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Review

Instrumentation for capillary electrochromatography

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Abstract

One of the reasons for the immense interest in capillary electrochromatography (CEC) is its feature to combine chromatographic selectivity with the high efficiency and the miniaturization potential of capillary electrophoresis (CE). The capability of commercial CE instruments to run CEC has enforced the readiness of users and researchers to work on this separation technique. Nevertheless, to fully exploit the potential of CEC, a routine CE device can certainly not fulfill all requirements. Two different approaches have been made to overcome this problem. The first was to modify commercial CE instruments for various demands. Pressurization of the packed capillary to prevent "air" bubble formation, gradient elution capabilities and thermostating devices allowing a greater flexibility in column designs have been implemented in CE instruments of several manufacturers. A completely different approach is the development of modular laboratory-made instrumentation dedicated to special CEC requirements. In order to increase mobile phase velocity and thus the speed of analysis the availability of voltages higher than 30 kV was accomplished in some of these devices. Gradient elution was achieved by either coupling of gradient LC systems or an electroosmotic generation of the changing eluent composition. When a pressure gradient is applied between both column ends in addition to the voltage gradient, a hybrid between capillary HPLC and CEC results. This chromatographic mode is named pressure-assisted electrochromatography (PEC). Either CE instruments equipped with additional HPLC pumps or modular laboratory-made devices are suitable for PEC. In CEC, sensitivity for UV detection is rather poor due to the short optical path length for on-column detection in capillary separation techniques. A special cell design with enhanced light path is presented and further principles like, e.g., fluorescence detection and coupling to mass spectrometry are discussed. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Reviews; Electrochromatography; Instrumentation

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1. Instrumentation for initial experiences in capillary electrochromatography

In 1974, Pretorius et al. introduced a new concept for high-speed liquid chromatography by using electroosmosis to ''pump'' eluents through a narrow bore packed column [1]. At that time, high-performance liquid chromatography (HPLC) was a young emerging technique and it was difficult to predict the development of commercially available instruments for electroosmotic flow (EOF)-driven separation techniques. Capillary electrophoresis (CE) was introduced in the 1980s and routine devices became commercially available some years later. These instruments were applied in capillary electrochromatography (CEC) later on.

Initially, laboratory-made equipment was used to study the fundamentals of capillary electrochromatography. Pretorius et al. [1] used for their EOF studies a vertically mounted packed glass column (5 cm×1 mm I.D.). They applied very high field strengths up to 2000 V cm^{-1} between a graphite electrode at the inlet solvent reservoir and a steel tube at the outlet connector of the column. The experimental set-up is depicted in Fig. 1. Linear velocities up to 0.2 mm s^{-1} in methanol–benzene (1:9) on bare silica were measured, a very low value compared to HPLC. The mobile phase movement was observed by the migration of the liquid surface in the vertically mounted open tube connected to the column end. For characterizing the ratio of column plate numbers between pressure- and EOF-driven chromatography in columns of 50 cm length, an injection device, a pump and a UV detector have been installed to extend the system. Although the detection details were not precisely described in the paper, Pretorius obtained with electroosmotic flow plate heights less than 50% as in the case of laminar flow.

The first real CEC separation was reported by Jorgenson and Lukacs in 1981 [2]. A 30 kV power supply delivered the voltage to the inlet and outlet reservoirs via graphite electrodes. Analytes were introduced electrokinetically into the packed capil-

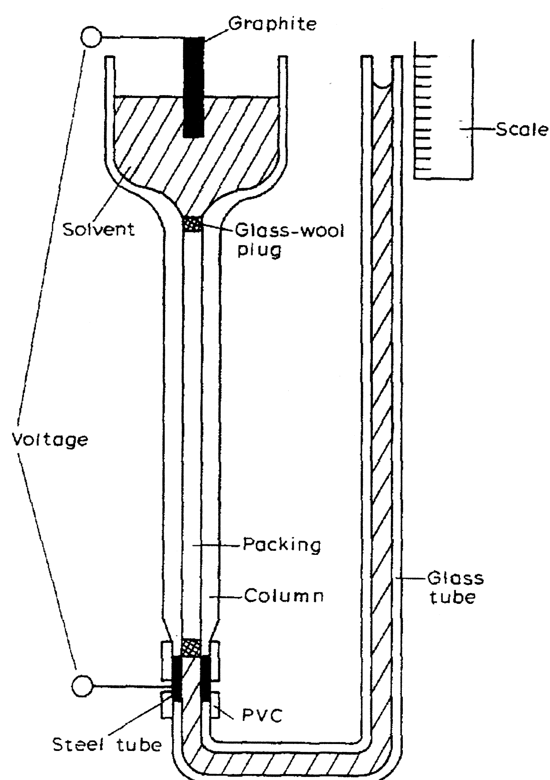

Fig. 1. Apparatus for measuring flow through microparticulate packing. From Ref. [1] with permission.

lary (Partisil-10 ODS-2 in 58 cm×170 μm I.D.) after switching from the buffer reservoir to a sample container. For detection, fluorescence was used. Due to safety reasons the high-voltage end of the system was set up in a plexiglass box with a voltage interlock mechanism. The authors reported reduced plate heights of 1.9 for 9-methylanthracene and 2.5 for perylene on stationary phases with 10-μm particle diameters.

In CEC the mobile phase is propelled by the EOF which depends on a variety of parameters. Compared to HPLC the hydraulic force on the solvent is now replaced by an electrical force that moves the solvent which is generated by the applied voltage. The resulting solvent velocity depends on the surface charge of the stationary phase, the mobile phase composition and the temperature. Thus, it is definitely more difficult to control mobile phase velocity in CEC than in HPLC. The possibility to adjust the actual value of the flow-rate directly is highly desirable for any chromatographic system. In CEC, this is only possible when the EOF is continuously measured and corrected. When gradient elution is applied in electrochromatography an additional problem arises. The mobile phase velocity changes automatically with the composition of the eluent, since the EOF is influenced by the organic modifier content, the buffer concentration and its pH value (see last paragraph of Section 4.2.2). Therefore, an EOF correction would be required in order to keep the linear velocity constant during the gradient.

Wanders et al. [3] published solutions for EOF measurement and control. One approach was the application of a microbalance in connection with a computer to quantify the EOF by the mass increase in the outlet vessel. A more complex method was the application of a kind of a post-column derivatization detector. A constant pressure-driven or electrically-driven reference flow of a solution with a UV marker was mixed with the eluent emerging from the separation capillary and the EOF was recalculated from the UV absorbance of the resulting combined flows. The software could control the EOF by coupling the data system to the high-voltage power supply. The precise mobile phase velocity could then be set by the software, similar to the control of eluent pumping in HPLC. However, when charged analytes are separated in CEC, the resulting fluctuations of the electrical field influence the proportional contribution of electrophoretical migration to the whole separation process. These problems do not arise when neutral solutes are separated.

2. Capillary electrochromatography column technology

The compatibility of CEC column technology with miniaturized instrumentation is a mandatory requirement. For fused-silica capillary columns no end fittings with retaining sieves or frits are available that would fit together with the electrodes into the buffer reservoirs of most commercial CE(C) instruments. Consequently, different approaches are used for immobilization of the packed bed within the capillary. The most common method has been the sintering of frits out of the packing material. As will be discussed in Section 3.1, bubble formation is a further hindrance in CEC practice. A laboratory-made apparatus for basic experiments to understand the origin of the problem has been described by van den Bosch et al. [4]. They concluded that the design of the packing retainers is of great importance for column plate numbers and suppression of bubble formation. The instrument has been designed to screen several approaches on frit and filter materials and techniques. No pressurization was applied to the column ends so that critical conditions in terms of bubble formation were established.

The schematic of the whole instrumental set-up is illustrated in Fig. 2. Inlet (5) and outlet (7) vials, separation column (6), power supply (2) and UV detector (3) were contained in a plexiglass box (10). Temperature control was carried out by a thermostated water bath (8). Air convection in the box was achieved by a fan (9) coupled to the water bath. The high voltage was connected to the open inlet glass buffer vial (5) via a graphite electrode. The closed stainless steel outlet vial (7) is depicted in an exploded view in Fig. 2 and appears to be quite promising. Ground was directly connected to the vial housing. A HPLC pump was attached to that vessel to facilitate the rinsing of the packed capillary for equilibration. This principle was described by Rebscher and Pyell [5] earlier in the same year and the related instrument will be presented in Section 4. At

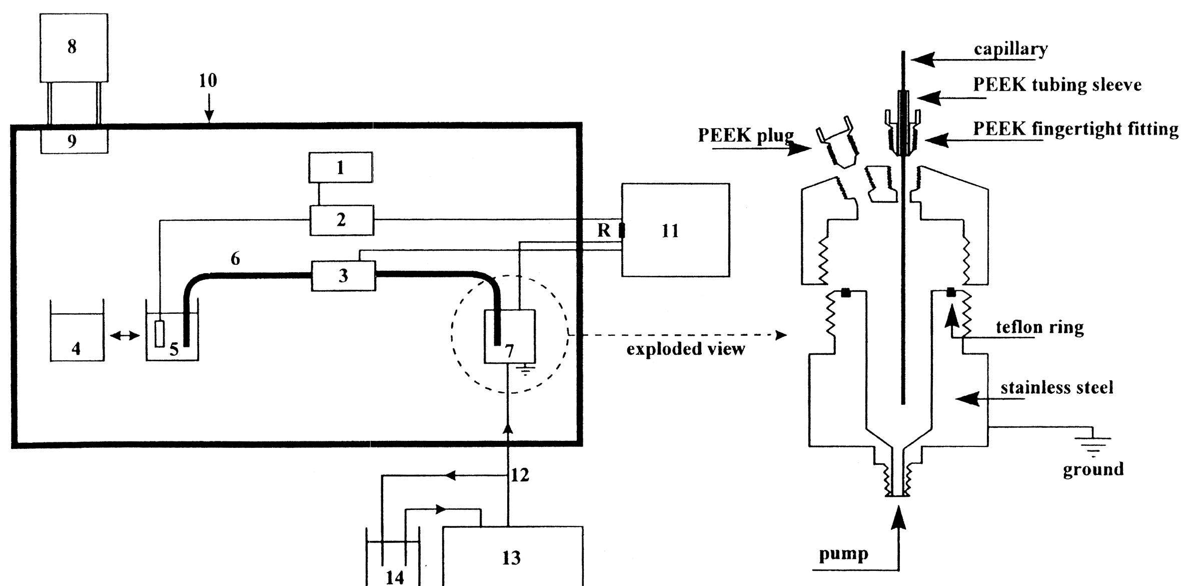

Fig. 2. Schematic representation of the CEC system: 1=timer; 2=high-voltage supply; 3=UV detector; 4=glass sample vial; 5=glass buffer vial; 6=packed capillary; 7=stainless steel buffer vial; 8=water bath; 9=fan; 10=safety box; 11=recorder; 12=split; 13=LC pump; 14=buffer reservoir. From Ref. [4] with permission.

the upper end of the container depicted in the exploded view (Fig. 2) a polyether ether ketone (PEEK) plug could be opened for flushing, when the buffer was changed.

3. Adaptation of commercial CE instruments for capillary electrochromatography

With the increasing availability of CE instruments, interest in packed column CEC started to grow for two reasons: the higher efficiencies achievable with electrically-driven flow and the increased loadability of chromatographic systems compared with capillary electrophoresis. The quality and performance of these fully automated instruments has gained a high level. The question to be discussed is however, whether they are really well-suited for CEC.

On the other hand, it is an obvious advantage of CEC that it can be run on a CE instrument without major modification, except that the open capillary is replaced by one at least partially filled with a stationary phase. This was outlined by Poppe as the simplest experimental set-up for CEC in his review on modern chromatographic methods [6]. The major problem also reported by him is the occurrence of bubbles under these conditions which initiated further modifications.

3.1. Bubble formation and pressurization of the packed capillary in capillary electrochromatography

Unlike in capillary electrophoresis, bubble formation is a practical problem in CEC, as already mentioned in Section 2. Generally, when bubbles occur, a very noisy baseline and spikes appear in the chromatogram. Moreover, bubbles can even cause interruption of electrical current and consequently of the electroosmotic flow leading to an abortion of the chromatographic process. These facts have been discussed by a variety of authors [7–9] and different suggestions have been made for solutions. The term ''air'' bubbles is used very often, as ''there is no practical way of finding out what the bubbles consist of'' [6]. At the introduction of CEC the aspects of bubble formation have been a steady focus of discussion.

CEC capillaries usually consist of a packed section where the separation process takes place and an open

section for UV detection. Between these two sections of the capillary the retaining frit is located. Due to this particular design CEC capillaries have discontinuities of the electric field strength and the flow velocity at the interface of the packed and open segments of the column. Similar discontinuities in flow velocity and electric field strength can originate from diverse zeta potentials in different parts of the column. This leads to the development of a flow-equalizing intersegmental pressure with concomitant bubble formation [10].

Common suggestions to prevent bubble formation are based on pressurization of the packed capillary. The pressurization techniques can be divided into two different modes. One possibility is the application of a hydraulic pressure gradient, sufficiently high to cause an additional pressure-driven flow through the column. The resulting mixed mode method may be called pressure-assisted electrochromatography (PEC) [11] or pseudo-electrochromatography [12] and will be described in Section 4.3. The second way is a simultaneous pressurization to both column ends at relatively low pressures of 10 to 20 bar. In this case no pressure gradient is generated and the plug like flow velocity profile of the EOF in the interparticulate space is not overlaid by a parabolic profile due to pressure gradient contributions.

The feasibility of this approach was first demonstrated in pharmaceutical analysis by Smith and Evans [8] by modification of a commercial instrument. However, little detail of the modification was given, except that the authors adjusted a pressure of 500 p.s.i. at both ends of the column prior to switching on the applied voltage (1 p.s.i.=6894.76 Pa). A year later Boughtflower et al. [7] described an application of obviously the same instrument (ABI 270A). However, the extension of the inlet tubing device in the apparatus, was not described in detail. A nitrogen pressure up to 15 bar was set to both ends of the capillary, which was applied or removed by a "manually switched valve operating a common vent to the inlet tubing manifold". In a later paper Smith and Evans [13] mention the additional protection of the device by a pressure relief valve set at 32 bar.

