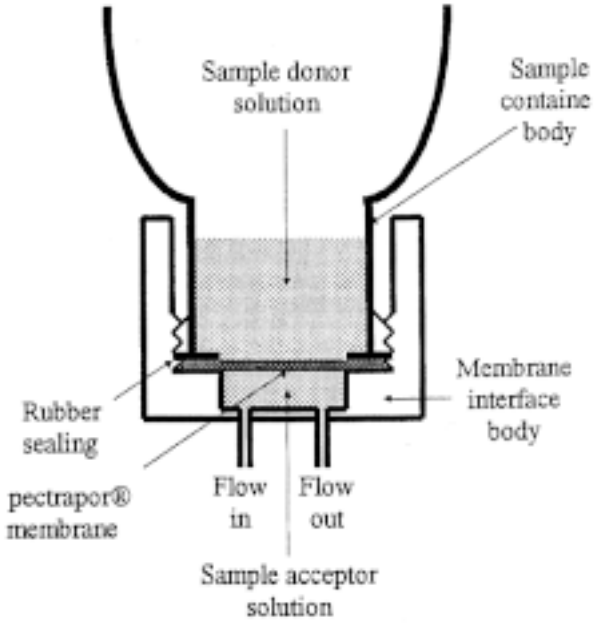


FIGURE 4. Membrane interface for CE.

brane using a bottomless glass bottle. This bottle forms the donor reservoir of the sample. In this way membrane covers the acceptor reservoir and separates it from the donor reservoir. The dialysis unit was connected with the inlet channel of the sampling device via PTFE tubing. The system made possible to analyse milk and juice without any sample preparation. The present FIA-CE interface allows to perform highly reproducible experiments, as demonstrated below in Section V. The kinetics of distribution of different analytes and the influence of the concentration and osmotic pressure of the system is under study.

B. Sorbents

Despite the fact that CE permits determining very wide variety of molecules the analysis of trace components in real samples with complex matrixes often is a problem. In this case the common solution in analytical chemistry is an implementation of a sorbent that enables to discriminate between analytes and sample matrix. This approach has been used successfully in CE as well. Morita et al. have designed a three-capillary system that allowed of direct pretreatment of a biological sample.[22] The system enabled to determine low drug concentration in serum by separating the drug prior to the electrophoretic separation step from interfering serum proteins. The system consisted of three pieces of fused silica capillary (namely, injection, drain, and separation capillary) connected to each other via a T-type connector (see Figure 5).

Into the inlet end of the injection capillary a short gel-filled capillary was inserted. The outlet ends of drain and separation capillaries were tipped into buffer reservoirs, while the solution at the inlet end of the injection capillary was changed according to the different steps of the procedures. The system also consisted of two alternatively applied power supplies. During the injection step high voltage was applied between the ends of injection and drain capillaries. The sample flowed by electromigration through the gel-filled bed. The analyte was kept in the gel bed by hydrophobic interactions, while serum proteins flowed through drain capillary to waste. After washing the injection capillary by running buffer to ensure the sample matrix removal, the system was washed with elution buffer (which contained high concentration of organic solvent) to elute analyte from the gel-bed. At the next step elution buffer was again changed for running buffer and the separation process started.

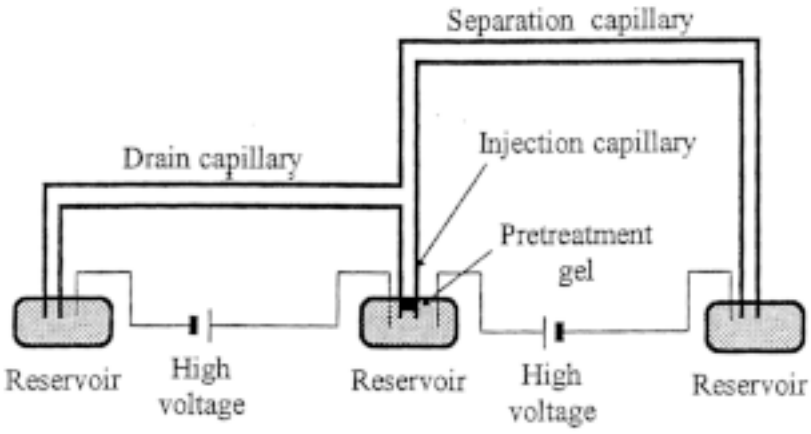


FIGURE 5. Three-capillary system for direct pretreatment of a biological sample.[23]

During last two steps high voltage was applied between the ends of injection and separation capillaries.

The system allowed to separate one single analyte from complex biological matrix without remarkable decrease in zone efficiency, and it also allowed to lower the detection limits by preconcentration of the analyte about two orders of magnitude. The recovery of the analyte was good. However, using the gel-bed on the top of injection capillary changed the migration time of the analyte about 10%, and there was no data presented about how good was the reproducibility of the experiments in terms of peak areas and migration times. It is also not clear how good that kind of system could be for separating multianalyte systems. The system was not computer controlled.

C. Sample Stacking and Isotachphoresis Sample Preparation

Capillary electrophoresis because of the "availability" of a high strength electric field in the equipment permits the use a specific sample concentration method as an alternative to the common sample concentration by sorption. The method is called sample stacking. The method implements difference in analyte velocities in solvents with different conductivity. Sample stacking has been demonstrated to be an efficient sample preparation technique.[24,25,26,27] The technique is usually performed just filling the capillary inlet manually or automatically (or even whole volume of the capillary) with sample solution that has lower conductivity than running buffer. Less common are the samplers that implement stacking as an essential component of their performance. Nevertheless, several ingenious and convenient devices have been presented.

Kaniansky et al. demonstrated that isotachphoresis (ITP) can be excellent sample preparation technique.[28,29] Using three capillaries connected by a "tee" unit to the CE. This is especially useful when the analyte is to be separated from the excess of environmental matrix. Applying high voltage to the ends of a capillary and with correct timing,

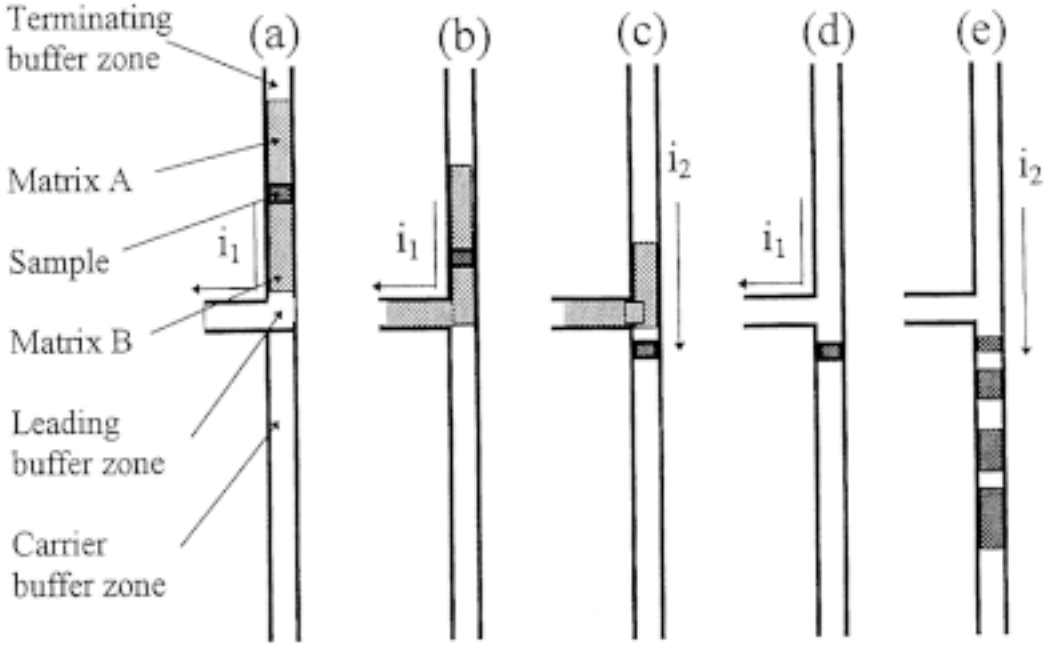


FIGURE 6. Sample matrix removal stages in ITP-CE coupled system.

it is possible to bypass matrix flow from the separation capillary and focus sample zone its' end. In Figure 6, different stages of the ITP-CE sample preparation are represented. First in stage (a) isotachphoresis takes place in direction of the driving current i_1 and matrix B is flushed out of system as shown in stage (b). Then voltage is applied in such manner that current i_2 flows in direction shown in stage (c). Sample stacks into the separation column head. In the next stage (d) voltage is applied again in direction of the current flow i_1 that causes the second matrix component A to be flushed out of column. At the final stage the electrophoresis separation is performed.

Stegehuis et al. presented a system where separate isotachophoresis (ITP) system was coupled with separate CE system.[30] The coupling interface was a Plexiglas box with two cross-channels. Into one end of the "horizontal" channel the ITP capillary was positioned. The CE capillary was inserted into the ITP capillary from the other end of the "horizontal" channel. The one arm of the "vertical" channel of the interface expanded to the buffer reservoir (the buffer reservoir served as leading buffer vial for ITP), the other arm directed the excess of the stream exiting from ITP capillary to waste. The interface allows one to perform both electrokinetic and hydrodynamic injections. The need for exact timing made electrokinetic injection less favorable then the hydrodynamic injection (especially in terms of reproducibility of experiments). Coupling two abovementioned separate systems enabled to perform on-line sample concentration and reduce the detection limit of the CE method by three orders of magnitude. The reproducibility of the system was also improved by factor of two. Hydrodynamic sample injection technique to transfer ITP zones to the capillary has to be done very carefully not to disturb either ITP or CE processes. The authors achieved the goal by applying small difference in level (4 cm) between the terminating and electrophoresis buffer vials, which generates a small hydrodynamic flow. Low detection limit achieved was, however, due to LIF detection used. The system was rather sample consuming, for only 10% of the sample was introduced into the capillary in the case of hydrodynamic sampling.

Kuldvee and Kaljurand have reported a pneumatically operated interface performing mixed hydrodynamic/electrokinetic sample introduction. Sampler is capable to perform head column field amplified sample stacking (HCFASS). Sampler scheme is shown in Figure 7.

Sample operates by applying pressures to the sample/buffer vials and sampling logic is presented in Table 1.

In all nonconventional samplers, the sample is thought to be introduced electrokinetically. However, in most cases there is a pressure used in the systems to force the sample pass or reach the inlet end of the capillary. Therefore, the sample could partially be introduced by hydrodynamic phenomena. However, the pressures used are quite low and the rinse/flush times short and the hydrodynamic part (if it exists) is negligible. However, in the case of the abovementioned pneumatically driven autosampler, the hydrodynamic part was big enough to be taken into account if the sample flow time in the inlet channel was longer than 0.5 s. To maintain the hydrodynamic part insignificant and allow pure electrokinetic sampling, the sample flow time was kept as short as 0.25 s. This time was long enough to completely fill the inlet channel with fresh sample. Using sample flow times and pressures higher than 1.5 atm. gave remarkably higher signal to noise ratio for sample peaks (if the sample was dissolved in water) and therefore lower the detection limits of the method three orders of magnitude. The reason for that phenomenon was believed to be partly due the phenomena of head column FASS, for example, little amount of the low conductivity sample solution was introduced

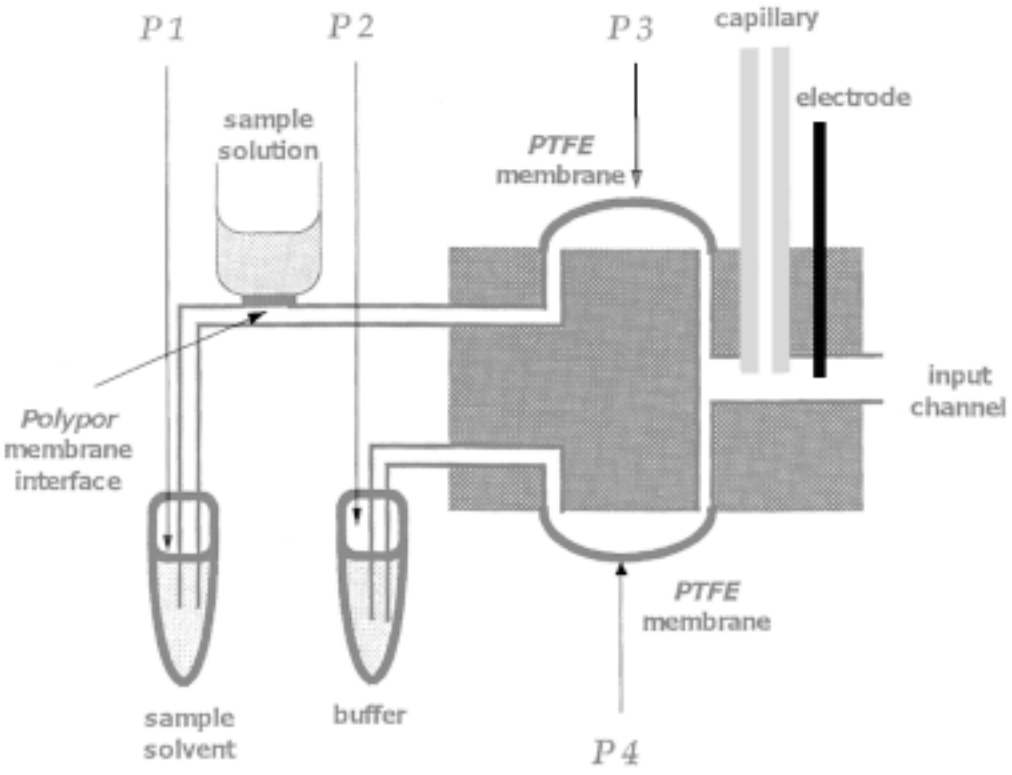


FIGURE 7. Schematics of pneumatic sampler.