Once the pressurization technique has been accepted in CEC instrumentation, the manufacturers incorporated it into their instruments. In the case of Hewlett-Packard (HP) (Waldbronn, Germany) the application of 10 to 12 bar to both vials is mentioned by Dittmann and Rozing in 1997 in their paper on CEC stationary and mobile phases [14]. Two years earlier Hewlett-Packard (now Agilent Technologies) released the CEC pressurization option for their HP ^{3D}CE instrument. Euerby et al. [15] described the application of the modified HP ^{3D}CE instrument in pharmaceutical analysis by applying a pressure of only 8 bar to both vials. In the meantime, Beckmann/Coulter (Fullerton, CA, USA) also provides a CEC option for pressurization in their P/ACE System MDQ.

3.2. Modifications for special capillary electrochromatography requirements

The pressurization option in CE instruments described in Section 3.1 is a minor modification for their adaptation to CEC. A fundamental question however is, whether CE instruments with minor adaptations can fulfill all CEC requirements. The concrete CEC requirements though are still a matter of debate. Nevertheless, according to the authors the following options should be available:

1. CEC instrumentation should provide gradient elution and a pressure-driven flow assistance capability
2. Separation column holding and cooling devices should pose no restriction on column dimensions and designs
3. The system should require no frits to retain column packing and high voltage options up to at least 50 kV should be available
4. The possibility to apply more sensitive detection techniques than UV is required.

It seems impossible to achieve all this just by a simple modification of commercial CE instruments. Therefore, there is still a great interest in developing specialized home made devices to fully evaluate the potential of CEC and the ideal design of instrumentation.

Some very interesting additional approaches of versatile modifications of commercial CE instruments beyond pressurization have been described in literature. Dittmann et al. [16] have reported an additional modification of the HP ^{3D}CE instrument in

1997. They have shown that the instrument allows six different modes of operation: CEC, micro-liquid chromatography (LC) and CEC–LC mixed mode, each in either isocratic or gradient mode. A schematic of the modified components is given in Fig. 3. A low-pressure gradient HPLC pump and an autosampler were used to provide the mobile phase gradient and sample injection. A standard capillary cassette designed for CE–MS coupling was used. Consequently, the cassette has to be functioned in the reversed flow mode, which means that the normal inlet vial used in the CE mode operated as outlet vial. This was possible, since no regular CE injection was required. The high-voltage end was connected to the column outlet with reverse polarity, while the inlet is set to ground. Thus, all devices outside the CE instrument were set to ground potential. Due to the change of the vial purpose the distance from detection cell to column outlet was extended to 20 cm, as can be deduced from the capillary length ratios given in the figure legends of separations in gradient mode in the original paper.

The only addition of originally existing parts is the introduction of a special T-piece into the cassette. An exploded view of this part is added in Fig. 3. Since the column inlet is usually set as anode, a commercially available titanium insert was mounted into the T-piece to minimize corrosion. The T-piece was additionally used as split for both the flow of the HPLC pump and the injection volume from the autosampler. The possibility to switch between pure CEC and the pressure-assisted (PEC) mode is advantageous. The outlet vial was always set to 10 bar which is usually not the case in other PEC devices. When operating in pure CEC mode, the restriction capillary 1 was selected (see Fig. 3). Due to its permeability, it provided a column inlet pressure of about 10 bar at a pump flow-rate of 200 μl min^{-1}. To adjust the exact pressure balance between the two vials, either the pump flow-rate could be altered or

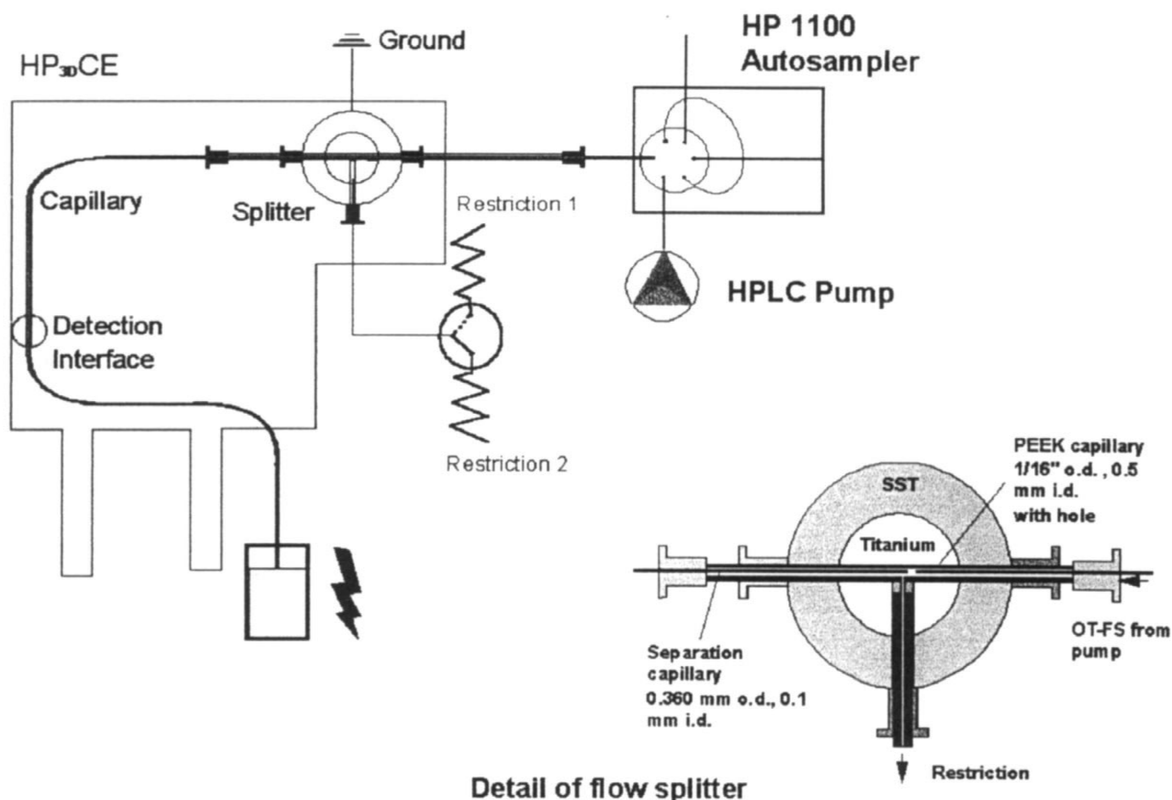

Fig. 3. Schematic of CEC–micro-LC instrument. From Ref. [16] with permission. Copyright (1997) ISC Technical Publications.

the outlet vial pressure in the CE system. The comparison of the separation of a standard parabene and polycyclic aromatic hydrocarbon (PAH) mixture in micro-HPLC (μ-HPLC) mode and CEC mode applying the same gradient profile from 20% to 80% acetonitrile at a similar flow-rate shows that the resulting separations are not identical. The relevant chromatograms are depicted in Fig. 4. The difference can be explained by the alteration of flow velocity in the electro-driven mode with changing organic modifier content. This demonstrates the intrinsic problem of gradient elution in electrochromatography already mentioned above. At constant field strength, the flow-rate in CEC cannot be kept constant when the composition of the mobile phase changes. It could be demonstrated that the peaks are sharper in CEC mode, even though plate numbers could not be calculated in the gradient mode.

To set the instrument to PEC mode, a valve had to be switched in connecting restriction capillary 2 instead of capillary 1. This capillary provided the required permeability to achieve a column head pressure of 110 bar at the standard pump flow-rate (200 μl min^{-1}). It resulted in a pressure gradient of 100 bar leading to an additional pressure-driven flow. The influence of the additional EOF is demonstrated in isocratic CEC–LC mixed mode compared with pure micro-HPLC mode for a standard separation. It can be deduced from the chromatograms in Fig. 5 that the additional electroosmotic flow does not contribute to band broadening, since the plate number remains constant although the linear velocity is doubled.

A similar instrumental set-up has been reported by Taylor et al. [17]. It was based on a Thermo Unicam Crystal 300 CE instrument and a variable-wave-

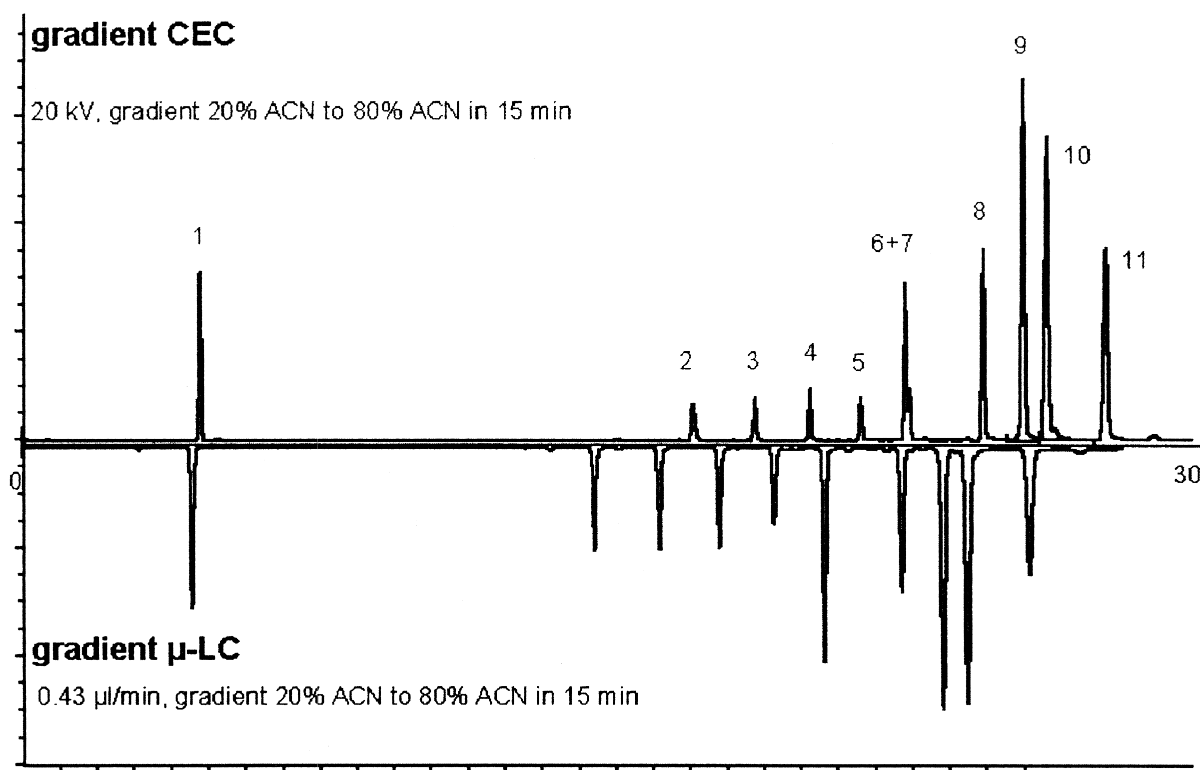

Fig. 4. Comparison of gradient micro-LC and gradient CEC. Column: CEC Hypersil C_{18} 3 μm, 250 (450)×0.1 mm; mobile phase: acetonitrile–water–Tris·HCl, 25 m*M*, pH 8; temperature 20°C. Compounds: (1) thiourea, (2) ethylbarabene, (3) propylparabene, (4) butylparabene, (5) pentylparabene, (6) naphthalene, (7) hexylparabene, (8) fluorene, (9) phenanthrene, (10) anthracene and (11) fluoranthene. From Ref. [16] with permission. Copyright (1997) ISC Technical Publications.

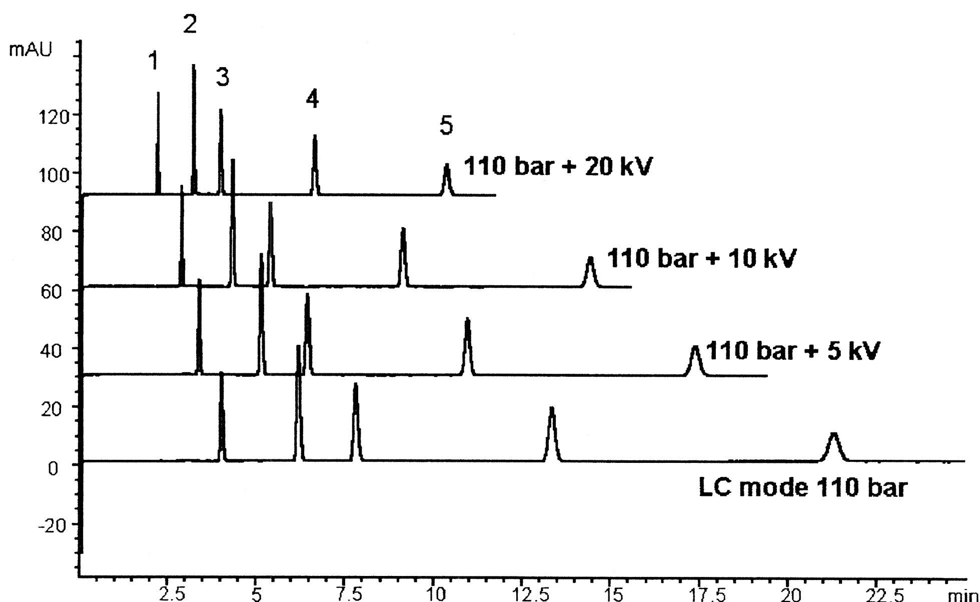

Fig. 5. Operation of a capillary column in LC–CEC mixed mode. Column: CEC Hypersil C_{18} 3 μm, 250 (450)×0.1 mm; mobile phase: acetonitrile–Tris·HCl, 25 m*M*, pH 8–water (70:20:10); 20°C. Compounds: (1) thiourea, (2) dimethylphthalate, (3) diethylphthalate, (4) biphenyl, (5) *o*-terphenyl. Plate count for *o*-terphenyl in all chromatograms: 25 000 plates. From Ref. [16] with permission. Copyright (1997) ISC Technical Publications.

length UV detector. Their apparatus was only designed for pure CEC mode with gradient elution facility. The schematic of the instrumentation is depicted in Fig. 6. A low-pressure gradient HPLC pump (6) and an autosampler (7) were connected to the column inlet device (4) to achieve solvent delivery and injection. The Crystal 300 instrument was used to provide the high-voltage power supply (8). The high voltage was connected to the inlet while the outlet was set to ground potential as in capillary electrophoresis. Two stainless steel T-pieces at both column ends acted as anode (4) and cathode (9). As can be deduced from Fig. 6, they also served to connect a quasi non-restrictive bypass capillary (1) for outlet pressurization in balance with the inlet. The pressure restriction to the HPLC pump is provided by the capillary (2) connecting the outlet T-piece (9) to the waste reservoir. Operating at a pump flow-rate of 100 μl min^{-1} the resulting pressure, which the authors name the "column head pressure", is 12 bar. The dimensions of the capillaries used are not given in the paper. This principle of pressurization looks very promising, since no adjustment of the outlet pressure is required for equilibrium. Applications of steroid analysis in equine urine and plasma extracts were reported. Samples have been injected after purification by solid-phase extraction. The authors quote this as the first CEC analysis of biological fluids published.

Huber et al. [18] have presented a further approach, where a high-pressure HPLC gradient device is combined with an extensively modified ABI 270A-HT capillary electrophoresis instrument. The principal experimental set-up is illustrated in Fig. 7. For gradient generation the flow of two HPLC pumps (14) was combined in a stainless steel jet mixer (12) connected to ground (13). The mixed flow ran through a fused-silica restrictor capillary (11) and a six-port valve (7) to the inlet reservoir (2) of the separation column (4). The six-port valve opened the possibility to bypass the column inlet and connect the HPLC pumps directly to the inlet waste reservoir. In this position the injection port was connected to the column inlet reservoir. The sample solution could be introduced by a syringe (10). A PEEK cross was used as inlet reservoir (2) with a split ability and via the fourth port (besides the three for flow splitting) a platinum electrode was inserted

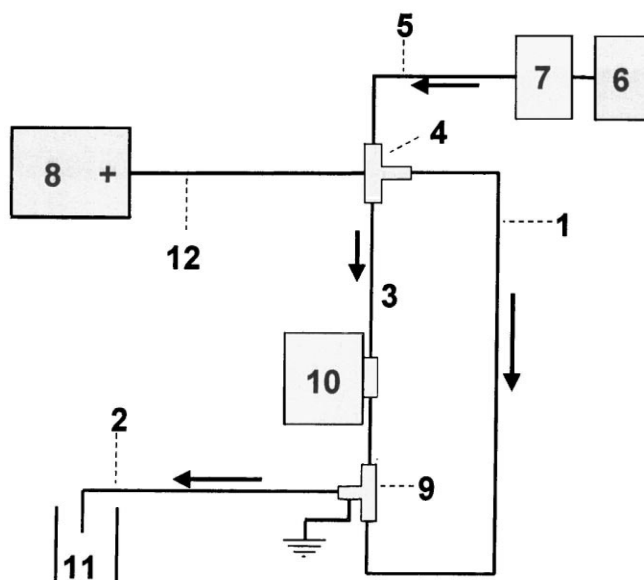

Fig. 6. Schematic of gradient CEC system employed to pressurize both ends of the CEC column and prevent bubble formation. The two waste capillaries 1 and 2 are of small diameter and of sufficient length to cause a restriction to flow and hence pressurization of both ends of the CEC column 3. The head pressure on the CEC column, resulting from the back pressure generated by the connecting waste capillary 1, was typically 10–15 bar. This was not sufficient to generate a significant hydrodynamic flow within the CEC column. Arrows indicate direction of flow: 4=high-voltage sampling interface, 5=loading capillary, 6=gradient HPLC pump, 7=HPLC autosampler, 8=HV power supply, 9=grounded waste interface, 10=UV detector, 11=waste reservoir and 12=HV power cable. From Ref. [17] with permission. Copyright (1997) American Chemical Society.

for connection to the unmodified high-voltage power supply (1). Injection onto the column was carried out electrokinetically from the PEEK cross after filling it with sample solution using the syringe. The inlet section could be rinsed with buffer solution applying the syringe, prior to applying high voltage to start the electrochromatographic run. The split outlet of the PEEK cross was connected to the inlet waste container (20) either directly or via a restrictor (9) in order to be able to apply a pressure gradient along the column for equilibration or μ-HPLC mode. A so-called purge valve (8) permitted one to switch between these two modes.

As can be seen in Fig. 8, the whole inlet section was mounted on a plexiglass plate, which allows one to adjust the position of the inlet cross. The plate was installed in the column oven of the ABI instrument, and the temperature control system was extended by an additional heating device for the detector compartment in order to avoid temperature differences between the part of the column in the column oven and in the detector compartment. The whole set-up permits accommodation of even straight columns between 80 and 180 mm length. Coiled columns can be used without upper length limitation.

The outlet reservoir (3) was a laboratory-made PEEK vessel and provided four connections. The first was attached to the column outlet, the second to the outlet waste reservoir (21), the third provided a connection to a syringe for easy buffer change and the fourth served to connect the high-voltage power supply via a platinum electrode. Both column ends could be pressurized simultaneously by nitrogen pressure of up to 14 bar on the outlet and inlet waste reservoir.

For further details on the experimental set-up and the operation of this rather complex system, the original paper should be considered [18]. The main differences to the system introduced by Dittmann et al. [16] should be summarized once again. A high-pressure gradient is used instead of a low-pressure device. Injection can be performed electrokinetically from the inlet reservoir instead of sample loop injection with split, which might be more convenient

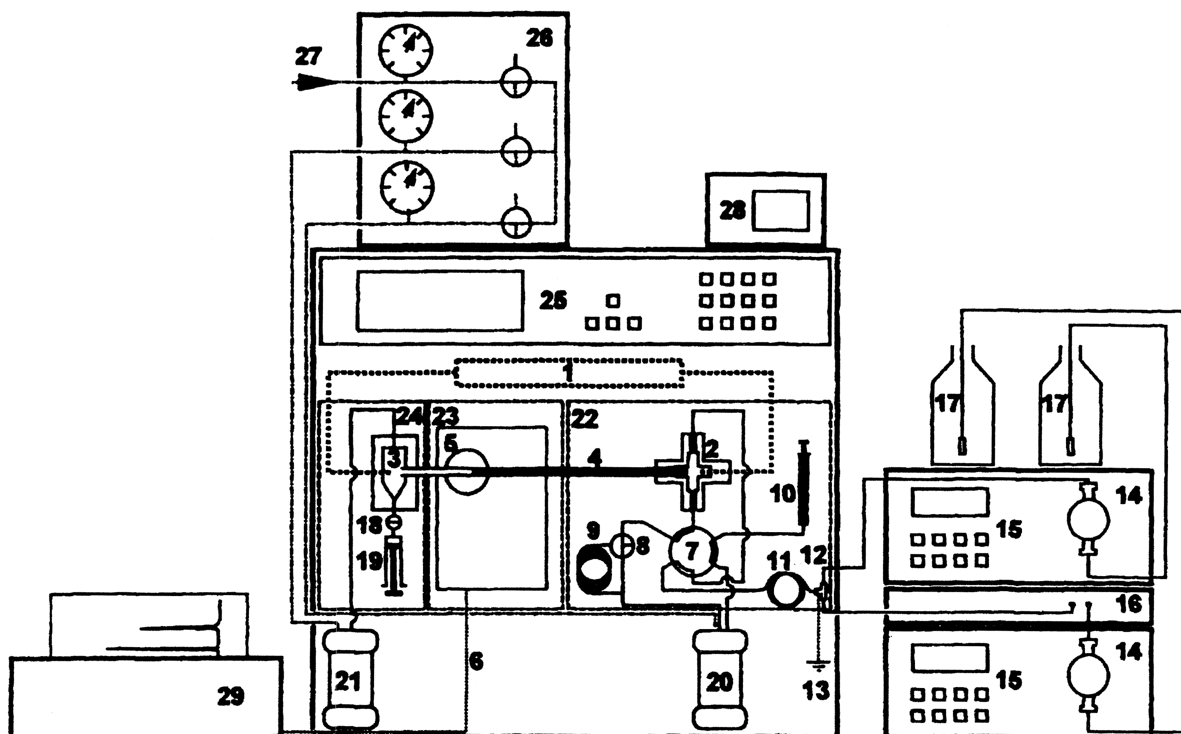

Fig. 7. Capillary electrochromatograph with gradient elution capability: 1=high-voltage power supply; 2=inlet reservoir with electrode; 3=outlet reservoir with electrode; 4=packed capillary column; 5=on-line sensing unit of UV detector; 6=detector output, 0–1 V; 7=sample injection valve; 8=purge valve; 9=restrictor; 10=syringe for introduction of sample or buffer; 11=capillary resistor; 12=static mixing tee; 13=grounding; 14=pumps; 15=pump control panels and readouts; 16=manometer; 17=eluent reservoirs; 18=switching valve; 19=syringe for buffer introduction; 20=waste reservoir at the inlet; 21=waste reservoir at the outlet; 22=thermostated inlet compartment; 23=detector compartment; 24=outlet compartment; 25=CEC instrument control panel; 26=gas pressure control; 27=gas inlet, 1.4 MPa nitrogen; 28=temperature control; 29=data acquisition. Line symbols: · · ·, electric wiring; —, liquid lines; – · –, gas lines; - - -, separating lines between instrument compartments. From Ref. [18] with permission. Copyright (1997) American Chemical Society.

for the μ-HPLC mode. Both column inlet and outlet can be pressurized by nitrogen to assure pressure balance instead of outlet gas pressurization and inlet pressurization by the HPLC pump.

The applicability of the system in gradient CEC mode has been demonstrated in a separation of phenylthiohydantoin (PTH)-amino acids which is depicted in Fig. 9. Principally, applications in μ-HPLC mode or pressure-assisted CEC mode are possible on the introduced apparatus, but have not yet been described. Since all three liquid phase microseparation techniques, namely CZE, CEC and μ-HPLC are unified, Horváth proposed the Greek name *triskelion* for this analytical device. The *triskelion* should "provide a reading on the sample composition by three different methods in a three dimensional fashion" [18].

4. Laboratory-made capillary electrochromatography devices

"CEC really needs a dedicated instrument, designed specifically for CEC and not modified from CE." This was the general consensus in a discussion of leading CEC scientists [19]. The instrumental deficiencies of commercially available instruments were summarized [20]: "Although development laboratories have performed stepwise gradient elution successfully, commercial instruments do not

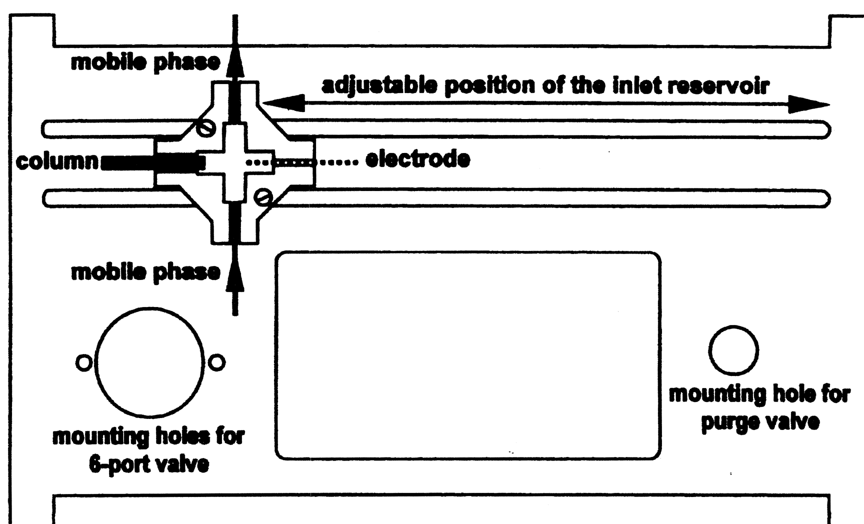

Fig. 8. Illustration of the inlet mobile phase reservoir with mounting plate to allow for the adjustment of axial position. From Ref. [18] with permission. Copyright (1997) American Chemical Society.

have this capability – a considerable deficiency. Minor shortcomings in instrumentation include the lack of voltages higher than 30 kV and inflexible column ovens with regard to heating or use of variable capillary lengths.''

As CEC is an emerging technique where the influence of all of the parameters affecting separation is not yet completely understood, there is a need for research on fundamental aspects of the method. Rebscher and Pyell [5] described a system for measurements of the dependence of EOF, flow profile and separation efficiency on a counterpressure applied by an HPLC pump at the outlet of the packed capillary. Injection was performed electrokinetically, detection by a spectrofluorometer (2) modified for detection in the packed section of the capillaries as well as after the packing. Temperature control of the separation capillary (1) was not possible. The whole system is depicted schematically in Fig. 10. The column inlet was kept at ambient pressure and the high voltage (6) is applied to the inlet buffer reservoir (7). The grounded stainless steel T-piece (5) at the column outlet is connected via valve A (4) to a microsyringe (8). The other branch is connected to an HPLC pump (11) and to waste (10) via valve B (9). The microsyringe serves either to measure the EOF when no pressure is applied while valve B is closed or to rinse the T-piece and the stainless steel outlet connector when both valves are open. When both valves are closed, the separation column can be equilibrated at reversed flow direction by means of the HPLC pump, prior to CEC application. This appears to be advantageous compared with the common practice of column equilibration outside of the CEC instrument. The main purpose for this special instrumental set-up was the possibility to apply a variable counterpressure from the HPLC pump to the column outlet during the separation in CEC mode. As it is discussed in the paper, the results of efficiency measurements at various counterpressures are very helpful to study fundamental phenomena in EOF-driven chromatographic systems.