TABLE 1
Sampling Sequence Logic Used in Pneumatic Sampler

Step #	Action	P_1	P_2	P_3	P_4	Duration
1	Sample rinse	Off	On	On	Off	0.125–10 s
2	Electrokinetic sampling	Off	Off	On	On	0.25–10 s
3	Buffer rinse	On	Off	Off	On	0.125–0.25 s
4	Pherogram run	Off	Off	On	On	5 min–1 h

into the inlet end of the capillary creating an electric field much higher than in the rest of the capillary. This high electric field acted as a trap for sample molecules in the inlet channel sucking them into the capillary. On the boundary of low conductivity zone and sample zone the sample zone was stacked into a narrow zone. The other reason for the signal-to-noise ratio improvement was probably the fact that during long sample flow the amount of the sample before the inlet end of the capillary is always the same (e.g., fresh sample is provided continuously), while during long electrokinetic sampling from stagnant solution the sample can get exhausted. The sampler enables easily to control the ratio of sample amounts introduced hydrodynamically and electrokinetically.

The reproducibility of the experiments was about 2% for peak areas and less than 1% for migration times if the sample flow time was kept constant. Changing the flow

time affects the migration times of the sample remarkably and an internal standard can be needed. The length of the sample flow time has a impact also at the separation efficiency decreasing with increasing the flow time.

IV. HYPENATED TECHNIQUES

Capillary electrophoresis is famous for its high power of resolution. On the other hand, being an instrumental technique, it lets itself easily to "hyphenate" with, for example, mass spectrometry to facilitate the detection of analytes. Hyphenation with the sampling side is less common. Kuban et al.[19,20,21] proposed the use of CE as a possible detection device for flow injection analysis (FIA). Relevant interface used by those authors for the FIA-CE coupling was described already in part IIIA. Fang et al.[6] proposed similar simple flow-through arrangement that could used for FIA-CE coupling: carrier flow from flow injection analyzer continuously flushes the separation capillary inlet and if analyte zone is carried by the FIA reagent flow it is electrokinetically introduced to the column when the analyte zone passes by the capillary (see Figure 8). Both authors introduced their interface as a FIA-CE coupling devices. However, in fact, an essential feature of FIA — an analyte reaction with carrier reagent — was not implemented in works described above. This is, of course, a matter of terminology, the devices itself appeared to be very useful for the analysis of real samples without the complicated sample preparation step.

The analysis of complex mixtures can require more than one separation process in one dimension can achieve in order to resolve all the sample components. This recognition has caused an ample interest in the area of two-dimensional (2D) separation techniques to join the resolution capacity of two (or more) methods.

Giddings and Davis are responsible for laying the conceptual foundation for the 2D separations.[32,33] The 2D methods are based on different separation mechanisms. For example, the separation in reversed phase liquid chromatography is based on the of hydrophobicity of analytes, while in the case of

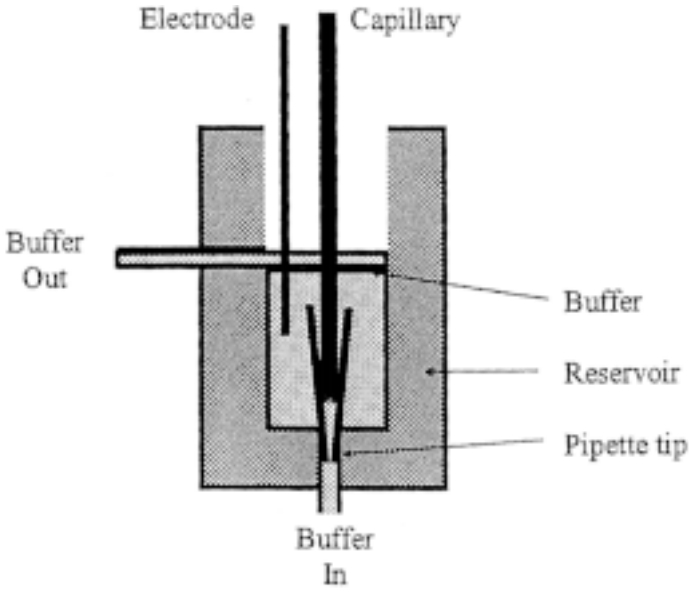


FIGURE 8. Schematic of the flow-through reservoir interface for FIA-CE coupling.

capillary zone electrophoresis it is a function of solute's charge-to-size ratio. This fact can be implemented successfully aiming almost orthogonal separations. Works of many groups of researchers proves the efficiency of 2D systems. The early developments of 2D separation systems were capable of analysing only a few number of samples in second dimensions, that is, performing only a few "heart cuts" of chromatographic zones eluting from the first dimension column. The reason for this was the slowness of the separation in the second column. Currently, the methods of high-speed chromatography and electrophoresis have been developed to the level that separation can be achieved within minutes or even seconds and one can talk about *comprehensive* 2D chromatography. In comprehensive 2D chromatography the effluent from the first column is subjected to the separation in the second column after every fraction of a minute. Remarkable results have been obtained in comprehensive 2D GC[34] and in 2D HPLC.[35,36] Coupling capillary electrophoresis systems with different modes of liquid chromatography is of high interest because of the interrelated nature and remarkable resolution power of both methods. However, coupling CE with another separation method needs an interface with specific characteristics: for sample volumes acceptable in CE are generally few orders of magnitude lower than those used in HPLC.

Kok et al. developed a capillary zone electrophoresis-liquid chromatography (CZE-LC) system where the capillary electrophoresis part was used for on-line clean up for liquid chromatography.[37] The system consisted of three PTFE capillaries (i.d. of the capillaries was 0.5 mm) that were connected to each other and to the liquid chromatography part by three six-port valves. In the start of experiment all three capillaries were filled with buffer solution. Then the first capillary (sample capillary) was filled with sample and HV was applied. The sample components were separated in the electric field according to their different charge-to-size ratio (the low molecular mass components moved faster) while traveling through transfer capillary to the injection capillary. When the zones of interest reached the injection capillary, HV was switched off and LC eluent flushed the sample component zones to LC column. The system was used to separate low molecular mass ionic components (which were of interest) from neutral compounds and from high molecular mass sample components.

The method proved to be a promising technique for on-line clean up of biological samples for LC. However, due to the comparatively large i.d.'s of the capillaries the heat produced in capillaries may influence the reproducibility and efficiency of the process. The reproducibility of the system was not studied. Also, because of the complexibility of the system the automatization of the process seems desirable.

Jorgenson's group has contributed to the 2D HPLC-CE the most. Bushey and Jorgenson introduced the first automated "comprehensive" 2D system that coupled column liquid chromatography with CZE.[38] In this system all sample constituents separated in reversed phase liquid chromatography (RPLC) were subjected to separation by CZE. The coupling of the two methods was facilitated by the use of the conventional column and sample loop technology. Jorgenson designed another 2D separation system based on size exclusion chromatography (SEC) and CZE.[39,40] SEC was carried out in packed capillary columns of 250 and 100 μm i.d. Because of their small volumes and operating flow rates, micro-columns pose an serious engineering puzzle in terms of designing a suitable 2D interface for use with CZE. Lemmo and Jorgenson have worked out two approaches to couple microcolumn SEC with CZE. The first approach is a further development of the valve/loop design used previously by Bushey and Jorgenson. In this a

six-port electrically actuated valve was fitted with a 300 nl collection loop. The loop was made in house from a piece of 50 μm i.d. fused-silica capillary. The microcolumn end was connected with short silica capillary by a piece of Teflon® tubing. The other end of the short capillary was connected with the valve by PEEK tubing. This helped to protect the fragile end of the microcolumn and therefore prolong its lifetime. It also made the insertion and removal of the microcolumn easier. The electrophoresis capillary was connected to the valve by Delrin "Tee" and PEEK tubing. Into the Delrin T-connection a stainless steel tubing was also inserted. This tubing served both as waste line and a ground electrode for CE system. However, in this way only limited success was accomplished. The main problem with the valve/loop interface was that it needed a certain amount of the effluent to be collected. The volume of the effluent collected during one run of electrophoresis was about 900 nl. The volume was too big to allow using optimum SEC flow rate or column inner diameter. The effluent volume needed was not determined only by the volume of the loop but also by volumes inside the valve (like the volumes of six connection ports of the valve and engravings on the rotor). The attempts solve the problem by underfilling the loop were not successful because underfilling diluted the sample and enlarged the dispersion that made detection limits for the studied proteins impractical.

The second approach was realized by circumventing the collection of the sample in a loop. This new interface (see Figure 9) overcomes the shortcomings associated with sample collection.

The in-house built flow gating interface consisted of two stainless steel plates with a Teflon® gasket between them. Into one plate the outlet end of SEC microcolumn was inserted. The inlet end of electrophoresis capillary was connected into the other plate just across the outlet end of the microcolumn. Into the Teflon® gasket a channel with 1 mm diameter was cut to allow the liquid flow through the plates. Normally, the capillary electrophoresis buffer run through the channel and carried the effluent from the microcolumn to the waste. An electrically activated valve controlled the flow. When

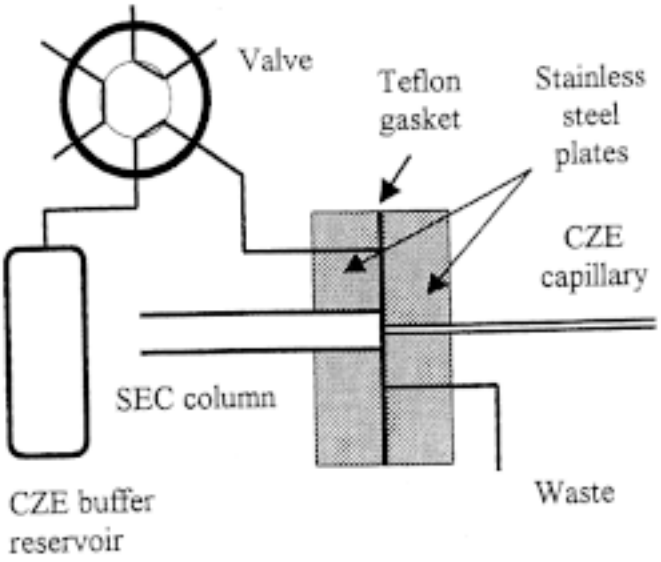


FIGURE 9. Set-up for 2D micro HPLC-CE.

the injection was desired the buffer flow was interrupted and the sample flowed into the narrow gap that separated microcolumn and CE capillary. The sample was introduced into the capillary by electromigration. When the desired sampling time was elapsed the transverse flow resumed and the SEC effluent was carried to waste until the next injection. Consequently, the amount of sample injected did not depend any more on the diameter of the microcolumn or the rate of the eluent. The system was completely computer controlled.