4.1. Capillary electrochromatography at voltages above 30 kV

The application of voltages higher than 30 kV and the concomitant higher field strengths result in an increased mobile phase velocity and reduce the runtime of CEC analyses.

In their paper on electroosmotic flow and conductance studies Choudhary and Horváth described a laboratory-made CEC device which could be oper-

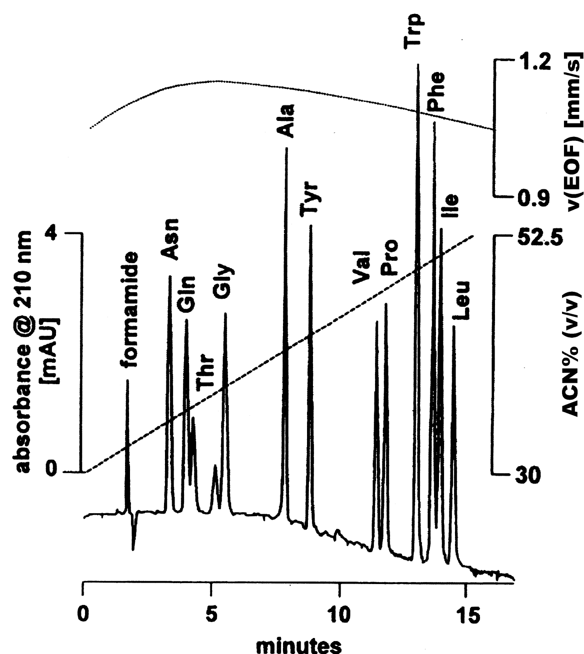

Fig. 9. Capillary electrochromatography of phenylthiohydantoin (PTH)-amino acids with gradient elution. Column, 20.7 cm (effective length 12.7 cm)×50 μm packed with 3.5 μm Zorbax ODS particles having a mean pore size of 80 Å. Starting eluent (A), 5 m*M* phosphate, pH 7.55, 30% acetonitrile; gradient former (B), 5 m*M* phosphate, pH 7.55, 60% acetonitrile; flow-rate of mobile phase through inlet reservoir, 0.1 ml min^{-1}; gradient, 0–100% B in 20 min; voltage, 10 kV; current, 1 μA; temperature, 25°C; UV detection at 210 nm; electrokinetic injection, 0.1 s, 1 kV. Peaks in order of elution: formamide; PTH-asparagine; PTH-glutamine; PTH-threonine, PTH-glycine; PTH-alanine; PTH-thyrosine; PTH-valine; PTH-proline; PTH-tryptophan; PTH-phenylalanine; PTH-isoleucine, PTH-leucine. The concentration of the PTH-amino acids dissolved in the mobile phase was 30–60 μg ml^{-1}. From Ref. [18] with permission. Copyright (1997) American Chemical Society..

ated at up to 90 kV [21]. The laboratory-built CEC device is presented in Fig. 11.

The 90 kV voltage power supply was composed of a bipolar 30 kV power supply (2) and a unipolar 60 kV power supply (1). Consequently, this experimental set-up allows the application of up to 90 kV for a chromatographic system with a cathodic EOF and a voltage drop up to 30 kV is available for a chromatographic system with an anodic EOF. In order to reduce the problem of arcing, a 1.4 cm thick poly(vinyl chloride) (PVC) protective spacer block has been mounted between the UV detector (11) and

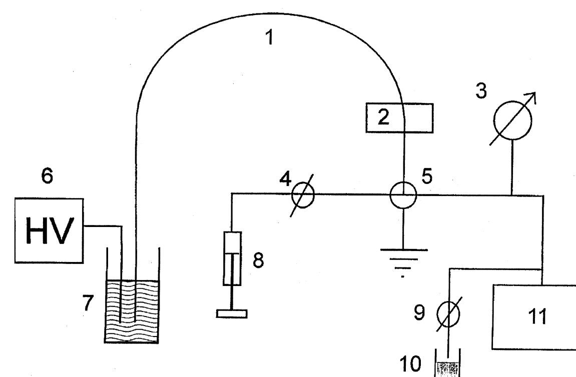

Fig. 10. Modular CEC system with pressurized detection side. 1=Packed capillary; 2=detector; 3=pressure gauge; 4=valve A; 5=T-piece; 6=high-voltage supply; 7=inlet buffer reservoir; 8=micro-syringe; 9=valve B; 10=waste; 11=LC-pump. From Ref. [5] with permission. Copyright (1996) Vieweg.

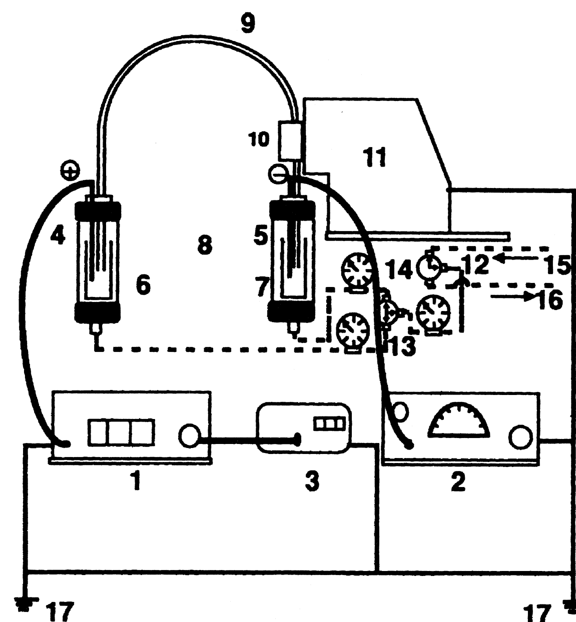

Fig. 11. Schematic of the modular capillary electrochromatograph with a 90 kV dual power supply and pressurizable chambers for the column inlet and outlet. 1=60 kV power supply; 2=30 kV power supply; 3=digital electrometer; 4, 5=electrodes; 6, 7=reservoir for mobile phase or the sample; 8=pressurizable chambers; 9=packed capillary column; 10=cell for on-column detection; 11=detector; 12=four-port two-way valve; 13=four-port three-way valves; 14=pressure gauges; 15=from nitrogen cylinder; 16=vent; 17=ground. From Ref. [21] with permission.

the cell for on-column detection (10). Electrolyte reservoirs (6, 7) were placed in glass chambers (50 mm×17 mm I.D.), which consisted of commercially available chromatographic columns. Pressurization from the column bottom was possible by nitrogen pressure of up to 20 bar.

Yan et al. [22] developed an apparatus that could be operated in both CEC and μ-HPLC modes. The high-voltage power supply of this instrument could provide up to 60 kV. A stainless steel six-port rotary valve (as used in HPLC) including an injection port served as the injection manifold, which was included in the electric circuit (in case of electrochromatography mode). In both, μ-HPLC and CEC modes, the volume of the sample loop was only partially injected to avoid volume overloading. The residual sample volume had to be flushed out before the separation was initialized. The instrument was partially housed within a Faraday cage for safety reasons.

The instruments described so far are not the only devices that allow the application of voltages above 30 kV. Some modular instruments used for gradient elution CEC or pressure-assisted electrochromatography can also be operated at elevated voltages (see Sections 4.2 and 4.3). Although important advantages of the application of such high voltages can be deduced from theory, working under these conditions requires utmost care and precaution. Obviously for this reason all commercial CE instruments are equipped with power supplies providing 30 kV maximum voltage.

On the other hand Hutterer and Jorgenson [23] described the modification of a commercial 30 kV power supply to provide voltages up to 120 kV in their paper on ultra-high voltage capillary zone electrophoresis. Separations of proteins were carried out at 28 and at 120 kV. Several millions of theoretical plates could be achieved with the ultrahigh voltage of 120 kV applied across the separation capillary.

4.2. Gradient elution in capillary electrochromatography

Gradient elution techniques increased the separation range in HPLC tremendously. Consequently, gradient elution should also be established in CEC to fully exploit the potential of the method.

The simplest approach to gradient elution is the so-called step-gradient technique where CEC separation is carried out with different inlet vials containing buffers of increasing elution power. Ding et al. [24] employed the technique on a rather simple laboratory-made CEC device consisting of a high-voltage power supply, a UV detector and two electrolyte reservoirs. The authors stated that no additional band broadening occurred due to flow interruption while changing inlet vials. Euerby et al. [25] analyzed six diuretics of widely differing hydrophobicities using the step-gradient technique on a commercial instrument.

Even though the step-gradient approach may be useful for certain applications, one should not forget that high efficiency separations in HPLC are performed with continuous gradients. Basically, continuous mobile phase gradients in CEC can be generated in two different ways. On the one hand, HPLC gradient techniques can be transferred to CEC. On the other hand, elution gradients are accessible by electroosmotic pumping from two diverse mobile phase reservoirs.

4.2.1. Eluent mixing by HPLC gradient techniques

The easiest way to generate a continuous mobile phase gradient in CEC is to increase the elution power by dosage of a stronger eluent into the inlet vial. This approach was published by Zhang et al. [26,27]. A schematic diagram of the CEC device used in this work is shown in Fig. 12.

A magnetic stirrer was used to assist the mixing process in the inlet vial. In this case, the separation was started with a relatively weak eluent and the stronger eluent was added continuously by means of a HPLC pump. The feasibility of this rather simple instrumental approach could be demonstrated by the comparison of two chromatograms (see Fig. 13).

Unfortunately, gradient and elution parameters were not described precisely. In detail this means that the composition of the solution delivered by the dropping pipette and the related flow-rate were not clearly specified. The authors stated that ionic strength and pH were kept constant during the separation. In addition to this CEC device, Zhang et al. presented in the same paper a further promising

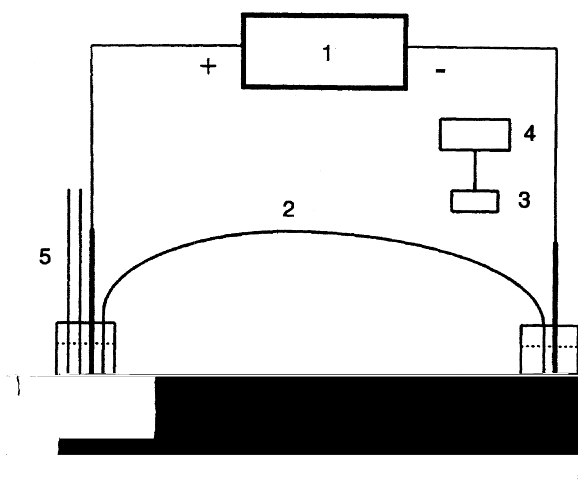

Fig. 12. Apparatus for gradient elution by continuous addition of the stronger eluting component into the inlet vial. 1=High-voltage power supply; 2=CEC column; 3=UV detector; 4=data collector; 5=pipette or pump; 6=magnetic stirrer. From Ref. [27] with permission. Copyright (1998) ISC Technical Publications.

instrumental set-up. Apart from CEC, PEC and μ-HPLC could also be performed on the later apparatus. A detailed description of the instrument will be given in Section 4.3.

In a comparable approach Wu et al. [28] placed a buffer vial containing solvent A on a magnetic stirrer and solvent B was added continuously by means of a syringe pump at a volume flow predetermined by the gradient.

Another approach to gradient elution electrochromatography was published by Lister et al. [29]. Solvent gradients were generated with a micro-LC system which was connected to the CEC column via a flow-injection analysis (FIA) interface. A diagram of the FIA–CEC interface is shown in Fig. 14. This interface has three connections and an electrode port. One is connected to the injector and micro-LC system. The two remaining channels are attached to the CEC column and waste. When the voltage was turned off and the micro-LC delivering solvent, the complete incoming solvent flowed through the waste channel. Under these conditions the authors reported no pressure-driven flow along the CEC column. With the voltage turned on the separation was purely electroosmotic. The complete instrumental set-up is depicted in Fig. 15. To avoid any damage to the low-pressure rotary injection valve, the Pt electrode on the inlet side of the separation capillary was set to ground potential. The high-voltage power supply was used in reversed polarity mode allowing to apply up to −30 kV at the Pt electrode located at the outlet reservoir. UV absorbance at 254 nm was used for detection.

A comparison of two isocratic and a gradient separation of a test mixture is presented in Fig. 16. The authors state that the relatively high dwell volume of their system (~250 μl) may cause early peaks to be eluted isocratically (see Fig. 16c). In order to observe the full advantage of gradient elution CEC peak tailing and band broadening effects due to the interface have to be minimized.

A further approach to gradient elution in CEC was published by Behnke and Bayer [30]. They combined a HPLC pump with a gradient mixer for their studies on pressure-assisted electrochromatography (see Section 4.3).

4.2.2. Eluent mixing by electroosmotic pumping

Mobile phase gradients in CEC can also be obtained without using any HPLC devices. Yan and co-workers [31,32] could demonstrate the feasibility of an electroosmotic flow generated elution gradient. The apparatus developed by Yan et al. is shown in Fig. 17.