Compared with the valve/loop interface, the flow gating interface enabled improvement in SEC resolution and an increase in the separation efficiency due to operating at a lower flow rate and decreasing the microcolumn diameter, respectively. The interface performed very reproducible experiments in terms of sample transfer. The RSDs for peak area and peak height were 1.9 and 3.1%, respectively. The RSD for migration time was 0.18%. The sensitivity of the measurements was increased about eight times compared with the loop/valve interface, but still the LOD was not very low. The flow gating interface was further developed by Hooker and Jorgenson. This interface was used for coupling microcolumn high-performance liquid chromatography (micro-HPLC) with capillary zone electrophoresis (CZE). The interface was similar to the above described interface but was now made from transparent polycarbonate (trade name Lexan). The material was relatively well machined, which made the fabrication of the channels and the designing of the interface quite easy. This homemade interface consisted of a 1-in diameter Lexan disk into which channels for microcolumn and CE capillary were drilled. Perpendicular to these channels the transverse flow channel was bored. The outlet end of the microcolumn and the inlet end of the capillary extended out of their channels into the flow channel. The interface operated similarly to the previously described flow gating (FG) interface: In the normal mode the running buffer flowed through the transverse flow channel and swept the effluent from microcolumn into waste. High voltage was simultaneously applied. For the injection the run voltage was dropped, while the transverse flow continued until the voltage was equal to zero. Then the transverse flow stopped and high-voltage was applied again. The short period between high voltage dropping and rising was called the "slew down" time. The slew down time was needed to prevent sampling during the high-voltage drop time that would cause peaks fronting. After the desired injection time was over, high voltage was switched off again. Then, first the transverse flow was switched on, to wash the remains of the sample out from the gap between the ends of capillary and microcolumn (this short period of time was called "slew up" time), and after a short time interval (2 to 0.5 s) high voltage was switched on again and the CE experiment started.

The experiments showed the right timing in operation was very important for influencing both the fronting and tailing of peaks but also the zone broadening due to diffusion. The last was the case if the slew up time was too long (2 s). Longer slew up times also lessened the amount of sample injected because part of the injected sample diffused out of the capillary. The other very important parameter was the gap distance the ends of capillary and microcolumn. If the gap was too big (more than 100 μm), then the sample band eluted from microcolumn got too diluted and the amount of analyte injected into capillary was too small. On the other hand, if the gap distance was less 40 μm, the sample tended to be electroinjected during the run and to avoid that rather high buffer flow rates were needed.

Compared with the former interface the transparent flow gating (TFG) interface has several advantages: (1) Due to its transparency, TFG more easily allowed connecting

the columns and capillaries into the interface. This was especially important in the case of connecting the microcolumn, which was connected into former FG interface via a capillary tubing. The additional tubing contributed to the overall zone broadening. (2) The transparency of the TFG interface made possible to reduce time for troubleshooting because several problems (e.g., air bubble between two capillaries) were now easily diagnosed. (3) It was easier and less time consuming to change the parameters of operation (e.g., the distance between the outlet of the microcolumn and the inlet of the capillary; the time duration of "slew down" and "slew up" periods) to optimize the injections. (4) The material was chemically inert. Therefore, no reactions between the interface and analytes may occur.

Both above-described flow gating interfaces are impressive in terms of the high separation power, high reproducibility (the % RSDs of the peak height and peak area were 2.5 and 3.5%, respectively). The reproducibility of the migration times of TFG interface was improved further being 0.07%. The system introduced very little extra-column band broadening allowing the achievement of a plate number of 480,000 in less than 35 s. These interfaces have already been used for coupling microdialysis and on-line immunoassays to CZE.[42,43] The systems need tuning before operation, but the transparency of the later developed interface made the tuning easy. Despite the impressive performance the transverse flow gating interface could be subjected to certain criticism. "Slew up/down" time value seems to be critical for the interface performance; however, one could wonder why high voltage has to be switched off at all. The other weak side of these systems seemed to be the relatively low concentration sensitivity despite the fact that in both cases a laser-induced fluorescence detection was used. The detection limits were not reported in the case of the last interface.

Because the speed of the separation in the second dimension is critical in performance of 2D systems so is the functioning speed of the device that introduces the sample to the second dimension. Lasers can be implemented successfully. Moore and Jorgenson designed a 2D reversed phase liquid chromatography–capillary zone electrophoresis (RPLC-CZE) system using a unique optical-gating injection system. This arrangement can be used even more advantageously by extending the dimensionality of separation system. The authors used the same system for designing comprehensive three-dimensional (3D) separation using size exclusion chromatography (SEC) as a first dimension for the system[44,45] (HPLC being the second and CE the third). The CZE part was in both cases connected to RPLC system via a simple stainless steel T-interface. The inner volume of the T-interface served as the inlet buffer reservoir for CE system (the interface was connected to ground). Into one arm of the interface the inlet end of a fused silica capillary was inserted, the other arms were connected to a waste valve and to RPLC column (via a flush valve) by PEEK tubing. The waste and flush valve were used for preconditioning and cleaning of the CE system. The waste valve also served as an outlet for RPLC effluent. During the experiment part of the RPLC effluent was continuously introduced into CE capillary (i.d. 10 μm) electrokinetically (HV was applied). The polyimide coating of the CE capillary was partly removed and a beam from a laser was split into two beams — into gating beam containing 95% of the laser power (focused near the inlet end of the capillary) and into the probe beam containing the remaining 5% of the laser power (focused near the outlet end of the capillary). The gating beam acted as a injector for the CE system, while the probe beam served as a detector. The "injection" of the sample was done as follows: because the HV was continuously applied the sample

zone was constantly migrating through the capillary. As long as the gating beam was focused on the capillary most of the sample was photo-degrated by the intense light of the beam and only a residual background fluorescence was seen at the probe beam. To make an "injection" the gating beam was blocked for several milliseconds. The blocking was computer controlled. As a result of the blocking a very narrow unbleached sample zone passed through into the region between two beams where it separates into corresponding component zones before reaching to the probe beam. Using the optically gated injection system allowed the introduction of very narrow sample zones and performed very fast CE separations in narrow and short-fused silica capillaries, which enabled complete 2D and 3D separations to be done within less than 10 min instead of 1 h (or even longer). The "injection" system of the system was ingenious and very effective, the coupling of CE with other separation methods was done via simple and reliable interface. The separation power of the systems was very high and the time of the experiments short. However, the narrowness of the sample zone caused also the increase of detection limits for the amount of the sample analyzed was very small. In the 3D system very concentrated samples were used because of the dilution factors caused by the design of SEC/RPLC interface. Coupling three dimensions made the computer controlling of the system and the control of temperature rather complicated. The control of temperature in all dimensions used was very important to keep elution/migration times in all parts of the systems strictly comparable. The reproducibilities of the systems were not reported. The systems used need expensive components (a fast and powerful compute, a laser) and therefore are not available for every laboratory. Also, it is not quite clear how general is such a photodegradative "sampling" method.

Although the comprehensive 2D separations are impressive in appearance with the results in 2D planes as a colorful contour or stack plots, the method might have still problems with high detection limits, as has been already mentioned. When 2D comprehensive HPLC-CE is ideal for the analysis of very complicated mixture, even more important could be the 2D separation with a low detection limit of some target compounds without an attempt at comprehensive separation of the rest of the sample. Palmarsdóttir and Edholm used coupling of column liquid chromatography (CLC) with CZE to enhance the selectivity and concentration sensitivity of the method.[46] To combine CLC with CZE they used a commercially available μ-Dumper interface and a Fused Silica Tee Adapter (Valco). The system also consisted of a Valco valve with a loop volume of 31 μl. The problem that the volume was far too large for CZE system was solved using a double stacking procedure. The double stacking procedure needed exact timing and the stacking time length impacts the recovery of the sample. However, reduction of the detection limits by 400 times was achieved and the reproducibility of the system in terms of peak areas, migration times, and resolution was quite good, ranging from 0.7 to 3.5, 0.5 to 1.5, and 1.1 to 1.9% RSD, respectively. The reproducibility of the experiments was influenced by the concentration of the sample. The extent of the impact of using CLC in this system was not clear. The sample injected into the CLC column was previously preconcentrated. Further development of the previously mentioned system was made by Palmarsdóttir et al.[47] To the micro-CLC/CZE system an on-line membrane unit was added. After minimal preparation step the sample was introduced into the membrane unit. The pH of the sample solution was adjusted so that the analytes were predominantly uncharged and therefore able to penetrate through membrane into acceptor phase. The pH of the acceptor phase was regulated

so that the analytes turned to ionic form. In this way not only the disturbing matrix elements were eliminated, but the sample components under study were concentrated several times. The outlet of the acceptor phase was connected to a 50 µl injection loop by a Valco valve. The inlet of the micro-CLC column was also connected to the valve. The sample was then further concentrated in the column and directed to CZE system via a µ-Dumper interface. The sample volume introduced to the CZE system reached to about 2 µl. In the CZE system the sample was double stacked before it reached the detection window. The all together sample concentration was about 40,000 times allowing to measure sub-nanomolar quantities (0.15 to 0.25 n*M*). The selectivity and concentration sensitivity enhancement of the system was tremendous. The reproducibility of experiments was about 6 to 7% RSD. The extraction efficiency was around 65%. The duration of one single analysis was about 1 h, which may be a problem when large number of samples have to be analyzed. Improvements in the system were made to shorten the analysis time and the duration of 30 min for a single analysis was achieved with the cost of slightly (twice) increased detection limits and decreased recovery (still more than 50%).

Kar and Dasgupta coupled ion chromatography (IC) with suppressed conductometric capillary electrophoresis.[48] The authors used two different interfaces to join these above-mentioned separation methods. First, interface used just a six-port sampling valve and enabled to perform discontinuous sampling from IC outlet, therefore allowing the injection of any segment of IC effluent into CE system. The other interface performed continuous valveless sampling of IC effluents to CE column. The specific problem here was the fact that suppressed IC effluent is nonconductive and as such not suitable for CE separation. The authors solved the problem by directing CE buffer from a separate vessel into the IC effluent through a Nafion membrane. Then the resulting solution (now contained sample components) passed the CE capillary inlet, and the sample components were introduced by electroosmosis/electro-migration into the capillary in a rather similar "split" arrangement presented, for example, in Figure 3. High voltage was continuously applied. The significant feature here was that the fundamentally nonionic IC effluent was on-line turned to appropriate running buffer for CE.

Both the valve based and the valveless coupling of IC-CE made possible achieving separation that was hardly reachable by the IC or CE method only. The separation power and peak efficiencies were higher in the case of the valveless interface. The latter did not need exact timing to find the optimum operating conditions. Both systems allowed rapid analysis with quite high reproducibility in terms of migration time (<1% RSD). The interfaces would be useful for fast screening purposes. The interfaces could also be effective if IC system would be used for on-line preconcentration or sample clean-up and CE system for final separation. However, the valve-based system cannot be used favorably for the separation of unresolved peaks in IC system, because of the disagreement between the greatest applicable loop volume and the maximum base width of an unresolved elute band. The detection limits of the systems were not very good because of many parameters that add to the overall physical dispersion of the elute band like the connecting conduits of the interface, non-loop volumes of the valve, the higher diameter of the Nafion tubing, etc. Although consisting several smart solutions, the whole experimental set up seems to be rather complicated. Ion analysis can be performed nowadays very efficiently by means of IC or CE alone, thus, although the authors successfully achieved their goal: 2D IC-CE, it might be difficult to justify the extra complications involved in their set up.

V. MONITORING

One of the most important goals in life sciences is the monitoring of biological processes. The study of kinetics and pathways of different processes like adsorption, biotransformation, and elimination are of the utmost importance in estimating the safety of different compounds to living organisms. For some of the reactions the time scale is very short and needs temporal resolution of seconds to minutes. To get this type of information, post-mortem analysis or biosensors were classically used. Post-mortem analysis has obvious disadvantages in terms of ambiguous results and the number of test animals. Biosensors are often limited to monitor a single analyte. During recent years microdialysis sampling has been coupled to different separation methods for the purpose of monitoring biochemical reactions *in vivo* using both off-line and on-line sample collection. On-line sampling has proven to be more successful mostly because it allows smaller volumes of samples to be collected and it enables shorter analysis time.[49,50,51] One of the most successful combination was on-line coupling of capillary electrophoresis with microdialysis. Hogan et al. designed a system of on-line coupling of microdialysis with micellar electrokinetic chromatography method.[52] The interface used to combine microdialysis unit to CE system consisted of a CE buffer reservoir into which two capillaries (transfer and separation capillary) were inserted. The capillaries were adjusted so that the inlet end of the separation capillary was across the outlet end of the transfer capillary. The optimal gap distance between the two ends was 50 μm or less. The interface was connected to an electrically actuated HPLC valve via transfer capillary. The microdialysis probe was inserted into living rat. The microdialysis was carried out by a syringe pump. The other syringe pump was used to continuously pump fresh running buffer to CE buffer reservoir. The interface used in the above-described system had to perform several functions. First, it had to modify the continuous microdialysis flow into discrete pulses of sample for CE analysis. Second, it had to change the microdialysis flow rate so that it would be acceptable for CE. Finally, it had to protect the test animal from the high electric field of CE system. The interface was able to accomplish all these functions. The microdialysis flow rate (1 μl/min) was converted to plugs of volume of 60 nl (the volume of the valve loop was 60 nl). The plugs were transported at desired time to the interface by the flow of running buffer. The microdialysis part of the system was not under the high voltage for the interface consisted also a grounded electrode. The CE part of the system and the interface were continuously under high voltage. Therefore, if the sample plug was transported into the interface the analytes were instantly captured by the separation capillary. The rest of the plug was swept away by the fresh portion of the running buffer.

The interface was able to perform *in vivo* monitoring via coupling on-line microdialysis with MECC. The interface was easy to construct, and it allowed at optimum conditions reasonably good reproducibility of the peak heights (2.6% RSD). The advantage of the design of the interface was that it was easy to control and optically determine the position of the capillaries relative to each other.

The disadvantages of the sample are relatively high detection limits and comparatively low zone efficiency. Compared to off-line electrokinetic injections the sample was about four times diluted using on-line injections. The low peak efficiency was probably mostly due to the gap between the ends of the capillaries in the interface. The gap exposed the analytes zone to the dispersion and this resulted in increased peak heights. Using shorter injection times and shorter flow rates improved the peak width but also decreased the peak height (threefold improvement in peak width caused a 50% decrease

in peak height), therefore increasing the detection limits of the method. If the injection time of the valve was more then 3 s, a considerable tailing of the CE peaks was noted. This resulted from "non-loop" volumes of the valve. To reduce the peak tailing the operation of the microinjection valve was modified by decreasing the time the valve was in the injection position. Using injection time 3 s the peak tailing was strongly minimized, but the short injection time also causes problems because only very limited volume of the sample can be injected into the CE system and this increase again the detection limits of the method.

Most of CE interfaces consisting of membrane and/or interfaces allowing rapid consequent injections that were described above so far[17–20,31,71] could be used for monitoring purposes. Because there are not so many examples available from the literature, the authors of this article prepared two examples of monitoring using pneumatic sampling device, which was presented in Figure 7. In the first example changes in a orange juice sample (purchased from the local grocery store) was poured into the sample vessel donor part. During a 2-h experiment acceptor part of the sample vessel was flushed with the buffer after every 20 min. In Figure 10 the resulting pherograms are presented.

Despite the similarity of all the pherograms, minor changes in areas of peaks (a) and (b) can be noticed. Because the experiment was performed for demonstration purposes only, no attempts of identification of peaks as well as processes were taken. In another experiment with the same device, L-ascorbic acid was kept in a sample vessel and the membrane interface was removed from the sample line. With no antioxidant in sample, L-ascorbic acid degrades according to the complicated kinetics that can be monitored by periodic sampling from the reaction vessel. If a stabilizer (L-cyteine) is added to the sample vessel the L-ascorbic concentra-

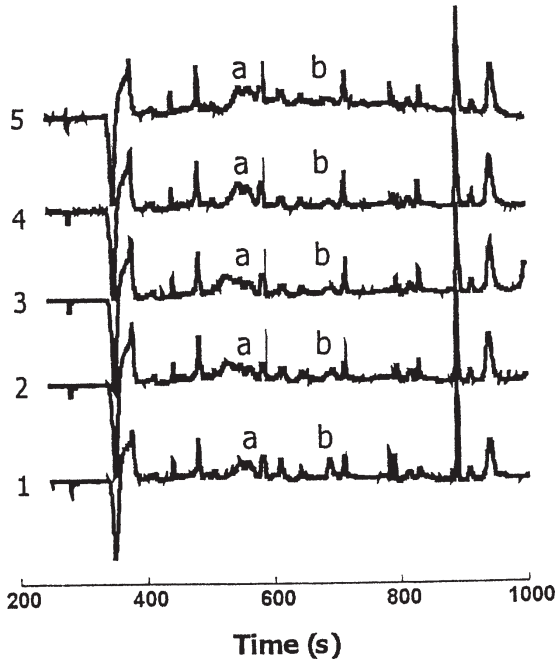


FIGURE 10. Monitoring changes in orange juice using pneumatic autosampler. Conditions: Running buffer was 50 m*M* SDS in phosphate buffer, I = 0.05; pH = 9. 0; Isco CV[4] UV detector; λ = 215 nm. Sampling interval between pherograms was 20 min.

tion remains almost constant during the monitoring. Typical pherograms are presented in Figure 11, where the stabilizer effect is clearly evident. Again, no interpretation of the processes were undertaken.

VI. CE ON MICROCHIPS

Production technology of the silicon microchips for the computer industry has reached such a high level and is so wide spread that it is not surprising that it has its influence in chemical analysis as well. Talking of CE, one can say that several groups are intensively investigating the possibilities of producing CE analyzers on monolithic silicon plates using current microchip technology. A number of publications concerned with the problems of preparing microchips has grown rapidly in recent years (see Ref. 53 and references therein). The most attractive feature of such analyzers seems to be the speed of separation that can be obtained. Separations have been completed within seconds[54] or recently even within submilliseconds.[55] This is especially attractive in view of the possibility of the involving CE into the Human Genome Project, where the throughput, and thus speed of analysis of the DNA fragments by CE, is critical. Also, because the separation is performed in much shorter channels than common in ordinary CE, the applied high voltage is lower.

A typical microchip that has been designed for CE involves a standard photolithographic procedure. A microchip is a glass plate with the dimensions $100 \times 100 \times 3$ mm. Sampling and separation channels are cut on the surface of the plate by wet chemical etching. Channels height and width size are usually about tens of micrometers, and geometry of their location is dictated by the requirements and convenience of the analysis mode. The current technology of the production of the microchips is rather sophisticated. At the first stage it involves design of the chip geometry, that is, design of the CE analyzer. Then the mask of the design is transformed to the raw glass plate on which has been deposited photo-resistive material and metal layers using UV light. After this the photoresistivity is developed, that is, the irradiated part of the mask will be removed. Then the plate is subjected to the chemical treatment, which, in fact, etches channels with necessary dimensions into the plate, and finally the metal layer is removed. In

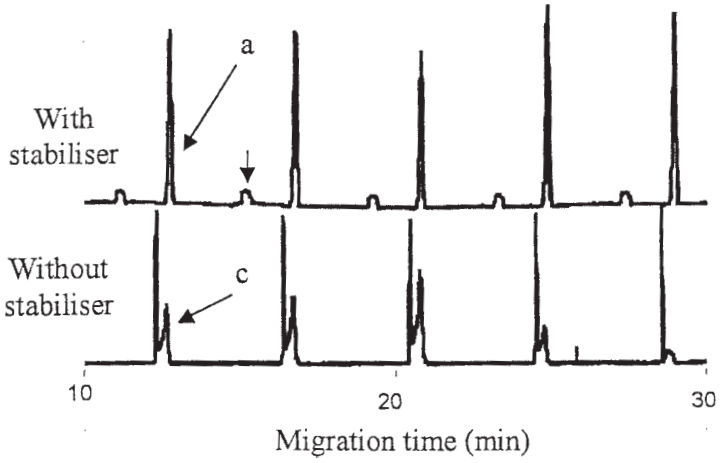


FIGURE 11. Monitoring L-ascorbic acid degradation. Five consecutive sample injections. Peaks a-ascorbic acid, b-stabilizer (cycteine), c-degradation product(s?). Conditions: Running buffer was phosphate buffer, I = 0.1; pH = 7.0; Isco CV^4 UV detector; λ = 245 nm. Sampling interval between pherograms was 7 min.

order to seal the capillary manifold, a second glass plate with the same dimensions is thermally bonded (4 h at 600°C) on the top of the glass plate containing capillary microstructures. Holes have been drilled into the cover plate to provide access to the capillary structure and pipette tips have been glued into these holes that serve as the reservoirs.

It follows that the equipment required for production of the microchips is rather specific and is not commonly available in every regular CE laboratory. Also, the sealing process of two plates together is not trivial. There are reports[56] of low device yield when closing channels. Much interest exists to investigate the possibilities of manufacturing CE microanalyzators by a more simple technology. Polymeric substrates are promising alternatives to the silicon and glass plates. Microchips for CE were produced by molding a poly(dimethylsiloxane)[57] silicon elastomer or poly(methyl metacrylate)[56] against a microfabricated master template.

A microfabrication facility has been developed involving the photoablation of commercially available polymers.[58,59] A laser is used like a pen to draw capillaries between 30 to 200 mm wide and 40 mm deep. Photoablated surfaces were studied by several microscopic techniques, and after the study the channels were then closed with a low-temperature lamination process. This very flexible method allows the development of complex networks of microchannels that can be filled by capillary flow. It has been shown that depending on the laser fabrication conditions, the channel surface can be either hydrophilic, exhibiting a fast capillary flow, or hydrophobic, exhibiting a very slow capillary flow.

Plate manufacturing was further simplified by Martynova et al.[56] by implementing template ("positive" inverse of the channel geometry) made on silicon wafer. This chip is pressed against Plexiglas plate to produce an CE microanalysator ("negative"). This plate is later covered by another plate pressing it against negative under medium pressure at a carefully controlled temperature (T = 107°C). The technology of producing CE microanalyzers can be developed to the ultimate simplicity as reported in the same paper.[56] Instead of a template, just a piece of Nichrom wire with a diameter of 25 μm can be used. This indeed opens opportunities for manufacturing CE analyzators on plate for every researcher who is interested, especially those with limited funds. Channels pattern that can be manufactured in such a way is a simple cross of two channels, which serve as sampling and separation capillaries.

Detection is the critical part of such devices because of the very tiny amounts of the sample available for detection. Also, microchip is not as convenient as capillary, for example, UV detection. So far, laser fluorescence detection is the prevailing in a straightforward mode: laser beam is focused in a certain spot on the plate and fluorescence is detected by microscope and photomultiplier. Use of other detectors is rare.

Sophisticated procedure of producing micro-CE devices on the chips might explain why only a few groups have been involved in the research in such prospective research so far. Because the etching of channels appears to happen preferably only in certain directions dictated by the silicon crystalline structure, it is relatively simple to produce channels with linear geometry on the microchips. Simple cross structures of channels are very popular.[60] Sampling can be performed simply by first applying voltage to the sample channel, which will be filled with the sample by electroosmosis, and next the voltage is applied to the separation channel and it drives part of the that part of the sample that was located in the area where the channels are crossing to the separation capillary (see Figure 12). Later on, more sophisticated channels geometry was proposed for the CE analysis of the reaction products at the same microchip involving reaction chamber on the chip.[61] This system

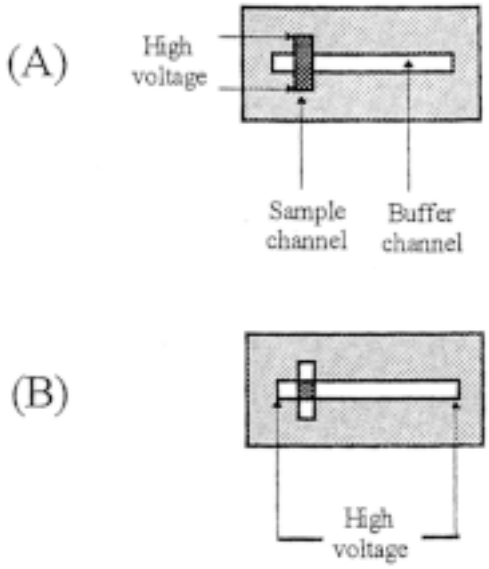


FIGURE 12. Sampling on the silicon microchip. (A) Filling channel with sample. (B) Sampling.

allowed the integration of a reaction stage to the CE separation.

Molen[62] proposed a more elaborate sampler on a silicon microchip that can perform multiple injections from the same sample vessel. The sample has similar cross-channel construction that is represented in Figure 13. Sampling is performed differently, however. In his design (Figure 14) one end of the sample channel is connected to the buffer reservoir and the other end to the sample reservoir. High voltage is applied either between sample reservoir and the other end of the separation capillary (sampling mode) or between buffer reservoir and end of the separation capillary (separation mode). Separation is not performed on the microchip itself but in the capillary that is connected to the end of the separation channel. Thus, common UV detection was possible. This sampler was constructed to perform multiple injection experiments in correlation chromatography. Some applications are described below.

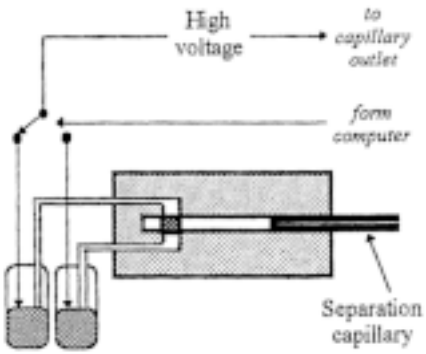


FIGURE 13. Sampler on a microchip.