The gradient delivery system consists of two independent high-voltage power supplies controlled by a computer. The gradient mixing is performed in a T-piece, which connects two independent mobile phase reservoirs to the separation column. This experimental set-up requires three electrodes, two of them are placed in the mobile phase reservoirs and the third is placed in the outlet reservoir. High voltage is applied to the electrodes in the mobile phase reservoirs and the electrode in the outlet reservoir is put to ground. Injection was carried out electrokinetically into the separation column by disconnecting it from the T-piece and placing its inlet into the sample vial. The whole CEC apparatus was mounted in a plexiglass box for safety purposes. Heat dissipation was assisted via air convection by a fan installed inside. Mobile phase reservoirs and outlet reservoir could not be pressurized in this instrumentation. The application of the equipment described is demonstrated for the separation of 16

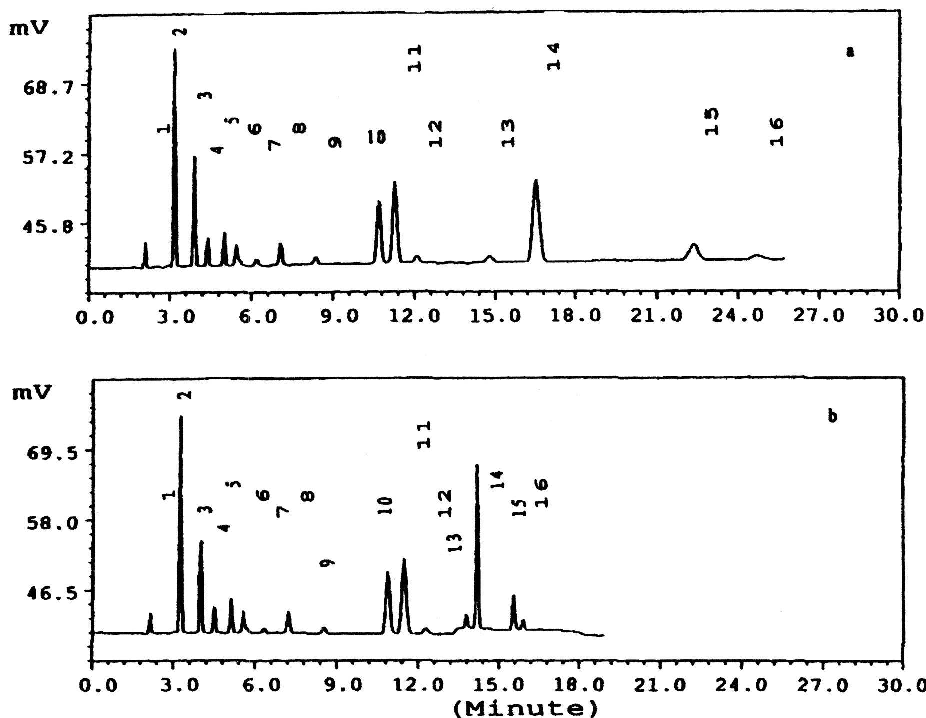

Fig. 13. Separation of aromatic compounds by CEC. Experimental conditions: instrument, laboratory-made apparatus; column, packing/total length, 15.8/43.5 cm with 3 μm Spherisorb-ODS$_2$; detection wavelength, 200 nm; electrokinetic injection, 5 kV, 5 s; applied voltage, 20 kV; operating current, 1.0 μA. For other experimental condition, see text. (a) Isocratic elution with acetonitrile (ACN)–buffer (60:40, v/v), containing 4 m*M* Tris, pH 9.2. (b) Gradient elution: mobile phase, ACN–buffer (60:40, v/v), containing 4 m*M* Tris, pH 9.2, from 0 to 12.25 min, then titrated to a mobile phase of ACN–buffer (80:20, v/v), containing 4 m*M* Tris, pH 9.2, Peaks: 1=thiourea, 2=phenol, 3=phenylpropanol; 4=2,3-dimethylphenol; 5=nitrobenzene; 6=2,4-dinitrotoluene; 7=benzene; 8=ethyl benzoate; 9=toluene; 10=naphthalene; 11=ethylbenzene; 12=*p*-dichlorobenzene; 13=1,2,3-trimethylbenzene; 14=*n*-propylbenzene; 15=1,2,4,5-tetramethylbenzene and 16=*n*-butylbenzene. From Ref. [26] with permission.

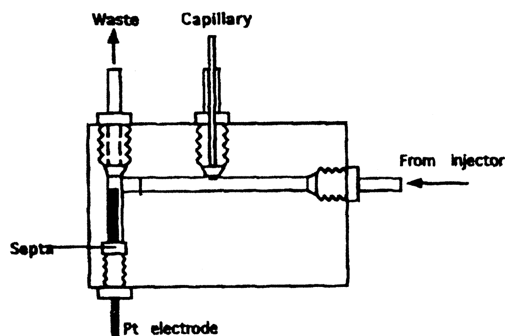

Fig. 14. Diagram of FIA–CEC interface. From Ref. [29] with permission.

PAHs (see Fig. 18). In addition to the gradient elution chromatogram, two separations performed isocratically with the initial and final eluent composition are presented.

In HPLC, eluent composition can easily be programmed via the volumetric flow of the individual pumps. Consequently, it is known what eluent composition leaves the mixing device at a defined time, provided that the system works properly. In the case of creating mobile phase gradients by means of electroosmotic pumping, the system must be calibrated to get information about the eluent composition in the column in dependence of the voltage program. The question is whether even slight changes of the buffer composition (pH, ionic strength, viscosity, dielectric constant) require a

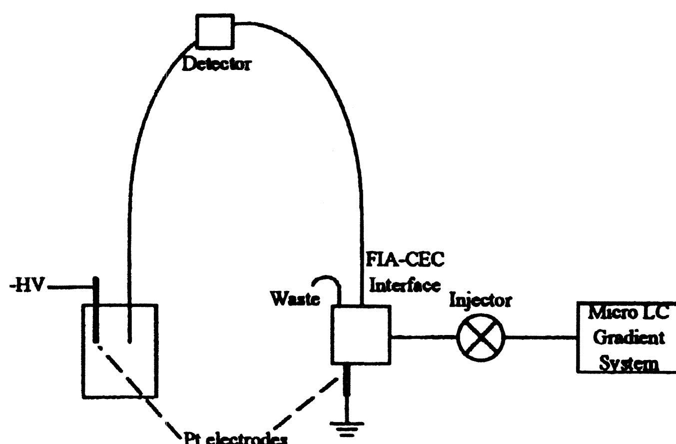

Fig. 15. Schematic diagram of instrument set-up. From Ref. [29] with permission.

recalibration of the voltage profiles which would be very time consuming. It has to be taken into account that the mobile phase velocity changes with the composition of the eluent, since the zeta potential and the ratio of the dielectric constant to the viscosity and thus the EOF are dependent on the solvent composition.

4.2.3. Eluent velocity gradient by voltage programming

Apart from a variation of the eluent composition, the mobile phase velocity can be increased during the separation. On the one hand, voltage programming enables short analysis times, and on the other hand, it does not oblige special requirements on voltage isolation, sample injection and other concerns inherent in gradient systems.

Xin and Lee [33] developed a laboratory-built CEC apparatus depicted in Fig. 19. The apparatus for the voltage programming experiments consisted of a computer controlled high-voltage power supply (0–50 kV), two pressurized electrolyte reservoirs and a fiber optic assembly for UV detection. The performance of the system could be demonstrated by the fast separation of 14 polycyclic aromatic hydrocarbons (see Fig. 20). Due to the higher reproducibility and more stable baseline compared with elution gradient methods reported by the authors, voltage programming appears to be a further optimization option in CEC, at least if voltages above 30 kV can be mastered. With decreasing diameter of the packing particles the minimum of the H/u curve is shifted towards higher linear velocities. Because of the small packing particles used in CEC ($d_p \leq 5$ μm), the initial value of the voltage gradient has to be in the region around 30 kV to achieve highly efficient separations. Consequently, high-voltage power supplies providing maximum voltages of at least 50 kV are required.

4.3. Pressure-assisted electrochromatography/pseudo-electrochromatography

In another instrumental approach to electrochromatography pressure is applied only at the inlet vial. The resulting hybrid between pressure- and electro-driven liquid chromatography has been introduced by Tsuda [11] and is referred to as pressure-assisted electrochromatography. Verheij et al. [12] proposed the name pseudo-electrochromatography to this technique. Both names, pressure-assisted and pseudo-electrochromatography are used as synonyms nowadays.

The instrumental set-up of the laboratory-made PEC apparatus developed by Kitagawa and Tsuda [34] is shown schematically in Fig. 21.

A HPLC pump, an injector, a laboratory-made capillary column, a UV detector and a high-voltage power supply (0–50 kV) were arranged as shown in Fig. 21. A fused-silica capillary served as a restriction tube to achieve flow splitting. The high voltage was applied at the stainless steel T-piece (A) while

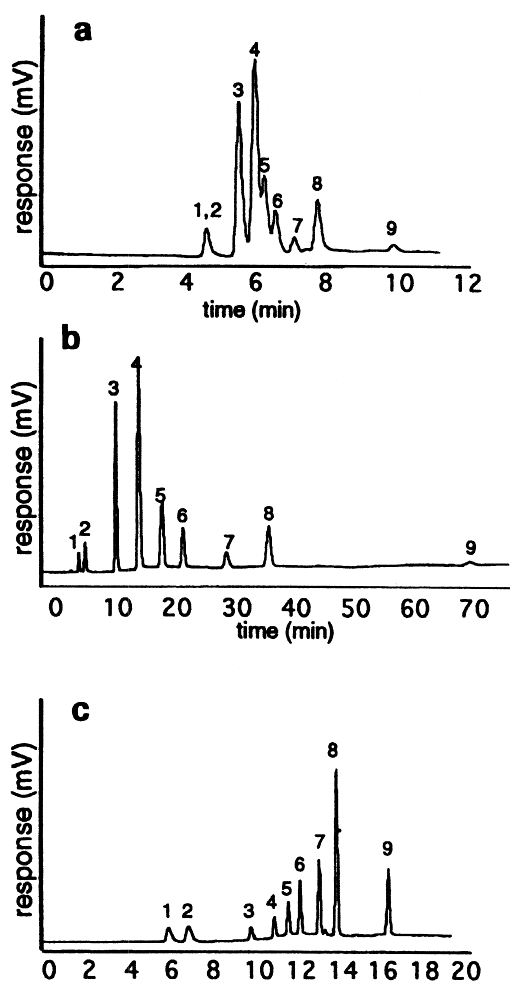

Fig. 16. Comparison of isocratic and gradient separation of test mixture. Gradient program length: 5 min. Flow-rate: 200 μl min^{-1}. Potential: 15 kV. Capillary: 20 cm packed with Shandon 5 μm ODS Hypersil, 50 cm×75 μm I.D. (a) Acetonitrile–water (90:10) isocratic, (b) acetonitrile–water (60:40) isocratic, (c) acetonitrile–water (60:40) to (90:10) gradient. Peaks: (1) acetone; (2) phenol; (3) benzene; (4) toluene; (5) naphthalene; (6) acenaphthylene; (7) fluorene; (8) anthracene; (9) 1,2-benzanthracene. From Ref. [29] with permission.

the outlet electrolyte reservoir (B) was grounded. The authors stated that the system could be operated free from bubble formation even though no pressure was applied at the column outlet.

Eimer et al. presented a similar laboratory-made system [35]. Unlike the instrument described above, high voltage was applied in this case to the outlet reservoir and the splitting T-piece was set to ground potential. Thus the pump was secure from any damage caused by the high voltage. Eimer operated the instrument not only in PEC mode, but also in μ-HPLC mode. It could be demonstrated that in PEC, selectivity control was possible by adjusting pressure and voltage. Three organic acids which could not be separated in pure HPLC mode, could be baseline resolved due to electromigration with the additional electric field applied across the capillary. The corresponding chromatograms are shown in Fig. 22.

Zhang et al. also presented a comparable laboratory-made apparatus [26]. In this approach the polarity of the electrodes was the same as described by Eimer et al. [35]. Whereas Eimer et al. grounded the T-piece used for eluent splitting, Zhang et al. set a stainless steel capillary, which was connected to the T-piece, to ground potential. In this way, the HPLC pump was also set to ground. The authors state that the operation mode of their laboratory-made instrument could be changed from PEC to CEC or μ-HPLC without dismantling the separation column. Considering the fragility of the packed capillary columns, this must be considered as really convenient.

The three laboratory-made PEC devices described so far were all equipped with a post-injection splitting technique. Therefore, conventional HPLC injectors can be utilized in combination with this instrumental set-up.

In his earlier investigation of PEC Tsuda introduced laboratory-made PEC instruments that worked with a pre-injection split [11,36]. In order to avoid overloading of the separation systems, in this technique, microinjectors had to be used in combination with separation capillaries of relatively large inner diameters.

Hugener et al. [37] developed a laboratory-made PEC apparatus that also used a pre-injection splitting technique. The scheme of the apparatus is shown in Fig. 23. A syringe pump (1) was combined with a T-piece (3) to obtain an appropriate flow-rate. A guard column (2) filled with reversed-phase material was placed between the pump and the pre-injector split (3) to retain eluent impurities. The separation capillary (6) was connected to a microinjector (150 nl) (5), which was set to ground potential. The high-voltage power supply (8) was connected to the

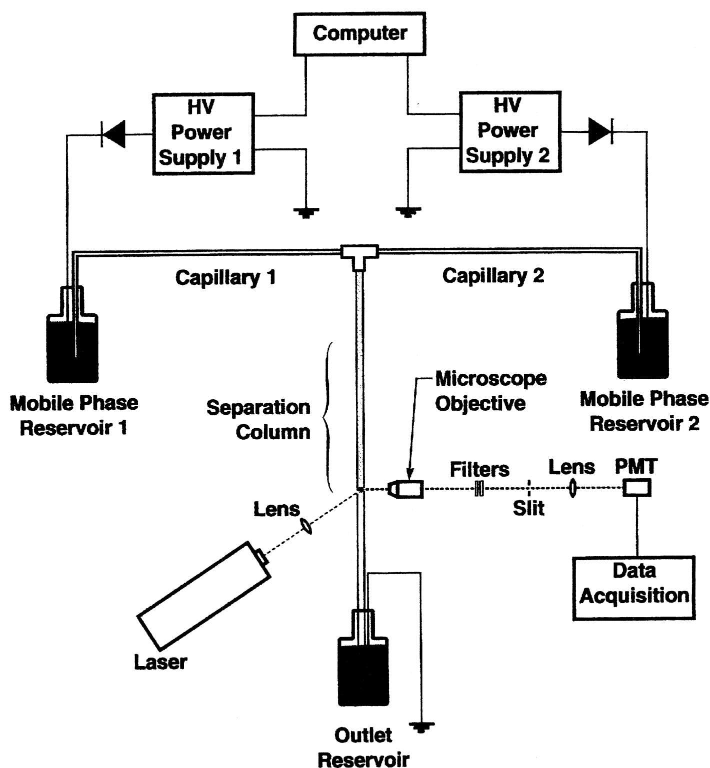

Fig. 17. Schematic of the solvent gradient elution CEC apparatus. From Ref. [31] with permission. Copyright (1996) American Chemical Society.