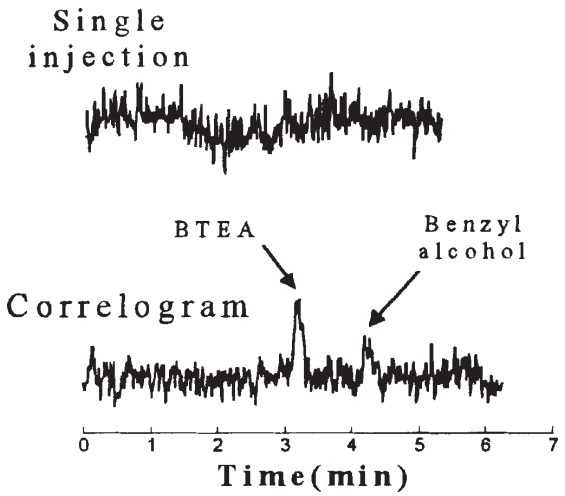


FIGURE 14. Comparison of correlogram and single injection pherograms. Conditions: sample: benzyltrimethylamine and benzyltriethylamine solved in buffer; running buffer: 0.01 *M* phosphate buffer, pH = 7; fused silica capillary (i.d. 75 μm, o.d. 375 μm , length to detector 50 cm, total length 90 cm); detector ISCO CV^4, λ = 204 nm. During the experiment 511 randomly spaced injections were made with mean interval 0.5 s between two consecutive injections. (Adapted from Ref. 63.)

Although the efforts of the investigators have been directed toward to the miniaturization CE analyzer in such a size that the whole system could be manufactured on the plate, this should not always be the issue. Excluding the cases where speed is important, analysis of the plates seems not to have particular advantages over more common capillary column version. Moreover, rapid analysis can be performed using short capillaries as well. Absence of convenient detection schemes on plates might become a further obstacle in developing of CE analyzers on microchips. On the other hand, very many nonconventional samplers can very conveniently be built on plates using available technology. Separation capillary can be just connected to such input device. Molens' work is an example of such approach. Also, sample preparation by isotachophoresis described above (where several capillaries with different sizes are involved) could evidently be done much more conveniently on the plate.

VII. REDUCTION OF DETECTION LIMITS BY MULTIPLE INPUT SAMPLING

As was pointed out previousy, some of the nonconventional samples have been designed for performing multiple injection. Performing regularly spaced multiple injections can be used for monitoring, as demonstrated in Section III.C. Interesting results can be obtained by also performing randomly spaced injections with very short mean interval compared with the pherogram length. As an alternative to sample concentration and stacking methods, detection limits of a method may be lowered using mathematical methods and sampling sequence of special form. This approach is known as correlation CZE.[63,64] The correlation method used was introduced by Izawa et al.[65] in gas chromatography to continuously control the processes during the experiment. Later, several investigators have modified and improved the method. As the method has been mostly

used in chromatography,[66,67,68] it has been generally known under the name "correlation chromatography". The basic principle of the method is as follows: instead of performing one single injection and then waiting until all the analytes reached to the detector, in correlation chromatography the sample is introduced several (hundred) times during one experiment. The injections are made according to a special sequence, called pseudo-random binary sequence (PRBS). The resulting detector signal cannot be interpreted directly, but by using special deconvolution methods it can be transformed. This can be done using, for example, Fourier or Hadamard transformation. As the result of decon-volution a correlogram will be received, which is very like to ordinary chromatogram, however, with reduced noise. The reason for the noise reduction is the fact that correlation technique is a summation procedure where all the (hundreds of) single injections chromatograms performed during the experiment are added to each other. The summation causes the signal to increase n times (where n is the number of injections), but the noise increases only $\sqrt{n}$ times. That causes the reduction of signal to noise ratio and therefore lowers the detection limit of a method. However, the difficulty of applying correlation chromatography method is due to the fact that it needs very reproducible single injections. Irreproducibility in single injections lead to appearance of so-called "correlation noise" that is the result of the fact that correlation chromatography, which is an averaging procedure by its nature, averages all irregularities over whole correlogram. Therefore, nonlinear behavior, nonstationary (or systematic) errors may all cause correlation noise and destroy the signal to noise ratio improvement that would be expected to achieve from the summation procedure.

Despite a lot of impressive results obtained in correlation gas and liquid chromatography, it was not clear is the correlation method applicable to capillary electrophoresis. The reason for that was the lack of suitable injection devices. The injection device befitting correlation technique has to be able to perform rapidly short duration multiple injections (the duration of an injection and the interval between two injections can be several seconds but in some cases less than a second) without high-voltage interruption. Most of the sampling devices for capillary electrophoresis (both conventional and nonconventional) were not able to perform that kind of operation. The on-line sample gating method controlled by laser induced photolysis, introduced by Moore and Jorgenson (described in Section IV), was one of the systems theoretically suitable for correlation capillary electrophoresis. Still no attempts to apply the correlation technique in capillary electrophoresis were made until Molen et al. introduced it in 1995.[64]

The principles of injection device Molen et al. used for correlation capillary electrophoresis experiments is described in Section VI. In preliminary experiments, the authors proved that it is possible to reduce detection limits in CZE using correlation technique. For the experiments a homemade injection device was used.[64] The sample was introduced electrokinetically. The authors achieved the reduction of detection limits of 5.3 times using a sequence of 127 elements (which involved 63 sample injection). The peak area reproducibility was less than 2%. The reproducibility and detection limits were both improved by using correlation CZE, but the resolution between two sample component peak was poorer and the width of the peaks larger than in common CZE. To improve the results, Molen et al. fabricated a new injection device using microchip technology.[62] This device enabled achieving an improvement of detection limits about eight times, and it also enabled lowering the RSDs of migration times four times compared with common single injections. The reproducibility of the peak areas was 1% of RSD, being

approximately the same as in the case of single injections. However, in the correlograms appeared a so-called ghost peak referring to a systematic injection error. The cause of the injection error was not clear. The influence of using correlation technique on the efficiency of peaks was not reported; however, judging from the pherograms, efficiency was not discarded compared with the common correlation CZE. Correlation CZE electrophoresis was developed further by proposing simultaneous correlation chromatography that enabled running at the same time pherograms of sample and internal standard.[69] Because both the standard and sample are analyzed in the same conditions the precision was considerably improved.

In 1998, Kuldvee et al. used another autosampler to perform multiple injections analysis, correlation chromatography.[70] This was an automated pneumatically driven sampling device described by Kaljurand et al. The sampler is very similar to that represented in Figure 7. It was made of Plexiglas with dimensions of $3 \times 1 \times 1$ cm. Two L-shaped channels and one T-shaped channel was drilled into the box. The ends of L-shaped channels were closed by actuators consisting of small Teflon® membranes and enclosing small volumes in the ends of the channels. The actuators were computer controlled via solenoid valves. The other ends of L-shaped channels were connected to buffer and sample reservoirs. Into the long path of the T-shaped channel the inlet end of the capillary and one electrode were mounted. In the system two pressures were used. One pressure was applied to the actuators so that the membranes closed the ends of L-shaped channels. The other pressure, somewhat lower, was applied to the buffer and sample reservoirs. To fill the long path of the L-shaped channel (inlet channel) with buffer or sample, the particular actuator pressure was released to the atmosphere. The sampler was totally computer controlled and enable very rapid (down to 0.1 s) changes for buffer to sample solution and vice versa. The inlet channel served both as buffer inlet reservoir and a sample vial. The sample was introduced electrokinetically into the capillary. The sampler was able to perform multiple injections without high-voltage interrupting, therefore avoiding from one possible source of experiment irreproducibility that would result from the voltage rise/drop time irreproducibility and would cause correlation noise.

The correlation CZE experiment was done similarly with Molen et al. using PRBS as an input sequence. The reproducibility of experiments was quite good (1% RSD for peak heights). The RSD for migration times was improved two to three times compared with single injections. Using the correlation chromatography method with the CZE method, the injection device enabled lowering the detection limits about six times. The advantages of the particular sampler compared with the above-described sampler were as follows: the sampler would be easy to build in every laboratory where chromatographs are available. The sampler was convenient to operate and it was easy to modify the operating pressures and injection times. The disadvantages of the sampler were that the sampler consumes relatively large amounts of sample, and it is not very convenient in the cases of numerous samples.

Both systems described above were suitable for applying a multiple injections techniques. The repeatability of injections was good. However, in both cases the efficiency of the peaks was moderate. It was noticed that the efficiency of peaks was lower when the correlation technique was used compared with peaks in common single pherogram. One possible reason for that could be that even the sample introduction was believed to be only electrokinetic there could also exist a hydrodynamic part during the buffer/sample rinse stage that may broaden the peaks. To study it the authors applied backpressure to the system. The influence of

the backpressure is under the study. The other reason for poorer efficiency could be related to the change of the ionic strength of the buffer. The latter is unlikely the case if samples with very low concentration are analyzed.

It was also observed that the migration times shortened in the case of using the correlation method. This may be caused by the fact that during the correlation experiment the whole capillary is filled with multiple buffer and sample zones distributed randomly along the capillary. The resulting "buffer" composition and its ionic strength are certainly different from that of the buffer used in single injection experiments and may have influence on the migration speed of analytes.

As it was pointed out above, the correlation technique is very sensitive to all injection/detection disturbances. Therefore, detector signal preprocessing is essential. Spikes and baseline drift can harm decorrelation procedure severely and must be removed before decorrelation. This can be considered as kind of "prewhitening" of the detector noise. A typical correlogram is compared with a single injection pherogram in Figure 14. While no peaks appear on single injection pherograms, appearance of peaks on the correlogram is self-evident.

VIII. MISCELLANEOUS

A. Falling Drop

Liu and Dasgupta have introduced an unique and interesting sampling device.[74] The inlet of a fused silica capillary (250 μm i.d.) was tightly sandwiched between two pieces of glass microscope slides. The two pieces were jointed with epoxy. The outlet end of the capillary was inserted into a buffer reservoir as usual. The "sandwich" was fixed in such an orientation that its wider plane was vertical and its longer and narrower side (where was the inlet end of the capillary) faced up with a tilt of 45° (see Figure 15). The surface of the latter was made wettable using sandpaper. The system also involved a dual-channel syringe pump with two syringes: S_1 and S_2. Syringe S_1 delivered constantly buffer to the top end of the wettable surface. Therefore a fresh stream of buffer continuously passed the inlet end of the capillary. Instead of the inlet buffer vial the grounding electrode was inserted in the centre of the stream. A constant voltage was applied to the other end of the capillary. Syringe S_2 was used to deliver the sample solution to the tip of a PTFE drop-tube. The location and height of the tube was adjustable. When a drop of sample fell from the narrow tube into the buffer stream it was carried by the stream to the capillary inlet and part of it was introduced electro-kinetically into the capillary, the rest of the sample was quickly washed away by the buffer flow. The injection moment was marked via two extra electrodes that sensed the conductance of the stream and that were situated into the stream near to the capillary inlet. When the sample reached those extra electrodes, the conductance between them changes and the change was registered.

The study of the sampling device showed that the location of the drop falling had a significant influence on all important performance criteria (peak height, peak area, the number of theoretical plates and asymmetry factor). The other essential operating parameters were the buffer flow rate during the injection and the voltage applied. The best results in terms of peak area and peak height (concentration sensitivity) were achieved if drop tube was shifted 1 mm to an upstream position relative to the capillary inlet. The best results in terms of peak asymmetry and the plate numbers were achieved shifting the drop tube 1mm downstream relative to the capillary inlet. The height of the drop tube did not influence the results significantly. Increasing the buffer flow rate caused a reduction of concentration sensitivity (both

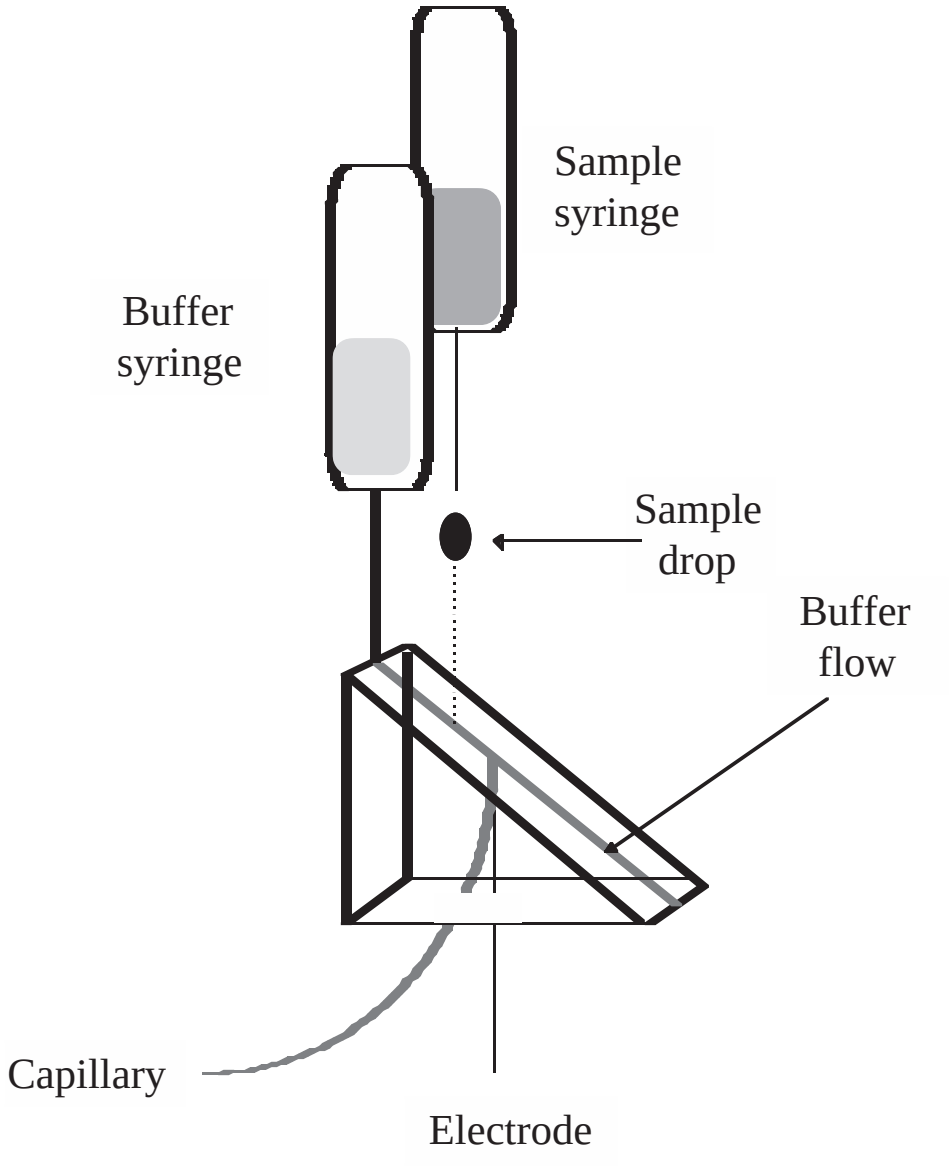


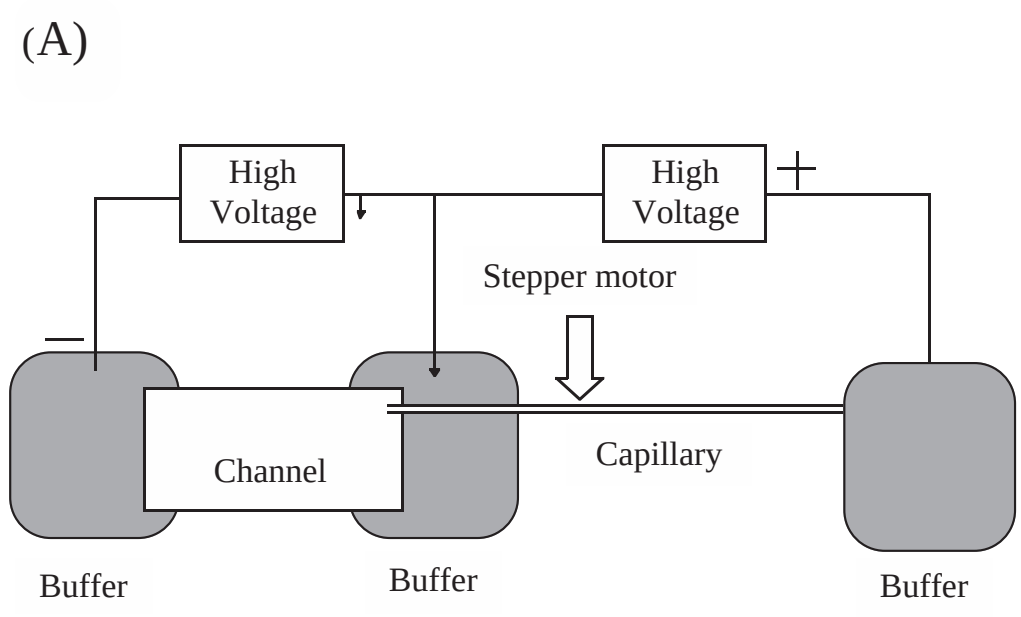
FIGURE 15. "Falling drop" sampler.

peak area and height decreased significantly) and as expected the increase of the plate number. Therefore, in cases where resolution and efficiency are the top priorities, a high, buffer flow rate should be used. If the resolution of analytes of interest is good a low flow rate should be used to reduce the consumption of the buffer and rise the concentration sensitivity. The asymmetry of peaks increased with the increasing flow rate but it reached a plateau if the flow rate was above 100 µl/min. Increasing the voltage applied both enhanced the concentration sensitivity and increased the plate number. The effect of the drop size was not clear — increasing the sample drop size by some factor resulted in higher peak heights and larger peaks but by a considerably smaller factor. The effect of the drop size on the peak efficiency and peak asymmetry was insignificant. The relative standard deviation of the system for peak height was 2.3%.

The sampling device allowed to perform consequent injections without high voltage interruption (thus being suitable for multiple injection techniques), no adjustment of voltage was necessary in the injection process. The method was simple, easy to perform and easy to automate, the reproducibility of experiments was good. The system designed seemed to have a good potential for coupling of another analytical system with a capillary electrophoresis system. For example, coupling with a liquid chromatography system would be easy — just a small volume of effluent from liquid chromatography system would be dropped on the capillary inlet and no search for compromise between the buffers of two systems would be needed. On the other hand, it seemed that the system was not the most suitable for analysis of dilute samples. The injection process itself had a diluting effect on the sample. No data about detection limits of the method were shown. The other weak point in the system may be the difficulty of exact positioning of the sample drop tube positioning. No data were published on this subject.

B. Continuous Electrophoretic Separation

During recent years, several analytical techniques has been developed to sample and separate analytes from microenvironments. Some processes, for example, the processes at single-cell level need methods that could manage very small quantities of analytes. Capillary zone electrophoresis has been among the most successful techniques that have been developed and used. Among the sampling devices described above only few are able to monitor processes as short discrete impulses. However, even these sampling devices have not proved to enable continuos sampling from microenvironments over long time periods. Mesaros et al. demonstrated continuous electrophoretic separation in narrow channels coupled to small-bore capillaries. In this system the narrow-bore fused silica capillary (38-41) (m i.d.) was used for sampling but not for separation of the analytes. The inlet end of the capillary was inserted into the sample reservoir. The other end of the capillary reached to a narrow rectangular channel that was attached across two buffer reservoirs (see Figure 16). The rectangular channel was constructed using quartz plates. The edges of the plates were bevelled, which allowed the end of the capillary to be inserted and held in the channel entrance. The thickness of the separation gap between the channel plates was controlled using glass microspheres mixed in UV cure adhesive. Adhesive was also used to attach plastic strips onto the channel sides and bottom. That created a plastic enclosed region on the outside of the channel. When a channel was fitted into the reservoirs, hot glue was applied to the plastic region and the channel was pressed into the reservoirs. The use of the plastic was necessary to avoid leaks across the channel reservoirs. The capillary was attached to a sliding platform witch was controlled by a stepper motor. Therefore, the capillary could move across the width of the channel entrance. There were two power supplies applied on the system. The first one was used to create electric field between the sample reservoir and the first buffer reservoir. The other one was used to create electric field between the first and second buffer reservoir. Before each experiment the capillary was first filled with buffer and then with sample. When a high potential field was applied across the sample and first buffer's reservoir, the capillary continuously sampled the material from sample reservoir by electromigration and introduced it into the channel. During the sampling the capillary moved across the entrance of the channel. At the same time a high-potential field was applied across first and second buffer's reservoir which resulted in a net flow of all species toward the cathode due to elec-



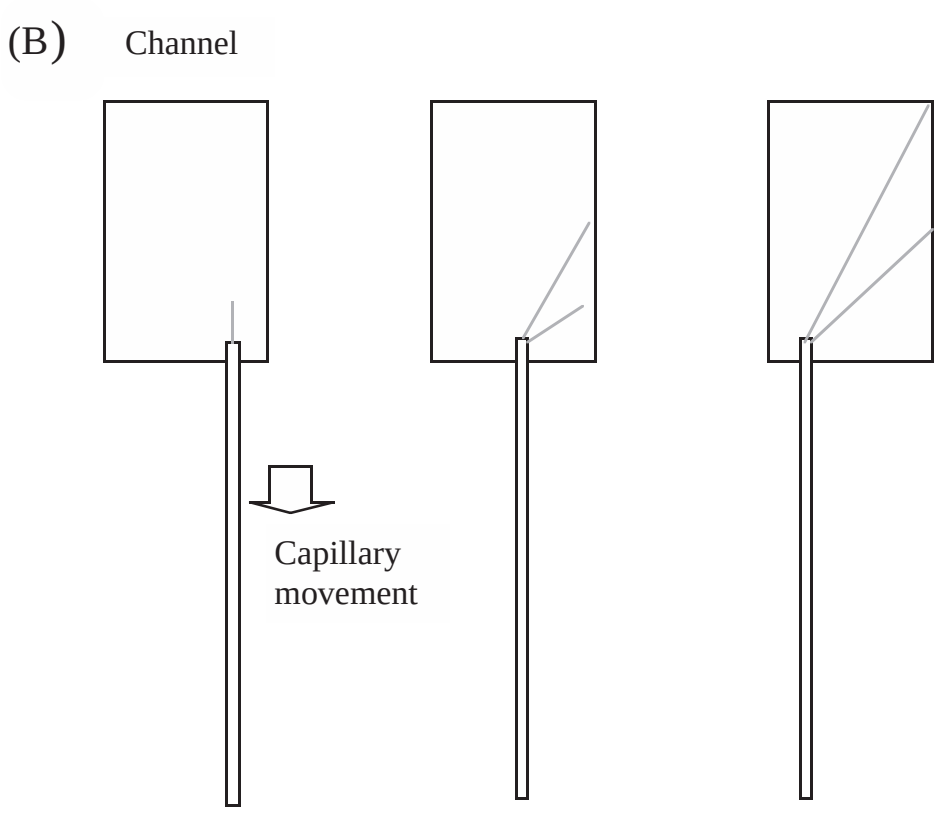


FIGURE 16. Experimental set up for continuous electrophoresis (A). Image of sequential stages of separation process (B). In (B) the image on the left shows analytes first deposited into the channel. The middle image shows the migration path after the capillary has moved to the middle of the channel. The image on the right shows the capillary at the end of the channel entrance. (Adapted from J. Measros et al., *Anal. Chem.* 193, 65 3313–3319.)

troosmosis. Ionic compounds were separated in the channel due to their electrophoretic mobility. An important component of the system was the detection system. For as the analytes come out from the channel, they must be detected in a way that retains their spatial integrity across the width of the channel exit. Fluorescence detection utilising two fibre optic arrays was employed. The system enabled to perform continuos sampling from the environment during up to 400 s with high separation power and good peak efficiencies. The system allowed also to get quantitative information (the simultaneous determination of the time of analyte contact and its duration of contact with the sampling capillary, identification of analytes based on electrophoretic mobility and concentration changes with time). The detection limit of the method was approximately 30 μ*M*. The detection limit achieved was reasonably good even may be not low enough for investigation of processes at the single-cell level. One of the weak sides of the method seemed to be the complexibility of the system, for it needed every-day disassembling and reassembling of channels. The disassembling/reassembling procedure might also cause scratches on the surface of channel plates. The system also needed improving of the detection scheme to achieve increased consistency in signal transfer and uniform illumination across the channel exit. The reproducibility of the system was not studied. Despite of the criticism, the system offered a promising tool for continuous analysis.

C. Robots

A coupling of flow injection system with commercial capillary electrophoresis system by two laboratory made programmable arms has developed by Válcarcel and Arce.[4,5] The outlet of the flow injection system (not described here) was connected to one programmable arm. The arm was fitted with two injection needles of different length, both connected to the flow injection system. With these needles the sample vials of capillary electrophoresis system were filled and drained. The task of the arm was to automate the filling of capillary electrophoresis sample vials. The other programmable arm, built-in into capillary electrophoresis system was used to control the inlet end of the capillary and the electrode. The arms were controlled by a microcomputer via an electronic interface and customised software.

As the emphasis of the authors of this interface seemed to be on the flow injection part of the system the advantages and disadvantages of the coupling interface were not pronounced.