Valco zero dead volume union that joined the separation capillary with the detection capillary (see Fig. 24). The authors showed that charged analytes could be both accelerated and retarded depending on the sign of the voltage applied.

The first approach of gradient elution in pressure assisted electrochromatography was published by Behnke and Bayer [30]. The instrumentation used is shown in Fig. 25. A gradient mixer and a HPLC pump were combined with a modular CE system. A post-injection split technique was employed and a conventional HPLC six-port injector was used for sample introduction. The stainless steel T-piece used for splitting of eluent and sample was grounded to avoid damage to the pump. The electrolyte reservoir on the inlet side of the separation capillary was connected to the splitter by a laboratory-made interface which was not further specified. With this system injection was performed in two different ways. Firstly, the HPLC six-port injector was used and due to the splitter only a minor fraction of the sample entered the separation capillary. In another mode, the electrolyte reservoir on the inlet side of the separation capillary was filled with 5 μl of sample and pressurized for 15 s at 200 bar before the reservoir was flushed with eluent. For flushing, the CEC column had to be disconnected, since there was no valve for switching to a waste channel. The separation power of the laboratory-made instrument could be demonstrated by the analysis of an oligonucleotide mixture.

Compared to pure electrochromatography, PEC has several advantages. There are less problems with bubble formation resulting in an increased stability of the mobile phase flow. A further advantage is the fact that the separation capillaries can be operated at low pH values with reasonable mobile phase flow velocities. Finally, the combination of a pressure-driven flow with an electric field may yield accelerated speed of analysis.

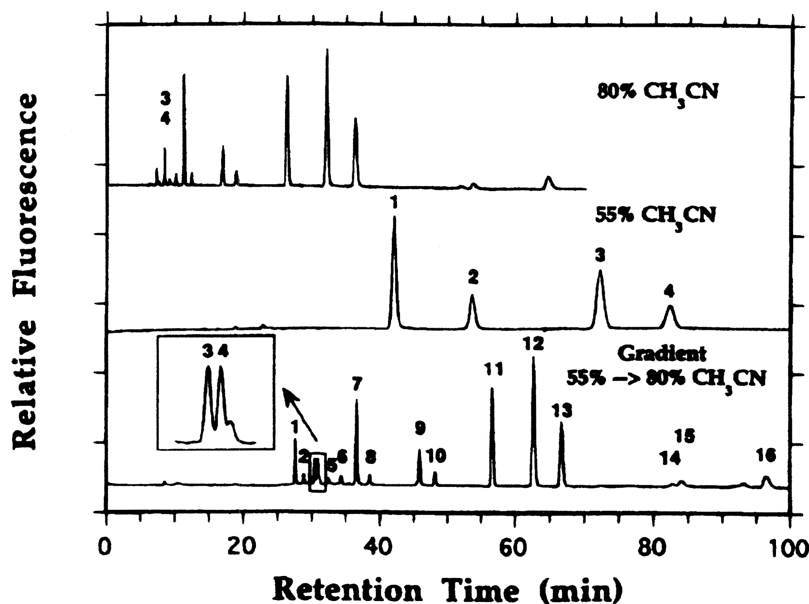

Fig. 18. Electrochromatograms showing the comparison of isocratic and gradient elution for the separation of 16 PAHs. The column dimensions were 26 cm packed length×75 μm I.D. The applied voltage for the isocratic separations was 20 kV. The buffer was 4 m*M* sodium tetraborate. The injection was performed electrokinetically at 5 kV for 5 s. From Ref. [31] with permission. Copyright (1996) American Chemical Society.

5. Miniaturization/capillary electrochromatography on a chip

Modern microfabrication technology permits the inexpensive mass production of miniaturized chemical separation systems. These microdevices are suitable for fast analysis of very small sample volumes (pl–nl) and high throughput analysis. Most research efforts up to now have been dedicated to electrically-driven separation systems, especially to capillary electrophoresis on microchips [38].

Open channel electrochromatography on a microchip has been reported by Jacobson et al. [39].The schematic of the high-voltage power supply in connection with the microchip is presented in Fig. 26. A glass made microchip substrate was chemically etched and the resulting channels (5.6 μm in height, 66 μm wide) were surface modified via chemical bonding of a reversed-phase coating. Detection was carried out on chip via laser-induced fluorescence with a photomultiplier tube (PMT) to collect the fluorescence signal. The geometry of the separation channel was serpentine like to reduce the chip size. A 171 mm long separation channel was located in an 8 mm×8 mm area.

Sample injection was performed in a ‘‘pinched sample loading’’ described previously [40]. During the injection procedure high voltage is applied to the analyte reservoir, the buffer reservoir and the waste reservoir, whereas the analyte waste reservoir is grounded. The amount of sample injected into the separation channel is defined by electroosmotic pumping of the buffer from the mobile phase reservoir and the waste reservoir towards the analyte waste reservoir. In separation mode, the analyte reservoir and the analyte waste reservoir were set at 57% of the potential applied to the buffer reservoir in order to prevent bleeding of the excess analyte. This led to a three-way flow that forced the excess analyte to move back into the analyte reservoir and the analyte waste reservoir.

Kutter et al. [41] could also transfer gradient techniques to a microchip. They separated four coumarin dyes in less than 20 s using solvent-programmed microchip open channel electrochromatography. A T-piece connected to the two eluent

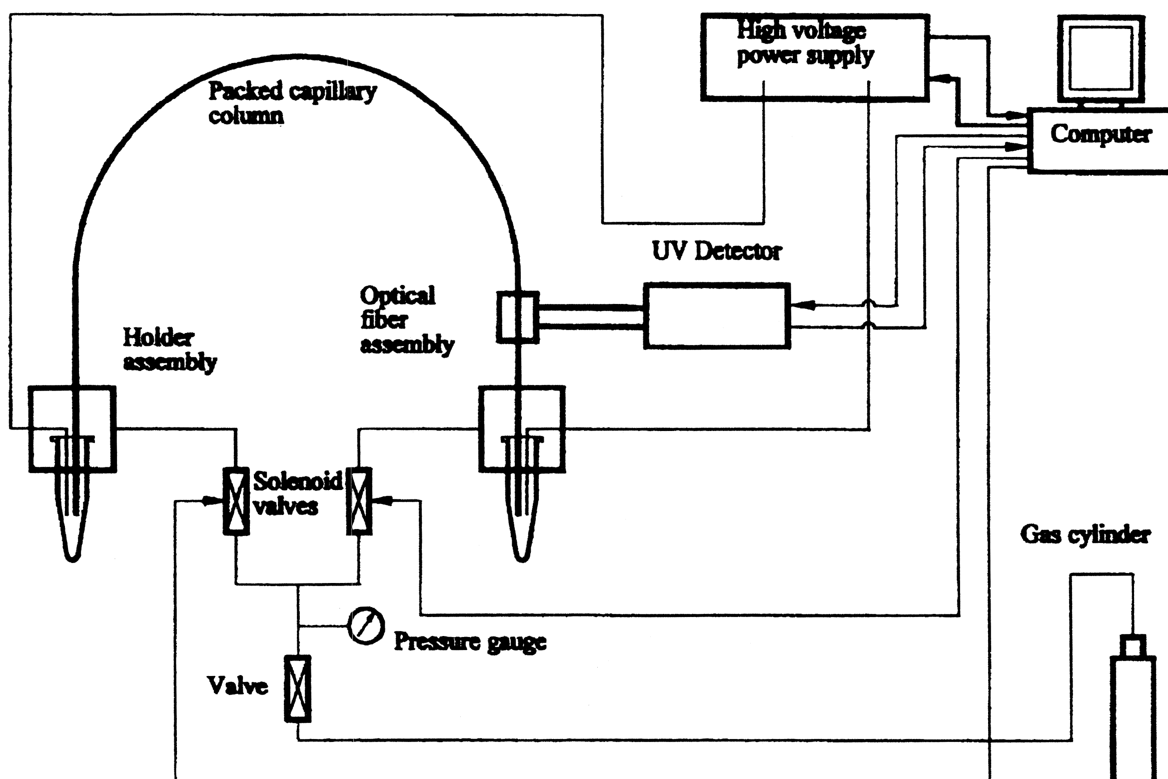

Fig. 19. Schematic diagram of the laboratory-built CEC system with voltage programming capability. From Ref. [33] with permission. Copyright (1999) Wiley.

reservoirs and the separation channel served as a micro-mixing chamber. Elution gradients were generated by computer-controlled application of voltages on the reservoirs of the microchip in a similar way as described by Yan et al. [31].

6. Detection in capillary electrochromatography

The quality of analytical separation techniques depends on both the separation efficiency and the detection sensitivity. In CEC, due to the low peak volumes and short optical path lengths, small volume and sensitive detection devices are required. In capillary electrophoresis, UV, fluorescence and conductivity detection are commercially available, besides coupling to mass spectroscopy. Since CEC can be operated on the same instruments as CE, the same detection principles are applied. Nevertheless, conductivity detection, which is mainly devoted to ion-exchange separations in HPLC and rarely used in CE, has not yet been described in combination with electrochromatography, so far.

6.1. UV detection

The great success of HPLC has driven the development of UV detection devices in terms of reliability and performance to a very high level. According to Beer–Lambert's law, the sensitivity in photometric detection is a function of the optical pathlength. Both in CE and CEC, on-column detection is used and thus the detection pathlength corresponds to the capillary I.D., usually 50–100 μm in CEC. This is an important drawback of capillary separation techniques compared with standard HPLC detection (usually 1 cm pathlength). Moreover, the incident light does not enter the detection zone of the capillary through a flat surface, which leads to light intensity loss. An improvement of the UV detection

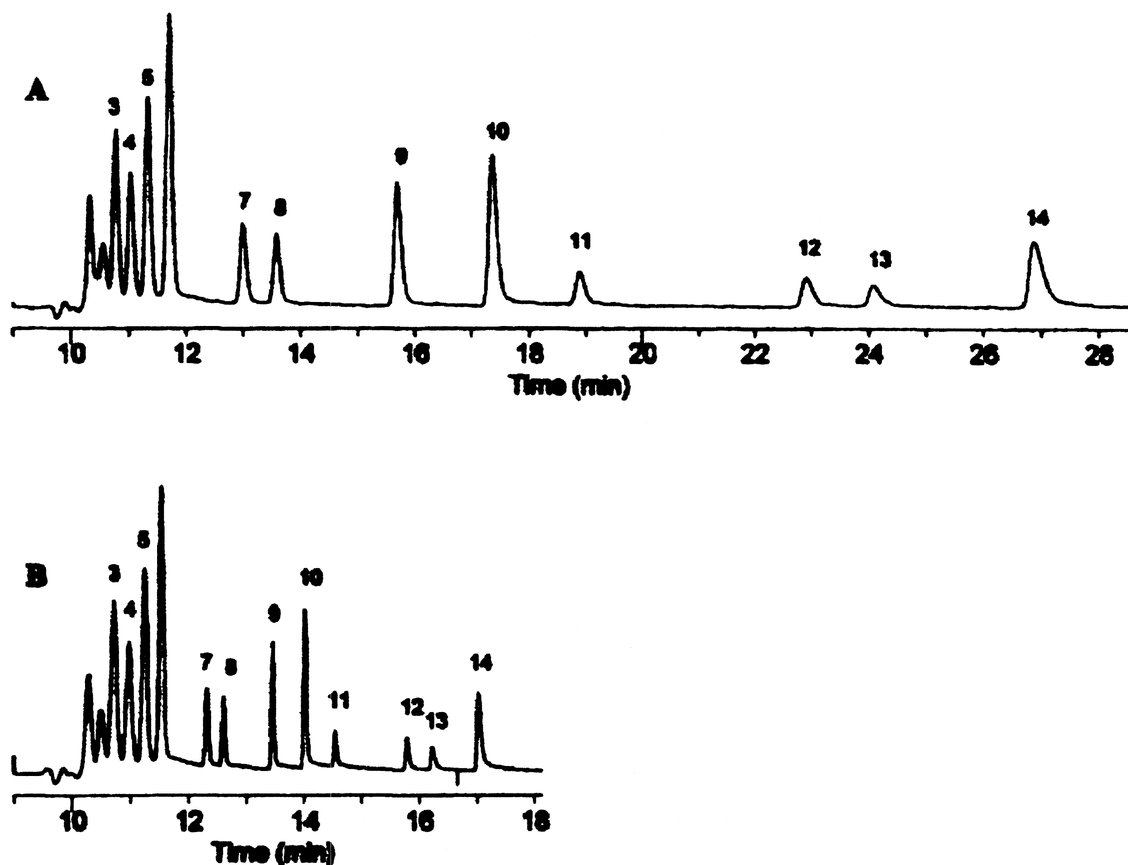

Fig. 20. Comparison of the analysis of PAHs (A) without and (B) with voltage programming. Conditions: 43 cm (effective length 35 cm)×50 μm I.D. fused-silica capillary column packed with 1.5-μm Micra nonporous ODS1 particles; 230 nm UV detection; (a) 15 kV applied voltage, (b) constant voltage at 15 kV for 11 min, then linearly programmed to 40 kV at a rate of 10 kV min^{-1}; 5 kV, 5 s electrokinetic injection; acetonitrile–Tris buffer (50 m*M*, pH 8.1) (80:20, v/v). Peak identifications: 1=acenaphthylene; 2=fluorene; 3=phenanthrene; 4=anthracene; 5=fluoranthene; 6=pyrene; 7=benz[*a*]anthracene; 8=chrysene; 9=benzo[*b*]fluoranthene; 10=benzo[*k*]fluoranthene, 11=benzo[*a*]pyrene; 12=dibenz[*a*,*h*]anthracene; 13=benzo[*ghi*]perylene; 14=indeno[1,2,3-*cd*]pyrene. From Ref. [33] with permission. Copyright (1999) Wiley.

sensitivity in electrochromatography has been reported by Dittmann et al. [16]. They applied a novel high-sensitivity detection cell (HSC) design (Fig. 27), initially introduced for capillary electrophoresis [42]. The packed capillary can be coupled to this cell, increasing the effective optical pathlength to 1.2 mm. The authors have demonstrated the improved detection performance with the separation of the US Environmental Protection Agency (EPA) 16 PAH standard. The same separation has been carried out primarily on the packed 100 μm capillary with on-column detection. Then the unpacked part with the detection window was cut off and the packed part was connected to the HSC. The peak areas could be increased by a factor of more than 10. Since the noise also increased with detection pathlength, the resulting improvement in signal-to-noise was approximately 7 to 8. Peak symmetry was not affected, whereas the column plate numbers differed only around 5% from their values of between 50 000 and 80 000 plates per column for the different PAHs. The increased cell volume seems not to affect system efficiency.

In narrow columns made from transparent materials as applied in CEC, it is possible to carry out UV detection through the stationary phase. This principle

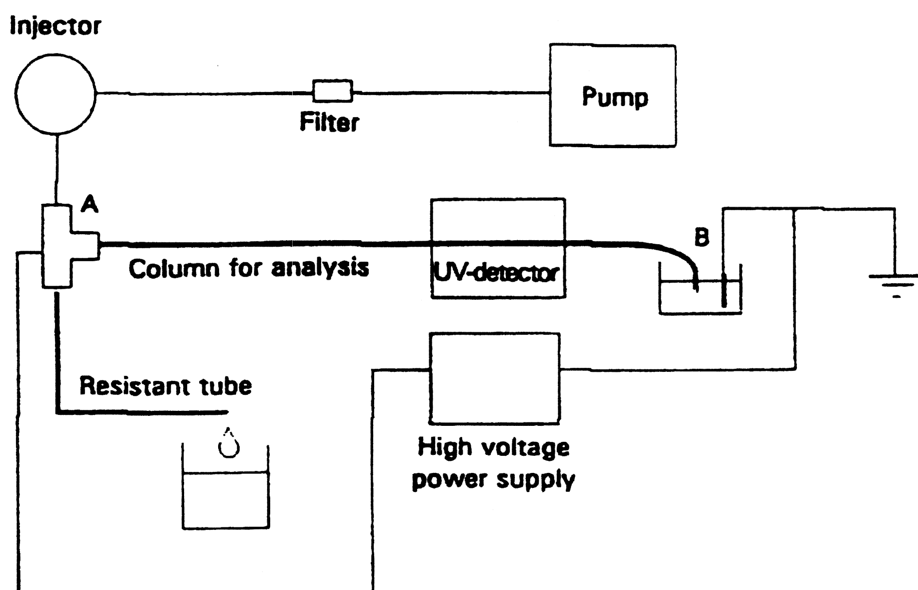

Fig. 21. Schematic diagram of the micro-electrochromatography system. From Ref. [34] with permission. Copyright (1994) Wiley.

is named "in column detection" and was first published by Chen and Horváth [43].

The performance of in-column photometric detection (ICPD) versus on-column photometric detection (OCPD) in CEC has been discussed by Banholczer and Pyell [44]. According to the authors, ICPD means the measurement of UV absorbance across a part of the packed capillary, whereas on-column detection means the common principle of detecting through the unpacked part behind the end frit. A simple laboratory-made instrumentation with no pressurization facility or temperature control has been used to carry out the experimental studies. Some preliminary photometric studies had been made to assess photometric detection in a slurry. A quartz cell of 1.0 mm was filled with a slurry prepared from stationary and mobile phase and measured in a spectral photometer. Due to light scattering phenomena the apparent absorption at 300 nm was 4.8 absorbance units (AU) and the slurry appeared non transparent. On the other hand, ICPD was performed successfully applying a capillary of 180 μm I.D. packed with the same stationary phase. The resulting baseline noise in ICPD was twice that of OCPD. Nevertheless, the limit of detection was found to be lower in ICPD, when a retention factor of 1.9 is exceeded. The reason for this is the signal enhancement due to the enrichment of solute in the stationary phase compared with the eluted zone passing through the detection window behind the end frit. The linear working range for the determination of alkyl benzoates exceeded two-orders of magnitude and was the same as in OCPD. The precision in ICPD was found to be poorer. For 10 repeated injections RSD values of 0.8 to 2.8% were calculated instead of 0.7 to 1.3% for OCPD.

6.2. Fluorescence detection

An alternative to UV detection in CEC is fluorescence detection. An advantage of fluorescence detection is the increased sensitivity compared to UV detection. But it has to be taken into account that fluorescence detection is limited to certain analytes. Rebscher and Pyell [45] compared in-column and on-column fluorescence detection in CEC. The apparatus used for this investigation was laboratory-made and equipped with a commercial spectrofluorimetric detector, which was modified for capillary applications. In comparison to on-column detection, with in-column fluorescence detection, an enhanced signal could be obtained and the enhancement factor

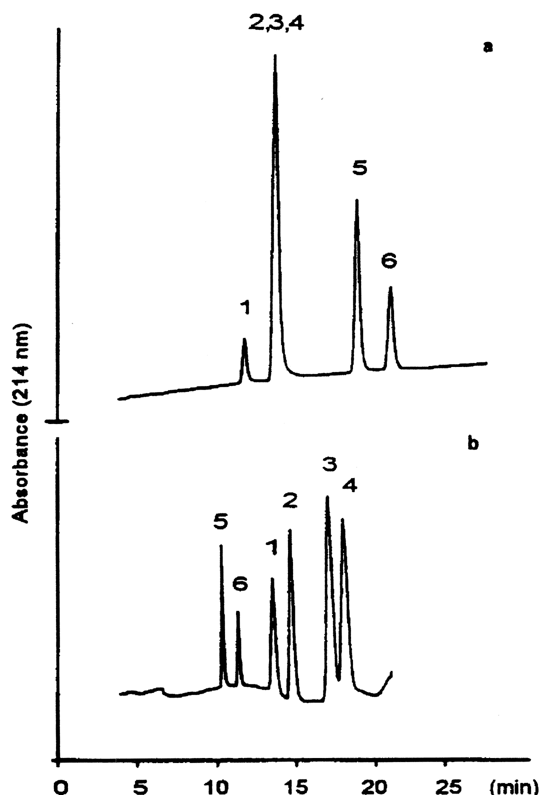

Fig. 22. Separation of carboxylic acids and hydrophobic compounds with capillary LC (a) and PEC (b); analytes in chromatogram (a) and (b): folic acid (1), *p*-hydroxybenzoic acid (2), acetylsalicylic acid (3), nicotinic acid (4), thiourea (5) and nicotinamide (6). Concentration of analytes: 0.5–1 mg ml^{-1}; 0.01 a.u.f.s. Capillary column: 150 mm×0.1 mm I.D. with Nucleosil 100 3-C_{18} (3 μm); mobile phase: methanol–20 mmol l^{-1} disodium tetraborate (pH 8.5) (75:25, v/v); pressure: 63 bar (a, b); field strength for (b): 21.4 kV m^{-1} (−6 kV). From Ref. [35] with permission. Copyright (1995) Springer.

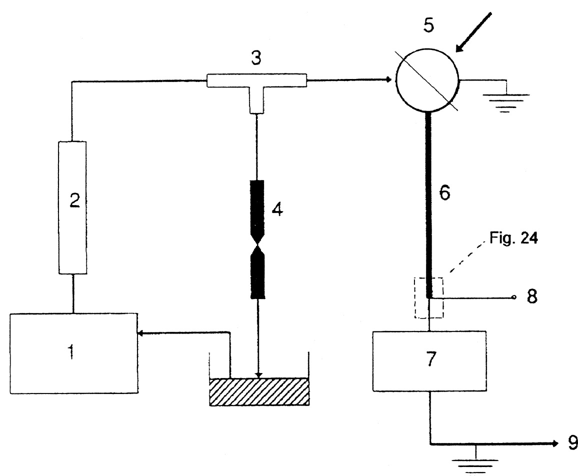

Fig. 23. Scheme of the PEC system. 1=Syringe pump; 2=1-cm guard column filled with 5-μm ODS stationary phase; 3=preinjector split; 4=restrictor; 5=150-nl microinjector; 6=microcolumn; 7=UV detector; 8=high voltage; 9=electrospray mass spectrometer. From Ref. [37] with permission.

could be calculated by means of a derived equation. Due to light scattering from the packing particles, the baseline noise was higher for in-column detection. Thus, in the case of in-column fluorimetric detection, lower limits of detection were only accessible if the signal enhancement could overcompensate the increased baseline noise compared to on-column detection.

6.2.1. Laser-induced fluorescence detection

Dadoo et al. [32,46] reached sub-attomole detection limits for polycyclic aromatic hydrocarbons with laser-induced fluorescence (LIF) detection. The CEC instrument used was essentially the same as described by Yan et al. [22], but the UV detector was replaced by a LIF detection system (see Fig. 28).

The excitation of the PAHs was achieved using the 257 nm line from an intracavity-doubled argon ion laser. An arrangement consisting of a prism, an aperture and a focusing lens removed the 514 nm light and focused the 257 nm line onto the separation capillary. The emitted fluorescence light was collected by means of a high numerical-aperture microscope objective and sent to a photomultiplier tube connected to a lock-in amplifier that was combined with a chopper in the path of the laser beam. Thus, the output of the photomultiplier tube could be measured. In order to avoid interference of the fluorescence signal with scattered excitation light, a set of optical filters and a variable slit were mounted between the microscope objective and the photomultiplier tube. Dadoo et al. investigated two different filter systems. A 70 nm bandpass filter centered at 400 nm was compared with a 280 nm longpass filter in combination with a 600 nm shortpass filter. It could be shown that better limits of detection were reached for most PAHs when the bandpass filter was employed. Reproducibility studies showed that the variation in the peak heights was less than 5%. The

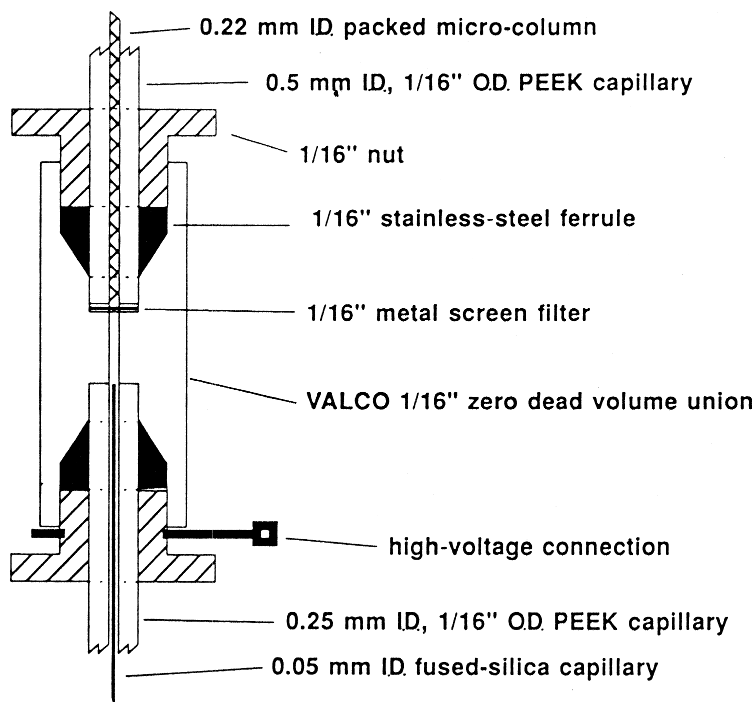

Fig. 24. Connection between microcolumn and detector (″=in.; 1 in.=2.54 cm). From Ref. [37] with permission.

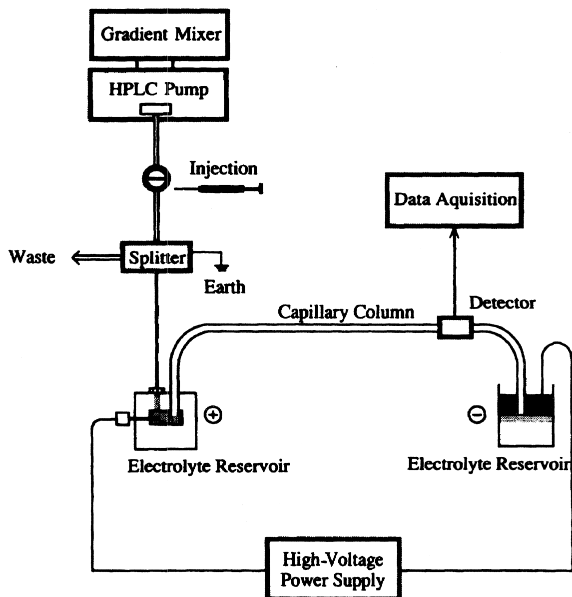

Fig. 25. Schematic representation of an electrochromatographic system. From Ref. [30] with permission.

signal for benzo[*k*]fluoranthene was linear over four orders of magnitude.

Bruin et al. [47] also applied a laser-induced detection system in their fundamental studies on electrically-driven open tubular liquid chromatography. According to the demands of the analytes, an argon ion laser or a helium–cadmium laser could be used as light sources. A more detailed description of the same LIF unit was published by Tock et al. [48]. The helium–cadmium laser beam was sent through a 325 nm bandpass filter and focussed onto the separation capillary by a quartz lens. The emitted light was collected by a fresnel lens and a photomultiplier tube perpendicular to the excitation beam.