D. Quantitative Injections from Microloop

Pressure-driven sample introduction depends on the viscosity of sample and buffer, and therefore also on temperature. The reproducibility and representativeness of electrokinetic sample injection depends on the mobility of sample components and the composition of sample matrix. Using loop type injectors would enable to overcome these difficulties. However, the volumes of commercial and also home-designed loop type injectors are generally too big for capillary electrophoresis sampling. The smallest loop injector was 20 nl. If sample stacking would be combined with injecting that size of sample, the loop volume would be small enough, but in that case the channel geometry created problems that inhibited electrostacking.[74] Dasgupta et al. have developed a sampling system that used a wire loop deployed at the tip of a capillary electrophoresis system[75] (see Figure 17). The sample solution was placed as a thin film formed over the loop (this should not be confused with the loop encountered, for example, in

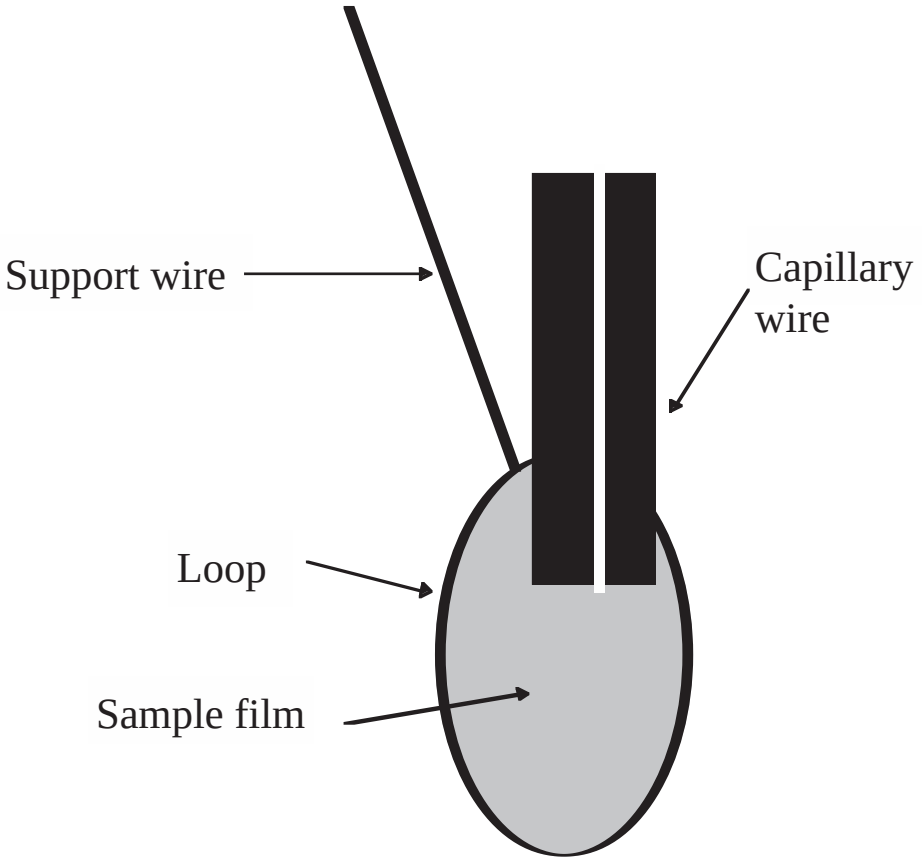


FIGURE 17. Electrokinetic microloop injector.

HPLC, which is just a piece of tubing containing sample).

The system enabled to perform quantitative injection of small amounts of sample both hydrodynamically[76] and electrokinetically.[77] The loop was fabricated under a microscope, wrapping a wire of various metals (Pt, Nichrome, and stainless steel) around the tip of a pin. The diameter of the loops varied also. When hydrodynamic injection was performed the loop was affixed at the capillary tip by wrapping around and applying some Epoxy adhesive. Then the capillary together with the loop was mounted into a movable (by pneumatically operated cylinders) Plexiglas head. Therefore, the injection end of the capillary could be addressed to various sample vials placed into a fraction collector. To perform an injection from a sample vial, the head was vertically lifted, translated to the top of an empty polypropylene enclosure equipped with a pressurization port and then lowered. The underside of the Plexiglas head housing the capillary was lined with a silicone gasket so that a pneumatic seal was formed when it rested on the polypropylene enclosure. Before every injection the capillary tip and the loop were carefully rinsed first with buffer and then with sample to avoid contamination. The described injection mode showed less dependence on sample surface tension and viscosity than common hydrodynamic injection. The reproducibility of experiments fell into the same range than in case of hydrodynamic injection (app. 2 to 4% RSD). The

amount of sample introduced by this technique depends on the size of the loop, yet the size of the loop has its limits. For bigger loop, the longer injection times or higher pressures are needed but injection times longer than 7 to 8 s did not cause any increase in peak area, probably due to evaporation. Using pressures higher than 0.5 psi caused air introduction into capillary that destroyed the reproducibility. Still the sample injection time can be significantly longer (up till minute) if the same kind of loops were used to perform quantitative electrokinetic sampling. In that case the sample loop was also used as a high-voltage electrode and instead of wrapping it around the tip of capillary, it was connected to a supporting wire that was united to a high voltage supply. The support wire and capillary were fixed a few centimetres above the tip by a small Plexiglas jig. The loops used were with larger diameter than in case of hydrodynamic injection. The experiments show remarkable improvement in terms of representativness of the sample. The mobility-induced bias of analytes was dramatically lowered. The explanation for that phenomenon was that due to the geometry of the electric field created inside the loop, the sample components could be almost exhaustively electromigrated from small sample volumes. The time needed was less than 1 min. The method described has great potential to vastly improve sampling in capillary electrophoresis. The method is relatively easy to applicate and the fabrication of the loops is simple. The detection limits of the method were relatively low. The probable limitation for further reduction of the detection limits might be the increasing amount of hydroxide. Because hydroxide is forming as a result of electric field, it is injected into the capillary and if the amount will be big enough it can influence the results. Also, it was not clear how much the length of the injection can influence the resolution between analytes. The reproducibility of experiments was not reported.

E. Microdrop Injector

Sziele et al. presented a micro injector for capillary electrophoresis based on a microdrop ink-jet system.[78] The device permitted the injection of small droplets into capillaries. The ejector supplied with a storage bottle as sample reservoir was placed so that the tip of the glass capillary of the ejector was exactly over the inlet end of the capillary. The positioning was done with help of a microscope. Prior to every injection the capillary was thoroughly washed with sodium hydroxide and buffer solutions. Then the capillary holder was removed from the capillary electrophoresis instrument and the capillary was positioned towards the ejector. Subsequently the injection was done via aiming a drop of sample into the capillary. After the injection the capillary holder was positioned back as quickly as possible and the measurement was carried out in common way. The system enabled to achieve quite reproducible results (the reproducibility for peak areas was about 3%), however, with the cost of careful adjustment of the injection system. The comparative experiments done with common pressure injection gave somewhat better results both in terms of peak area reproducibility and detection limits. The sampling procedure did not seem to be much comfortable because it needed washing and conditioning of the capillary between every injection and positioning off and on the capillary holder.

F. Sample Matrix Switching

Increasing the concentration sensitivity of the method may often result in decreasing of resolution power or repeatability of the method. To overcome this Zhao et al. have been developed an on-line sample concentration method using sample matrix switching and field amplification peak stacking.[79] The method was designed for on-line concentration and separation of ionizable com-

pounds present in low concentrations in physiological high ionic strength fluids. The heart of the system were four fused silica capillaries (C_1, C_2, C_3, C_4) connected to each other via a Valco cross microadapter. Capillaries C_2 and C_4 were linked to two-way switching valves. The other end of C_1 was connected to the liquid chromatography (LC) column. The column was not used for separation or concentration of sample components, it was rather used to slightly retain the analytes in the column, while the high ionic strength sample matrix (the physiological fluid) was flushed through the column by a weak LC buffer. The other ends of capillaries C_2, C_3, and C_4 were inserted into CE buffer reservoirs. All the reservoirs included electrodes. The operation of the system was as it is seen on**.** First the sample was introduced into LC column. Then the sample matrix was flushed out of the column while sample components were a little retained in the column (a). Then analytes were eluted by a low ionic strength LC buffer through the capillary C_1 into the capillary C_3 (b). Elution of the analytes was monitored by first UV detector (D). When the detector show maximum absorbency (i.e., when the peak of sample reached to the detector), the flow from LC system was stopped. The capillary C_3 now consisted of analytes, sample matrix and LC buffer. Capillaries C_2, and C_4 (which were cross to capillaries C_1, and C_3) were filled with CE buffer. In the next step high voltage was applied at the ends of capillaries C_2 and C_3 and the analytes were stacked at the interface between the high ionic strength CE buffer in C_2 and the low ionic strength LC buffer in C_3 (c). In the fourth step the low ionic strength buffer was removed from capillary C_3 (d) and the analytes were separated in capillary C_2 by a normal CE process (e). The above system allows to increase the concentration sensitivity more than 500 times compared to a normal hydrodynamic injection. The improvement in detection limits was achieved without loss in resolution. The fact that even long-time stacking (80 s) did not ruin the resolution between closely migrating ions made the method very attractive. The detection limit received was 10 n*M* that was very acceptable detection limit if using common UV detection. The entire analysis time was less than 15 min. The method had also its disadvantages. First of all, the system was a bit complicated and many factors had to be controlled to achieve successful results: the choice of capillaries with right diameter and length; the choice of right pH values and ionic strength for both liquid chromatography and capillary electrophoresis buffers; right timing of trapping the liquid chromatography buffer peak. The system was not automated and that made the timing more complicated and less exact, which resulted in poorer reproducibility. The reproducibility of migration times less than 6% RSD but the reproducibility of the peak height was more than 10% RSD. The other weak point in the system is that it is not always easy to find a suitable liquid chromatography column — especially if the range of analytes of interest is wide.

CONCLUSIONS

CE has many weak points that can be attributed to the sample introduction (e.g., many samples cannot introduce small quantities of sample, but if they can then the detection becomes the problem). As follows from the text above lot of ingenious sampling devices have been designed to solve variety of problems encountered in CE analysis. As every method has its advantages and disadvantages every new sampling device has been designed to overcome at least some of the disadvantages of the previous devices. However, excellent results have frequently obtained at the expense of sacrificing the performance of some other parameters.

The driving force has mainly been the extending of CE applications to monitoring, hyphenating, easing of sample preparations

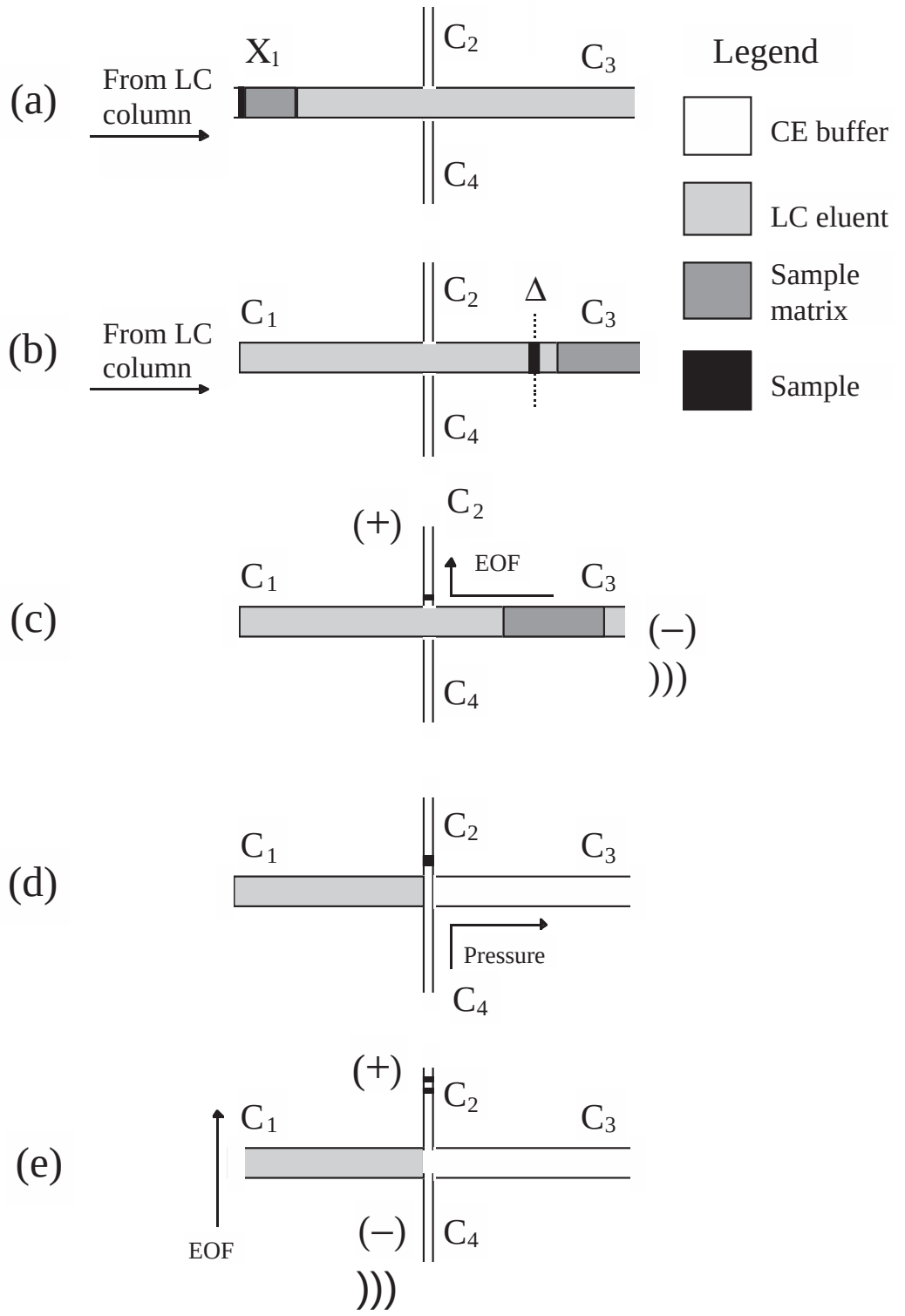


FIGURE 18. Schematic picture of different steps during matrix switching method. (a) Separation of the sample from matrix and matrix switching; (b) trapping the sample peak; (c) stacking; (d) replacing low ionic strength LC buffer by CE buffer; (e) separation of analytes by electric field in CE capillary.