7. Coupling of capillary electrochromatography to mass spectrometry

On-line coupling of analytical separations with spectroscopic procedures in order to obtain an orthogonal analytical system is the focus of interest for many years. Since the introduction of electrospray

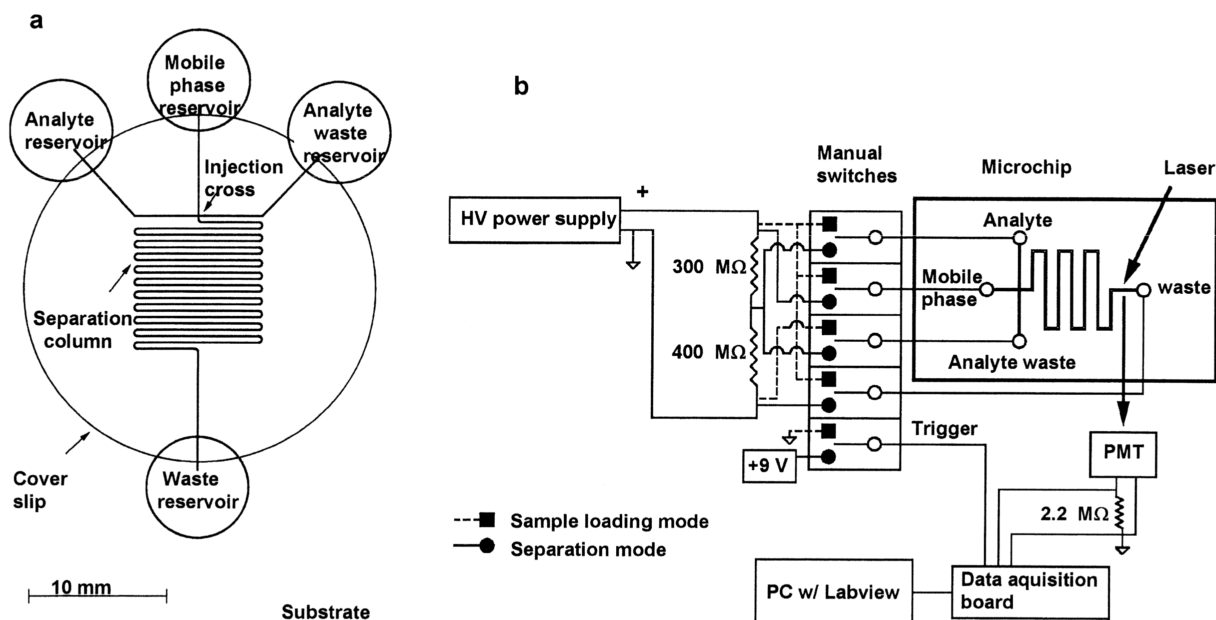

Fig. 26. (a) Schematic of the microchip with a serpentine column geometry. (b) Diagram of the high-voltage switching apparatus and detection/data acquisition system. From Ref. [39] with permission. Copyright (1994) American Chemical Society.

ionization (ESI) and the corresponding interfaces, the coupling of HPLC to mass spectroscopy (MS) has reached the status of a well established routine method. Although the flow-rate range of capillary separation techniques must be regarded as ideal for electrospray ionization, LC–MS interfacing cannot be simply used if the solvent is electrically driven. The reasons are the required current circuit while no outlet buffer vial is present and incompatibility of ESI with the mostly nonvolatile buffers commonly applied in CZE and CEC. The coupling of CE to MS by ESI interfacing was introduced by Olivares et al. in 1987 [49] and has undergone a rapid development in the early 1990s [50–53] but is not yet generally accepted as a routine method. An approach to the coupling of open tubular electrochromatography to ESI-MS–MS has been reported by Jakubetz et al. in 1996 [54]. They applied a Prince CE system coupled to a Sciex API II triple stage quadrupole mass spectrometer for feasibility studies on miniaturized enantiomeric separation.

The combination of pure, non pressure-assisted packed column electrochromatography with MS has been described by Gordon et al. [55]. They have coupled an ISCO model 3140 electropherograph to a Kratos Concept II HH mass spectrometer. A continuous flow fast atom bombardment (CF-FAB) ionization was used to interface both instruments. The authors considered CEC–MS as follows: "The technique is a viable alternative to micellar electrokinetic capillary chromatography (MECC) for the separation of neutral compounds, surmounting the problems in coupling this method to MS, whilst retaining its major virtues. These include the advantages of using capillaries and the ability to deal with extremely small sample quantities, but in addition CEC offers higher loadability when required, and would seem to offer considerable potential". Lord et al. published 1 year later the combination of the same CE instrument with a VG quadrupole MS via an electrospray interface [56] for CEC analysis of textile dyes. For generating the electrospray, they linked the separation column via a commercial co-axial interface probe. It comprised two concentric stainless steel capillaries, each one fitted into a separate stainless steel T-piece. To provide a make-up flow, a methanol–water (1:1) solution containing 1% acetic acid was delivered at a flow-rate of 10 μl min^{-1} by a microgradient syringe pump and was incorporated via the capillary at the first T-piece. The outer

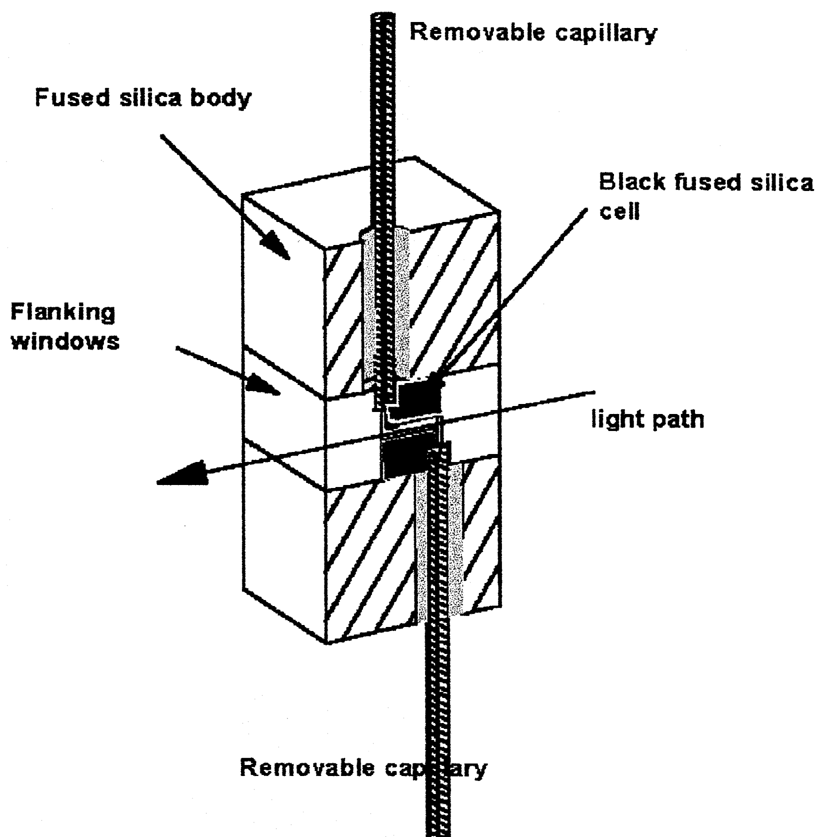

Fig. 27. Schematic drawing of the high-sensitivity cell (HSC). From Ref. [16] with permission. Copyright (1997) ISC Technical Publications.

capillary supplied nitrogen gas via the second T-piece for nebulization. To initiate the electrospray process, voltage of 4 kV was applied to both T-pieces. A similar instrumental set-up has been applied by Lane and co-workers [57,58]. They have also coupled the ISCO instrument to a VG Platform ESI-MS for applications in pharmaceutical analysis. Unlike Gordon et al., they delivered the sheath flow by a HP 1050 HPLC pump with split from a microflow processor and used 0.3% formic acid as make-up additive.

Verheij et al. [12] had reported for the first time the coupling of pseudo-electrochromatography to mass spectrometry in 1991, before MS combined with pure CEC was introduced. They applied a CF-FAB ion source like Gordon et al. [55]. Further papers on the coupling of pseudo-electrochromatography with ESI-MS [59] and ESI-MS–MS [37] followed from the same group. In both cases the electrospray needle was set to ground potential and a sheath flow was applied.

Another approach to combine PEC with MS–MS without applying a sheath flow has been reported by Schmeer et al. [60] in 1995. Their laboratory-made electrochromatographic instrumentation with pressurization facility was coupled to a Sciex API III triple-quadrupole spectrometer. A packed 100 μm I.D. capillary was inserted into the ESI steel needle, where a nitrogen flow and a voltage of 4.8 kV was applied in order to generate the electrospray. Obviously, the current circuit was closed via the ground of both power supplies (CE and ESI voltages). The chromatographic flow was enhanced by a supplementary pressure gradient of 180 bar resulting in a flow-rate of 1–2 μl min^{-1}, almost ideal for ESI-MS. The system has been applied for peptide analysis.

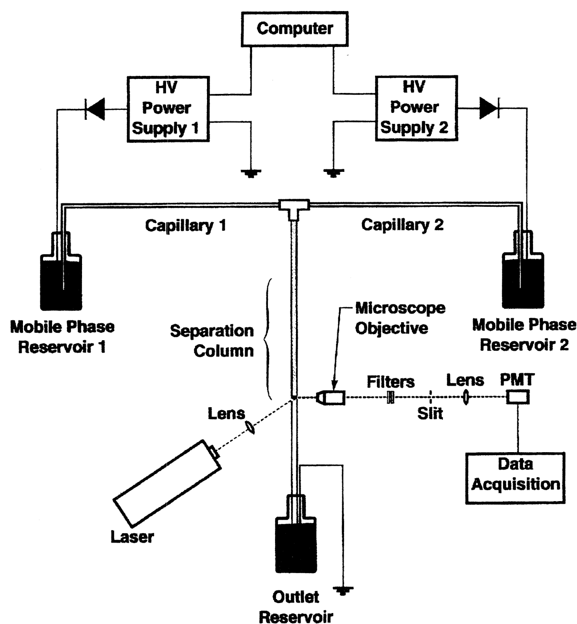

Fig. 28. Schematic of the CEC–LIF apparatus. From Ref. [31] with permission. Copyright (1996) American Chemical Society.

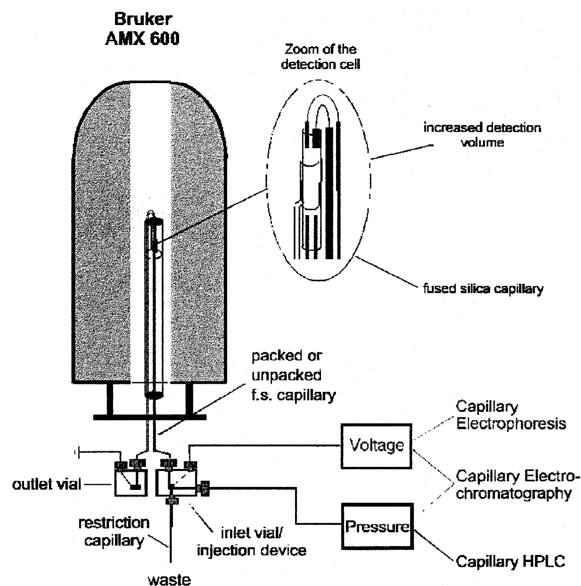

Fig. 29. Coupling of capillary separation techniques with NMR. From Ref. [63] with permission. Copyright (1999) American Chemical Society.

In a more recent paper, Bayer et al. [61] presented a new ionization technique for PEC coupled to MS. The method is named coordination ion spray (CIS). They introduced cations like Li^+ or Ag^+ dissolved in a sheath liquid to form either negatively or positively charged complexes with the analytes and applied a nebulizer gas. Provided that the ion source fulfils the requirements for CIS which are specified in the paper, the ESI voltage can be switched off.

8. Coupling of capillary electrochromatography to NMR spectrometry

Bayer and co-workers [62,63] developed a further coupling device for the combination of CZE, μ-HPLC and CEC with nuclear magnetic resonance spectrometry. The authors stated, that due to the higher sample capacity of CEC compared to CZE and due to higher separation efficiencies and shorter analysis times compared to μ-HPLC, CEC seems to be very promising for coupling to NMR spectrometry.

The laboratory-made apparatus used for the separation is, as far as the high-voltage power supply and HPLC gradient system are concerned, the same as already presented in Fig. 25. The coupling of this apparatus to a 600 MHz NMR spectrometer is shown schematically in Fig. 29.

Due to the relatively high limits of detection in NMR spectrometry, a detection capillary with an enlarged detection cell had to be used. The detection capillary was connected to the separation capillary via PTFE tubing and placed parallel to the magnetic field in order to avoid any effects by the current induced magnetic field on the original magnetic field from the spectrometer coil.

9. Outlook

The application of CEC and related techniques in routine analysis is generally limited due to the lack of appropriate instrumentation. The adaptation of CE instruments to CEC is mainly hindered by the unfulfilled need for gradient elution required for HPLC-like separations. Column technology is restricted by the length of capillaries fitting into the CE cartridges and the means necessary to immobilize the

packed bed in the capillary via frits or similar technologies.

At the present time, with our knowledge on the acceptance and the potential of CEC hardly any instrument company will invest in developing dedicated instruments for CEC. Nevertheless, at the HPCE '99 conference exhibition in Palm Springs, the Micro-Tech Scientific Company presented the Ultra-Plus II Capillary LC, CE & CEC Integrated System. Moreover, the Unimicro Technologies Inc. introduced their Trisep CEC apparatus, to fulfill the application of the three different separation modes. Both instruments must be quoted as dedicated devices for CEC, providing most of the features discussed above.

Yet it is not clear whether such instruments can really enter the market and improve the situation for CEC as a routine method. Even though, they provide a potential to break the existing vicious circle described in the following. On the one hand, instrument companies accuse the lack of relevant applications for CEC and, on the other hand, possible CEC or PEC routine analysis tasks accuse the lack of optimized dedicated instrumentation.

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