and reduction of detection limits. However, many of those samplers have rather special and limited use. On the other hand, some smart solutions seemed to have been motivated just by joy of play (like some inventions of Dasgupta's group), which obviously is one of the strongest driving force of science. Even it is not easy to predict the future of all those smart inventions (will they survive and become widely accepted or not) they all enrich the field of CE. One of the most attractive features of CE is the fact that the technique is still immature and there is lot of room left for further developments, so is the situation with the sampling in CE.

REFERENCES

1. **R. Kuhn; S. Hoffstetter-Kuhn** Capillary Electrophoresis. Principles and Practice, Springlet Verlag, Germany, 1993.
2. **S. Hjerten** Free Zone Electrophoresis, Almquist & Wiksells Boktrykery AB, Uppsala, 1967.
3. **Verheggen, Th. P. E. M.; Beckers, J. L.; Everaets, F. M.** *J. Cromatogr*. **1979**, 169, 11–20.
4. **Jorgenson, J. W.; Lukacs, K. D.** *Anal. Chem*. **1981**, 53, 1298–1302.
5. **Albin, M.; Grossman, P. D.; Moring, S. E.** *Anal. Chem*. **1993**, 65, 489A.
6. **Fang, Z.-L.; Liu, Z.-S.; Shen, Q.** *Anal. Chim. Acta* **1997**, 346, 135–143.
7. **Zhao, Y.; McLaughlin, K.; Lunte, C. E.** *Anal. Chem*. **1998**, 70, 4578–4585.
8. **Verheggen, Th. P. E. M.; Beckers, J. L.; Everaets, F. M.** *J. Cromatogr*. **1979**, 169, 21–38.
9. **Deml, M.; Foret, F.; Bočcek, P.** *J. Cromatogr*. **1985**, 320, 159–165.
10. **Tsuda, T.; Mizuno, T.; Akiyama, J.** *Anal. Chem*. **1987**, 59, 799–800.
11. **Verheggen, Th. P. E. M.; Beckers, J. L.; Everaets, F. M.** *J. Cromatogr*. **1988**, 452, 615–622.
12. **Tsuda, T.; Zare, R. N.** *J. Cromatogr*. **1991**, 559, 303–310.
13. **Honda, S.; Iwase, S.; Fujiwara, S.** *J. Cromatogr*. **1987**, 404, 313–320.
14. **Rose, D. J., Jr.; Jorgenson, J. W.** *Anal. Chem*. **1988**, 60, 642–648 .
15. **Wallingford, R. A.; Ewing, A. G.** *Anal. Chem*. **1987**, 59, 678–681.
16. **Lindgren, P. F.; Dasgupta, P. K.** *Anal. Chem*. **1989**, 61, 19–24.
17. **Bao, L.; Dasgupta, P. K.** *Anal. Chem*. **1992**, 64, 991–996 .
18. **Melcher, R. G.** *Anal. Chim. Acta* **1988**, 214, 299–313.
19. **Kuban, P.; Karlberg, B.** *Anal. Chem*. **1997**, 69, 1169–1173.
20. **Kuban, P.; Engström, A.; Olsson, J. C.; Thorsén, G.; Tryzell, R.; Karlberg, B.** *Anal. Chim. Acta* **1997**, 337, 117–124.
21. **Kuban, P.; Pirmohammadi, R.; Karlberg, B.** *Anal. Chim. Acta* **1999**, 378, 55–62.
22. **Morita, I.; Sawada, J.** *J. Chromatogr*. **1993**, 641, 375–381.
23. **Morita, I.; Sawada, J.** *J. Chromatogr*. **1993**, 641, 375–381.
24. **Zhang, C.-X.; Thormann, W.** *Anal. Chem*. **1996**, 68, 2523–2532.
25. **Chien, R. L.; Burgi, D. S.** *Anal. Chem*. **1992**, 64, 489A-496A.
26. **Albin, M; Grossman, P. D.; Moring, S. E.** *Anal. Chem*. **1993**, 65, 489A-497A.
27. **Burgi, D. S.; Chien, R. L.** *Microcolumn Sep.* **1991**, 3, 199–202.
28. **D. Kaniansky; J. Marak**, *J. Chromatogr*. **1990,** 498, 191–204.
29. **D. Kaniansky; Zelinsky, I.; Hybenova, A.; Onuska, F. I.** *Anal. Chem*. **1994**, 66, 4256–4264.
30. **Stegehuis, D. S.; Tjaden, U. R.; van der Greef, J.** *J. Chromatogr*. **1992**, 591, 341–349.
31. **Kuldvee, R.; Kaljurand, M.** *Anal. Chem*. **1998**, 70, 3695–3698.
32. **Giddings, J. C.** *Anal. Chem*. **1984**, 56, 1258A.

33. **Davis, J. M.; Giddings, J. C.** *Anal. Chem.* **1985**, 57, 2168–2177.

34. **Phillips, J. B.; Xu, J.** *J. Chromatogr*. **1995,** 703, 327–334.

35. **Bushey, M. M.; Jorgenson, J. W.** *Anal. Chem*. **1990**, 62, 161–167.

36. **Murphy R.; Shure M. R.; Foley J. P**., *Anal. Chem*. **1998**, 70, 1585–1594.

37. **Kok, W. Th.; Hype, K. P.; Frei, R. W.** *J. Chromatogr*. **1988**, 436, 421–427.

38. **Bushey, M. M.; Jorgenson, J. W.** *Anal. Chem*. **1990**, 62, 978–984.

39. **Lemmo, V. L.; Jorgenson, J. W.** *Anal. Chem*. **1993**, 65, 1576–1581.

40. **Monning C. A.; Jorgenson J. W.** *Anal. Chem.* **1991**, 63, 802–807.

41. **Hooker, T. F.; Jorgenson, J. W.** *Anal. Chem*. **1997**, 69, 4134–4142.

42. **Lada, M. W.; Kennedy, R. T.** *Anal. Chem.* **1996**, 68, 2790–2797.

43. **Tao, L.; Kennedy, R. T.** *Anal. Chem*. **1996**, 68, 3899–3906.

44. **Moore, A. W., Jr.; Jorgenson, J. W.** *Anal. Chem*. **1995**, 67, 3448–3455.

45. **Moore, A. W., Jr.; Jorgenson, J. W.** *Anal. Chem*. **1995**, 67, 3456–3463.

46. **Palmarsdóttir, S.; Edholm, L.-E.** *J. Chromatogr*. *A* **1995**, 693, 131–143.

47. **Palmarsdóttir, S.; Mathiasson, L.; Jönsson, J. Å.; Edholm, L.-E.** *J. Cap. Elec.* **1996**, 003:5, 255–260.

48. **Kar, S.; Dasgupta, P. K.** *J. Microcolumn Sep.* **1996**, 8, 561–568 .

49. **Church, W. H.; Justice, J. B.** *Anal. Chem.* **1987**, 59, 712–716.

50. **Boutelle, M. G.; Fellows, L. K.; Cook, C.** *Anal. Chem.* **1992**, 64, 1790–1794.

51. **Deterding, L. J.; Dix, K.; Burka, L. T.; Tomer, K. B.** *Anal. Chem*. **1992**, 64, 2636–2641.

52. **Hogan, B. L.; Lunte, S. M.; Stobaugh, J. F.; Lunte, C. E.** *Anal. Chem.* **1994**, 66, 596–602.

53. **Jacobson S. C.; Ramsey J. M.** *Anal Chem.* **1996**, 68, 720.

54. **Wooley A.; Mathies R. A.** *Proc. Natl. Acad. Sci. USA* **1994**, 91, 11348–11352.

55. **Jacobson S. C.; Culbertson C. T.; Daler J. E.; Ramsey J. M.** *Anal. Chem*. **1998,** 70, 3476–3480.

56. **Martynova, L., Locascio, L. E., Gaitan, M., Kramer, G. W., Christensen, R. G.; MacCrechan, W. A.** *Anal. Chem*. **1997,** 69, 4783–4789.

57. **Effenhauser C S., Bruin G. J. M., Paulus A.; Erhart M.** *Anal. Chem*. **1997**, 69, 3451–3457.

58. **Schwarz A. B.; Rossier J. S.; Roberts M. A.; Roulet E.; Mermod N.; Girault H. H.** *Langmuir* **1998**,14, 5526–5532.

59. **Roberts M. A., Bercier P., Girault H. H.** *Anal. Chem.* **1997,** 69, 2035–2046.

60. **Effenhauser C. S.; Manz A.; Widmer M.** *Anal. Chem.* **1993,** 65, 2637–2642.

61. **Jacobson S. C.; Hergenrröder R.; Moore A. W.; Ramsey J. M.** *Anal. Chem*. **1994,** 66, 4127–4132.

62. **van der Molen J. N.; Poppe H.; Smit H. C**., *Anal. Chem*. **1997** 69, 4420–4427 .

63. **Kuldvee, R.; Kaljurand, M.; Smit, H. C.** *J. High Resol. Chromatogr*. **1998**, 21,169–174.

64. **van der Moolen, J. N.; Louwerse, D. J.; Poppe, H.; Smit, H. C.** *Chromatographia* **1995**, 40, 368.

65. **Izawa, K.; Furuta, K.; Fujiwara, T.; Suyama, N.** *Ind. Chim. Belge* **1967**, 32, 223.

66. **Smit, H. C.** *TrAC* **1983**, 2, 1.

67. **Kaljurand, M; Kullik, E** *J. Chromatogr*. **1979**, 171, 243.

68. **Kaljurand, M.; Elomaa, M.; Plit, L.** *Anal. Chim. Acta* **1991**, 248, 271.

69. **van der Molen**, **J. N.** Correlation Techniques and Micromachinery in CZE; Improving Precision and Accuracy, Dissertation, University of Amsterdam, 1998, 95 p.

70. **Kaljurand, M.; Kullik, E.** *Computerized Multiple Input Chromatography*, Ellis Horwood Ltd, Chichester, UK, **1989.**

71. **Kaljurand, M.; Ebber, A.; Somer, T.** *J. High Resol. Chromatogr*. **1995**, 18, 263–265.

72. **Arce, L; Ríos, A.; Válcarcel, M.** *J. Chromatogr. A* **1997**, 791, 279–287.

73. **Válcarcel, M.; Ríos, A.; Arce, L.** *Critical Reviews in Analytical Chemistry* **1998**, 28, 63–81.

74. **Liu, H.; Dasgupta, P. K.** *Anal. Chim. Acta* **1993**, 283, 739–745.

75. **Dasgupta, P. K.; Kar, S.** *Anal. Chem.* **1995**, 67, 3853–3860.

76. **Dasgupta, P. K.; Surowiec, K.** *Anal. Chem.* **1996**, 68, 1164–1168.

77. **Dasgupta, P. K.; Surowiec, K.** *Anal. Chem.* **1996**, 68, 4291–4299.

78. **Sziele, D.; Brüggemann, O.; Döring, M.; Freitag, R.; Schügerl** *J. Chromatogr. A* **1994**, 669, 254–248.

79. **Zhao, Y.; McLaughlin, K.; Lunte, C. E.** *Anal. Chem.* **1998**, 70, 4578–4585.